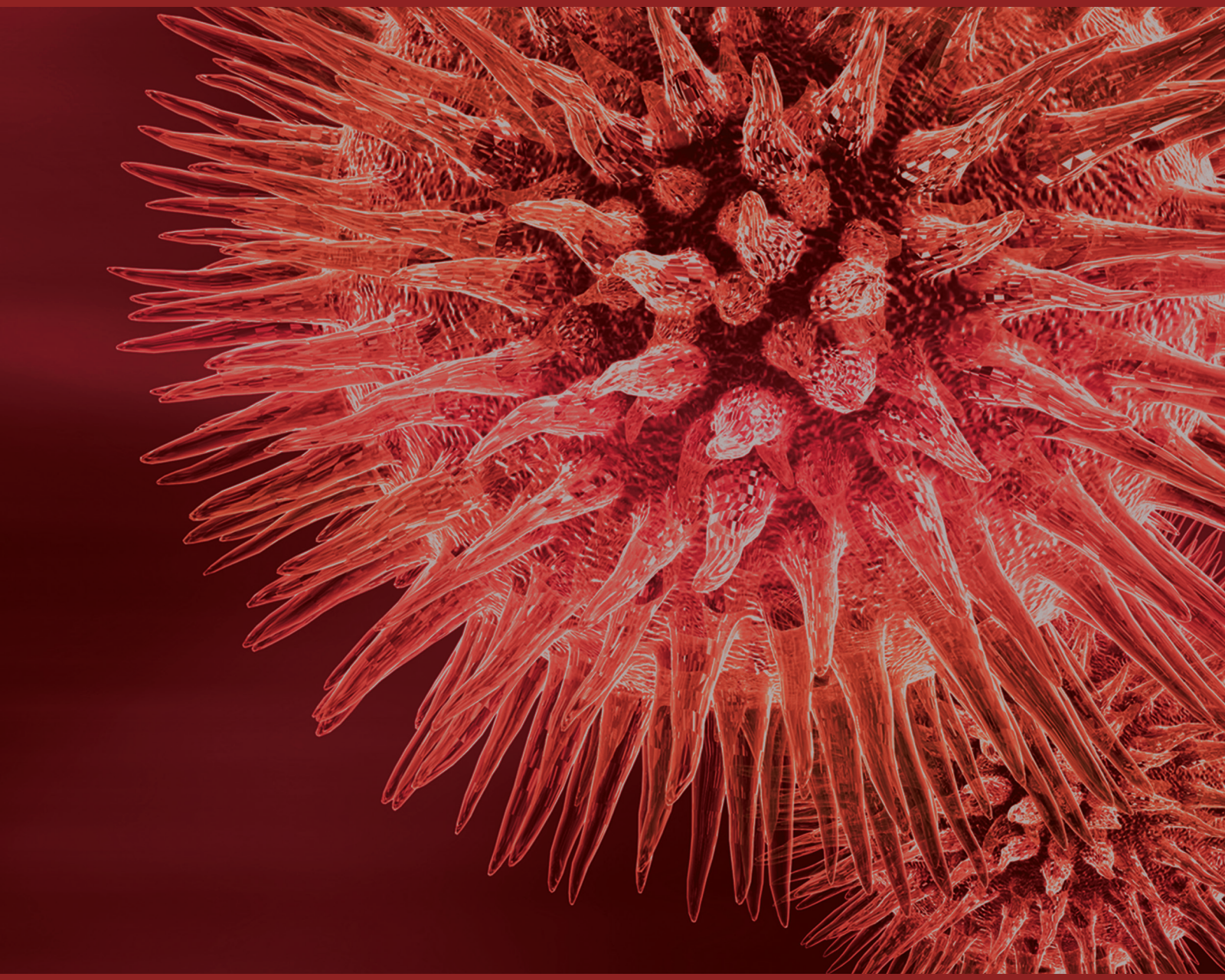
BioMed Research International

Hijacking of Endocrine and Metabolic Regulation in Cancer and Diabetes

Guest Editors: Eileen M. McGowan, Ann Simpson, James McManaman, Viroj Boonyaratanakornkit, and Anandwardhan A. Hardikar



Hijacking of Endocrine and Metabolic Regulation in Cancer and Diabetes

BioMed Research International

Hijacking of Endocrine and Metabolic Regulation in Cancer and Diabetes

Guest Editors: Eileen M. McGowan, Ann Simpson, James McManaman, Viroj Boonyaratanakornkit, and Anandwardhan A. Hardikar

Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 386203, 2 pages
http://dx.doi.org/10.1155/2015/386203

Editorial

Hijacking of Endocrine and Metabolic Regulation in Cancer and Diabetes

Eileen M. McGowan,[1] Ann Simpson,[1] James McManaman,[2] Viroj Boonyaratanakornkit,[3] and Anandwardhan A. Hardikar[4]

[1] *Medical and Molecular Biosciences, University of Technology Sydney, Sydney, NSW 2007, Australia*
[2] *Division of Basic Reproductive Sciences, Graduate Program in Integrative Physiology, University of Colorado School of Medicine, Denver, CO 80045, USA*
[3] *Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, 154 Rama I Road, Wangmai, Pathumwan, Bangkok 10330, Thailand*
[4] *NHMRC Clinical Trials Centre, University of Sydney, NSW 2006, Australia*

Correspondence should be addressed to Eileen M. McGowan; eileen.mcgowan@uts.edu.au

Received 31 December 2014; Accepted 31 December 2014

This issue features articles related to endocrine and metabolic regulation in cancer and diabetes. The modern diet, rich in fat and refined carbohydrates, is creating worldwide obesity and diabetes epidemics. As early as 1959, Joslin and colleagues speculated about an association between diabetes and cancer, and current epidemiologic evidence indicates an increased risk of various types of cancer, with increased morbidity, in diabetes patients. The underlying mechanisms for this association have not been fully elucidated as yet.

Cancer, diabetes, and obesity are all chronic diseases with common discernible alterations in metabolic processes. A sweet tooth and alterations in the glucose metabolism are common underlying themes. As scientists uncover more evidence to support this idea, the current research focuses on preventative strategies and treatments targeting disturbances in common cellular metabolic pathways. Some of the drugs used clinically to treat diabetes have been shown to have antitumour effects, as highlighted in the review of N. Turner et al. (University of New South Wales, Australia).

However, targeted treatments are not so easily implemented and have some inherent complexities, which derive from the possibility that some diabetes treatments may alter cancer risk. Alternatively, some cancer treatments may be incompatible with diabetes therapies. Thiazolidinediones and metformin are two common diabetes treatments which show promise as cancer therapeutics. In this edition, E. Fröhlich and R. Wahl (University of Tuebingen, Germany) discuss thiazolidinediones as treatments for multiple cancer types whilst the review by D. Hatoum and E. M. McGowan (University of Technology Sydney, Australia) summarizes recent advances in the use of metformin in comorbidity of diabetes and breast cancer. Although the *in vitro* evidence is compelling, retrospective evidence for comorbidity therapy is controversial. As another option, contemporary research directed at targeting the sphingolipid rheostat holds great promise for diabetes and cancer therapy. The paper by K. Marzec et al. (Kolling Institute, Sydney, Australia) strongly supports a role for targeting sphingosine kinase 1 (SphK1) in combinational treatments for the hard to manage triple negative breast cancers (TNBC), whereas the corresponding paper by N. K. Haass et al. (University of Queensland/University of Technology Sydney, Australia) focuses on the complexities inherent in the use of sphingosine kinase/sphingosine-1-phosphate modulators in the treatment of comorbidity of cancer and diabetes.

It is becoming clear that obesity and cancer is not a random association. A. J. Hoy et al. (University of Sydney, Australia) emphasize that key pathways of fatty acid metabolism are altered in cancer. Associations between obesity, ovarian steroid hormones, and cancer are common themes in two

complementary papers by V. Boonyaratanakornkit and P. Pateetin (Chulalongkorn University, Thailand) and K. H. Joung et al. (Chungnam National School of Medicine, North Korea). These papers describe the strong prevalence of cancer in postmenopausal obese women. V. Boonyaratanakornkit and P. Pateetin raise the importance of developing a better understanding of the molecular mechanisms and signalling pathways associated with alterations in endocrine and metabolic regulation in obese women for improved breast cancer treatment.

IGF-1 and insulin are strongly associated with most diabetes related cancers. Several of the papers in this issue focus on the importance of the insulin-like growth factors (IGFs) as novel targets for cancer therapies. K. Marzec et al. concentrate on IGF binding protein 3 (IGFP-3) as a critical target for TNBCs. The original paper authored by the R. Pietras group (UCLA, USA) showed that elevated IGF-2 was concomitant with stimulated estrogen receptor-β (ERβ) and poor overall survival. Furthermore, the paper by H. S. Atreya et al. (Indian Institute of Science, India) advanced the concept of blocking IGF and IGFBPs in cancer therapies.

The scope of this special issue highlights the growing awareness of the link between altered cellular metabolism and the risk of developing diabetes and cancer. Understanding and targeting altered cellular metabolism in individuals with or without diabetes, so as to reduce the risk of developing cancer, form the basis for future combinational treatments.

Acknowledgments

Thanks are due to all the authors for their contributions in this special issue. We would especially like to acknowledge Dr. Lee Goodglick for his 25 years of dedication to cancer research. Dr. Goodglick recently passed away of pancreatic cancer during the preparation of this special issue.

Eileen M. McGowan
Ann Simpson
James McManaman
Viroj Boonyaratanakornkit
Anandwardhan A. Hardikar

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 638526, 8 pages
http://dx.doi.org/10.1155/2015/638526

Review Article

Targeting Insulin-Like Growth Factor Binding Protein-3 Signaling in Triple-Negative Breast Cancer

Kamila A. Marzec, Robert C. Baxter, and Janet L. Martin

Hormones and Cancer Division, Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, St Leonards, NSW 2065, Australia

Correspondence should be addressed to Janet L. Martin; janet.martin@sydney.edu.au

Received 20 July 2014; Accepted 29 September 2014

Academic Editor: Eileen M. McGowan

Insulin-like growth factor binding protein-3 (IGFBP-3) is a key regulatory molecule of the IGF axis and can function in a tissue-specific way as both a tumor suppressor and promoter. Triple-negative breast cancer (TNBC) has high tumor expression of IGFBP-3 associated with markers of poor prognosis and, although accounting for 15–20% of all breast cancers, is responsible for disproportionate rates of morbidity and mortality. Because they lack estrogen and progesterone receptors and overexpression of HER2, TNBC are resistant to treatments that target these molecules, making the development of new therapies an important goal. In addition to frequent high expression of IGFBP-3, these tumors also express EGFR highly, but targeting EGFR signaling alone in TNBC has been of little success. Identification of a functional growth-stimulatory interaction between EGFR and IGFBP-3 signaling prompted investigation into cotargeting these pathways as a novel therapy for TNBC. This involves inhibition of both EGFR kinase activity and a mediator of IGFBP-3's stimulatory bioactivity, sphingosine kinase-1 (SphK1), and has shown promise in a preclinical setting. Functional interaction between EGFR and IGFBP-3 may also promote chemoresistance in TNBC, and delineating the mechanisms involved may identify additional targets for development of therapies in cancers that express both IGFBP-3 and EGFR.

1. An Introduction to the Insulin-Like Growth Factor Binding Proteins in Cancer

The insulin-like growth factor (IGF) system is fundamental to normal growth and development, and by virtue of their potent proliferative and antiapoptotic effects, the polypeptide hormones IGF-I and IGF-II have also been shown to play an important role in tumorigenesis. The IGF binding proteins (IGFBPs) are key regulatory molecules of the IGF system, and aberrations in their expression or function have been associated with a range of cancers [1]. The IGFBP family comprises six IGFBPs (IGFBP-1 to IGFBP-6) that bind the IGFs with high affinity. They vary in length, ranging from 216 amino acids to 289 amino acids [2], and each consists of three regions: the highly conserved N-terminal and C-terminal domains which contain binding sites for the IGFs and the variable central or linker domain, which is also the region most commonly subject to posttranslational modification such as glycosylation, phosphorylation, and limited proteolysis and probably contributes to differences in IGFBP function [2].

The IGFBPs were first identified for their function as serum proteins that bind IGF-I and IGF-II, thereby extending the circulating half-life of IGFs from minutes to hours, regulating the hypoglycaemic potential of IGF-I and IGF-II, and controlling extravasation of the growth factors to target tissues [1]. IGFBPs also act in the pericellular environment to regulate IGF/IGF-receptor interaction, because the affinity of the IGFs for IGFBPs is similar to that for the IGF and insulin receptors [3]. This is important in the context of cancer, because IGF activation of these receptors elicits mitogenic and survival signals that promote tumor growth.

IGFBP-3 is responsible for carrying the vast majority of IGFs in the blood and is the most abundant circulating IGFBP. In this environment its role is clear: together with the acid-labile subunit (ALS), it stabilizes IGF-I and IGF-II in ternary complexes that have very slow dissociation rates and therefore long circulating half-lives [4, 5]. Release of

bioavailable IGFs from these complexes is generally thought to result from limited proteolytic cleavage of IGFBP-3, which reduces its binding affinity for IGFs [6–8].

IGFBP-3 is expressed by most tissues of the body and as an antagonist of IGF binding to the signal-transducing type 1 IGF receptor (IGF1R), it blocks the proliferative and cell-survival effects elicited by its activation [3]. Consistent with this, loss of IGFBP-3 expression and consequent derepression of IGF1R signaling have been suggested to account for acquired resistance to the EGFR tyrosine kinase inhibitor gefitinib [9]. *In vitro* studies in breast cancer cells have also indicated that the efficacy of some anticancer agents, including retinoic acid, antiestrogens, and tumor necrosis factor-alpha (TNFα), is in part mediated by IGFBP-3 [10–13]. Not all of these effects depend solely on inhibition of IGF action, and "IGF-independent" growth inhibitory or apoptotic effects of IGFBP-3 have been attributed to a variety of mechanisms, including its interaction with nuclear hormone receptors such as retinoid X receptor [14, 15] and the vitamin D receptor [16], TGFβ/SMAD signaling pathways [17, 18], and upregulation of apoptotic effectors [19].

By contrast with these inhibitory and apoptotic effects of IGFBP-3, however, it appears that in some tissues IGFBP-3 functions as a tumor promoter as it is associated with poor patient outcomes. Overexpression of IGFBP-3 has been shown in renal clear cell carcinoma [20] and head and neck squamous cancers [21], and expression is higher in primary tumor than adjacent normal tissue or benign disease tissue in pancreatic ductal adenocarcinoma [22] and oesophageal cancer [23]. There is also evidence of IGFBP-3 being associated with metastatic disease, with IGFBP-3 expression elevated in metastatic tissue compared with primary tumor in melanoma [24], and higher in metastatic than nonmetastatic tumors in pancreatic endocrine neoplasms [25]. However, perhaps the earliest and best-documented association of IGFBP-3 with poor patient outcome is in breast cancer.

2. IGFBP-3 Is Highly Expressed in Aggressive Breast Cancer

Early studies investigating the expression of IGFBP-3 in breast cancer cell lines revealed a negative correlation between expression of estrogen receptor (ER) and IGFBP-3 [26, 27]. This is also seen in breast tumor tissue where many, though not all, studies have shown that expression of IGFBP-3 mRNA and protein is higher in ER-negative tumors compared to ER-positive tumors [28–31]. The clinical significance of these findings was underscored by two independent groups showing that high expression of IGFBP-3 in breast tumor tissue is associated with markers of aggressiveness and poor prognosis, including ER- and progesterone receptor- (PR-) negativity, high S-phase fraction, and aneuploidy [32, 33]. Other studies showed that while there was no significant association of high tissue IGFBP-3 protein levels with breast cancer recurrence, long-term survival was reduced [34, 35].

Both cell culture and xenograft tumor models have shown that overexpression of IGFBP-3 can indeed result in enhanced growth of breast cancer cells. T47D, an ER-positive breast cancer cell line that normally expresses low IGFBP-3, was initially growth-inhibited *in vitro* when IGFBP-3 was expressed ectopically but eventually developed resistance to its inhibitory effects and grew faster than vector-transfected cells [36]. This was reiterated *in vivo* using IGFBP-3-expressing T47D cells to establish tumors in nude mice [37], where it was found that IGFBP-3-expressing T47D tumors grew faster and larger compared to those that did not express the protein.

3. Mechanisms Underlying IGFBP-3's Growth-Stimulatory Actions

It seems counterintuitive that IGFBP-3, a protein that has been shown in numerous breast cancer cell studies to be growth inhibitory and proapoptotic, is associated with aggressive forms of breast cancer. However, growth-stimulatory effects of IGFBP-3 have been documented in many cell types and contexts, and a variety of mechanisms, both IGF-dependent and -independent, have been described as underlying this bioactivity. Studies in fibroblasts and mammary epithelial cells indicated that IGFBP-3 can potentiate the actions of IGFs [38–40], possibly through modulation of IGF1R activation and AKT signaling pathways [39, 40]. A "switch" in the actions of IGFBP-3 from apoptotic to antiapoptotic was shown to be dependent on the presence of matrix components, with exogenous IGFBP-3 promoting breast cancer cell survival in the presence of fibronectin, but accentuating apoptosis triggered by ceramide in the absence of fibronectin [41]. Similarly, dual effects of IGFBP-3 as both pro- and antiapoptotic molecule have been reported in human umbilical vein endothelial cells (HUVEC), with IGFBP-3 potentiating apoptosis in the presence of doxorubicin, but promoting cell survival in its absence [42]. This was shown to correlate with IGFBP-3 differentially regulating proapoptotic ceramide production by the HUVECs, with IGFBP-3 stimulating ceramide production in the presence of doxorubicin, but reducing it in the absence of doxorubicin [42].

Investigation into possible mechanisms underlying the development of resistance to IGFBP-3's growth inhibitory effects in breast cells and pathways involved in its tumorigenic bioactivity revealed that phenotypically normal MCF-10A breast epithelial cells are growth-inhibited by exogenous IGFBP-3 but become refractory to its inhibitory effects when cells express HRas, an oncogenic form of the Ras protein which is constitutively active [43]. Hs578T breast cancer cells, which also express HRas, were similarly resistant to the inhibitory effects of IGFBP-3 but were resensitized to it in the presence of PD98059, an inhibitor of p44/42MAPK [43].

Although activating Ras mutations occur relatively rarely (<5%) in breast cancer, aberrant activation of Ras signaling pathways downstream of growth factor receptors is considered an important driver of the tumorigenic process. As noted above, IGFBP-3 can enhance the growth-promoting effects of IGFs, and this has also been shown for other growth factors that activate receptors upstream of Ras, such as EGF and

TGFβ [38, 44–47]. In MCF-10A cells, preincubation with exogenous IGFBP-3 enhanced EGF-stimulated cell proliferation, and this was associated with increased EGFR and p44/42 MAPK activation [47]. As in HRas-expressing cells, inhibition of p44/42 MAPK restored IGFBP-3's inhibitory activity [47]. Similarly, in the IGFBP-3-transfected T47D cell model described above, the acquisition of growth-stimulation associated with IGFBP-3 expression was accompanied by enhanced EGFR signaling, and pharmacological blockade of EGFR tyrosine kinase activity resensitized cells to inhibition by IGFBP-3 [37].

4. Potentiation of EGFR Signaling by IGFBP-3 Is Mediated by Sphingosine Kinase Signaling

Potentiation of EGF signaling by estrogen in breast cancer cells has been shown to involve transactivation of EGFR by receptors for sphingosine 1-phosphate (S1P), a bioactive phospholipid generated by the phosphorylation of sphingosine by sphingosine kinases 1 and 2 (SphK1 and SphK2) [48]. Sukocheva and coworkers showed in MCF-7 breast cancer cells that estradiol increased SphK1 activity and the formation of S1P, with subsequent binding of S1P to one of its receptors, $S1P_3$, which in turn transactivated EGFR [49, 50]. IGFBP-3 had been shown to stimulate SphK1 expression and activity in HUVECs [42], raising the possibility that IGFBP-3 might enhance EGFR phosphorylation and signaling in breast cells *via* upregulation of SphK and S1P, and transactivation of EGFR. Investigation of this in MCF-10A cells showed that SphK1 expression and activity were increased by IGFBP-3 and that silencing of SphK1 expression blocked IGFBP-3's enhancement of ligand-stimulated EGFR phosphorylation [51]. The underlying mechanism involved transactivation of EGFR by $S1P_1$ or $S1P_3$ because pharmacological inhibition or siRNA-mediated silencing of either S1P receptor prevented the effects of IGFBP-3. IGF1R was similarly subject to transactivation by S1P receptors in cells preincubated with IGFBP-3 and stimulated with IGF-I [51]. Collectively, these data suggested that, in cells that express both IGFBP-3 and EGFR (or IGF1R), IGFBP-3 may promote growth *via* its potentiation of EGFR signaling secondary to increasing SphK1 activity and formation of S1P.

5. Triple-Negative Breast Cancer: A Clinical Challenge

The clinical significance of these findings in the context of breast cancer is that IGFBP-3 and EGFR are relatively highly expressed in some aggressive ER-negative and PR-negative tumors that lack amplification of HER2, which are now referred to as triple-negative breast cancers, or TNBC [31, 52, 53]. Because TNBC lack estrogen and progesterone receptors and HER2 is not amplified, these breast cancers are refractory to anticancer therapies that target these molecules. TNBC accounts for 15–20% of all breast cancer cases and often occurs in younger premenopausal women, including those of African ancestry, and is further characterized by high recurrence and high metastatic and mortality rates [53, 54]. Despite these tumors expressing EGFR, making them potentially susceptible to anti-EGFR therapies, studies have shown either no benefit or, in some instances, a worsening of clinical outcomes, associated with their use as single-line agents [55–57]. The default treatment for TNBC remains cytotoxic chemotherapy or radiation [53, 58] therapies that damage the cancer cell's DNA. The use of such therapies is associated with serious side effects [59], and the development of new treatments for TNBC that show increased efficacy but reduced toxicity is the subject of considerable interest among cancer biologists and oncologists.

6. Targeting IGFBP-3 Signaling through Sphingosine Kinase in TNBC

As alluded to above, TNBC typically express IGFBP-3 and EGFR, and Kaplan-Meier analysis of gene expression data [60] revealed that high expression of either IGFBP-3 or EGFR in ER-negative breast cancers is associated with shorter recurrence-free survival [61], and when both are highly expressed the hazard ratio is even higher (Figure 1). In light of the observed interactions between EGFR and IGFBP-3 signaling pathways, it was feasible that cotargeting these systems might be of potential benefit in the treatment of TNBC. While the most obvious way of inhibiting IGFBP-3 signaling would be to target the protein itself, its importance as an endocrine regulator of IGF metabolic activity makes such an approach unlikely to be clinically practical. An alternative approach in the setting of TNBC is to target IGFBP-3's effector pathway, the SphK system, in conjunction with EGFR inhibition.

An oncogenic role for SphK1 was first proposed more than a decade ago [62] and was supported by subsequent work showing the protective effect of SphK1 knockout against the development of tumors in a range of animal models [63–65]. Interest in SphK1 as a therapeutic target in various cancers has grown enormously [66], and a number of inhibitors of SphK have been developed, some of which have reached clinical evaluation (as reviewed in [48]). The availability of such inhibitors made it feasible that if successful, a combinatorial approach of targeting this axis with EGFR could be rapidly implemented.

As in normal breast epithelial cells, endogenous and exogenous IGFBP-3 enhanced EGF-stimulated EGFR phosphorylation in four TNBC cell lines, and this was dependent on the expression and activity of SphK1 [31]. Under conditions where inhibition of EGFR or SphK1 alone had little effect on the growth of these cell lines *in vitro*, the combination of gefitinib and SKi-II (2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl)thiazole), an inhibitor of SphK1, essentially abolished cell proliferation [31]. Importantly, when the combination was tested *in vivo* using TNBC cells grown as xenograft tumors in nude mice, gefitinib in combination with SKi-II significantly attenuated tumor growth when used at concentrations that had no significant effect as single agents [31]. These promising proof-of-principle studies indicate that the approach of cotargeting EGFR and SphK1 has potential

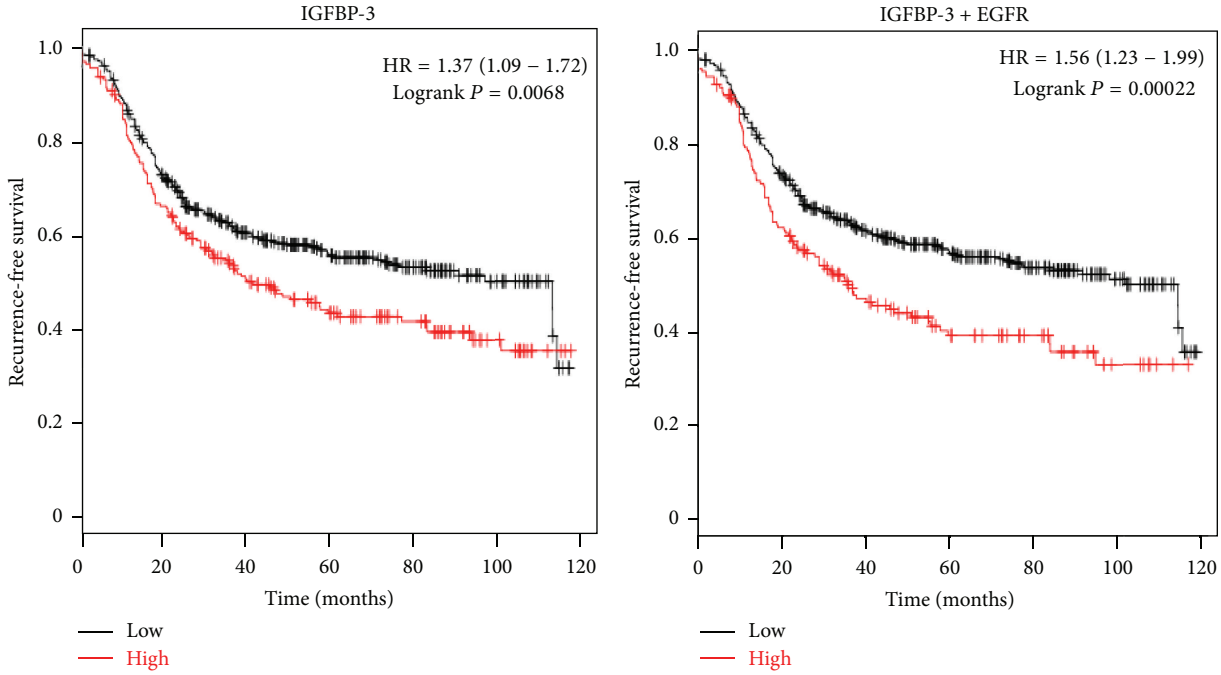


Figure 1: Kaplan-Meier analysis of gene expression data from 690 patients with ER-negative breast tumours shows that 10-year recurrence-free survival is significantly worse if tissue IGFBP-3 is high (red) compared to low (black), and this difference is even greater if expression of both *IGFBP3* and *EGFR* is high. HR: hazard ratio.

for the treatment of TNBC. In view of the known heterogeneity of TNBC, ongoing studies are now focussing on whether all subtypes of TNBC respond similarly to this combination treatment.

7. IGFBP-3 and the DNA Damage Response: Can IGFBP-3 Alter Responsiveness to Chemo- and Radiotherapy?

Activation of the tumor suppressor p53 in response to chemotherapy or radiotherapy plays an important part in the cytotoxic effects of these therapies [67]. Since the discovery of *IGFBP3* as a p53-inducible gene in 1995 [68], studies in many cancer cell lines have demonstrated an increase in the expression of IGFBP-3 in response to treatment by chemotherapeutic drugs [69–71]. Because exogenous IGFBP-3 has proapoptotic activity in many cell types, including breast cancer cells, either when used alone [72] or in combination with other apoptotic agents such as C_2 ceramide [73], chemotherapy drugs [70], or radiation [19], the induction of IGFBP-3 in response to DNA-damaging therapies has been assumed to contribute to the cytotoxicity of these treatments. Consistent with this idea, IGFBP-3 is more highly expressed in certain chemo- or radiosensitive cancer cell lines than in matched resistant cell lines, as shown in cervical carcinoma and ovarian and lung cancer cells [74–76]. Similarly, when examined in patient NSCLC tumors, loss of IGFBP-3, mediated by promoter hypermethylation, has been found to be associated with decreased chemosensitivity [77].

However, the association of high IGFBP-3 expression with poor outcome in aggressive breast cancer [32, 35, 78] may, in addition to reflecting enhanced tumor growth as described above, reflect altered responsiveness to anticancer therapies. Thus in some breast cancers high IGFBP-3 expression might be associated with treatment resistance rather than sensitivity. Skog et al. [79] speculated that the relatively high expression of IGFBP-3 in ER-negative compared to ER-positive breast cancer cells and tumor tissue [26, 29] might contribute to enhanced DNA damage repair, which might in turn lead to relative resistance to DNA-damaging therapies. Although based on a very limited study, this speculation has turned out to have some experimental support in ER-negative breast cancer cell lines.

The authors have recently reported that IGFBP-3 has an integral role in the non-homologous end-joining (NHEJ) repair response to DNA double strand breaks (DSB) caused by the topoisomerase II poisons, etoposide, and doxorubicin [80]. Of the two major mechanisms of DSB repair (homologous recombination and NHEJ), NHEJ is relatively error-prone but can occur at any stage of the cell cycle, while homologous recombination is more faithful but is generally restricted to S and G2 phase [81, 82]. NHEJ has been shown to involve EGFR which, in response to DNA-damaging agents, forms a nuclear complex with the catalytic subunit of one of the key kinases involved in NHEJ, DNA-dependent protein kinase (DNA-PKcs) [83]. In TNBC cell lines that have high IGFBP-3 expression, siRNA-mediated downregulation of IGFBP-3 was shown to inhibit the formation of this EGFR-DNA-PKcs complex [80]. Further, IGFBP-3 itself formed DNA damage-dependent complexes with both EGFR and

DNA-PKcs, suggesting the possibility of a nuclear ternary complex in the DSB repair process. IGFBP-3 downregulation also directly inhibited DNA DSB repair as measured in an *in vitro* NHEJ assay [80]. This suggests a previously unrecognized involvement of IGFBP-3 in chemotherapy-induced DNA DSB repair, which may in some circumstances lead to cancer cell recovery rather than apoptosis in response to cytotoxic drugs. IGFBP-3-dependent breast cancer cell chemoresistance, while contrary to studies in some other cancers showing IGFBP-3-dependent chemosensitivity, might in part explain the association between high IGFBP-3 (and EGFR) expression and poor patient outcomes in women with ER-negative breast cancer.

8. Concluding Remarks

It is clear that IGFBP-3 functions as a tumor promoter in some cancers, including TNBC. In TNBC it does so, at least in part, by potentiating growth-stimulatory signaling through the EGFR, and this requires SphK1. Understanding the mechanism involved in growth-stimulatory signaling by IGFBP-3 has led to the design of a combination treatment that cotargets both pathways involved and may therefore have improved efficacy not only in TNBC but in other cancers in which EGFR is highly expressed and IGFBP-3 functions as a tumor promoter. Further dissection of the molecular interactions and pathways by which IGFBP-3 may confer chemoresistance in TNBC also has the potential to identify other targets for the development of novel therapies to treat this aggressive disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. C. Baxter, "IGF binding proteins in cancer: mechanistic and clinical insights," *Nature Reviews Cancer*, vol. 14, no. 5, pp. 329–341, 2014.

[2] B. E. Forbes, P. McCarthy, and R. S. Norton, "Insulin-like growth factor binding proteins: a structural perspective," *Frontiers in Endocrinology*, vol. 3, article 38, 2012.

[3] S. M. Firth and R. C. Baxter, "Cellular actions of the insulin-like growth factor binding proteins," *Endocrine Reviews*, vol. 23, no. 6, pp. 824–854, 2002.

[4] R. C. Baxter, "Circulating levels and molecular distribution of the acid-labile (α) subunit of the high molecular weight insulin-like growth factor-binding protein complex," *Journal of Clinical Endocrinology and Metabolism*, vol. 70, no. 5, pp. 1347–1353, 1990.

[5] J. Beattie, K. Phillips, J. H. Shand, M. Szymanowska, D. J. Flint, and G. J. Allan, "Molecular interactions in the insulin-like growth factor (IGF) axis: a surface plasmon resonance (SPR) based biosensor study," *Molecular and Cellular Biochemistry*, vol. 307, no. 1-2, pp. 221–236, 2008.

[6] H. B. Boldt and C. A. Conover, "Overexpression of pregnancy-associated plasma protein-a in ovarian cancer cells promotes tumor growth in vivo," *Endocrinology*, vol. 152, no. 4, pp. 1470–1478, 2011.

[7] M. Nakamura, S. Miyamoto, H. Maeda et al., "Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability," *Biochemical and Biophysical Research Communications*, vol. 333, no. 3, pp. 1011–1016, 2005.

[8] X. Yan, L. D. Payet, R. C. Baxter, and S. M. Firth, "Activity of human pregnancy insulin-like growth factor binding protein-3: determination by reconstituting recombinant complexes," *Endocrinology*, vol. 150, no. 11, pp. 4968–4976, 2009.

[9] M. Guix, A. C. Faber, S. E. Wang et al., "Acquired resistance to EGFR tyrosine kinase inhibitors in cancer cells is mediated by loss of IGF-binding proteins," *The Journal of Clinical Investigation*, vol. 118, no. 7, pp. 2609–2619, 2008.

[10] J. L. Martin, J. A. Coverley, S. T. Pattison, and R. C. Baxter, "Insulin-like growth factor-binding protein-3 production by MCF-7 breast cancer cells: stimulation by retinoic acid and cyclic adenosine monophosphate and differential effects of estradiol," *Endocrinology*, vol. 136, no. 3, pp. 1219–1226, 1995.

[11] S. E. Pratt and M. N. Pollak, "Estrogen and antiestrogen modulation of MCF7 human breast cancer cell proliferation is associated with specific alterations in accumulation of insulin-like growth factor-binding proteins in conditioned media," *Cancer Research*, vol. 53, no. 21, pp. 5193–5198, 1993.

[12] H. Huynh, X. Yang, and M. Pollak, "Estradiol and antiestrogens regulate a growth inhibitory insulin-like growth factor binding protein 3 autocrine loop in human breast cancer cells," *The Journal of Biological Chemistry*, vol. 271, no. 2, pp. 1016–1021, 1996.

[13] F. Rozen, J. Zhang, and M. Pollak, "Antiproliferative action of tumor necrosis factor-α on MCF-7 breast cancer cells is associated with increased insulin-like growth factor binding protein-3 accumulation," *International Journal of Oncology*, vol. 13, no. 4, pp. 865–869, 1998.

[14] B. Liu, H.-Y. Lee, S. A. Weinzimer et al., "Direct functional interactions between insulin-like growth factor-binding protein-3 and retinoid X receptor-α regulate transcriptional signaling and apoptosis," *The Journal of Biological Chemistry*, vol. 275, no. 43, pp. 33607–33613, 2000.

[15] L. J. Schedlich, M. K. O'Han, G. M. Leong, and R. C. Baxter, "Insulin-like growth factor binding protein-3 prevents retinoid receptor heterodimerization: implications for retinoic acid-sensitivity in human breast cancer cells," *Biochemical and Biophysical Research Communications*, vol. 314, no. 1, pp. 83–88, 2004.

[16] T. Ikezoe, S. Tanosaki, U. Krug et al., "Insulin-like growth factor binding protein-3 antagonizes the effects of retinoids in myeloid leukemia cells," *Blood*, vol. 104, no. 1, pp. 237–242, 2004.

[17] S. Fanayan, S. M. Firth, A. J. Butt, and R. C. Baxter, "Growth inhibition by insulin-like growth factor-binding protein-3 in T47D breast cancer cells requires transforming growth factor-β (TGF-β) and the type II TGF-β receptor," *The Journal of Biological Chemistry*, vol. 275, no. 50, pp. 39146–39151, 2000.

[18] S. Fanayan, S. M. Firth, and R. C. Baxter, "Signaling through the Smad pathway by insulin-like growth factor-binding protein-3 in breast cancer cells. Relationship to transforming growth factor-β1 signaling," *The Journal of Biological Chemistry*, vol. 277, no. 9, pp. 7255–7261, 2002.

[19] A. J. Butt, S. M. Firth, M. A. King, and R. C. Baxter, "Insulin-like growth factor-binding protein-3 modulates expression of Bax and Bcl-2 and potentiates p53-independent radiation-induced

apoptosis in human breast cancer cells," *The Journal of Biological Chemistry*, vol. 275, no. 50, pp. 39174–39181, 2000.

[20] M. Takahashi, V. Papavero, J. Yuhas et al., "Altered expression of members of the IGF-axis in clear cell renal cell carcinoma," *International Journal of Oncology*, vol. 26, no. 4, pp. 923–931, 2005.

[21] A. Marimuthu, S. Chavan, G. Sathe et al., "Identification of head and neck squamous cell carcinoma biomarker candidates through proteomic analysis of cancer cell secretome," *Biochimica et Biophysica Acta—Proteins and Proteomics*, vol. 1834, no. 11, pp. 2308–2316, 2013.

[22] A. Xue, C. J. Scarlett, C. J. Jackson, B. J. Allen, and R. C. Smith, "Prognostic significance of growth factors and the urokinase-type plasminogen activator system in pancreatic ductal adenocarcinoma," *Pancreas*, vol. 36, no. 2, pp. 160–167, 2008.

[23] M. Takaoka, H. Harada, C. D. Andl et al., "Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3," *Cancer Research*, vol. 64, no. 21, pp. 7711–7723, 2004.

[24] Y. Xi, G. Nakajima, T. Hamil, O. Fodstad, A. Riker, and J. Ju, "Association of insulin-like growth factor binding protein-3 expression with melanoma progression," *Molecular Cancer Therapeutics*, vol. 5, no. 12, pp. 3078–3084, 2006.

[25] D. E. Hansel, A. Rahman, M. House et al., "Met proto-oncogene and insulin-like growth factor binding protein 3 overexpression correlates with metastatic ability in well-differentiated pancreatic endocrine neoplasms," *Clinical Cancer Research*, vol. 10, no. 18, pp. 6152–6158, 2004.

[26] D. R. Clemmons, C. Camacho-Hubner, E. Coronado, and C. K. Osborne, "Insulin-like growth factor binding protein secretion by breast carcinoma cell lines: correlation with estrogen receptor status," *Endocrinology*, vol. 127, no. 6, pp. 2679–2686, 1990.

[27] J. A. Figueroa, J. G. Jackson, W. L. McGuire, R. F. Krywicki, and D. Yee, "Expression of insulin-like growth factor binding proteins in human breast cancer correlates with estrogen receptor status," *Journal of Cellular Biochemistry*, vol. 52, no. 2, pp. 196–205, 1993.

[28] Z.-M. Shao, M. S. Sheikh, J. V. Ordonez et al., "IGFBP-3 gene expression and estrogen receptor status in human breast carcinoma," *Cancer Research*, vol. 52, no. 18, pp. 5100–5103, 1992.

[29] F. Pekonen, T. Nyman, V. Ilvesmaki, and S. Partanen, "Insulin-like growth factor binding proteins in human breast cancer tissue," *Cancer Research*, vol. 52, no. 19, pp. 5204–5207, 1992.

[30] J. L. Martin and R. C. Baxter, "Expression of insulin-like growth factor binding protein-2 by MCF-7 breast cancer cells is regulated through the phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin pathway," *Endocrinology*, vol. 148, no. 5, pp. 2532–2541, 2007.

[31] J. L. Martin, H. C. De Silva, M. Z. Lin, C. D. Scott, and R. C. Baxter, "Inhibition of insulin-like growth factor-binding protein-3 signaling through sphingosine kinase-1 sensitizes triple-negative breast cancer cells to egf receptor Blockade," *Molecular Cancer Therapeutics*, vol. 13, no. 2, pp. 316–328, 2014.

[32] R. L. Rocha, S. G. Hilsenbeck, J. G. Jackson, A. V. Lee, J. A. Figueroa, and D. Yee, "Correlation of insulin-like growth factor-binding protein-3 messenger RNA with protein expression in primary breast cancer tissues: detection of higher levels in tumors with poor prognostic features," *Journal of the National Cancer Institute*, vol. 88, no. 9, pp. 601–606, 1996.

[33] H. Yu, M. A. Levesque, M. J. Khosravi, A. Papanastasiou-Diamandi, G. M. Clark, and E. P. Diamandis, "Associations between insulin-like growth factors and their binding proteins and other prognostic indicators in breast cancer," *British Journal of Cancer*, vol. 74, no. 8, pp. 1242–1247, 1996.

[34] H. Yu, M. A. Levesque, M. J. Khosravi, A. Papanastasiou-Diamandi, G. M. Clark, and E. P. Diamandis, "Insulin-like growth factor-binding protein-3 and breast cancer survival," *International Journal of Cancer*, vol. 79, no. 6, pp. 624–628, 1998.

[35] S. M. Sheen-Chen, H. Zhang, C. C. Huang, and R. P. Tang, "Insulin-like growth factor-binding protein-3 in breast cancer: analysis with tissue microarray," *Anticancer Research*, vol. 29, no. 4, pp. 1131–1135, 2009.

[36] S. M. Firth, S. Fanayan, D. Benn, and R. C. Baxter, "Development of resistance to insulin-like growth factor binding protein-3 in transfected T47D breast cancer cells," *Biochemical and Biophysical Research Communications*, vol. 246, no. 2, pp. 325–329, 1998.

[37] A. J. Butt, J. L. Martin, K. A. Dickson, F. McDougall, S. M. Firth, and R. C. Baxter, "Insulin-like growth factor binding protein-3 expression is associated with growth stimulation of T47D human breast cancer cells: the role of altered epidermal growth factor signaling," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 4, pp. 1950–1956, 2004.

[38] J. S. M. de Mellow and R. C. Baxter, "Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-I-stimulated DNA synthesis in human skin fibroblasts," *Biochemical and Biophysical Research Communications*, vol. 156, no. 1, pp. 199–204, 1988.

[39] C. A. Conover, "Potentiation of insulin-like growth factor (IGF) action by IGF-binding protein-3: studies of underlying mechanism," *Endocrinology*, vol. 130, no. 6, pp. 3191–3199, 1992.

[40] C. J. Grill and W. S. Cohick, "Insulin-like growth factor binding protein-3 mediates IGF-I action in a bovine mammary epithelial cell line independent of an IGF interaction," *Journal of Cellular Physiology*, vol. 183, pp. 273–283, 2000.

[41] C. McCaig, C. M. Perks, and J. M. P. Holly, "Intrinsic actions of IGFBP-3 and IGFBP-5 on Hs578T breast cancer epithelial cells: inhibition or accentuation of attachment and survival is dependent upon the presence of fibronectin," *Journal of Cell Science*, vol. 115, no. 22, pp. 4293–4303, 2002.

[42] R. Granata, L. Trovato, G. Garbarino et al., "Dual effects of IGFBP-3 on endothelial cell apoptosis and survival: involvement of the sphingolipid signaling pathways," *The FASEB Journal*, vol. 18, no. 12, pp. 1456–1458, 2004.

[43] J. L. Martin and R. C. Baxter, "Oncogenic ras causes resistance to the growth inhibitor insulin-like growth factor binding protein-3 (IGFBP-3) in breast cancer cells," *The Journal of Biological Chemistry*, vol. 274, no. 23, pp. 16407–16411, 1999.

[44] C. A. Conover, "Glycosylation of insulin-like growth factor binding protein-3 (IGFBP-3) is not required for potentiation of IGF-I action: evidence for processing of cell-bound IGFBP-3," *Endocrinology*, vol. 129, no. 6, pp. 3259–3268, 1991.

[45] J.-C. Chen, Z.-M. Shao, M. S. Sheikh et al., "Insulin-like growth factor-binding protein enhancement of insulin-like growth factor-I (IGF-I)-mediated DNA synthesis and IGF-I binding in a human breast carcinoma cell line," *Journal of Cellular Physiology*, vol. 158, no. 1, pp. 69–78, 1994.

[46] P. Cohen, R. Rajah, J. Rosenbloom, and D. J. Herrick, "IGFBP-3 mediates TGF-β1-induced cell growth in human airway smooth muscle cells," *The American Journal of Physiology: Lung Cellular and Molecular Physiology*, vol. 278, no. 3, pp. L545–L551, 2000.

[47] J. L. Martin, S. M. Weenink, and R. C. Baxter, "Insulin-like growth factor-binding protein-3 potentiates epidermal growth

factor action in MCF-10A mammary epithelial cells. Involvement of p44/42 and p38 mitogen-activated protein kinases," *Journal of Biological Chemistry*, vol. 278, no. 5, pp. 2969–2976, 2003.

[48] H. Alshaker, L. Sauer, D. Monteil et al., "Therapeutic potential of targeting SK1 in human cancers," *Advances in Cancer Research*, vol. 117, pp. 143–200, 2013.

[49] O. A. Sukocheva, L. Wang, N. Albanese, S. M. Pitson, M. A. Vadas, and P. Xia, "Sphingosine kinase transmits estrogen signaling in human breast cancer cells," *Molecular Endocrinology*, vol. 17, no. 10, pp. 2002–2012, 2003.

[50] O. Sukocheva, C. Wadham, A. Holmes et al., "Estrogen transactivates EGFR via the sphingosine 1-phosphate receptor Edg-3: the role of sphingosine kinase-1," *The Journal of Cell Biology*, vol. 173, no. 2, pp. 301–310, 2006.

[51] J. L. Martin, M. Z. Lin, E. M. McGowan, and R. C. Baxter, "Potentiation of growth factor signaling by insulin-like growth factor-binding protein-3 in breast epithelial cells requires sphingosine kinase activity," *The Journal of Biological Chemistry*, vol. 284, no. 38, pp. 25542–25552, 2009.

[52] S. Kobayashi, "Basal-like subtype of breast cancer: a review of its unique characteristics and their clinical significance," *Breast Cancer*, vol. 15, no. 2, pp. 153–158, 2008.

[53] I. A. Mayer, V. G. Abramson, B. D. Lehmann, and J. A. Pietenpol, "New strategies for triple-negative breast cancer-deciphering the heterogeneity," *Clinical Cancer Research*, vol. 20, no. 4, pp. 782–790, 2014.

[54] P. Boyle, "Triple-negative breast cancer: epidemiological considerations and recommendations," *Annals of Oncology*, vol. 23, no. 6, Article ID mds187, pp. vi7–vi12, 2012.

[55] M. F. Press, R. S. Finn, D. Cameron et al., "HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer," *Clinical Cancer Research*, vol. 14, no. 23, pp. 7861–7870, 2008.

[56] R. S. Finn, M. F. Press, J. Dering et al., "Estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), and epidermal growth factor receptor expression and benefit from lapatinib in a randomized trial of paclitaxel with lapatinib or placebo as first-line treatment in HER2-negative or unknown metastatic breast cancer," *Journal of Clinical Oncology*, vol. 27, no. 24, pp. 3908–3915, 2009.

[57] T.-C. Hsia, C.-Y. Tu, Y.-J. Chen et al., "Lapatinib-mediated cyclooxygenase-2 expression via epidermal growth factor receptor/HuR interaction enhances the aggressiveness of triple-negative breast cancer cells," *Molecular Pharmacology*, vol. 83, no. 4, pp. 857–869, 2013.

[58] C. L. Griffiths and J. L. Olin, "Triple negative breast cancer: a brief review of its characteristics and treatment options," *Journal of Pharmacy Practice*, vol. 25, no. 3, pp. 319–323, 2012.

[59] Y. Chen, P. Jungsuwadee, M. Vore, D. A. Butterfield, and D. K. St. Clair, "Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues," *Molecular Interventions*, vol. 7, no. 3, pp. 147–156, 2007.

[60] B. Gyorffy, A. Lanczky, A. C. Eklund et al., "An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients," *Breast Cancer Research and Treatment*, vol. 123, no. 3, pp. 725–731, 2010.

[61] R. C. Baxter, "Insulin-like growth factor binding protein-3 (IGFBP-3): novel ligands mediate unexpected functions," *Journal of Cell Communication and Signaling*, vol. 7, no. 3, pp. 179–189, 2013.

[62] P. Xia, J. R. Gamble, L. Wang et al., "An oncogenic role of sphingosine kinase," *Current Biology*, vol. 10, no. 23, pp. 1527–1530, 2000.

[63] M. Kohno, M. Momoi, M. L. Oo et al., "Intracellular role for sphingosine kinase 1 in intestinal adenoma cell proliferation," *Molecular and Cellular Biology*, vol. 26, no. 19, pp. 7211–7223, 2006.

[64] T. Kawamori, T. Kaneshiro, M. Okumura et al., "Role for sphingosine kinase 1 in colon carcinogenesis," *The FASEB Journal*, vol. 23, no. 2, pp. 405–414, 2009.

[65] L. A. Heffernan-Stroud, K. L. Helke, R. W. Jenkins, A.-M. de Costa, Y. A. Hannun, and L. M. Obeid, "Defining a role for sphingosine kinase 1 in p53-dependent tumors," *Oncogene*, vol. 31, no. 9, pp. 1166–1175, 2012.

[66] L. A. Heffernan-Stroud and L. M. Obeid, "Sphingosine kinase 1 in cancer," *Advances in Cancer Research*, vol. 117, pp. 201–235, 2013.

[67] E. A. Bassett, W. Wang, F. Rastinejad, and W. S. El-Deiry, "Structural and functional basis for therapeutic modulation of p53 signaling," *Clinical Cancer Research*, vol. 14, no. 20, pp. 6376–6386, 2008.

[68] L. Buckbinder, R. Talbott, S. Velasco-Miguel et al., "Induction of the growth inhibitor IGF-binding protein 3 by p53," *Nature*, vol. 377, no. 6550, pp. 646–649, 1995.

[69] M. Giuliano, M. Lauricella, E. Vassallo, M. Carabillò, R. Vento, and G. Tesoriere, "Induction of apoptosis in human retinoblastoma cells by topoisomerase inhibitors," *Investigative Ophthalmology & Visual Science*, vol. 39, no. 8, pp. 1300–1311, 1998.

[70] C. A. Fowler, C. M. Perks, P. V. Newcomb, P. B. Savage, J. R. Farndon, and J. M. Holly, "Insulin-like growth factor binding protein-3 (IGFBP-3) potentiates paclitaxel-induced apoptosis in human breast cancer cells," *International Journal of Cancer*, vol. 88, no. 3, pp. 448–453, 2000.

[71] R. Kawabata, S. Oie, M. Takahashi, H. Kanayama, T. Oka, and K. Itoh, "Up-regulation of insulin-like growth factor-binding protein 3 by 5-fluorouracil (5-FU) leads to the potent anti-proliferative effect of androgen deprivation therapy combined with 5-FU in human prostate cancer cell lines," *International Journal of Oncology*, vol. 38, no. 6, pp. 1489–1500, 2011.

[72] T. Nickerson, H. Huynh, and M. Pollak, "Insulin-like growth factor binding protein-3 induces apoptosis in MCF7 breast cancer cells," *Biochemical and Biophysical Research Communications*, vol. 237, pp. 690–693, 1997.

[73] Z. P. Gill, C. M. Perks, P. V. Newcomb, and J. M. P. Holly, "Insulin-like growth factor-binding protein (IGFBP-3) predisposes breast cancer cells to programmed cell death in a non-IGF-dependent manner," *The Journal of Biological Chemistry*, vol. 272, no. 41, pp. 25602–25607, 1997.

[74] M. P. Achary, W. Jaggernauth, E. Gross, A. Alfieri, H. P. Klinger, and B. Vikram, "Cell lines from the same cervical carcinoma but with different radiosensitivities exhibit different cDNA microarray patterns of gene expression," *Cytogenetics and Cell Genetics*, vol. 91, no. 1–4, pp. 39–43, 2000.

[75] T. Goto, M. Takano, M. Sakamoto et al., "Gene expression profiles with cDNA microarray reveal RhoGDI as a predictive marker for paclitaxel resistance in ovarian cancers," *Oncology Reports*, vol. 15, no. 5, pp. 1265–1271, 2006.

[76] Y. Sun, S. Zheng, A. Torossian et al., "Role of insulin-like growth factor-1 signaling pathway in cisplatin-resistant lung cancer cells," *International Journal of Radiation Oncology, Biology, Physics*, vol. 82, no. 3, pp. e563–e572, 2012.

[77] I. I. de Caceres, M. Cortes-Sempere, C. Moratilla et al., "IGFBP-3 hypermethylation-derived deficiency mediates cisplatin resistance in non-small-cell lung cancer," *Oncogene*, vol. 29, no. 11, pp. 1681–1690, 2010.

[78] S. B. Vestey, C. M. Perks, C. Sen, C. J. Calder, J. M. P. Holly, and Z. E. Winters, "Immunohistochemical expression of insulin-like growth factor binding protein-3 in invasive breast cancers and ductal carcinoma in situ: implications for clinicopathology and patient outcome," *Breast Cancer Research*, vol. 7, no. 1, pp. R119–R129, 2005.

[79] S. Skog, Q. He, R. Khoshnoud, T. Fornander, and L.-E. Rutqvist, "Genes related to growth regulation, DNA repair and apoptosis in an oestrogen receptor-negative (MDA-231) versus an oestrogen receptor-positive (MCF-7) breast tumour cell line," *Tumor Biology*, vol. 25, no. 1-2, pp. 41–47, 2004.

[80] M. Z. Lin, K. A. Marzec, J. L. Martin, and R. C. Baxter, "The role of insulin-like growth factor binding protein-3 in the breast cancer cell response to DNA-damaging agents," *Oncogene*, vol. 33, no. 1, pp. 85–96, 2014.

[81] S. P. Jackson and J. Bartek, "The DNA-damage response in human biology and disease," *Nature*, vol. 461, no. 7267, pp. 1071–1078, 2009.

[82] I. Brandsma and D. C. Gent, "Pathway choice in DNA double strand break repair: observations of a balancing act," *Genome Integrity*, vol. 3, article 9, 2012.

[83] D. J. Chen and C. S. Nirodi, "The epidermal growth factor receptor: a role in repair of radiation-induced DNA damage," *Clinical Cancer Research*, vol. 13, no. 22, pp. 6555–6560, 2007.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 390863, 11 pages
http://dx.doi.org/10.1155/2015/390863

Review Article

Diabetes and Its Link with Cancer: Providing the Fuel and Spark to Launch an Aggressive Growth Regime

Sanket Joshi, Menghan Liu, and Nigel Turner

Department of Pharmacology, School of Medical Sciences, UNSW Australia, Kensington, NSW 2052, Australia

Correspondence should be addressed to Nigel Turner; n.turner@unsw.edu.au

Received 20 June 2014; Accepted 27 September 2014

Academic Editor: Anandwardhan Hardikar

Diabetes is a disease involving metabolic derangements in multiple organs. While the spectrum of diabetic complications has been known for years, recent evidence suggests that diabetes could also contribute to the initiation and propagation of certain cancers. The mechanism(s) underlying this relationship are not completely resolved but likely involve changes in hormone and nutrient levels, as well as activation of inflammatory and stress-related pathways. Interestingly, some of the drugs used clinically to treat diabetes also appear to have antitumour effects, further highlighting the interaction between these two conditions. In this contribution we review recent literature on this emerging relationship and explore the potential mechanisms that may promote cancer in diabetic patients.

1. Introduction

Type 2 diabetes (T2D) and cancer are two of the most prevalent diseases facing modern society. Recent estimates suggest that close to 400 million people worldwide have T2D [1], while 12.7 million cancer cases and 7.6 million cancer deaths are reported each year [2]. Both diseases are multifactorial in origin and cancer is recognized as being a particularly heterogeneous disease.

Both T2D and cancer are characterized by marked alterations in metabolic profile and recent epidemiological evidence suggests a close link between diabetes and some forms of cancer [3]. Indeed, individuals with diabetes have significantly higher likelihood of developing a range of different cancers including liver, pancreatic, colorectal, breast, endometrial, and bladder cancers [4, 5]. The molecular basis for this link has not been fully elucidated but likely relates to changes in several factors, including nutrient availability and growth factor signaling. In this review we will briefly describe the metabolic alterations that are present in T2D and cancer and will discuss some of the factors that may potentially link these two diseases. We will also examine emerging evidence around therapeutic agents that may have utility in treating aspects of both diseases.

2. Metabolic Features of Type 2 Diabetes

In healthy individuals, the variation of plasma glucose levels is kept minimal despite considerable fluctuations in nutrient intake (Figure 1(a)). The maintenance of circulating glucose levels under conditions of high nutrient availability is mainly mediated through the actions of insulin, a potent anabolic hormone secreted by the pancreatic β-cells in response to an increase in blood glucose level. Upon binding to its receptor, insulin initiates a cascade of downstream signaling events that influence a spectrum of enzymatic and transcriptional activities for the maintenance of glucose, lipid, and protein homeostasis [6]. Specifically, insulin promotes glucose uptake in skeletal muscles and adipose tissue by stimulating the membrane translocation of the GLUT4 transporter and activating enzymes involved in glycolysis [7]. In parallel, it facilitates carbohydrate disposal via both glycolysis and the nonoxidative pathways glycogen synthesis and *de novo* lipogenesis [8]. Meanwhile, insulin suppresses the processes generating circulating nutrients such as gluconeogenesis in the liver and lipolysis in the adipose tissue [6, 8]. The regulation of protein metabolism is another important aspect of insulin signaling, involving downregulation of proteolysis in skeletal muscles and

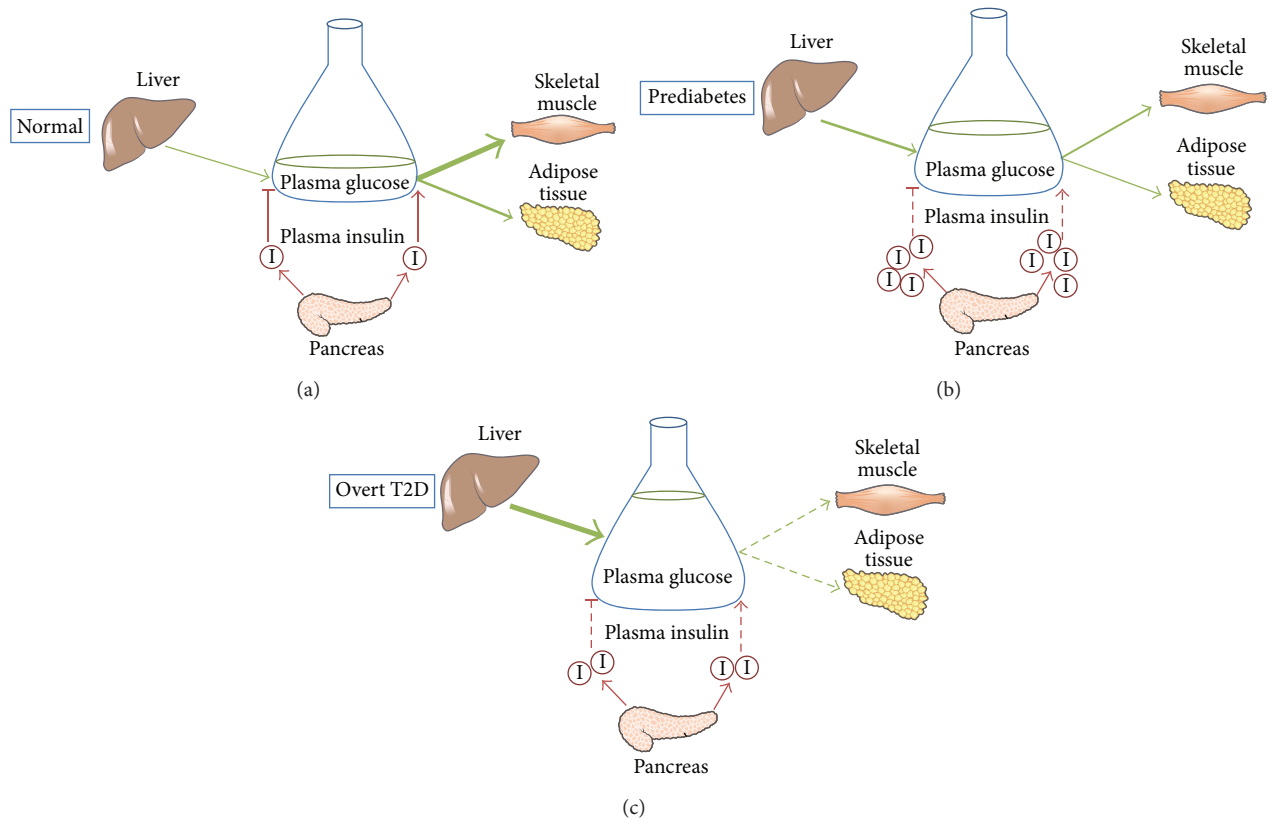


Figure 1: Under normal conditions, insulin is secreted from pancreatic β-cells in response to an increase in plasma glucose levels. It promotes glucose uptake into skeletal muscle and adipose tissue while suppressing hepatic glucose output, resulting in maintenance of blood glucose concentration to ~5 mM (a). In insulin resistant individuals, an increased amount of insulin is required to compensate for diminished effects on insulin-target organs, giving rise to hyperinsulinaemia. As insulin resistance worsens, blood glucose level gradually increases despite increased insulin secretion and a prediabetic state is established (b). In susceptible individuals, relative insulin deficiency progressively develops due to failure of β-cells to secrete adequate levels of insulin, resulting in loss of glucose homeostasis if exogenous insulin is not provided (c).

promotion of protein synthesis through the mTOR pathway [9].

T2D is a pathological condition involving defects in both insulin action and secretion. It is characterised by elevations in postprandial and fasting blood glucose levels. Insulin resistance (IR), which is defined as the diminished biological effects of insulin on target tissues, is a major early defect in the pathogenesis of T2D [10, 11]. In the state of IR, the regulatory actions of insulin action on carbohydrate metabolism are impaired in target tissues. Accordingly, a state of hyperinsulinemia ensues due to the requirement of increased amounts of insulin to suppress hepatic glucose output from the liver and promote clearance of glucose into peripheral tissues (Figure 1(b)) [8]. When IR becomes more severe and glucose homeostasis cannot be maintained despite increased insulin levels, mild hyperglycemia sets in and a prediabetic state begins to manifest (Figure 1(b)). In susceptible individuals, the sustained increase in insulin secretion leads to the failure of pancreatic β-cells and the progression to T2D and marked hyperglycemia. In T2D patients this relative insulin deficiency necessitates insulin from exogenous sources to maintain whole-body glucose control (Figure 1(c)).

Obesity, especially the visceral form where mesenteric, epididymal, perirenal fat depots surround internal organs, is a well-recognised predisposing factor for developing T2D [12, 13]. Research in the last two decades has clearly demonstrated that, in the obese state, deposition of lipid in insulin-sensitive tissues such as muscle and liver is a key driver of IR [8]. In particular, bioactive lipid metabolites such as diacylglycerol and ceramide are thought to be the key culprits antagonising insulin action [8]. The ectopic accumulation of lipid metabolites is primarily due to elevated influx of fatty acids (FAs) into nonadipose tissues, due to the high availability of circulating FA coming from excess lipid intake and/or impaired insulin action to suppress adipose tissue lipolysis [8]. Liver steatosis is also secondarily enhanced by the paradoxical maintenance of insulin-stimulated *de novo* lipogenesis, despite reduced insulin sensitivity in glucose metabolism pathways [14]. In addition to inappropriate lipid deposition, obese individuals display chronic low-grade inflammation, especially in white adipose tissue, as well as elevations in local and systemic oxidative stress. Both of these factors are thought to attenuate insulin action, in part, by activating pathways that interfere with or oppose insulin signaling and thus they have also been implicated in the development of obesity-induced IR

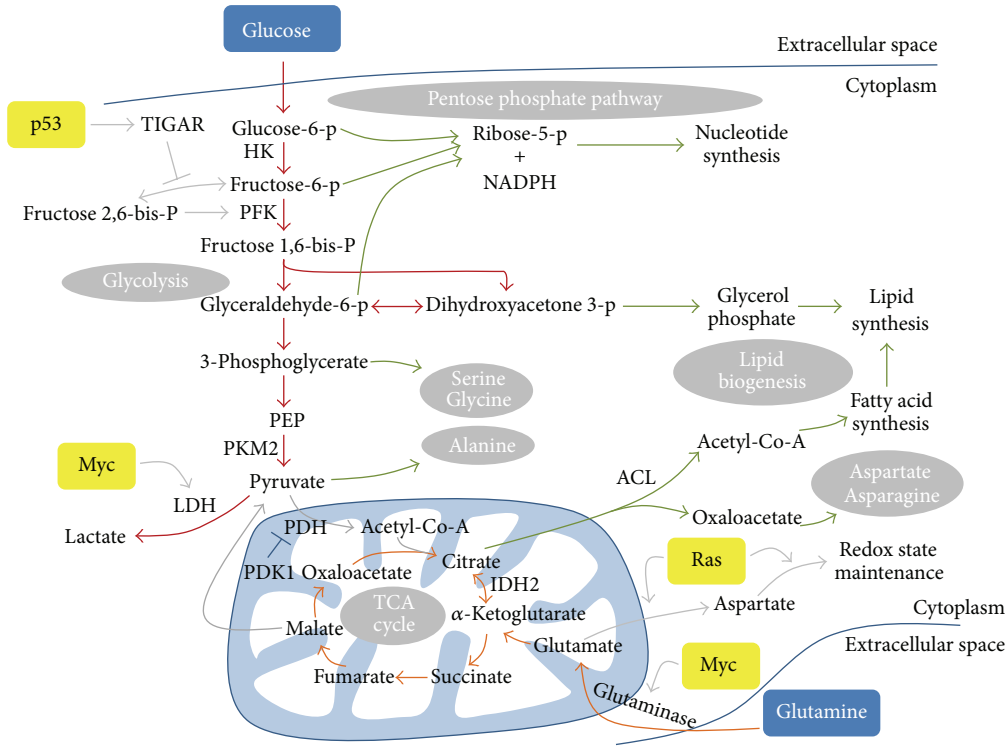


Figure 2: Glycolysis and glutaminolysis are two of the most important pathways for cancer cells. Increased glucose uptake, together with reduced glycolytic flux, accumulates glycolytic intermediates for synthesis of biomolecules such as nucleotides, amino acids, and lipids. Similarly, glutamine uptake is also increased. Glutamine is converted to glutamate by mitochondrial glutaminase. Glutamate is then converted to α-ketoglutarate which can be oxidised in the TCA cycle to generate ATP or reductively carboxylated to citrate. Citrate is exported to the cytoplasm where it is converted to acetyl-Co-A or oxaloacetate, which are used for synthesis of fatty acids or amino acids, respectively. Metabolic changes in cancer cells are driven by changes in the regulation of critical enzymes. Examples of these enzymes are shown in bold. Regulation of metabolic pathways by oncogenes (Myc and K-Ras) and tumour suppressor genes (p53) is also shown. Glycolysis is shown in red. Glutaminolysis is shown in orange. Biosynthetic pathways are shown in green. Other pathways are shown in grey.

[15–17]. Collectively, the coexistence of obesity and T2D suggests that patients with these conditions have a fertile whole-body environment saturated in growth factor signals (insulin), an abundance of circulating nutrients (glucose, FAs), inflammatory cytokines, and reactive oxygen species.

3. Altered Metabolism in Cancer

Cancer is a heterogenous disease, characterised by the acquisition of successive mutations in protooncogenes and tumour suppressor genes [18]. These mutations allow tumour cells to sustain their growth signaling pathways, evade cell death, and continue to proliferate in an uncontrolled manner. Despite diversity in the precise molecular origin of different cancers, most (but not all) tumours tend to converge on a common metabolic phenotype, which was first described by Otto Warburg. In his seminal work in this field, Warburg observed that tumour cells exhibit aerobic glycolysis (i.e., high rates of glycolysis even in the presence of abundant oxygen) where pyruvate is converted to lactate instead of entering mitochondria for the TCA cycle [19, 20]. This phenomenon, known as the Warburg effect, has been the subject of intense research in recent years. Warburg reasoned that defective mitochondria prevent pyruvate from entering the TCA cycle and this underlies the enhanced rate of conversion of glucose to lactate [19]. However, later studies found that mitochondria in many tumour cell types are functional [21–23] and it is now clear that the alterations in the uptake and metabolism of different nutrients are critical for meeting both the bioenergetics needs of tumour cells and more importantly the increased requirement for biosynthesis of macromolecules.

Glucose and glutamine constitute two of the most important sources for meeting synthetic and energetic needs of tumour cells (Figure 2) [24]. The rate of glucose uptake in tumours is profoundly increased and the glycolytic intermediates provide building blocks for the synthesis of nucleotides, lipids, and amino acids [25–28]. Nucleotides are synthesised via the pentose phosphate pathway (PPP) using the intermediates generated by glycolysis including glucose 6-phosphate, fructose 6-phosphate, and glyceraldehyde 3-phosphate. The PPP also generates NADPH which is necessary for lipid synthesis and for maintenance of cellular redox potential. Lipid biogenesis also requires glycerol phosphate which is converted from another intermediate of glycolysis, dihydroxyacetone phosphate. Furthermore,

the end product of glycolysis, pyruvate, is the substrate for synthesising nonessential amino acids alanine, whereas 3-phosphoglycerate is used for synthesising serine and glycine. The importance of aerobic glycolysis in cancer cells is highlighted by the fact that glucose withdrawal or inhibition of glucose uptake by small molecule inhibitors of PI3K signaling (discussed below) induces tumour cell death and tumour regression [29, 30] and that inhibition of lactate dehydrogenase (LDH) which converts pyruvate to lactate impairs cell proliferation [22, 31].

The increases in glucose uptake and glycolytic pathway activation are consequences of alterations in a range of metabolic enzymes and proteins. Tumour cells exhibit a marked increase in the expression of glucose transporters and their presence on the cell membrane, to achieve the required increase in glucose uptake [28, 32, 33]. This has been exploited in the clinic for the detection of tumours by imaging radioactive F-19-2-deoxyglucose uptake by positron emission tomography (PET). Once inside the cell, glucose is phosphorylated and trapped by hexokinase which is also hyperactivated in cancers. Cancer cells predominantly express the hexokinase II isoform [34], which is present on the outer membrane of mitochondria where it rapidly phosphorylates glucose. Despite the high rate of glucose uptake and phosphorylation, the overexpression of pyruvate kinase M2 (PKM2), which is less active than the M1 form in converting phosphoenolpyruvate (PEP) to pyruvate and subject to negative regulation by growth factor signaling, causes an overall reduction in glycolytic flux reaching the end-point pyruvate in cancer cells [26]. Overall, increased glucose uptake and reduced glycolytic flux going to completion result in the accumulation and channeling of glycolytic intermediates towards biosynthetic pathways.

Due to the diversion of most glucose-derived intermediates to biosynthesis, glutamine uptake is also increased in tumours to replenish the depletion of TCA cycle intermediates which are normally supplied from glucose sources and to fuel mitochondrial ATP production. Additionally, recent work has shown that under certain conditions glutamine can also play another important role in the growth of tumour cells, providing acetyl-CoA for lipid synthesis through a process known as reductive carboxylation [35–38]. Similar to glucose, the expression of membrane glutamine transporters (e.g., ASCT2), in particular the high affinity isoforms, is elevated in cancer [39]. Furthermore glutaminase, the enzyme responsible for the metabolism of glutamine, is markedly increased in many cancers, consistent with an addiction of tumours to the use of this nutrient [40].

The biosynthesis of lipids is another key aspect of the tumour metabolic program. Cancer cells perform *de novo* fatty acid synthesis extensively from glucose and glutamine-derived precursors and NADPH to supply materials for the production of membranes and signaling molecules, as opposed to the majority of normal cells that rely mainly on lipids from the environment [41]. Several proteins involved in lipogenesis including ATP citrate lyase (ACL) [42], acetyl-CoA carboxylase (ACC) [43], fatty acid synthase (FAS) [44], and sterol response element binding protein (SREBP) [45] have been shown to be intimately related to cancer cell growth and survival. In addition, a subset of cancers also scavenges lipids from adipocytes [46] and the circulation [47] by upregulating FA transporters fatty acid binding protein 4 and CD36. Together, the coordinated upregulations of aerobic glycolysis, glutamine uptake, and biosynthetic processes represent a fundamental shift in cellular metabolic landscape to support tumour growth and rapid expansion.

4. Factors Driving Metabolic Changes in Cancer

A major factor leading to alterations in metabolic enzymes and pathways in cancer is the presence of tumour hypoxia. Hypoxia leads to the stabilisation of hypoxia-inducible factors 1 and 2 (HIF-1 and HIF-2) and HIF-1 is known to upregulate 9 out of 10 enzymes of glycolysis [48, 49]. HIF-1 also prevents entry of pyruvate into the TCA cycle, firstly by upregulating LDH which converts pyruvate to lactate and secondly by upregulating pyruvate dehydrogenase kinase (PDK1) which inhibits PDH thus blocking the conversion of pyruvate to acetyl-CoA [50, 51]. HIF-2, on the other hand, increases Myc function (discussed below) allowing cells to proliferate under hypoxia [52]. HIF transcription factors themselves are under regulation of the TCA cycle enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH). Although mitochondria are not typically defective in tumour cells, SDH and FH enzymes are mutated in some types of cancers [53]. Mutations in these enzymes cause an accumulation of fumarate and succinate, which results in the inhibition of prolyl-hydroxylases that mediate degradation of HIF proteins, thereby enhancing glycolysis [53].

The changes in metabolic enzymes in cancer are not always an adaptation to hypoxia, as cancers such as leukaemia and lung cancer have abundant oxygen supply during tumourigenesis but still operate aerobic glycolysis [54–56]. There is increasing evidence that oncogenes and tumour suppressor genes directly regulate metabolic pathways in tumourigenesis. Not only do mutations in these genes reprogram metabolic pathways for progression of the tumours, but also metabolic changes induced by them may be primary events in cellular transformations [57]. Myc, an oncogene frequently mutated in many cancers, was one of the first to be linked to metabolism, as it directly activates expression of LDH [31, 58]. Myc target genes include enzymes of glycolysis, glutaminolysis, and fatty acid synthesis [39, 59, 60]. The enhanced expressions of membrane glutamine transporters and mitochondrial glutaminase and the consequent increase in glutaminolysis are mediated by the Myc oncogene [39, 40]. Conversely, inhibition of mitochondrial glutaminase by pharmacological inhibitors impairs tumour growth of Myc-expressing B cells in xenograft models [61]. Similarly, glutamine removal from culture media results in cell death in Myc overexpressing cancer cells [62].

Another well characterised oncogene, Ras, can also promote changes favouring tumour growth and proliferation. For example, oncogenic K-Ras, which is associated with over 90% of pancreatic ductal adenocarcinoma (PDAC), mediates changes in both glucose and glutamine metabolism that are essential for PDAC maintenance. K-Ras stimulates glycolytic

flux and diverts glycolytic intermediates to hexosamine biosynthesis pathway (HBP) and PPP [63]. This effect appears to be dependent on Myc, as its knockdown significantly downregulated the expression of metabolic genes involved in glycolysis, HBP and PPP [63]. PDAC cells also operate a distinct metabolic pathway for glutamine metabolism where glutamine is metabolized through the noncanonical pathway to produce aspartate which is subsequently transported to the cytoplasm for the maintenance of NADPH/$NADP^+$ ratio and cellular redox state [64]. Downregulation of enzymes in this pathway leads to suppression of PDAC growth *in vitro* and *in vivo* [64].

Mutation of tumour suppressor genes, such as p53, is a critical event in many cancers, but their emerging roles in metabolism have been elucidated only recently [65]. One of the most discussed links between p53 and metabolism is via TIGAR-dependent inhibition of glycolysis [25]. The p53 target gene TIGAR lowers intracellular concentration of fructose 2,6-bisphosphate (FBP), an allosteric activator of phosphofructokinase, thus inhibiting glycolysis and diverting glucose to PPP. Additionally, p53 represses transcription of glucose transporters GLUT1 and GLUT4 [66]. Apart from suppressing glycolysis, p53 also influences mitochondrial metabolism by increasing the transcription of synthesis of cytochrome c oxidase 2 (SCO2) which assembles into oxidative phosphorylation complex and enhances mitochondrial respiration [67]. Therefore, loss of tumour suppressors confers growth advantage to tumours from a metabolic angle, by favouring a metabolic profile conductive to rapid cell proliferation.

5. Metabolic Changes in Diabetes Can Facilitate Tumourigenesis

As noted above, epidemiological evidence shows that individuals with diabetes have significantly higher likelihood of developing multiple types of cancers [3]. Amongst these, organs associated with energy metabolism such as liver and pancreas have the strongest association with diabetes. Furthermore, diabetic patients with colorectal, breast, and endometrial cancers have significantly higher chances of dying of cancer than normal individuals [68]. The mechanisms driving cancers in diabetic patients are still not entirely clear, but some possibilities are discussed below.

In early stages of diabetes, pancreatic β-cells produce excess amount of insulin, resulting in hyperinsulinemia. While insulin-target organs are resistant to the actions of insulin in diabetes, hyperinsulinemia may have progrowth effects on a nascent tumour by allowing the tumour to overcome an important early barrier in tumourigenesis, that is, lack of growth factor signaling. There is epidemiological data to suggest that insulin secretion rate and insulin-like growth factor 1 (IGFI) levels influence cancer risk and/or cancer progression [69, 70]. Insulin and IGFI stimulate the proliferation of tumour cells *in vitro* [71] and promote glucose uptake in the subset of tumours that are insulin-dependent [72, 73]. The IGFI receptor (IGF1R) is necessary for the transforming ability of several oncogenes, suggesting that parallel growth factor signaling-mediated metabolic changes are crucial for cellular transformation [74]. In line with the above observations, reduced growth factor signaling leads to decreased tumour growth in mouse models [69]. The above observations indicate that hyperinsulinemia or administration of synthetic insulin in diabetes may enhance growth factor signaling and promote glucose usage to promote tumour growth. As many tumours devise means to evade regulations of growth factor signaling, we propose that insulin may serve as the spark to initiate cancer development at early stages when self-sufficiency of growth factors has not yet been established.

Hyperglycemia, another characterising feature of diabetes, may also contribute to enhanced cancer risk [75]. Given the central role that glycolysis plays in tumour development, elevated glucose levels in the circulation are likely to provide abundant glucose resources and a concentration gradient for convenient usage by cancer cells. Indeed, epidemiological evidence suggests that hyperglycemia in cancer patients contributes to increased likelihood of tumour recurrence, metastasis, or fatal outcome compared to patients with hyperglycemia [75]. Additionally to the direct metabolic role, hyperglycemia in a subset of tumour cells can lead to increased production of ROS from mitochondrial respiration, which below certain levels can lead to DNA damage that are not severe enough to induce apoptosis [75, 76] but may give rise to mutations in protooncogenes and tumour suppressor genes or other changes that are beneficial for the tumour. For example, hyperglycemia-related increased ROS production in pancreatic cancer cell lines such as Panc-1 and BxPC-3 increases cell motility and invasiveness, indicating hyperglycemia may contribute to pancreatic cancer metastasis [9]. Enhanced glucose metabolism may also prevent cytochrome c mediated-cell death in cancer cells [77] and confer resistance to chemotherapy [78, 79], both favouring continued tumour growth.

At the systemic level in diabetes, the excess availability of nutrients and local changes in tissues, including adipose tissue, leads to chronic low-grade inflammation. For instance, the levels of cytokines such as TNFα and IL-6 are increased, as a result of both the stimulation of monocytes and macrophages by excess nutrients and the increased expression and release from inflamed adipose tissue [80–83]. Inflammation is important in tumourigenesis as it contributes to all stages of tumourigenesis, including angiogenesis and metastasis [84]. Both TNFα and IL-6 have been shown to promote tumour invasiveness and metastasis by secretion of matrix remodelling proteins matrix metalloproteinases [85]. IL-6-deficient mice are resistant to multiple myeloma, while neutralization of TNFα switches inflammation-driven metastatic growth to inflammation-induced tumour regression [86–88]. Thus, diabetes associated hyperglycemia and hyperlipidaemia can promote tumourigenesis by inducing inflammation.

There may also be a more direct link between the obesity that is commonly observed in diabetes and the development of some tumours. Recent work has shown that there is cross talk between adipocytes and certain types of tumours, whereby signals from tumours can lead to enhanced provision of FA from the surrounding adipocytes for use in

energy production [46]. The generality of this mechanism for tumours that exist in regions with high levels of adipocytes (e.g., breast) remains to be elucidated.

6. Treatments for Diabetes Can Impact Cancer Progression

There are a range of glucose-lowering therapeutic agents currently prescribed for T2D. The most widely used front-line drug is metformin, which alters intracellular metabolism in insulin-target tissues (liver, skeletal muscle, and adipose tissue) to reduce end-organ resistance to the actions of insulin [89]. Other therapies are designed to increase endogenous insulin secretion by directly acting on pancreatic β-cells (sulfonylurea) or by enhancing the action of insulin secretion-promoting gut peptides (incretin mimetics) [10, 89]. At late stage of T2D, relative insulin deficiency due to heightened IR and pancreatic β-cell failure makes the administration of exogenous insulin a necessity. As noted above, high level of circulating insulin may facilitate cancer propagation, and thus insulin secretagogues and exogenous insulin are likely to increase cancer risk. Metformin, on the other hand, has been observed to reduce incidence and mortality of several cancer types compared to other diabetes medications, based on numerous population-based epidemiological studies and meta-analysis [90–93].

There are multiple aspects of diabetes that are improved by metformin, including suppression of hepatic overproduction of glucose and improvement of peripheral insulin sensitivity. One mechanism by which metformin achieves these effects is through activation of the energy sensor AMP-dependent protein kinase (AMPK). AMPK has versatile functions in the regulation of cellular energy metabolism, some of which overlap with and enhance the effects of insulin, such as the augmentation of glucose uptake in peripheral tissues [94]. Furthermore, AMPK inhibits endogenous lipid synthesis and promotes fatty acid oxidation, contributing to diminished lipid storage in nonadipose tissues and improved insulin sensitivity [95]. The way that metformin activates AMPK is thought to be through alterations in nucleotide levels. As a positively charged drug, metformin is taken into the mitochondrial matrix due to the inner membrane electrical gradient where it inhibits complex I of the respiratory chain in a time-dependent and self-limiting manner [96, 97]. The blockage of mitochondrial energy production through oxidative phosphorylation leads to changes in the AMP/ATP and ADP/ATP ratios, which signal energetic crisis that activates AMPK. Independent of its effects to activate AMPK it was recently shown that metformin suppresses hepatic glucose production by restraining glucagon-dependent gluconeogenesis [98].

With respect to cancer, several mechanisms have been proposed to underlie the beneficial antitumour effects of metformin and the more potent member of the biguanide class of drugs, phenformin. Given the tumour-promoting roles of plasma insulin and glucose, the alleviation of IR in insulin-target organs and the resulting reduction in glucose and insulin concentration in the circulation likely contribute to metformin-mediated tumour-suppressive effects in diabetic patients [99]. In addition, the accumulation of biomass in neoplastic cells is attenuated by metformin, which inhibits mTOR (mammalian target of rapamycin) signaling via Rag and Rac1 GTPase [100, 101].

The more well-described mechanism proposed to mediate the effects of metformin is activation of AMPK, which as noted above reprograms nutrient metabolism in response to energetic stress, favouring catabolic over anabolic pathways. AMPK signaling is downregulated in breast and ovarian cancers [102, 103] and its upstream activator LKB1, a well-known tumour suppressor, is nonfunctional in subsets of endometrial and lung cancers [104, 105]. Activation of AMPK through metformin treatment inhibits breast cancer growth through inducing cell-cycle arrest and opposing protein synthesis [106, 107]. Metformin-induced activation of AMPK is also associated with reduced growth of a number of other tumour types [108, 109]. Many of the effects of metformin are also seen when tumour cells are treated with the AMPK activator AICAR, which promotes oxidative metabolism and favours lipid utilization [110]. To further substantiate the tumour-suppressive role of AMPK in opposing cancer-related metabolic alterations, Faubert et al. showed that inactivation of the AMPK α catalytic subunit in both transformed lymphoma cells and nontransformed counterparts resulted in a shift towards aerobic glycolysis, increased incorporation of glucose-derived carbons into lipids, and biomass production while mice deficient in AMPKα had accelerated rate of lymphomagenesis [111]. Collectively these studies highlight the important role AMPK likely plays in the efficacy of metformin and suggest that the development of agents mimicking some of the effects (e.g., inhibition of lipogenesis and promotion of fat oxidation) of AMPK activators may have therapeutic relevance.

In 2004, Shaw and colleagues reported the paradoxical observation that tumour suppressor LKB1-deficient mammalian cells are resistant to oncogene-induced transformation but more prone to apoptotic cell death in response to cellular energy stress [112]. This intriguing finding raises the possibility that oblation of the energy-sensing LKB1-AMPK axis, while conferring biosynthetic and proliferative advantages, also imparts vulnerability to the cells so that they are hypersensitive to energetic crisis-induced killing. Indeed, non-small cell lung cancer (NSCLC) mice harbouring Kras and Lkb1 mutations, compared to those with Kras and p53 mutations, are selectively targeted by phenformin, leading to prolonging of survival [113]. Another potential application of biguanides as cancer-metabolism based therapies could be for tumours that have greater reliance on mitochondrial oxidative metabolism rather than the classical aerobic glycolysis. For example, a subset of human melanoma tumours was recently characterized to overexpress the master regulator of mitochondrial biogenesis PGC1α and exhibit increased mitochondrial energy metabolism [114]. For negative PGC1α melanoma cells, it was demonstrated that inhibition of BRAF, the most frequently overexpressed oncogene in melanoma, switched on a mitochondrial phosphorylation gene program including PGC1α and rendered the cells addicted to oxidative metabolism for a window of period before resistance developed [115]. A separate study reported synergistic

tumour-suppressive effects of combining phenformin and a BRAF inhibitor in melanoma on attenuating mTOR signaling and inducing apoptosis, which were attributed to cross talk between AMPK and BRAF signaling pathways [116]. It is unknown if the cooperation between BRAF inhibition and phenformin also acts via the induction of addiction to oxidative phosphorylation by the former and inhibition of mitochondrial respiratory chain complex I by the latter, but if the idea of synthetic lethality involving biguanide can be generalised to other cancer types, the impact on rational therapeutic design will be considerable.

Another prominent class of diabetic drug is the thiazolidinediones (TZDs) including pioglitazone and rosiglitazone. TZDs are agonists of peroxisome proliferator-activated receptor γ (PPARγ) which are predominantly expressed in adipose tissue [117]. They function primarily by inducing adipocyte proliferation and increasing adipose tissue lipid storage capacity to reduce fatty acid overflow to ectopic sites such as muscle, liver, and pancreas, along with exerting transcriptional control of genes involved in glucose and lipid metabolism [117]. TZDs have been shown to induce cell-cycle arrest, apoptosis, differentiation, and metastasis in a range of *in vitro* and *in vivo* cancer models [118]. Interestingly, some of the anticancer effects such as inhibitions of cell-cycle progression and invasiveness have been suggested to be independent of PPARγ activation [119, 120]. Despite these *in vitro* effects, epidemiological studies and meta-analysis over the past few years investigating the association between TZD use and cancer risk generated mixed results with the overall conclusion that TZDs reduce or do not affect the incidence of most cancer types but may increase the likelihood of developing bladder cancer [121–126]. The mechanisms responsible for these disparate findings are still under investigation.

7. Conclusions and Future Perspectives

T2D is increasing in prevalence across the world and with it comes the well-described complications, as well as an increased risk of many other diseases (e.g., cardiovascular disease). There is growing evidence that diabetes can also increase the risk of certain types of cancers. This relationship is not fully understood and there are many unanswered questions. For example, what are the exact features of diabetes that promote these types of cancer? What is the relative importance of different circulating nutrients, given the high level of glucose and lipids in diabetes and the recently described branched-chain amino acid signature [127]? Why does diabetes only increase the risk of certain types of cancers, but not all of them? Since compounds such as metformin appear to be beneficial for both T2D and cancer, we suggest that developing further compounds with dual effectiveness in both diseases, along with the pursuit of the unresolved questions above, should be the focus of future research in this area.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Sanket Joshi and Menghan Liu contributed equally to this work. All authors conceptualised and wrote the paper together.

Acknowledgments

Work in the laboratory of the authors is supported by funding from the National Health and Medical Research Council of Australia (NHMRC). Menghan Liu is supported by a University International Postgraduate Award from UNSW Australia. Nigel Turner is supported by an Australian Research Council Future Fellowship.

References

[1] International Diabetes Federation, *IDF Diabetes Atlas*, International Diabetes Federation, Brussels, Belgium, 2013.

[2] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, "Global cancer statistics," *CA Cancer Journal for Clinicians*, vol. 61, no. 2, pp. 69–90, 2011.

[3] E. Giovannucci, D. M. Harlan, M. C. Archer et al., "Diabetes and cancer: a consensus report," *Diabetes Care*, vol. 33, no. 7, pp. 1674–1685, 2010.

[4] E. Giovannucci, D. M. Harlan, M. C. Archer et al., "Diabetes and cancer: a consensus report," *CA: A Cancer Journal for Clinicians*, vol. 60, no. 4, pp. 207–221, 2010.

[5] S. S. Coughlin, E. E. Calle, L. R. Teras, J. Petrelli, and M. J. Thun, "Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults," *American Journal of Epidemiology*, vol. 159, no. 12, pp. 1160–1167, 2004.

[6] A. R. Saltiel and C. R. Kahn, "Insulin signalling and the regulation of glucose and lipid metabolism," *Nature*, vol. 414, no. 6865, pp. 799–806, 2001.

[7] R. Noguchi, H. Kubota, K. Yugi et al., "The selective control of glycolysis, gluconeogenesis and glycogenesis by temporal insulin patterns," *Molecular Systems Biology*, vol. 9, p. 664, 2013.

[8] D. B. Savage, K. F. Petersen, and G. I. Shulman, "Disordered lipid metabolism and the pathogenesis of insulin resistance," *Physiological Reviews*, vol. 87, no. 2, pp. 507–520, 2007.

[9] P. Tessari, D. Cecchet, A. Cosma et al., "Insulin resistance of amino acid and protein metabolism in type 2 diabetes," *Clinical Nutrition*, vol. 30, no. 3, pp. 267–272, 2011.

[10] M. Stumvoll, B. J. Goldstein, and T. W. van Haeften, "Type 2 diabetes: principles of pathogenesis and therapy," *The Lancet*, vol. 365, no. 9467, pp. 1333–1346, 2005.

[11] V. T. Samuel and G. I. Shulman, "Mechanisms for insulin resistance: common threads and missing links," *Cell*, vol. 148, no. 5, pp. 852–871, 2012.

[12] M. Qatanani and M. A. Lazar, "Mechanisms of obesity-associated insulin resistance: many choices on the menu," *Genes and Development*, vol. 21, no. 12, pp. 1443–1455, 2007.

[13] H. E. Lebovitz and M. A. Banerji, "Point: visceral adiposity is causally related to insulin resistance," *Diabetes Care*, vol. 28, no. 9, pp. 2322–2325, 2005.

[14] N. Kumashiro, D. M. Erion, D. Zhang et al., "Cellular mechanism of insulin resistance in nonalcoholic fatty liver disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 39, pp. 16381–16385, 2011.

[15] C. N. Lumeng and A. R. Saltiel, "Inflammatory links between obesity and metabolic disease," *The Journal of Clinical Investigation*, vol. 121, no. 6, pp. 2111–2117, 2011.

[16] S. Furukawa, T. Fujita, M. Shimabukuro et al., "Increased oxidative stress in obesity and its impact on metabolic syndrome," *Journal of Clinical Investigation*, vol. 114, no. 12, pp. 1752–1761, 2004.

[17] M. F. Gregor and G. S. Hotamisligil, "Inflammatory mechanisms in obesity," *Annual Review of Immunology*, vol. 29, pp. 415–445, 2011.

[18] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[19] O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[20] O. Warburg, K. Posener, and E. Negelein, "On metabolism of tumors," *Biochemische Zeitschrift*, vol. 152, pp. 319–344, 1924.

[21] S. Weinhouse, "The Warburg hypothesis fifty years later," *Zeitschrift für Krebsforschung und Klinische Onkologie*, vol. 87, no. 2, pp. 115–126, 1976.

[22] V. R. Fantin, J. St-Pierre, and P. Leder, "Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance," *Cancer Cell*, vol. 9, no. 6, pp. 425–434, 2006.

[23] R. Moreno-Sánchez, S. Rodríguez-Enríquez, A. Marín-Hernández, and E. Saavedra, "Energy metabolism in tumor cells," *FEBS Journal*, vol. 274, no. 6, pp. 1393–1418, 2007.

[24] C. V. Dang, "Links between metabolism and cancer," *Genes and Development*, vol. 26, no. 9, pp. 877–890, 2012.

[25] K. Bensaad, A. Tsuruta, M. A. Selak et al., "TIGAR, a p53-inducible regulator of glycolysis and apoptosis," *Cell*, vol. 126, no. 1, pp. 107–120, 2006.

[26] H. R. Christofk, M. G. Vander Heiden, M. H. Harris et al., "The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth," *Nature*, vol. 452, no. 7184, pp. 230–233, 2008.

[27] H. R. Christofk, M. G. Vander Heiden, N. Wu, J. M. Asara, and L. C. Cantley, "Pyruvate kinase M2 is a phosphotyrosine-binding protein," *Nature*, vol. 452, no. 7184, pp. 181–186, 2008.

[28] M. L. Macheda, S. Rogers, and J. D. Best, "Molecular and cellular regulation of glucose transporter (GLUT) proteins in cancer," *Journal of Cellular Physiology*, vol. 202, no. 3, pp. 654–662, 2005.

[29] M. G. V. Heiden, D. R. Plas, J. C. Rathmell, C. J. Fox, M. H. Harris, and C. B. Thompson, "Growth factors can influence cell growth and survival through effects on glucose metabolism," *Molecular and Cellular Biology*, vol. 21, no. 17, pp. 5899–5912, 2001.

[30] J. A. Engelman, L. Chen, X. Tan et al., "Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers," *Nature Medicine*, vol. 14, no. 12, pp. 1351–1356, 2008.

[31] H. Shim, C. Dolde, B. C. Lewis et al., "c-Myc transactivation of LDH-A: implications for tumor metabolism and growth," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 13, pp. 6658–6663, 1997.

[32] L. Szablewski, "Expression of glucose transporters in cancers," *Biochimica et Biophysica Acta—Reviews on Cancer*, vol. 1835, no. 2, pp. 164–169, 2013.

[33] K. Adekola, S. T. Rosen, and M. Shanmugam, "Glucose transporters in cancer metabolism," *Current Opinion in Oncology*, vol. 24, no. 6, pp. 650–654, 2012.

[34] S. P. Mathupala, Y. H. Ko, and P. L. Pedersen, "Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria," *Oncogene*, vol. 25, no. 34, pp. 4777–4786, 2006.

[35] R. J. Deberardinis and T. Cheng, "Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer," *Oncogene*, vol. 29, no. 3, pp. 313–324, 2010.

[36] D. R. Wise, P. S. Ward, J. E. S. Shay et al., "Hypoxia promotes isocitrate dehydrogenasedependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 49, pp. 19611–19616, 2011.

[37] A. R. Mullen, W. W. Wheaton, E. S. Jin et al., "Reductive carboxylation supports growth in tumour cells with defective mitochondria," *Nature*, vol. 481, no. 7381, pp. 385–388, 2012.

[38] C. M. Metallo, P. A. Gameiro, E. L. Bell et al., "Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia," *Nature*, vol. 481, no. 7381, pp. 380–384, 2012.

[39] D. R. Wise, R. J. Deberardinis, A. Mancuso et al., "Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 48, pp. 18782–18787, 2008.

[40] P. Gao, I. Tchernyshyov, T.-C. Chang et al., "c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism," *Nature*, vol. 458, no. 7239, pp. 762–765, 2009.

[41] E. Currie, A. Schulze, R. Zechner, T. C. Walther, and R. V. Farese Jr., "Cellular fatty acid metabolism and cancer," *Cell Metabolism*, vol. 18, no. 2, pp. 153–161, 2013.

[42] D. E. Bauer, G. Hatzivassiliou, F. Zhao, C. Andreadis, and C. B. Thompson, "ATP citrate lyase is an important component of cell growth and transformation," *Oncogene*, vol. 24, no. 41, pp. 6314–6322, 2005.

[43] V. Chajès, M. Cambot, K. Moreau, G. M. Lenoir, and V. Joulin, "Acetyl-CoA carboxylase α is essential to breast cancer cell survival," *Cancer Research*, vol. 66, no. 10, pp. 5287–5294, 2006.

[44] J. A. Menendez and R. Lupu, "Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis," *Nature Reviews Cancer*, vol. 7, no. 10, pp. 763–777, 2007.

[45] K. J. Williams, J. P. Argus, Y. Zhu et al., "An essential requirement for the SCAP/SREBP signaling axis to protect cancer cells from lipotoxicity," *Cancer Research*, vol. 73, no. 9, pp. 2850–2862, 2013.

[46] K. M. Nieman, H. A. Kenny, C. V. Penicka et al., "Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth," *Nature Medicine*, vol. 17, no. 11, pp. 1498–1503, 2011.

[47] R. A. DeFilippis, H. Chang, N. Dumont et al., "CD36 repression activates a multicellular stromal program shared by high mammographic density and tumor tissues," *Cancer Discovery*, vol. 2, no. 9, pp. 826–839, 2012.

[48] J. A. Bertout, S. A. Patel, and M. C. Simon, "The impact of O_2 availability on human cancer," *Nature Reviews Cancer*, vol. 8, no. 12, pp. 967–975, 2008.

[49] G. L. Semenza, "Targeting HIF-1 for cancer therapy," *Nature Reviews Cancer*, vol. 3, no. 10, pp. 721–732, 2003.

[50] J.-W. Kim, I. Tchernyshyov, G. L. Semenza, and C. V. Dang, "HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia," *Cell Metabolism*, vol. 3, no. 3, pp. 177–185, 2006.

[51] I. Papandreou, R. A. Cairns, L. Fontana, A. L. Lim, and N. C. Denko, "HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption," *Cell Metabolism*, vol. 3, no. 3, pp. 187–197, 2006.

[52] J. D. Gordan, J. A. Bertout, C.-J. Hu, J. A. Diehl, and M. C. Simon, "HIF-2α promotes hypoxic cell proliferation by enhancing c-Myc transcriptional activity," *Cancer Cell*, vol. 11, no. 4, pp. 335–347, 2007.

[53] E. Gottlieb and I. P. M. Tomlinson, "Mitochondrial tumour suppressors: a genetic and biochemical update," *Nature Reviews Cancer*, vol. 5, no. 11, pp. 857–866, 2005.

[54] S. Gottschalk, N. Anderson, C. Hainz, S. G. Eckhardt, and N. J. Serkova, "Imatinib (STI571)-mediated changes in glucose metabolism in human leukemia BCR-ABL-positive cells," *Clinical Cancer Research*, vol. 10, no. 19, pp. 6661–6668, 2004.

[55] R. L. Elstrom, D. E. Bauer, M. Buzzai et al., "Akt stimulates aerobic glycolysis in cancer cells," *Cancer Research*, vol. 64, no. 11, pp. 3892–3899, 2004.

[56] K. B. Nolop, C. G. Rhodes, L. H. Brudin et al., "Glucose utilization in vivo by human pulmonary neoplasms," *Cancer*, vol. 60, no. 11, pp. 2682–2689, 1987.

[57] P. S. Ward and C. B. Thompson, "Metabolic reprogramming: a cancer hallmark even warburg did not anticipate," *Cancer Cell*, vol. 21, no. 3, pp. 297–308, 2012.

[58] S. V. Tavtigian, S. D. Zabludoff, and B. J. Wold, "Cloning of mid-G1 serum response genes and identification of a subset regulated by conditional myc expression," *Molecular Biology of the Cell*, vol. 5, no. 3, pp. 375–388, 1994.

[59] C. V. Dang, K. A. O'Donnell, K. I. Zeller, T. Nguyen, R. C. Osthus, and F. Li, "The c-Myc target gene network," *Seminars in Cancer Biology*, vol. 16, no. 4, pp. 253–264, 2006.

[60] P. Gao, I. Tchernyshyov, T.-C. Chang et al., "C-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism," *Nature*, vol. 458, no. 7239, pp. 762–765, 2009.

[61] A. Le, A. N. Lane, M. Hamaker et al., "Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in b cells," *Cell Metabolism*, vol. 15, no. 1, pp. 110–121, 2012.

[62] M. Yuneva, N. Zamboni, P. Oefner, R. Sachidanandam, and Y. Lazebnik, "Deficiency in glutamine but not glucose induces MYC-dependent apoptosis in human cells," *The Journal of Cell Biology*, vol. 178, no. 1, pp. 93–105, 2007.

[63] H. Ying, A. C. Kimmelman, C. A. Lyssiotis et al., "Oncogenic kras maintains pancreatic tumors through regulation of anabolic glucose metabolism," *Cell*, vol. 149, no. 3, pp. 656–670, 2012.

[64] J. Son, C. A. Lyssiotis, H. Ying et al., "Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway," *Nature*, vol. 496, pp. 101–105, 2013.

[65] K. H. Vousden and K. M. Ryan, "p53 and metabolism," *Nature Reviews Cancer*, vol. 9, no. 10, pp. 691–700, 2009.

[66] F. Schwartzenberg-Bar-Yoseph, M. Armoni, and E. Karnieli, "The tumor suppressor p53 down-regulates glucose transporters *GLUT1* and *GLUT4* gene expression," *Cancer Research*, vol. 64, no. 7, pp. 2627–2633, 2004.

[67] S. Matoba, J.-G. Kang, W. D. Patino et al., "p53 regulates mitochondrial respiration," *Science*, vol. 312, no. 5780, pp. 1650–1653, 2006.

[68] B. B. Barone, H. C. Yeh, C. F. Snyder et al., "Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis," *Journal of the American Medical Association*, vol. 300, no. 23, pp. 2754–2764, 2008.

[69] M. Pollak, "Insulin and insulin-like growth factor signalling in neoplasia," *Nature Reviews Cancer*, vol. 8, no. 12, pp. 915–928, 2008.

[70] M. Pollak, "The insulin and insulin-like growth factor receptor family in neoplasia: an update," *Nature Reviews Cancer*, vol. 12, no. 3, pp. 159–169, 2012.

[71] C. K. Osborne, G. Bolan, M. E. Monaco, and M. E. Lippman, "Hormone responsive human breast cancer in long term tissue culture: effect of insulin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 73, no. 12, pp. 4536–4540, 1976.

[72] C. Algire, L. Amrein, M. Bazile, S. David, M. Zakikhani, and M. Pollak, "Diet and tumor LKB1 expression interact to determine sensitivity to anti-neoplastic effects of metformin in vivo," *Oncogene*, vol. 30, no. 10, pp. 1174–1182, 2011.

[73] V. Venkateswaran, A. Q. Haddad, N. E. Fleshner et al., "Association of diet-induced hyperinsulinemia with accelerated growth of prostate cancer (LNCaP) xenografts," *Journal of the National Cancer Institute*, vol. 99, no. 23, pp. 1793–1800, 2007.

[74] C. Sell, M. Rubini, R. Rubin, J.-P. Liu, A. Efstratiadis, and R. Baserga, "Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 23, pp. 11217–11221, 1993.

[75] W. Duan, X. Shen, J. Lei et al., "Hyperglycemia, a neglected factor during cancer progression," *BioMed Research International*, vol. 2014, Article ID 461917, 10 pages, 2014.

[76] Y. Zhang, J. Zhou, T. Wang, and L. Cai, "High level glucose increases mutagenesis in human lymphoblastoid cells," *International Journal of Biological Sciences*, vol. 3, no. 6, pp. 375–379, 2007.

[77] A. E. Vaughn and M. Deshmukh, "Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c," *Nature Cell Biology*, vol. 10, no. 12, pp. 1477–1483, 2008.

[78] A. Brunello, R. Kapoor, and M. Extermann, "Hyperglycemia during chemotherapy for hematologic and solid tumors is correlated with increased toxicity," *American Journal of Clinical Oncology: Cancer Clinical Trials*, vol. 34, no. 3, pp. 292–296, 2011.

[79] L. Zeng, K. M. Biernacka, J. M. P. Holly et al., "Hyperglycaemia confers resistance to chemotherapy on breast cancer cells: the role of fatty acid synthase," *Endocrine-Related Cancer*, vol. 17, no. 2, pp. 539–551, 2010.

[80] S. K. Fried, D. A. Bunkin, and A. S. Greenberg, "Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 3, pp. 847–850, 1998.

[81] G. S. Hotamisligil, P. Arner, J. F. Caro, R. L. Atkinson, and B. M. Spiegelman, "Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance," *Journal of Clinical Investigation*, vol. 95, no. 5, pp. 2409–2415, 1995.

[82] Y. Gonzalez, M. T. Herrera, G. Soldevila et al., "High glucose concentrations induce TNF-α production through the down-regulation of CD33 in primary human monocytes," *BMC Immunology*, vol. 13, article 19, 2012.

[83] S. Devaraj, S. K. Venugopal, U. Singh, and I. Jialal, "Hyperglycemia induces monocytic release of interleukin-6 via induction of protein kinase C-α and -β," *Diabetes*, vol. 54, no. 1, pp. 85–91, 2005.

[84] S. I. Grivennikov, F. R. Greten, and M. Karin, "Immunity, inflammation, and cancer," *Cell*, vol. 140, no. 6, pp. 883–899, 2010.

[85] T. Kitamura, K. Kometani, H. Hashida et al., "SMAD4-deficient intestinal tumors recruit CCR1$^+$ myeloid cells that promote invasion," *Nature Genetics*, vol. 39, no. 4, pp. 467–475, 2007.

[86] D. R. Hodge, E. M. Hurt, and W. L. Farrar, "The role of IL-6 and STAT3 in inflammation and cancer," *European Journal of Cancer*, vol. 41, no. 16, pp. 2502–2512, 2005.

[87] E. Kastritis, A. Palumbo, and M. A. Dimopoulos, "Treatment of relapsed/refractory multiple myeloma," *Seminars in Hematology*, vol. 46, no. 2, pp. 143–157, 2009.

[88] J. L. Luo, S. Maeda, L. C. Hsu, H. Yagita, and M. Karin, "Inhibition of NF-κB in cancer cells converts inflammation-induced tumor growth mediated by TNFα to TRAIL-mediated tumor regression," *Cancer Cell*, vol. 6, no. 3, pp. 297–305, 2004.

[89] Z. T. Bloomgarden, "Exploring treatment strategies for type 2 diabetes," *Diabetes Care*, vol. 30, no. 10, pp. 2737–2745, 2007.

[90] J. M. M. Evans, L. A. Donnelly, A. M. Emslie-Smith, D. R. Alessi, and A. D. Morris, "Metformin and reduced risk of cancer in diabetic patients," *British Medical Journal*, vol. 330, no. 7503, pp. 1304–1305, 2005.

[91] A. DeCensi, M. Puntoni, P. Goodwin et al., "Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis," *Cancer Prevention Research*, vol. 3, no. 11, pp. 1451–1461, 2010.

[92] S. L. Bowker, S. R. Majumdar, P. Veugelers, and J. A. Johnson, "Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin," *Diabetes Care*, vol. 29, no. 2, pp. 254–258, 2006.

[93] C. J. Currie, C. D. Poole, and E. A. M. Gale, "The influence of glucose-lowering therapies on cancer risk in type 2 diabetes," *Diabetologia*, vol. 52, no. 9, pp. 1766–1777, 2009.

[94] D. G. Hardie, "The AMP-activated protein kinase pathway—new players upstream and downstream," *Journal of Cell Science*, vol. 117, no. 23, pp. 5479–5487, 2004.

[95] D. G. Hardie, "AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 10, pp. 774–785, 2007.

[96] M. R. Owen, E. Doran, and A. P. Halestrap, "Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain," *Biochemical Journal*, vol. 348, no. 3, pp. 607–614, 2000.

[97] M.-Y. El-Mir, V. Nogueira, E. Fontaine, N. Avéret, M. Rigoulet, and X. Leverve, "Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I," *Journal of Biological Chemistry*, vol. 275, no. 1, pp. 223–228, 2000.

[98] R. A. Miller, Q. Chu, J. Xie, M. Foretz, B. Viollet, and M. J. Birnbaum, "Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP," *Nature*, vol. 494, no. 7436, pp. 256–260, 2013.

[99] M. N. Pollak, "Investigating metformin for cancer prevention and treatment: the end of the beginning," *Cancer Discovery*, vol. 2, no. 9, pp. 778–790, 2012.

[100] A. Kalender, A. Selvaraj, S. Y. Kim et al., "Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner," *Cell Metabolism*, vol. 11, no. 5, pp. 390–401, 2010.

[101] B. Dirat, I. Ader, M. Golzio et al., "Metformin targets the GTPase Rac1 to inhibit prostate cancer cell migration," *Cancer & Metabolism*, vol. 2, supplement 1, article O24, 2014.

[102] S. M. Hadad, L. Baker, P. R. Quinlan et al., "Histological evaluation of AMPK signalling in primary breast cancer," *BMC Cancer*, vol. 9, article 307, 2009.

[103] C. Li, V. W. S. Liu, P. M. Chiu, K.-M. Yao, H. Y. S. Ngan, and D. W. Chan, "Reduced expression of AMPK-β1 during tumor progression enhances the oncogenic capacity of advanced ovarian cancer," *Molecular Cancer*, vol. 13, no. 1, article 49, 2014.

[104] D. B. Shackelford and R. J. Shaw, "The LKB1-AMPK pathway: metabolism and growth control in tumour suppression," *Nature Reviews Cancer*, vol. 9, no. 8, pp. 563–575, 2009.

[105] J. Carretero, P. P. Medina, R. Blanco et al., "Dysfunctional AMPK activity, signalling through mTOR and survival in response to energetic stress in LKB1-deficient lung cancer," *Oncogene*, vol. 26, no. 11, pp. 1616–1625, 2007.

[106] M. Zakikhani, R. Dowling, I. G. Fantus, N. Sonenberg, and M. Pollak, "Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells," *Cancer Research*, vol. 66, no. 21, pp. 10269–10273, 2006.

[107] Y. Zhuang and W. K. Keith, "Cell cycle arrest in Metformin treated breast cancer cells involves activation of AMPK, down-regulation of cyclin D1, and requires p27^{Kip1} or p21^{Cip1}," *Journal of Molecular Signaling*, vol. 3, article 18, 2008.

[108] L. Zheng, W. Yang, F. Wu et al., "Prognostic significance of AMPK activation and therapeutic effects of metformin in hepatocellular carcinoma," *Clinical Cancer Research*, vol. 19, no. 19, pp. 5372–5380, 2013.

[109] W. Y. Shi, D. Xiao, L. Wang et al., "Therapeutic metformin/AMPK activation blocked lymphoma cell growth via inhibition of mTOR pathway and induction of autophagy," *Cell Death and Disease*, vol. 3, article e275, 2012.

[110] C. Jose, E. Hébert-Chatelain, N. Bellance et al., "AICAR inhibits cancer cell growth and triggers cell-type distinct effects on OXPHOS biogenesis, oxidative stress and Akt activation," *Biochimica et Biophysica Acta: Bioenergetics*, vol. 1807, no. 6, pp. 707–718, 2011.

[111] B. Faubert, G. Boily, S. Izreig et al., "AMPK is a negative regulator of the warburg effect and suppresses tumor growth in vivo," *Cell Metabolism*, vol. 17, no. 1, pp. 113–124, 2013.

[112] R. J. Shaw, M. Kosmatka, N. Bardeesy et al., "The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 10, pp. 3329–3335, 2004.

[113] D. B. Shackelford, E. Abt, L. Gerken et al., "LKB1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin," *Cancer Cell*, vol. 23, no. 2, pp. 143–158, 2013.

[114] F. Vazquez, J.-H. Lim, H. Chim et al., "PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress," *Cancer Cell*, vol. 23, no. 3, pp. 287–301, 2013.

[115] R. Haq, J. Shoag, P. Andreu-Perez et al., "Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF," *Cancer Cell*, vol. 23, no. 3, pp. 302–315, 2013.

[116] P. Yuan, K. Ito, R. Perez-Lorenzo et al., "Phenformin enhances the therapeutic benefit of BRAF^{V600E} inhibition in melanoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 45, pp. 18226–18231, 2013.

[117] H. Hauner, "The mode of action of thiazolidinediones," *Diabetes/Metabolism Research and Reviews*, vol. 18, no. 2, pp. S10–S15, 2002.

[118] A. Elnemr, T. Ohta, K. Iwata et al., "PPARgamma ligand (thiazolidinedione) induces growth arrest and differentiation markers of human pancreatic cancer cells," *International Journal of Oncology*, vol. 17, no. 6, pp. 1157–1164, 2000.

[119] A. Galli, E. Ceni, D. W. Crabb et al., "Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARγ independent mechanisms," *Gut*, vol. 53, no. 11, pp. 1688–1697, 2004.

[120] J.-W. Huang, C.-W. Shiau, Y.-T. Yang et al., "Peroxisome proliferator-activated receptor γ-independent ablation of cyclin D1 by thiazolidinediones and their derivatives in breast cancer cells," *Molecular Pharmacology*, vol. 67, no. 4, pp. 1342–1348, 2005.

[121] I. N. Colmers, S. L. Bowker, S. R. Majumdar, and J. A. Johnson, "Use of thiazolidinediones and the risk of bladder cancer among people with type 2 diabetes: a meta-analysis," *Canadian Medical Association Journal*, vol. 184, no. 12, pp. E675–E683, 2012.

[122] C. Bosetti, V. Rosato, D. Buniato, A. Zambon, C. La Vecchia, and G. Corrao, "Cancer risk for patients using thiazolidinediones for type 2 diabetes: a meta-analysis," *The Oncologist*, vol. 18, no. 2, pp. 148–156, 2013.

[123] M. Monami, I. Dicembrini, and E. Mannucci, "Thiazolidinediones and cancer: results of a meta-analysis of randomized clinical trials," *Acta Diabetologica*, vol. 51, no. 1, pp. 91–101, 2014.

[124] C.-H. Chang, J.-W. Lin, L.-C. Wu, M.-S. Lai, L.-M. Chuang, and K. Arnold Chan, "Association of thiazolidinediones with liver cancer and colorectal cancer in type 2 diabetes mellitus," *Hepatology*, vol. 55, no. 5, pp. 1462–1472, 2012.

[125] R. Govindarajan, L. Ratnasinghe, D. L. Simmons et al., "Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes," *Journal of Clinical Oncology*, vol. 25, no. 12, pp. 1476–1481, 2007.

[126] M. E. Ramos-Nino, C. D. MacLean, and B. Littenberg, "Association between cancer prevalence and use of thiazolidinediones: results from the Vermont Diabetes Information System," *BMC Medicine*, vol. 5, article 17, 2007.

[127] C. B. Newgard, "Interplay between lipids and branched-chain amino acids in development of insulin resistance," *Cell Metabolism*, vol. 15, no. 5, pp. 606–614, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 925703, 15 pages
http://dx.doi.org/10.1155/2015/925703

Research Article

Biologic Roles of Estrogen Receptor-β and Insulin-Like Growth Factor-2 in Triple-Negative Breast Cancer

Nalo Hamilton,[1,2] Diana Márquez-Garbán,[2,3] Vei Mah,[2,4] Gowry Fernando,[1] Yahya Elshimali,[5] Hermes Garbán,[2,6] David Elashoff,[2,7] Jaydutt Vadgama,[2,3,5] Lee Goodglick,[2,4] and Richard Pietras[2,3]

[1] *UCLA School of Nursing, Los Angeles, CA 90095, USA*
[2] *UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA 90095, USA*
[3] *Division of Hematology-Oncology, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA*
[4] *Department of Pathology and Laboratory Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA*
[5] *Division of Cancer Research and Training, Department of Medicine, Charles Drew University School of Medicine and Science, Los Angeles, CA 90095, USA*
[6] *Division of Dermatology, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA*
[7] *Division of General Internal Medicine, Department of Medicine, UCLA David Geffen School of Medicine, Los Angeles, CA 90095, USA*

Correspondence should be addressed to Richard Pietras; rpietras@mednet.ucla.edu

Received 30 July 2014; Revised 10 December 2014; Accepted 16 December 2014

Academic Editor: Ann Simpson

Triple-negative breast cancer (TNBC) occurs in 10–15% of patients yet accounts for almost half of all breast cancer deaths. TNBCs lack expression of estrogen and progesterone receptors and HER-2 overexpression and cannot be treated with current targeted therapies. TNBCs often occur in African American and younger women. Although initially responsive to some chemotherapies, TNBCs tend to relapse and metastasize. Thus, it is critical to find new therapeutic targets. A second ER gene product, termed ERβ, in the absence of ERα may be such a target. Using human TNBC specimens with known clinical outcomes to assess ERβ expression, we find that ERβ1 associates with significantly worse 5-year overall survival. Further, a panel of TNBC cell lines exhibit significant levels of ERβ protein. To assess ERβ effects on proliferation, ERβ expression in TNBC cells was silenced using shRNA, resulting in a significant reduction in TNBC proliferation. ERβ-specific antagonists similarly suppressed TNBC growth. Growth-stimulating effects of ERβ may be due in part to downstream actions that promote VEGF, amphiregulin, and Wnt-10b secretion, other factors associated with tumor promotion. *In vivo*, insulin-like growth factor-2 (IGF-2), along with ERβ1, is significantly expressed in TNBC and stimulates high ERβ mRNA in TNBC cells. This work may help elucidate the interplay of metabolic and growth factors in TNBC.

Dedicated to the memory of the authors' distinguished colleague and friend Dr. Lee Goodglick (1960–2014) who made major contributions in cancer research during his career

1. Introduction

Breast cancer (BC) is the most common malignancy in women [1, 2]. About 70% of patients with breast cancer express estrogen receptor-α (ERα). Due to the success of endocrine therapies, the mortality of patients with ERα-positive tumors has declined significantly in the past decade. Similarly, about 15% of patients have tumors that overexpress HER2 receptor and thus are candidates for HER2-targeted treatments. In contrast, TNBC occurs in 10–15% of patients, yet this disease subtype accounts for about half of all breast cancer deaths. TNBCs lack clinical expression of ERα, progesterone receptor, and HER2 overexpression (ERα−/PR−/HER2−). TNBCs have incomplete overlap with

basal-like breast tumors, a subgroup of breast cancers defined by gene-expression profiling that express specific cytokeratins, and with some hereditary breast cancers. Though heterogeneous, TNBCs typically occur in younger women and African American women as well as among some patients with BRCA1 gene defects [1, 2]. Population-based data show that African American women have a higher incidence of TNBC and present with more advanced stages than Caucasian women [3]. This cancer subtype also associates with adverse biological features including high mitotic count and very aggressive behavior. Of note, some recent reports indicate that the incidence of ERα-negative BC and TNBC, high-risk breast cancer subtypes, may correlate with the extent of African ancestry [4]. Though initially responsive to chemotherapy, TNBCs tend to relapse and metastasize early and have a prognosis worse than other subtypes. Currently specific therapies for TNBC are unavailable [1–3].

Estrogens promote progression of ERα-positive cancers, effects exerted by binding of estradiol to ERα, a ligand-activated transcription factor [5]. ERα is predominantly a nuclear-localized protein. Immunohistochemical (IHC) detection of nuclear ERα in tumors is a standard clinical assay used to plan patient management [6]. Of special note, recent reports show that a second type of estrogen receptor, termed estrogen receptor-beta (ERβ), is expressed in TNBC cells [7, 8]. ERα and ERβ are encoded by two different genes, yet ERβ has 96% homology with ERα at the DNA-binding domain and 60% homology at the ligand-binding domain (LBD). However, it is important to note that ERβ is not identified in standard assays for ERα. The role of ERβ in breast cancer remains to be elucidated but some studies show ERβ is a biomarker related to a more aggressive clinical course [8] and correlates with Ki-67, a marker of proliferation [7, 9]. Early studies demonstrate higher levels of ERβ in breast tumors of African American as compared to Caucasian women, suggesting that ERβ may play a critical role in TNBC development [10–14].

Based on current data, estradiol regulates gene expression by genomic and nongenomic inputs [15, 16]. Genomic signals involve direct action of nuclear-localized ERα as an estradiol-regulated transcription factor or coregulator. By contrast, nongenomic signaling involves extranuclear events mediated by extranuclear ERs often in cooperation with coactivator or adaptor proteins [17]; these then impact gene expression indirectly by modulating signaling cascades such as MAPK, PI3K/AKT, and mTOR [8, 14–19] to regulate transcription [5, 15, 16]. In target cells, extranuclear ERα forms are derived from the same transcript as nuclear ERα; however minor extranuclear ERα splice variants occur [15, 16]. In TNBC, less is known about the role of ERβ in cancer progression [7, 8, 11]. Several ERβ isoforms occur in breast cancers, including ERβ1, ERβ2, ERβ4, and ERβ5, but only ERβ1 retains an intact LBD to interact with specific ligands, thus ERβ1 is a preferred clinical target [17, 20–22]. ERβ forms occur in tumor cell nuclei but, as ERα forms, may also occur at extranuclear sites [15, 16, 23]. Like ERα, ERβ activates transcription by genomic pathways or nongenomic pathways by interaction with coactivators/coregulators [17] that in turn modulate signaling cascades to impact gene expression and tumor progression [5, 12, 14, 20, 21, 23]. Of note, ERβ target genes appear to be those that regulate cell death and survival, cell movement, and cell development, growth, and proliferation, as well as genes involved in the Wnt/β-catenin and the G1/S cell cycle phase checkpoint pathways [24].

Obesity and the metabolic syndrome are associated with multiple factors that may cause an increased risk for cancer and cancer-related mortality [25]. One example is the insulin family of proteins which have pleiotropic effects on metabolism and growth. A large body of evidence indicates the insulin/insulin-like growth factor (IGF-1, IGF-2) pathway in breast cancer progression [25–29]. Of note, IGF-2 occurs in an unprocessed (pIGF-2) and mature (mIGF-2) form and plays a role in breast cell proliferation and inhibition of apoptosis [28–33]. Under normal conditions, IGF-2 is tightly bound and sequestered [34–38], but overexpression of IGF-2 is associated with breast cancer development and increased tumor formation [39, 40]. Most human cancers overexpress both the IGF-1 receptor (IGF-1R) and insulin receptor (IR) isoforms, leading to the formation of hybrid IGF-1R/IRs. IGF-2 is a known ligand for these receptors as well as the mannose-6-phosphate/IGF-2R and high-affinity binding proteins [25–29, 32]. IGF-2 expression is strongly enhanced in invasive breast cancers and downstream mTOR signaling is stimulated [41] as is TNBC cell migration [29]. Of note, disparities in the expression of IGF-2 and its receptors are reported to occur in breast tissue samples from African American women as compared to Caucasian women and may contribute to differences in clinical outcomes [42]. IGF-2 is detected in both tumor stroma and epithelial breast cancer cells and correlates with both breast epithelial [27, 43–45] and stromal cell proliferation [46]. Westley and May [26] also report that estrogen signaling may cross communicate with IGF pathways, with estrogen promoting increased breast cancer production of IGF-2.

This report details interactions of ERβ with IGF-2 and other growth factor pathways in TNBC [46–52]. Our findings using TNBC models and archival specimens suggest that IGF-2 may regulate ERβ expression which in turn modulates metabolic and growth factor pathways in cancer progression.

2. Materials and Methods

2.1. Breast Cancer Cell Lines. For these studies, we used the following triple-negative breast cancer cell lines (ATCC) which have been previously well characterized as lacking expression of ERα and PR as well as overexpression of HER2 [47, 48]: MDA-MB-231, MDA-MB-435, BT549, HCC38, HCC1143, HCC1937, and HCC1806. As controls, we used MCF-7 (expressing abundant ERα and minimal/no ERβ) and T47D (expresses ERα and more abundant ERβ). Cell cultures were routinely maintained at 37°C in a 5% CO_2 incubator using RPMI 1640 media supplemented with penicillin/streptomycin (10,000 units/mL penicillin and 10,000 units/mL streptomycin sulfate) and 10% fetal bovine serum unless other specific media were recommended by the supplier (ATCC). For the MDA-MB-231 cell line, we created stable transfectants with a specific ERβ shRNA producing a knockdown of ERβ. As controls, we used a stable transfectant

with a scrambled shRNA and vector control prepared as detailed below (all reagents from Origene).

2.2. Reagents. ERβ ligands for use in these experiments included the following: (a) diarylpropionitrile (DPN), an ERβ agonist (Tocris), (b) 4-[2-phenyl-5,7-*bis*(trifluoromethyl)-pyrazolo[1,5-*a*]pyrimidin-3-yl]phenol (PHTPP), ERβ antagonist, and (c) 4,4′,4″-(4-propyl-[1*H*]-pyrazole-1,3,5-triyl)trisphenol (PPT), an ERα agonist [49, 50].

2.3. Assays for Cell Proliferation. In experiments to assess proliferative effects of ERβ ligands, cells were grown in phenol red-free, estrogen-free media with 0.1% dextran-coated charcoal-treated- (DCC-) FBS for 48 hours and then treated with selected doses of DPN, PPT, or PHTPP. Cell counts and viability tests (Trypan blue) were done every 24 hours for 3 days. After 72 hours, proliferation was assessed using the BrdU cell proliferation ELISA (Roche). Cell numbers were also assessed initially by cell counts to confirm ELISA data.

2.4. Assays for Growth Factor Secretion. Tumor cells were cultured in estrogen-free media and then treated 20–120 minutes with DPN, followed by harvest of particle-free media and application of established ELISA assays for VEGF, amphiregulin, WNT 10b/12 [51–53], signaling molecules that activate angiogenesis, EGFR, and WNT pathways, respectively, which promote TNBC [1, 52, 53].

2.5. Knockdown of ERβ Expression. To suppress ERβ expression, we used the HuSH 29 mer shRNA constructs (Origene) designed to target human ERβ (*ESR2*) and included positive and negative controls. Plasmids were designed and validated specifically to knockdown expression of specific genes by RNA interference and allow for enrichment of transfected cells. Each vector expresses a short hairpin RNA (shRNA) under control of the U6 promoter and puromycin resistance gene to select stably transfected cells. Cells were transfected with negative control, scrambled negative control, and shRNA plasmids for ERβ using MegaTran 1.0 transfection reagent (Origene). After 48 hours, cells were replated at low density in the presence of an effective concentration of puromycin. Culture medium was replaced every 2-3 days, with cells replated every week for 2 weeks. As stable transfectants were obtained, we isolated total RNA to identify colonies with optimal ERβ1 knockdown, as confirmed by qRT-PCR and immunoassays. Expression of transcripts was done as before [14] and protein levels of ERβ variants were determined (data not shown). After ERβ1 knockdown, cell proliferation was determined by established methods in the presence of vehicle or specific ERβ ligands.

2.6. ERβ Expression by Quantitative RT-PCR (QRT-PCR). To assess ERβ transcript levels in TNBC cells [54], total RNA was isolated by the Aurum total RNA mini kit (Bio-Rad). UV spectroscopy and RNA quality indicator (RQI) values obtained from the Experion automated electrophoresis system (Bio-Rad) were used to determine RNA integrity. Primer pairs used for ERβ (Qiagen SA Biosciences): ERβ forward: 5′-GCTCATCTTTGCTCCAGATCTTG-3′ and ERβ reverse: 5′-GATGCTTTGGTTTGGGTGATTG-3. cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) with 400 ng total RNA. The iQ SYBR Green Supermix (Bio-Rad) was used for PCR amplification. Each reaction was performed in triplicate and ribosomal protein 36B4 mRNA, a housekeeping gene unaffected by estrogen, was used as the internal control. The cycling conditions consisted of 95.0°C for 30 seconds followed by 39 cycles of 95.0°C for 5 seconds, 57.0°C for 15 seconds, and 72.0°C for 90 seconds in a CFX96 Touch Thermocycler (Bio-Rad). Transcript levels of ERβ were normalized to 36B4. Fold induction or repression was measured relative to controls and calculated after adjusting for 36B4 RNA (endogenous control) using $2^{-\Delta\Delta Ct}$, where ΔCt = Ct interested gene − Ct 36B4 RNA and $\Delta\Delta Ct$ = ΔCt treatment $-\Delta Ct$ vehicle control. For experiments that evaluated IGF-2 effects on ERβ, cell lines were seeded and cultured to 70–75% confluence in complete media followed by 24 hours in serum/phenol red-free media. Following serum starvation, cultures were treated with 100 ng/mL of human recombinant pIGF-2 or mIGF-2 (GroPep) for 24 hours in serum-free, phenol-red-free media before total RNA isolation.

2.7. Gel Electrophoresis and Immunoblotting. TNBC cells were maintained in estrogen-free conditions 48 hours before experiments. Cells were then incubated with vehicle control or 10 nM DPN for 15 minutes or 24 hours and then harvested and lysed. Total cell proteins were resolved by 4–15% SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with antibodies directed against phosphotyrosine-1068-EGFR (Cell Signaling Technology D7A5), total EGFR (Calbiochem, GR15), or HER-3 (Santa Cruz Biotechnology, C17). Phospho-p44/p42 MAPK (Thr202/Tyr204; #9101), total MAPK (#9102), phospho-mTOR (Ser-2448, clone D9C2), and total mTOR (7C10) antibodies were from Cell Signaling Technology. Proteins were detected by using horseradish peroxidase (HRP) conjugated secondary antibodies and Thermo Scientific Pierce ECL Western Blotting Substrate with enhanced chemiluminescent for detection of activity from HRP. Membranes were stripped and reprobed with β-actin as a loading control; anti-beta-actin antibody C4 was from Santa Cruz Biotechnology (sc-47778). Immunoblots shown in figures are a representative of at least three independent experiments.

2.8. Patients and Analyses of Archival TNBC Specimens. Overall 19 TNBC cases were available for this study, with 14 provided by tumor banks associated with the UCLA Early Detection Research Network [55, 56] and the Division of Cancer Research at Charles Drew University School of Medicine and Science (CDU) [57]. Patient specimens were obtained from archival breast cancer studies between 1995 and 2007. This study was approved by our Institutional Review Boards and written informed consent was obtained from all participants. TNBC was confirmed by surgical biopsy/pathology and follow-up data. Of the 14 UCLA-CDU cases, each is linked to deidentified clinical and outcome data. Follow-up time was performed up to 5 years. The specimens

include those from 11 non-Hispanic Caucasian and 3 non-Hispanic African American female patients. General characteristics of these patients were reported previously [55–57].

An additional 15 specimens were provided by the NCI-supported Cooperative Human Tissue Network (http://www.chtn.nci.nih.gov/). These archival specimens included 5 cases from female patients with TNBC as well as controls for comparison from female patients with ERα-negative breast cancer (non-TNBC) ($n = 5$) and ERα-positive breast cancer ($n = 5$). Overall, all histologies included cases with invasive tumors with some associated with metastases. Among the 15 NCI CHTN tumor cases, tissue samples were taken from neighboring regions of nonmalignant tissue ($n = 9$). All specimens were collected with the appropriate institutional human subject protection committee approvals and patient consent.

Formalin-fixed and paraffin-embedded tissue samples were stained using standard IHC protocols as before [55–62]. Antibodies used included ERα (clone 1D5, DAKO); ERβ, anti-ERβ (GeneTex); anti-ERβ-1 clone PPG5/10 (AbD Serotec); IGF-2 (Abcam); EGFR; and Ki-67 (DAKO). Appropriate controls were included to assess specificity and validate each antibody including (i) appropriate preimmune control, (ii) dose-dependent titration, (iii) known positive and negative tissues, (iv) use of specific peptide competitors, and (v) other established approaches in our laboratory [58, 62]. For IHC, antibody binding was detected by using the "Envision+" System-HRP (DAB) followed by chromogen detection with diaminobenzidine (DAKO). Sections were counterstained with Harris hematoxylin, followed by dehydration through graded alcohol solutions, and mounted. Images were captured by using an Olympus BX41 microscope hooked to a Pixera Pro 150 ES or Evos xl Core microscope. Care was taken to evaluate staining of specific target structures only. Both intensity of staining and percentage of cells were recorded for both neoplastic and normal cells expressing antigen in both nuclear and extranuclear localizations. Board-certified pathologists quantified expression as before [55–58]. We used an Allred scoring system to quantify expression in tumors [62]. Of note, stromal expression of growth factors or steroid receptors in endometrial cancer are reported to be biomarkers to predict response to hormonal therapy [63]. Hence, we explored stromal as well as tumor expression of selected biomarkers, particularly ERβ1 and IGF-2 (Abcam).

2.9. Statistical Analyses. For *in vitro* work, experiments were done in triplicate. Student's t-test, ANOVA, or Kruskal-Wallis test, if outcomes were nonnormally distributed, was used to compare intervention groups. Analyses were evaluated using bar and scatter graphs with means, SD, and SE. Time trend curves for agents under different conditions were obtained as appropriate. Repeated measures ANOVA were used to assess time, condition, and time by condition interaction effects. $P < 0.05$ was considered significant. For analyses of clinical TNBC specimens, we used methods and protocols for assessing novel associations with clinical/pathological variables as well as marker associations as detailed before [56–62]. The log-rank test was used to compare overall survival (OS) between those subjects with positive nuclear ERβ1 expression score versus those with negative scores.

Table 1: Demographic characteristics.

Variable	N (%)
Race	
African American	3 (21.4)
Caucasian	11 (78.6)
AJCC stage	
0-I	0
II	7 (50)
III-IV	7 (50)
Ki-67 status	
Low	3 (21.4)
High	11 (78.6)
Age, mean years (range)	
<50 yrs	8 (32–58)
>50 yrs	6 (53–65)

Table 2: ERβ1 biomarker distribution.

Variable	N (%)
Nuclear ERβ1 primary tumor	
Positive	8 (57.1)
Negative	6 (42.8)

3. Results

3.1. ERβ Expression in Archival TNBC Specimens. Several reports indicate that ERβ expression in node-positive breast cancer is a biomarker for more aggressive disease [8, 21]. For these studies, we used 14 archival TNBC specimens with demographic characteristics including American Joint Committee on Cancer (AJCC) stages provided in Table 1. Using immunohistochemistry, specific ERβ1 staining was observed and scored in nuclear sites, with our observations noted in Table 2. Representative immune staining patterns are shown in Figure 1. Of note, we also observed evidence of diffuse extranuclear ERβ1 staining in most specimens but this staining was not scored or analyzed for this report. In further analyses, we assessed the clinical outcome of 14 patients with a 5-year follow-up whose tumors expressed or did not express ERβ1. In this group of patients with advanced TNBC, overall survival (OS) was significantly worse for TNBC patients with high nuclear ERβ1 (positive) as compared to those with low (negative) ERβ1 ($P < 0.001$; see Figure 2). Finally, we note that, of the African American patients included in our sample of 14 TNBC patients (11 Caucasian and 3 African American women), all three had tumors that were ERβ1-positive.

3.2. ERβ Expression in Breast Cancer Cell Lines. We assessed ERβ expression in a panel of established TNBC [47, 48] and control breast cancer cell lines, MCF-7 and T47D. All TNBC cell lines were confirmed to express ERβ. Lysates of reported TNBC cells (MDA-MB-231, MDA-MB-435, BT549, HCC-38,

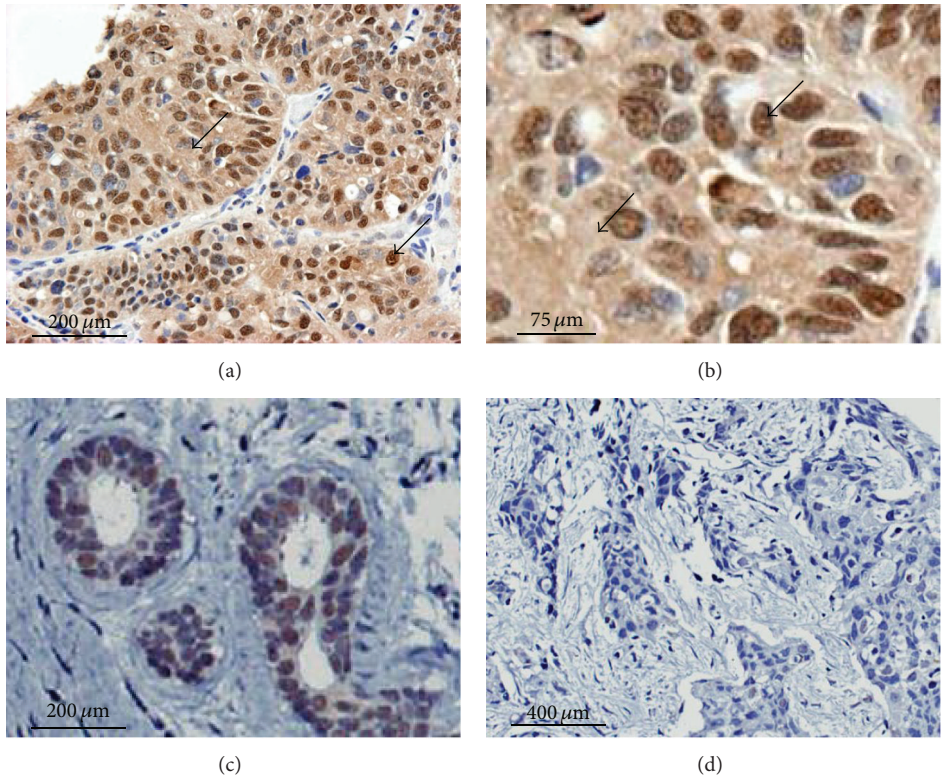


Figure 1: ERβ1 expression in archival TNBC specimens. Representative examples are shown of IHC staining of tumor and nonmalignant tissue specimens using anti-ERβ1 antibody (AbDSerotec PPG5/10). (a) TNBC specimen shows nuclear (and cytoplasmic) immunostaining of ERβ1 at low magnification. (b) The same TNBC specimen shows nuclear (and cytoplasmic) immunostaining of ERβ1 at higher magnification. (c) Expression of nuclear ERβ1 is also observed in neighboring nonmalignant mammary tissue from the clinical specimen used in panels (a) and (b). (d) Negative ERβ1 detected in a different clinical specimen as shown as comparison. Antibody binding was detected by using the "Envision+" amplification system followed by chromogen detection with diaminobenzidine (DAKO). Sections were counterstained with Harris hematoxylin followed by dehydration through graded alcohol solutions and mounting. See Table 2 for a summary of findings on all TNBC cases examined.

HCC-1143, and HCC-1937) and nuclear ERα-positive controls (MCF-7, T47D) were subjected to gel electrophoresis and immunoblots with anti-ERβ antibody and anti-ERα antibody. Results are shown in Figure 3. T47D cells were also used as a positive control for ERβ expression. These findings indicate significant expression of ERβ TNBC cell lines.

3.3. ERβ Regulates Breast Cancer Cell Proliferation. MDA-MB-231 TNBC cells were stably transfected with an empty control vector, nonspecific shRNA scrambled sequence control plasmid, or an ERβ-specific shRNA plasmid to suppress ERβ expression. Evidence of expected molecular alterations in ERβ protein expression in MDA-MB-231 cells is shown in Figure 4(a). Transfected cells were treated with known estrogen receptor agonists and/or antagonists [49, 50] including DPN (ERβ agonist), PPT (ERα agonist), and PHTPP (ERβ antagonist) alone. As shown in Figure 4(b), PHTPP decreased proliferation in ERβ-expressing cells but not in cells where ERβ expression was suppressed. Notably, DPN (ERβ agonist) increased proliferation only in cells expressing ERβ (Figure 4(b)). These results are consistent with the hypothesis that ERβ1 plays a significant role in modulating TNBC proliferation. This finding is consistent with reports that associate ERβ1 expression with Ki-67, a marker of cell proliferation, in ERα-null tumors [17].

3.4. ERβ1 Stimulates Secretion of Growth Factors in TNBC. To investigate potential cross talk between ERβ1 and growth factor signaling pathways [15] which may promote TNBC progression (such as VEGF, amphiregulin, and WNT 10b/12) [1, 15, 52, 53, 64], we cultured TNBC cells in the presence and absence of DPN. Using established ELISA methods as detailed before [51], levels of selected secreted growth factors were assayed in particle-free, extracellular media (Figure 5). DPN promoted secretion of several critical growth factors ($P < 0.001$).

3.5. Activation of ERβ1 Correlates with an Increase in EGFR Expression and Activation of Downstream Signaling Pathways. Since EGFR is known to be expressed and active in many TNBCs [1, 64], we explored cross communication between ERβ activation and EGFR in TNBC cells. MDA-MB-231 cells treated with DPN for 15 minutes showed increased phosphorylation of Tyr1068-EGFR as well as downstream signaling demonstrated by phosphorylation of p44/p42-MAPK and phospho-Ser2448-mTOR (see Figure 6(a)). Further, TNBC

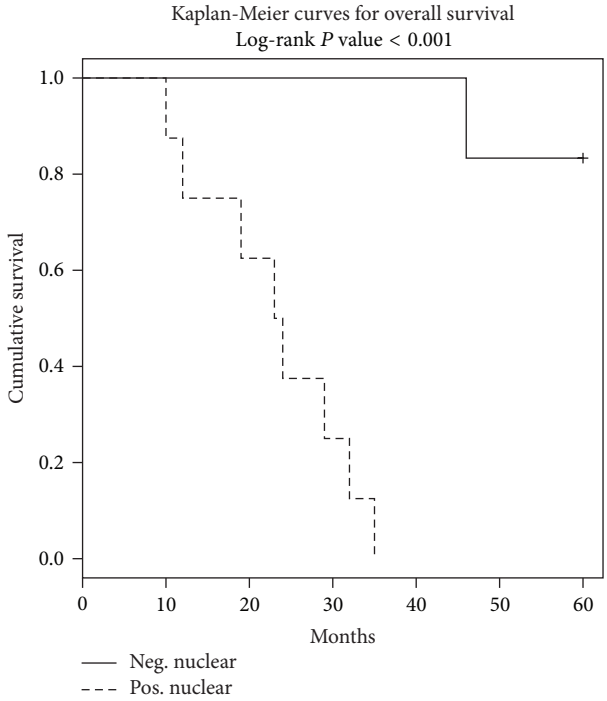


Figure 2: ERβ1 expression reduces overall survival (OS) in TNBC. TNBCs from 3 African American and 11 Caucasian women were scored for nuclear ERβ1 using IHC with validated antibody (see Table 2). Allred scores >2 are denoted as positive. In this group of patients with advanced TNBC, overall survival (OS) was significantly worse for TNBC patients with high nuclear ERβ1 (positive) as compared to those with low (negative) ERβ1 ($P < 0.001$). We noted that TNBCs from all 3 African American women were ERβ1-positive.

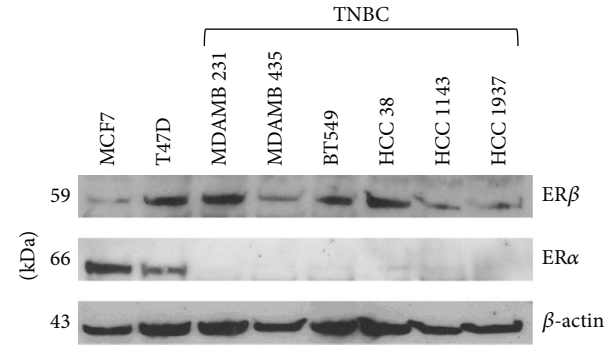


Figure 3: ERβ is expressed in TNBC cells. Lysates of reported TNBC cells (MDA-MB-231, MDA-MB-435, BT549, HCC38, HCC1143, and HCC1937) and nuclear ERα-positive controls (MCF-7, T47D) were subjected to polyacrylamide gel electrophoresis and immunoblots with anti-ERβ antibody (D7N, Zymed/Invitrogen; confirmed with GeneTex ERβ1 antibody (not shown)) and anti-ERα antibody (1D5, DAKO). T47D cells are also a positive control for ERβ expression. β-actin is used as a loading control. Methods were as before [60, 61]. Blot shown is a representative of at least three independent experiments.

cells treated with DPN for 24 hours had increased EGFR protein levels but levels of HER-3, a related EGFR family member, were not increased (Figure 6(b)). Our findings in Figure 5 that note ERβ-induced promotion of amphiregulin, a known EGFR ligand, as well as EGFR expression (Figure 6(b)) implicate a cascade that could potentially promote downstream EGFR signaling modules such as the Ras/Raf/MEK/ERK1/2 and mTOR pathway for TNBC progression [15, 65]. Hence, ERβ1 elicits increased phosphorylation of EGFR, as well as activation of MAPK and mTOR.

3.6. IGF-2 Stimulates ERβ Transcription. To evaluate potential effects of unprocessed or big (pIGF2) and processed (mIGF2) IGF-2 on ERβ transcription, we used a real-time PCR approach. As compared to T47D cells, which express both ERα and ERβ, higher transcript levels of ERβ were stimulated by mIGF-2 in HCC 1806 and MDA-MB-231 cell lines (Figure 7). These findings indicate a role of IGF-2 in ERβ transcription.

3.7. IGF-2 and ERβ1 Expression in Archival TNBC Specimens. IGF-2 is a secreted protein highly expressed in breast tumor tissue. To assess the association of elevated IGF-2 with a specific tumor subtype, we evaluated IGF-2 expression levels in epithelial and neighboring stromal components of ERα-positive (ER+), ERα-negative (ER−), and TNBC tumors. Following pathology review, we obtained archival tissue from 5 patients in each subtype. The patients selected ranged in age from 35 to 61 years (average age 50) with additional demographic characteristics shown in Table 3. In comparison to ER+ and ER− epithelium, TNBC epithelia expressed significantly higher levels of IGF-2 ($P < 0.01$; see Figure 8(a)). Additionally, IGF-2 staining in neighboring stromal cells was highest in TNBCs as compared to ER− tumors ($P = 0.05$) and ER+ tumors ($P < 0.01$; see Figure 8(b)). These results support a potential apocrine and/or paracrine role of IGF-2 in cancer progression.

Since the expression of IGF-2 and ERβ1 is associated with poor patient outcomes, we evaluated their coexpression in normal breast tissue and TNBC tumors (Figure 9). Using immunohistochemistry, archival TNBC tumors and adjacent nonmalignant breast specimens were evaluated for IGF-2 and ERβ1 expression (Figure 9(a)). In the samples evaluated and scored, notably increased expression of IGF-2 ($P < 0.01$) and ERβ1 ($P < 0.005$) is found in all TNBC tumors examined (Figure 9(b)).

4. Discussion

The discovery of ERβ and its expression in TNBC raised hope that targeting ERβ might offer new treatment options for TNBC patients where previously only aggressive chemotherapies were available [1, 2, 17]. However, these predictions have yet to be realized. Some investigators report that ERβ is a favorable prognostic factor [13] or tumor suppressor [10], while others find that ERβ correlates with aggressive phenotypes and worse prognosis [8, 14, 17, 58]. These differences may be due in part to use of nonspecific antibodies to assay ERβ and some studies of archival specimens

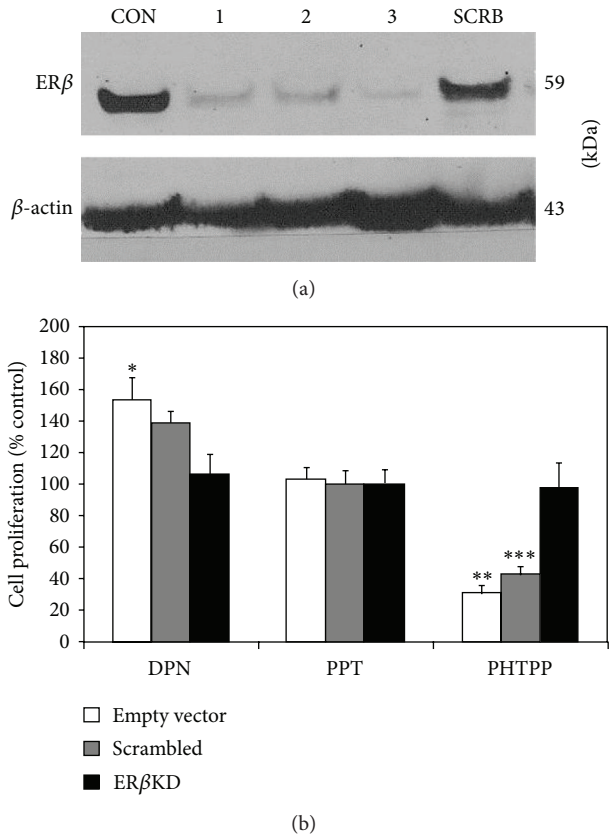


Figure 4: Blockade of ligand-induced proliferation of triple-negative MDA-MB-231 cells by ERβ antagonists and by ERβ shRNA. (a) Estrogen receptor-β knockdown. MDA-MB-231 TNBC cells were transfected with shRNA control (CON), ERβ-targeted shRNA (1, 2, 3), and scrambled shRNA vectors (SCRB) (Origene #TG320347). Stable transfectants were selected using puromycin. Cell lysates were processed for Western blot, with results shown for a negative control (CON), 3 different ERβ knockdown clones (1, 2, and 3), and scrambled shRNA (SCRB). β-actin was used as a loading control. (b) Blockade of ligand-induced proliferation of triple-negative MDA-MB-231 cells by ERβ antagonists and by ERβ shRNA. Cells were stably transfected with empty vector (white bars), nonspecific shRNA scrambled-sequence plasmid (grey bars), or an ERβ shRNA plasmid (black bars). As indicated in the figure, cells were treated with different ligands: DPN (ERβ1 agonist), PPT (ERα agonist), and PHTPP (ERβ antagonist) [49, 50]. Cell proliferation was determined after 72 hours using the BrdU cell proliferation ELISA (Roche) ($n > 3$). Graph shows percentage of surviving cells relative to untreated controls (vehicle control), defined as 100% for each transfection condition: empty vector, scrambled, and ERβ shRNA. $^{*}P = 0.007$, $^{**}P = 0.005$, and $^{***}P = 0.006$. Data represents at least three independent experiments.

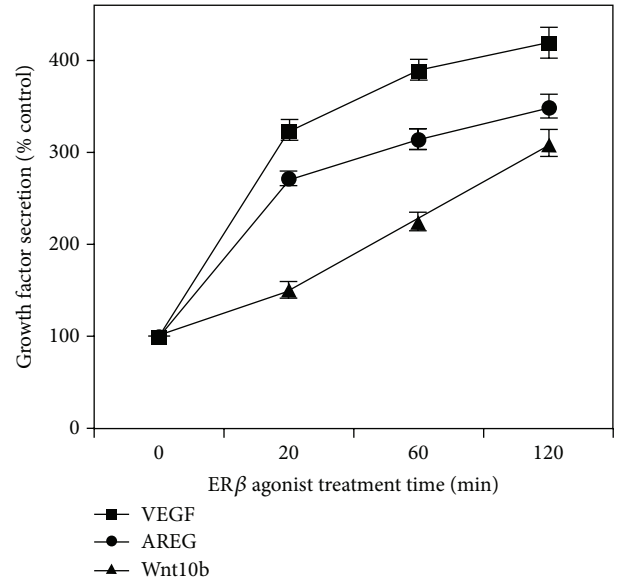


Figure 5: ERβ1 stimulates secretion of VEGF, amphiregulin, and WNT 10b/12. TNBC cells (MDA-MB-231) were cultivated in estrogen-free media and then treated for 20–120 minutes with ERβ agonist (10 nM DPN), followed by harvesting of medium and ELISA assays for VEGF, amphiregulin (AREG), and WNT 10b/12, signaling molecules that are each reported to contribute to the progression of TNBC [52, 53, 64]. Assays were done in triplicate in 3 independent experiments. DPN promoted secretion of several critical growth factors (all at $P < 0.001$) as compared to appropriate controls.

lack validation of a true TNBC phenotype [17, 20, 21]. To address these problems, we used validated ERβ antibodies [58, 67] and established TNBC specimens (ERα-negative/PR-negative/HER2-overexpression-negative). Our findings support earlier reports on the prognostic potential of ERβ isoforms in TNBC [7, 8, 11, 17, 20, 21, 68, 69], particularly ERβ1. Our data show specific staining for ERβ1 isoform in TNBC specimens, a finding consistent with other recent reports that both ERβ1 and ER coregulator SRAP are predictive biomarkers of tamoxifen-response/benefit in women with ERα-negative breast cancer [17, 70]. It is independently reported that high levels of nuclear ERβ2 are associated with lymph node involvement and serve as an independent biomarker for early tumor relapse in ERα-negative breast cancers, particularly in TNBC subgroups [69]. Since ERβ1 and ERβ2 tend to correlate positively, ERβ2 expression may contribute in part to this finding. This may be critical as ERβ2 can heterodimerize with ERβ1 and modulate gene expression [22, 68]. It will be important in future work to assess the ratio of ERβ1 : ERβ2 expression in a larger sample of TNBC specimens and to correlate receptor protein and transcript levels with treatment outcomes [21]. Expression of ERβ1 is of particular interest because it is the only ERβ isoform that contains an intact LBD, thereby serving as a potential drug target in the clinic [17, 21, 22].

In pursuit of new approaches to treat TNBC, it is important to consider results of some recent studies on TNBC and related ERα-negative disease. Our data appear consistent with independent reports of ERβ1 as a biomarker for improved survival in TNBC patients when treated with tamoxifen [13, 17, 21, 70]. Although previous work suggests that tamoxifen use only reduced the risk of ERα-positive breast cancer, Yan et al. [17] report that both ERβ1 and ER coregulator SRAP are predictive biomarkers of tamoxifen-response/benefit in women with ERα-negative breast cancer.

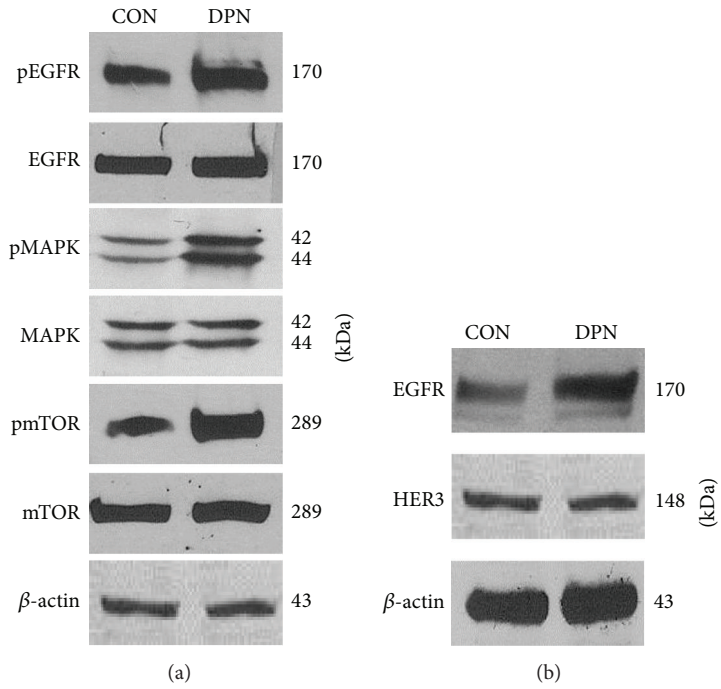


Figure 6: Estrogen receptor-β agonist DPN promotes expression and activation of EGFR and downstream signaling in MDA-MB-231 cells. (a) Cells were treated with DPN for 15 minutes. Thereafter, cells were lysed and processed for gel electrophoresis and Western immunoblot using antibodies against phosphotyrosine-1068- and total-EGFR, phospho-p44/42- and total-MAPK, and phosphoserine-2448- and total-mTOR. These data are consistent with independent reports on DPN activity [66]. (b) MDA-MB-231 cells were treated with DPN for 24 hours. Then, cells were lysed, processed for gel electrophoresis and Western immunoblot using antibodies to EGFR and HER-3. β-actin was the loading control. Blot shown is representative of at least three independent experiments.

In ERα-negative tumors, ERβ1 expression correlates with Ki-67 proliferation marker suggesting that ERβ1 may have a role in driving proliferation; thus antiestrogen treatment aimed at ERβ1 inhibition could slow tumor progression [17]. With the discovery of ERβ expression alone in TNBC, this presents the possibility that, in this ERα-negative cohort, antiestrogen strategies may potentially mediate activity via ERβ1, the full-length ligand-binding receptor isoform [57, 71, 72].

Although earlier work confirmed that ERα-positive breast cancer cells are more sensitive than ERα-negative breast cells to the growth-inhibitory effects of tamoxifen, moderate antiproliferative responses to tamoxifen and to ICI-164,384 are found in ERα-negative cells [73–75]. These effects may be modulated in part by a second unique binding site identified for hydroxytamoxifen in the coactivator-binding groove of ERβ that may disrupt ERβ-coactivator interactions [76]. Collectively, these reports have important implications since approved breast cancer treatments (tamoxifen, raloxifene) are largely well tolerated and orally administered. Such medications may be alternative treatments for ERβ-positive TNBC patients with generally few options other than cytotoxic chemotherapy [1, 17, 21]. A clinical trial to test this hypothesis in TNBC patients with ERβ-positive status was recently launched [77].

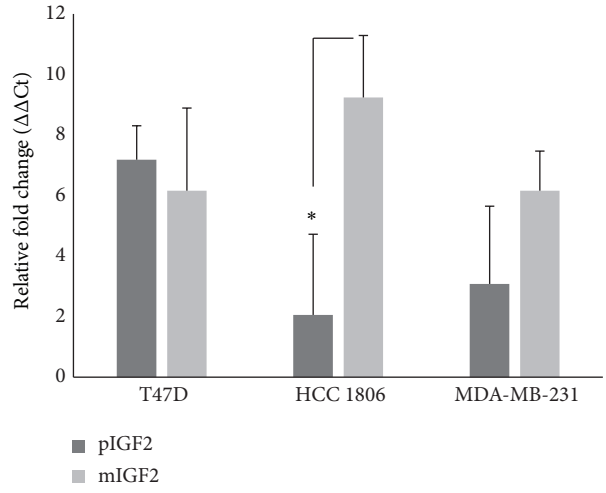


Figure 7: IGF-2 modulates the transcription of ERβ in HCC 1806 and MDA-MB-231 cell lines. Cells were seeded and then cultured for 24 hours in serum-free, phenol red-free media. Following serum starvation, cultures were treated with 100 ng/mL of pIGF-2 or mIGF-2 (GroPep). Following a 24-hour incubation period, total RNA was extracted and qRT-PCR was performed. Data presented are the result of at least six independent experiments performed in triplicate. Statistical significance was determined using Student's t-test (Graphpad). Error bars: SE. $^*P$ value = 0.05.

Table 3: Demographic characteristic of archival ER+, ER−, and TNBC breast samples.

Variable	ER+	ER−	TNBC
Race	**ERα+/PR+/HER2+**	**ERα−/PR−/HER2+**	**ER−/PR−/HER2−**
African American	3 (60%)	1 (20%)	2 (40%)
Caucasian	2 (40%)	4 (80%)	3 (60%)
AJCC stage			
0-I	2 (40%)	None	1 (20%)
II	2 (40%)	None	None
III-IV	1 (20%)	5 (100%)	4 (80%)
Ki-67 status			
Not recorded	4 (80%)	1 (20%)	1 (20%)
Low	1 (20%)	1 (20%)	1 (20%)
High		3 (60%)	3 (60%)
Metastasis			
Not recorded			1 (20%)
Positive	2 (40%)	3 (60%)	2 (40%)
Negative	3 (60%)	2 (40%)	2 (40%)
Age, mean years (range)			
<50 yrs	3 (45–49)	2 (35–49)	2 (45–49)
>50 yrs	2 (50–70)	3 (50–70)	3 (50–70)

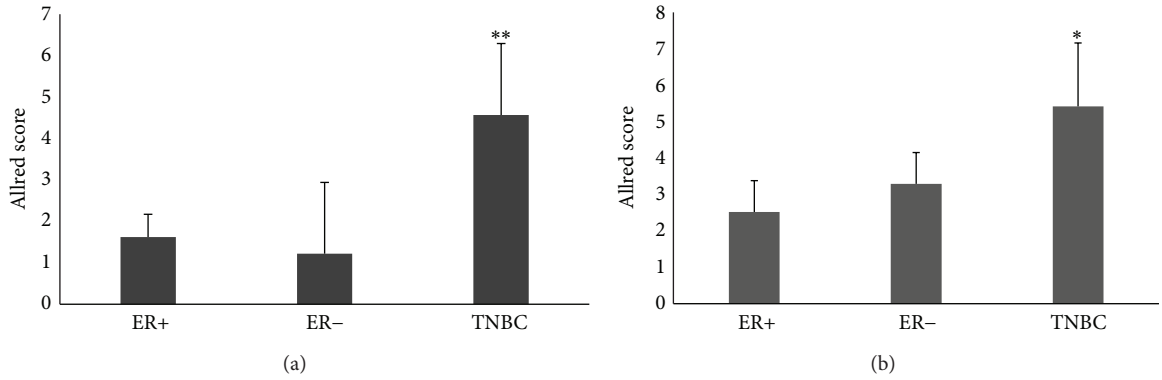


Figure 8: High levels of IGF-2 occur in archival TNBC tumors as compared to ERα+ and ERα− breast cancers. Archival breast tissues from patients with ERα-positive (ER+, $n = 5$), ERα-negative (ER−, $n = 5$), and TNBC ($n = 5$) breast cancer were evaluated for IGF-2 expression. Validated IHC staining methods were used to evaluate IGF-2 expression in tumor epithelium (a) and neighboring stroma (b). Scoring was done using an Allred-based criterion. (a) Significantly higher expression of IGF-2 is found in the epithelium of TNBC tumors as compared to ER+ ($^{**}P < 0.01$) and ER− breast cancers ($^{**}P < 0.01$). (b) Compared to ER+ and ER− archival breast tumors, stromal tissue adjacent to TNBC tumors expressed significantly higher levels of IGF-2 than stromal tissue neighboring non-TNBCs ($^{**}P < 0.01$ and $^{*}P < 0.05$ for ER+ and ER−, resp.). Error bars: SD.

We note that ERβ activity is generally considered antagonistic to that of ERα when both receptors occur together in a cell, but, in isolation, the role of ERβ forms is not well-documented. Molecular studies show that when both ERα and ERβ are present together in tumor cells, each ER restricts the binding site occupancy of the other, with ERα generally being dominant to ERβ. It is clear that ERβ binding and actions in gene regulation are different in the absence of ERα expression in breast tumor cells [78]. Indeed, correlation of ERβ with high proliferative biomarkers is reported in ERα-negative tumors but not in those with ERα expression [13, 17]. Among the first studies on stable transfection of ERβ in MDA-MB-231 cells, it was determined that the proliferation rate of the tumor cells positively correlates with the level of ERβ expression [23]. Results using stable ERβ clones in ERα-negative tumor cells demonstrate increased proliferation as ERβ expression increased. A confirmation of these experiments was published when MDA-MB-435 cells transfected with ERβ led to significant stimulation of tumor progression as well as metastasis *in vivo* [79]. These reports support ERβ stimulated tumor proliferation in ERα-negative breast cancer cells [23, 79] and complement the data presented in this study. In contrast, in other studies with ERβ transfection in ERα-low or -negative breast cancer cells, cell proliferation was inhibited [80, 81]. Several reasons may explain such contrasting results. For example, transfection of

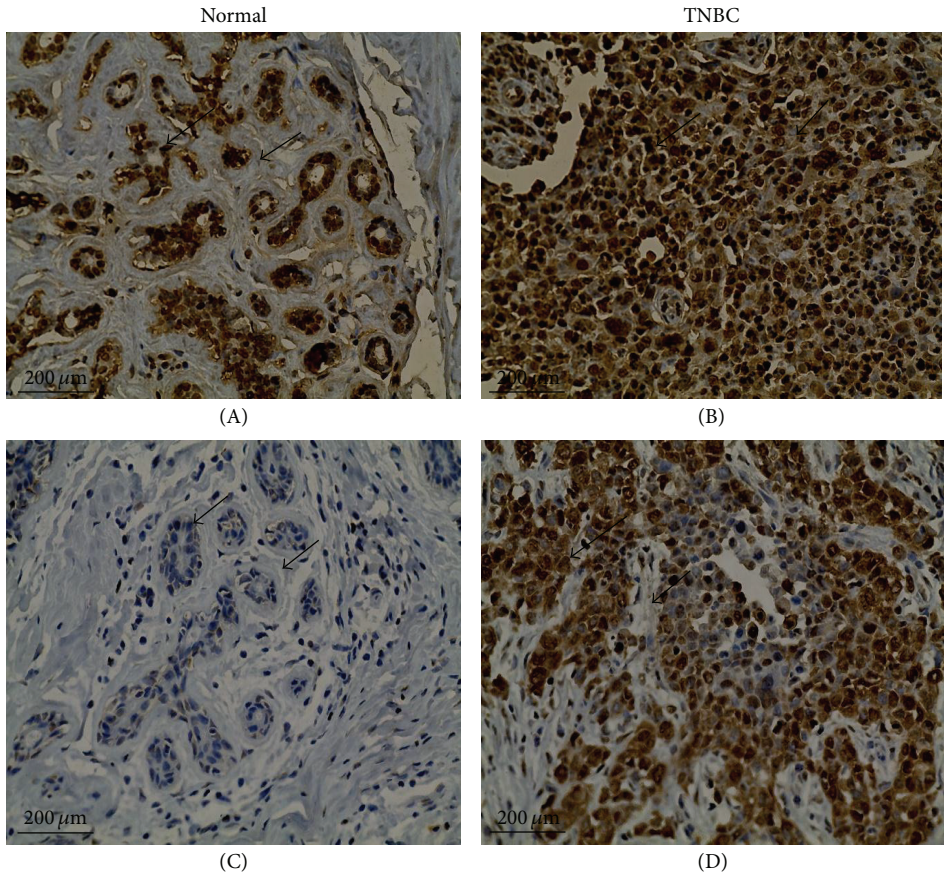


FIGURE 9: IGF-2 and ERβ1 are highly expressed in TNBC tumors. Archival breast tissue from patients with TNBC along with adjacent normal tissue adjacent to TNBC tumors was obtained from the NIH CHTN and UCLA Early Detection Research Network. Immunohistological assays were used to evaluate tissue samples for IGF-2 and ERβ1 expression (see Section 2). (a) Representation of IGF-2 and ERβ1 expression in normal and TNBC breast tissue. IGF-2 (A and B) and ERβ1 (C and D) expression were evaluated and visualized using Evos xl Core microscope. Representative images are presented. (b) Allred scoring of IGF-2 and ERβ1 in archival breast tissue. Samples from neighboring normal tissue ($n = 7$) and from TNBC tumors ($n = 11$) were stained for IGF-2 and ERβ1 then scored using Allred-based criterion (see Section 2). IGF-2 ($^{**}P < 0.01$) and ERβ1 ($^{***}P < 0.005$) were notably expressed in TNBC tumor samples. Statistical significance was determined using Student's t-test (Graphpad). Error bars: SE.

ERα led to the paradoxical finding that ERα was a growth inhibitor in breast cancer, a result that is clearly inconsistent with established clinical findings [82–84]. This unexpected result and similar ERβ transfection data may be due, in part, to excessive levels of ER expression in the model systems used or other complicating factors [23]. Further, conflicting data could be the result of differences in ERβ1 and ERβ2 expression/activity. High nuclear ERβ2 is an independent marker of early relapse in ERα-negative breast cancer and especially TNBC [69]. As noted above, it will be important to assess the impact of ERβ2 [22]. It is possible that ERβ1 and ERβ2 are both necessary partners in promoting aggressive TNBC [85]. Finally, there are conflicting reports on activity of ERβ-specific ligands in TNBC as well as other tumor types [86, 87]. ERβ1 expression correlates with tumor proliferation and progression in lung cancer [58, 87–89] but not in colon cancer [90]. An important new report with direct relevance to TNBC shows that under basal conditions ERβ agonists induced apoptosis in breast cancer cells [65]. However, when extracellular signal-regulated kinases 1 and 2 (ERK1/2) were activated by coincubation with epidermal growth factor (EGF), ERβ1 agonist DPN induced proliferation in breast cancer cells [65]. Such results indicate EGFR-induced signaling activity, known to be frequently overexpressed and active in TNBCs, can modulate ERβ growth-promoting effects [15, 91].

Emerging evidence highlights the increased frequency of obese patients afflicted with TNBC [25, 92–94] and a recent meta-analysis indicates that obese women are at a greater risk of presenting with a TNBC than nonobese women [95]. The insulin family of growth factors are notably critical mediators of metabolic factors [25, 93]. IGF-2 expression is strongly enhanced in invasive breast cancers and stimulates downstream mTOR signaling and TNBC cell migration [29, 41]. An IGF gene signature including those involved in cell growth, survival, metabolism, and biosynthesis is activated in TNBC and ERα-negative breast tumors [96, 97]. Of note, this IGF signature altered mRNA levels of numerous members of both the PI3K and ERK1/2 pathways with enrichment for transcriptional targets of PI3K/Akt/mTOR and EGFR/Ras/MAPK pathways.

Our findings show that ERβ transcripts are stimulated severalfold in TNBC cells treated with IGF2 and elevated IGF-2 expression occurs in both archival TNBC and neighboring stromal cells. These data are consistent with independent reports that detected IGF-2 in both tumor stroma and epithelial breast cancer cells and correlated IGF-2 expression with breast epithelial [27, 43–45] and stromal cell proliferation [46]. Westley and May [26] also report cross communication between estrogen and IGF pathways, with estrogen promoting increased production of IGF-2. Extensive cross talk between the IGF signaling pathway and two major growth regulators in breast cancer, namely, ER and EGFR, is detailed in numerous studies [15, 33]. Previous ideas about why patients with TNBC have more aggressive tumors that relapse earlier than patients with other types of breast cancer have focused only on tumor cell-intrinsic properties. Our results support the notion that the host systemic environment and local stromal cell niches may also play a role in tumor behavior [98]. Cross talk between epithelium and stroma is essential for neoplastic transformation in many hormonally regulated tissues [99, 100]. One way this cross talk occurs is through reciprocal signals regulating hormone receptor expression or activity in each cellular compartment. In patients with TNBC, progression of tumor cell populations may depend in part on complex interactions and the bioavailability of IGF-2 and ERβ [98].

5. Conclusions

Results of this study indicate that ERβ1 expression in archival TNBC specimens associates with significantly worse 5-year overall survival, a common characteristic of this disease. Significant expression of ERβ in a panel of TNBC cell lines is consistent with these findings. Of note, IGF-2, known to associate with breast cancer promotion, is expressed in TNBC and neighboring cells in archival clinical specimens and stimulates increased levels of ERβ mRNA in TNBC cells. Using shRNA to silence ERβ expression, we find that ERβ suppression significantly reduced TNBC proliferation. ERβ-specific antagonists similarly reduced TNBC growth. In TNBC, ERβ may stimulate growth through downstream actions that promote VEGF, amphiregulin, and Wnt-10b secretion which in turn activate specific receptor signaling pathways known to be associated with TNBC progression. This work may further our understanding on the interplay of metabolic and growth factors in TNBC, and, hopefully, lead to new therapeutics to manage patients afflicted with this deadly disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Nalo Hamilton and Diana Márquez-Garbán contributed equally to this work.

Acknowledgments

This work was supported by funds from the National Institutes of Health (NIH)/National Cancer Institute U54 CA14393 Partnership to Eliminate Cancer Health Disparities, NIH CA-86366 Early Detection Research Network, NIH/National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant no. UL1TR000124, California Breast Cancer Research Program IDEA Awards 16IB-0042 and 18IB-0034, UCLA Jonsson Cancer Center Foundation Transdisciplinary Research Grant, Stiles Program in Integrative Oncology, Hickey Family Foundation, and the Robert Wood Johnson Foundation Nurse Faculty Scholar Award 69352.

References

[1] W. D. Foulkes, I. E. Smith, and J. S. Reis-Filho, "Triple-negative breast cancer," *The New England Journal of Medicine*, vol. 363, no. 20, pp. 1938–1948, 2010.

[2] G. J. Morris, S. Naidu, A. K. Topham et al., "Differences in breast carcinoma characteristics in newly diagnosed African-American and Caucasian patients: a single-institution compilation compared with the national cancer institute's surveillance, epidemiology, and end results database," *Cancer*, vol. 110, no. 4, pp. 876–884, 2007.

[3] K. C. Amirikia, P. Mills, J. Bush, and L. A. Newman, "Higher population-based incidence rates of triple-negative breast cancer among young African-American women. Implications for breast cancer screening recommendations," *Cancer*, vol. 117, no. 12, pp. 2747–2753, 2011.

[4] A. Stark, C. G. Kleer, I. Martin et al., "African ancestry and higher prevalence of triple-negative breast cancer," *Cancer*, vol. 116, no. 21, pp. 4926–4932, 2010.

[5] Z. Madak-Erdogan, K. J. Kieser, H. K. Sung, B. Komm, J. A. Katzenellenbogen, and B. S. Katzenellenbogen, "Nuclear and extranuclear pathway inputs in the regulation of global gene expression by estrogen receptors," *Molecular Endocrinology*, vol. 22, no. 9, pp. 2116–2127, 2008.

[6] "Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group," *The Lancet*, vol. 351, no. 9114, pp. 1451–1467, 1998.

[7] G. P. Skliris, E. Leygue, L. Curtis-Snell, P. H. Watson, and L. C. Murphy, "Expression of oestrogen receptor-β in oestrogen receptor-α negative human breast tumours," *British Journal of Cancer*, vol. 95, no. 5, pp. 616–626, 2006.

[8] F. Novelli, M. Milella, E. Melucci et al., "A divergent role for estrogen receptor-beta in node-positive and node-negative breast cancer classified according to molecular subtypes: an observational prospective study," *Breast Cancer Research*, vol. 10, no. 5, article R74, 2008.

[9] E. V. Jensen, G. Cheng, C. Palmieri et al., "Estrogen receptors and proliferation markers in primary and recurrent breast cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 26, pp. 15197–15202, 2001.

[10] J. Hartman, A. Strom, and J. A. Gustafsson, "Estrogen receptor beta in breast cancer-diagnostic and therapeutic implications," *Steroids*, vol. 74, no. 8, pp. 635–641, 2009.

[11] J.-Q. Chen and J. Russo, "ERα-negative and triple negative breast cancer: molecular features and potential therapeutic approaches," *Biochimica et Biophysica Acta*, vol. 1796, no. 2, pp. 162–175, 2009.

[12] A. I. Phipps, R. T. Chlebowski, R. Prentice et al., "Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer," *Journal of the National Cancer Institute*, vol. 103, no. 6, pp. 470–477, 2011.

[13] N. Honma, R. Horii, T. Iwase et al., "Clinical importance of estrogen receptor-β evaluation in breast cancer patients treated with adjuvant tamoxifen therapy," *Journal of Clinical Oncology*, vol. 26, no. 22, pp. 3727–3734, 2008.

[14] I. Poola, S. A. W. Fuqua, R. L. de Witty, J. Abraham, J. J. Marshallack, and A. Liu, "Estrogen receptor α-negative breast cancer tissues express significant levels of estrogen-independent transcription factors, ERβ1 and ERβ5: potential molecular targets for chemoprevention," *Clinical Cancer Research*, vol. 11, no. 20, pp. 7579–7585, 2005.

[15] R. J. Pietras and D. C. Márquez-Garbán, "Membrane-associated estrogen receptor signaling pathways in human cancers," *Clinical Cancer Research*, vol. 13, no. 16, pp. 4672–4676, 2007.

[16] S. R. Hammes and E. R. Levin, "Extranuclear steroid receptors: nature and actions," *Endocrine Reviews*, vol. 28, no. 7, pp. 726–741, 2007.

[17] Y. Yan, X. Li, A. Blanchard et al., "Expression of both estrogen receptor-beta 1 (ER-β1) and its co-regulator steroid receptor RNA activator protein (SRAP) are predictive for benefit from tamoxifen therapy in patients with estrogen receptor-alpha (ER-α)-negative early breast cancer (EBC)," *Annals of Oncology*, vol. 24, no. 8, pp. 1986–1993, 2013.

[18] S. Wessler, C. Otto, N. Wilck, V. Stangl, and K.-H. Fritzemeier, "Identification of estrogen receptor ligands leading to activation of non-genomic signaling pathways while exhibiting only weak transcriptional activity," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 98, no. 1, pp. 25–35, 2006.

[19] K. Paech, P. Webb, G. G. J. M. Kuiper et al., "Differential ligand activation of estrogen receptors ERα and ERrβ at AP1 sites," *Science*, vol. 277, no. 5331, pp. 1508–1510, 1997.

[20] A. M. Shaaban, A. R. Green, S. Karthik et al., "Nuclear and cytoplasmic expression of ERβ1, ERβ2, and ERβ5 identifies distinct prognostic outcome for breast cancer patients," *Clinical Cancer Research*, vol. 14, no. 16, pp. 5228–5235, 2008.

[21] L. C. Murphy and E. Leygue, "The role of estrogen receptor-β in breast cancer," *Seminars in Reproductive Medicine*, vol. 30, no. 1, pp. 5–13, 2012.

[22] Y. K. Leung, P. Mak, S. Hassan, and S. M. Ho, "Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 35, pp. 13162–13167, 2006.

[23] D. A. Tonetti, R. Rubenstein, M. DeLeon et al., "Stable transfection of an estrogen receptor beta cDNA isoform into MDA-MB-231 breast cancer cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 87, no. 1, pp. 47–55, 2003.

[24] E. K. Shanle, Z. Zhao, J. Hawse et al., "Research resource: global identification of estrogen receptor β target genes in triple negative breast cancer cells," *Molecular Endocrinology*, vol. 27, no. 10, pp. 1762–1775, 2013.

[25] V. Belardi, E. J. Gallagher, R. Novosyadlyy, and D. Leroith, "Insulin and IGFs in obesity-related breast cancer," *Journal of Mammary Gland Biology and Neoplasia*, vol. 18, no. 3-4, pp. 277–289, 2013.

[26] B. R. Westley and F. E. B. May, "Role of insulin-like growth factors in steroid modulated proliferation," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 51, no. 1-2, pp. 1–9, 1994.

[27] C. K. Osborne, D. R. Clemmons, and C. L. Arteaga, "Regulation of breast cancer growth by insulin-like growth factors," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 37, no. 6, pp. 805–809, 1990.

[28] A. E. Richardson, N. Hamilton, W. Davis, C. Brito, and D. de León, "Insulin-like growth factor-2 (IGF-2) activates estrogen receptor-α and-β via the IGF-1 and the insulin receptors in breast cancer cells," *Growth Factors*, vol. 29, no. 2-3, pp. 82–93, 2011.

[29] M. Mancini, M. B. Gariboldi, E. Taiana et al., "Co-targeting the IGF system and HIF-1 inhibits migration and invasion by (triple-negative) breast cancer cells," *British Journal of Cancer*, vol. 110, pp. 2865–2873, 2014.

[30] D. LeRoith, R. Baserga, L. Helman, and C. T. Roberts Jr., "Insulin-like growth factors and cancer," *Annals of Internal Medicine*, vol. 122, no. 1, pp. 54–59, 1995.

[31] X.-F. Yang, W. G. Beamer, H. Huynh, and M. Pollak, "Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation," *Cancer Research*, vol. 56, no. 7, pp. 1509–1511, 1996.

[32] L. Sciacca, A. Costantino, G. Pandini et al., "Insulin receptor activation by IGF-II in breast cancers: evidence for a new autocrine/paracrine mechanism," *Oncogene*, vol. 18, no. 15, pp. 2471–2479, 1999.

[33] D. Yee and A. V. Lee, "Crosstalk between the insulin-like growth factors and estrogens in breast cancer," *Journal of Mammary Gland Biology and Neoplasia*, vol. 5, no. 1, pp. 107–115, 2000.

[34] D. M. Klotz, S. C. Hewitt, P. Ciana et al., "Requirement of estrogen receptor-α in insulin-like growth factor-1 (IGF-1)-induced uterine responses and in vivo evidence for IGF-1/estrogen receptor cross-talk," *Journal of Biological Chemistry*, vol. 277, no. 10, pp. 8531–8537, 2002.

[35] S. Vyas, Y. Asmerom, and D. D. De León, "Resveratrol regulates insulin-like growth factor-II in breast cancer cells," *Endocrinology*, vol. 146, no. 10, pp. 4224–4233, 2005.

[36] S. K. Singh, D. Moretta, F. Almaguel, N. R. Wall, M. de León, and D. de León, "Differential effect of proIGF-II and IGF-II on resveratrol induced cell death by regulating survivin cellular localization and mitochondrial depolarization in breast cancer cells," *Growth Factors*, vol. 25, no. 6, pp. 363–372, 2007.

[37] S. K. Singh, D. Moretta, F. Almaguel, M. de León, and D. D. de León, "Precursor Igf-II (proIGF-II) and mature IGF-II (mIGF-II) induce Bcl-2 and Bcl-X_L expression through different signaling pathways in breast cancer cells," *Growth Factors*, vol. 26, no. 2, pp. 92–103, 2008.

[38] C. F. Singer, M. Mogg, W. Koestler et al., "Insulin-like growth factor (IGF)-I and IGF-II serum concentrations in patients with benign and malignant breast lesions: free IGF-II is correlated with breast cancer size," *Clinical Cancer Research*, vol. 10, no. 12, part 1, pp. 4003–4009, 2004.

[39] M. Pacher, M. J. Seewald, M. Mikula et al., "Impact of constitutive IGF1/IGF2 stimulation on the transcriptional program of human breast cancer cells," *Carcinogenesis*, vol. 28, no. 1, pp. 49–59, 2007.

[40] C. Livingstone, "IGF2 and cancer," *Endocrine-Related Cancer*, vol. 20, no. 6, pp. R321–R339, 2013.

[41] C. A. Gebeshuber and J. Martinez, "MiR-100 suppresses IGF2 and inhibits breast tumorigenesis by interfering with proliferation and survival signaling," *Oncogene*, vol. 32, no. 27, pp. 3306–3310, 2013.

[42] S. Kalla Singh, Q. W. Tan, C. Brito, M. De León, and D. De León, "Insulin-like growth factors I and II receptors in the breast cancer survival disparity among African-American women," *Growth Hormone and IGF Research*, vol. 20, no. 3, pp. 245–254, 2010.

[43] M. Mathieu, H. Rochefort, B. Barenton, C. Prebois, and F. Vignon, "Interactions of cathepsin-D and insulin-like growth factor-II (IGF-II) on the IGF-II/mannose-6-phosphate receptor in human breast cancer cells and possible consequences on mitogenic activity of IGF-II," *Molecular Endocrinology*, vol. 4, no. 9, pp. 1327–1335, 1990.

[44] K. J. Cullen, A. Allison, I. Martire, M. Ellis, and C. Singer, "Insulin-like growth factor expression in breast cancer epithelium and stroma," *Breast Cancer Research and Treatment*, vol. 22, no. 1, pp. 21–29, 1992.

[45] D. D. de Leon, D. M. Wilson, M. Powers, and R. G. Rosenfeld, "Effects of insulin-like growth factors (IGFs) and IGF receptor antibodies on the proliferation of human breast cancer cells," *Growth Factors*, vol. 6, no. 4, pp. 327–336, 1992.

[46] C. Giani, D. Campani, A. Rasmussen et al., "Insulin-like growth factor II (IGF-II) immunohistochemistry in breast cancer: relationship with the most important morphological and biochemical prognostic parameters," *The International Journal of Biological Markers*, vol. 17, no. 2, pp. 90–95, 2002.

[47] R. M. Neve, K. Chin, J. Fridlyand et al., "A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes," *Cancer Cell*, vol. 10, no. 6, pp. 515–527, 2006.

[48] B. D. Lehmann, J. A. Bauer, X. Chen et al., "Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies," *Journal of Clinical Investigation*, vol. 121, no. 7, pp. 2750–2767, 2011.

[49] W. R. Harrington, S. Sheng, D. H. Barnett, L. N. Petz, J. A. Katzenellenbogen, and B. S. Katzenellenbogen, "Activities of estrogen receptor alpha- and beta-selective ligands at diverse estrogen responsive gene sites mediating transactivation or transrepression," *Molecular and Cellular Endocrinology*, vol. 206, no. 1-2, pp. 13–22, 2003.

[50] M. J. Meyers, J. Sun, K. E. Carlson, G. A. Marriner, B. S. Katzenellenbogen, and J. A. Katzenellenbogen, "Estrogen receptor-beta potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues," *Journal of Medicinal Chemistry*, vol. 44, no. 24, pp. 4230–4251, 2001.

[51] Z. Aguilar, R. W. Akita, R. S. Finn et al., "Biologic effects of heregulin/neu differentiation factor on normal and malignant human breast and ovarian epithelial cells," *Oncogene*, vol. 18, no. 44, pp. 6050–6062, 1999.

[52] P. Wend, S. Runke, K. Wend et al., "WNT10B/β-catenin signalling induces HMGA2 and proliferation in metastatic triple-negative breast cancer," *EMBO Molecular Medicine*, vol. 5, no. 2, pp. 264–279, 2013.

[53] S. Walsh, L. Flanagan, C. Quinn et al., "mTOR in breast cancer: differential expression in triple-negative and non-triple-negative tumors," *Breast*, vol. 21, no. 2, pp. 178–182, 2012.

[54] V. Mandusic, B. Dimitrijevic, D. Nikolic-Vukosavljevic, Z. Neskovic-Konstantinovic, K. Kanjer, and U. Hamann, "Different associations of estrogen receptor β isoforms, ERβ1 and ERβ2, expression levels with tumor size and survival in early- and late-onset breast cancer," *Cancer Letters*, vol. 321, no. 1, pp. 73–79, 2012.

[55] N. K. Yoon, E. L. Maresh, Y. Elshimali et al., "Elevated MED28 expression predicts poor outcome in women with breast cancer," *BMC Cancer*, vol. 10, article 335, 2010.

[56] N. K. Yoon, E. L. Maresh, D. Shen et al., "Higher levels of GATA3 predict better survival in women with breast cancer," *Human Pathology*, vol. 41, no. 12, pp. 1794–1801, 2010.

[57] Y. Wu, M. Sarkissyan, Y. Elshimali, and J. V. Vadgama, "Triple negative breast tumors in African-American and Hispanic/Latina women are high in CD44+, low in CD24+, and have loss of PTEN," *PLoS ONE*, vol. 8, no. 10, Article ID e78259, 2013.

[58] V. Mah, D. Marquez, M. Alavi et al., "Expression levels of estrogen receptor beta in conjunction with aromatase predict survival in non-small cell lung cancer," *Lung Cancer*, vol. 74, no. 2, pp. 318–325, 2011.

[59] X. Liu, V. Minin, Y. Huang, D. B. Seligson, and S. Horvath, "Statistical methods for analyzing tissue microarray data,"

Journal of Biopharmaceutical Statistics, vol. 14, no. 3, pp. 671–685, 2004.

[60] D. C. Márquez-Garbán, H.-W. Chen, M. C. Fishbein, L. Goodglick, and R. J. Pietras, "Estrogen receptor signaling pathways in human non-small cell lung cancer," *Steroids*, vol. 72, no. 2, pp. 135–143, 2007.

[61] O. K. Weinberg, D. C. Marquez-Garban, M. C. Fishbein et al., "Aromatase inhibitors in human lung cancer therapy," *Cancer Research*, vol. 65, no. 24, pp. 11287–11291, 2005.

[62] J. M. Harvey, G. M. Clark, C. K. Osborne, and D. C. Allred, "Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer," *Journal of Clinical Oncology*, vol. 17, no. 5, pp. 1474–1481, 1999.

[63] D. M. Janzen, M. A. Rosales, D. Y. Paik et al., "Progesterone receptor signaling in the microenvironment of endometrial cancer influences its response to hormonal therapy," *Cancer Research*, vol. 73, no. 15, pp. 4697–4710, 2013.

[64] B. Bilir, O. Kucuk, and C. S. Moreno, "Wnt signaling blockage inhibits cell proliferation and migration, and induces apoptosis in triple-negative breast cancer cells," *Journal of Translational Medicine*, vol. 11,article 280, 2013.

[65] C. Z. Cotrim, V. Fabris, M. L. Doria et al., "Estrogen receptor beta growth-inhibitory effects are repressed through activation of MAPK and PI3K signalling in mammary epithelial and breast cancer cells," *Oncogene*, vol. 32, no. 19, pp. 2390–2402, 2013.

[66] C. M. Klinge, K. A. Blankenship, K. E. Risinger et al., "Resveratrol and estradiol rapidly activate MAP kinase signaling through estrogen receptors α and β in endothelial cells," *The Journal of Biological Chemistry*, vol. 280, no. 9, pp. 7460–7468, 2005.

[67] X. Wu, M. Subramaniam, V. Negron et al., "Development, characterization, and applications of a novel estrogen receptor beta monoclonal antibody," *Journal of Cellular Biochemistry*, vol. 113, no. 2, pp. 711–723, 2012.

[68] Y. K. Leung, H. M. Lam, S. Wu et al., "Estrogen receptor beta2 and beta5 are associated with poor prognosis in prostate cancer, and promote cancer cell migration and invasion," *Endocrine-Related Cancer*, vol. 17, no. 3, pp. 675–689, 2010.

[69] N. I. Chantzi, D. G. Tiniakos, M. Palaiologou et al., "Estrogen receptor beta 2 is associated with poor prognosis in estrogen receptor alpha-negative breast carcinoma," *Journal of Cancer Research and Clinical Oncology*, vol. 139, no. 9, pp. 1489–1498, 2013.

[70] S. Mann, R. Laucirica, N. Carlson et al., "Estrogen receptor beta expression in invasive breast cancer," *Human Pathology*, vol. 32, no. 1, pp. 113–118, 2001.

[71] K.-A. Phillips, R. L. Milne, M. A. Rookus et al., "Tamoxifen and risk of contralateral breast cancer for *BRCA1* and *BRCA2* mutation carriers," *Journal of Clinical Oncology*, vol. 31, no. 25, pp. 3091–3099, 2013.

[72] M. M. Litwiniuk, K. Roznowski, V. Filas et al., "Expression of estrogen receptor beta in the breast carcinoma of BRCA1 mutation carriers," *BMC Cancer*, vol. 8, article 100, 2008.

[73] J. Stewart, R. King, J. Hayward, and R. Rubens, "Estrogen and progesterone receptors: correlation of response rates, site and timing of receptor analysis," *Breast Cancer Research and Treatment*, vol. 2, no. 3, pp. 243–250, 1982.

[74] K. von Maillot, H. H. Gentsch, and W. Gunselmann, "Steroid receptors and response to endocrine treatment and chemotherapy of advanced breast cancer," *Journal of Cancer Research and Clinical Oncology*, vol. 98, no. 3, pp. 301–313, 1980.

[75] R. R. Reddel, L. C. Murphy, R. E. Hall, and R. L. Sutherland, "Differential sensitivity of human breast cancer cell lines to the growth-inhibitory effects of tamoxifen," *Cancer Research*, vol. 45, no. 4, pp. 1525–1531, 1985.

[76] Y. Wang, N. Y. Chirgadze, S. L. Briggs, S. Khan, E. V. Jensen, and T. P. Burris, "A second binding site for hydroxytamoxifen within the coactivator-binding groove of estrogen receptor β," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 26, pp. 9908–9911, 2006.

[77] B. E. Kiely, K. A. Phillips, P. A. Francis et al., "ANZ1001 SORBET: study of oestrogen receptor beta and efficacy of tamoxifen, a single arm, phase II study of the efficacy of tamoxifen in triple-negative but estrogen receptor beta-positive metastatic breast cancer," *Journal of Clinical Oncology*, vol. 29, supplement, 2011, TPS1136.

[78] T. H. Charn, E. T.-B. Liu, E. C. Chang, Y. K. Lee, J. A. Katzenellenbogen, and B. S. Katzenellenbogen, "Genome-wide dynamics of chromatin binding of estrogen receptors alpha and beta: mutual restriction and competitive site selection," *Molecular Endocrinology*, vol. 24, no. 1, pp. 47–59, 2010.

[79] Y. F. Hou, S. T. Yuan, H. C. Li et al., "ERβ exerts multiple stimulative effects on human breast carcinoma cells," *Oncogene*, vol. 23, no. 34, pp. 5799–5806, 2004.

[80] C. Thomas, G. Rajapaksa, F. Nikolos et al., "ERβ1 represses basal-like breast cancer epithelial to mesenchymal transition by destabilizing EGFR," *Breast Cancer Research*, vol. 14, article R148, 2012.

[81] E. K. Shanle, Z. Zhao, J. Hawse et al., "Research resource: global identification of estrogen receptor β target genes in triple negative breast cancer cells," *Molecular Endocrinology*, vol. 27, no. 10, pp. 1762–1775, 2013.

[82] M.-H. Jeng, S.-Y. Jiang, and V. C. Jordan, "Paradoxical regulation of estrogen-dependent growth factor gene expression in estrogen receptor (ER)-negative human breast cancer cells stably expressing ER," *Cancer Letters*, vol. 82, no. 2, pp. 123–128, 1994.

[83] S.-Y. Jiang and V. C. Jordan, "Growth regulation of estrogen receptor-negative breast cancer cells transfected with complementary DNAs for estrogen receptor," *Journal of the National Cancer Institute*, vol. 84, no. 8, pp. 580–591, 1992.

[84] G. Lazennec and B. S. Katzenellenbogen, "Expression of human estrogen receptor using an efficient adenoviral gene delivery system is able to restore hormone-dependent features to estrogen receptor-negative breast carcinoma cells," *Molecular and Cellular Endocrinology*, vol. 149, no. 1-2, pp. 93–105, 1999.

[85] S. Navaratnam, G. Skliris, G. Qing et al., "Differential role of estrogen receptor beta in early versus metastatic non-small cell lung cancer," *Hormones and Cancer*, vol. 3, no. 3, pp. 93–100, 2012.

[86] H. B. Nair, N. B. Kirma, M. Ganapathy, R. K. Vadlamudi, and R. R. Tekmal, "Estrogen receptor-β activation in combination with letrozole blocks the growth of breast cancer tumors resistant to letrozole therapy," *Steroids*, vol. 76, no. 8, pp. 792–796, 2011.

[87] P. A. Hershberger, L. P. Stabile, B. Kanterewicz et al., "Estrogen receptor beta (ERβ) subtype-specific ligands increase transcription, p44/p42 mitogen activated protein kinase (MAPK) activation and growth in human non-small cell lung cancer cells," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 116, no. 1-2, pp. 102–109, 2009.

[88] V. Mah, D. B. Seligson, A. Li et al., "Aromatase expression predicts survival in women with early-stage non-small cell lung cancer," *Cancer Research*, vol. 67, no. 21, pp. 10484–10490, 2007.

[89] L. P. Stabile, A. L. Davis, C. T. Gubish et al., "Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen," *Cancer Research*, vol. 62, no. 7, pp. 2141–2150, 2002.

[90] A. Rudolph, C. Toth, M. Hoffmeister et al., "Expression of oestrogen receptor B and prognosis of colorectal cancer," *British Journal of Cancer*, vol. 107, no. 5, pp. 831–839, 2012.

[91] B. Corkery, J. Crown, M. Clynes, and N. O'Donovan, "Epidermal growth factor receptor as a potential therapeutic target in triple-negative breast cancer," *Annals of Oncology*, vol. 20, no. 5, pp. 862–867, 2009.

[92] A. A. Davis and V. G. Kaklamani, "Metabolic syndrome and triple-negative breast cancer: a new paradigm," *International Journal of Breast Cancer*, vol. 2012, Article ID 809291, 10 pages, 2012.

[93] R. Jain, H. D. Strickler, E. Fine, and J. A. Sparano, "Clinical studies examining the impact of obesity on breast cancer risk and prognosis," *Journal of Mammary Gland Biology and Neoplasia*, vol. 18, no. 3-4, pp. 257–266, 2013.

[94] L. A. Sturtz, J. Melley, K. Mamula, C. D. Shriver, and R. E. Ellsworth, "Outcome disparities in African American women with triple negative breast cancer: a comparison of epidemiological and molecular factors between African American and Caucasian women with triple negative breast cancer," *BMC Cancer*, vol. 14, no. 1, article 62, 2014.

[95] M. Pierobon and C. L. Frankenfeld, "Obesity as a risk factor for triple-negative breast cancers: a systematic review and meta-analysis," *Breast Cancer Research and Treatment*, vol. 137, no. 1, pp. 307–314, 2013.

[96] C. J. Creighton, A. Casa, Z. Lazard et al., "Insulin-like growth factor-I activates gene transcription programs strongly associated with poor breast cancer prognosis," *Journal of Clinical Oncology*, vol. 26, no. 25, pp. 4078–4085, 2008.

[97] B. C. Litzenburger, C. J. Creighton, A. Tsimelzon et al., "High IGF-IR activity in triple-negative breast cancer cell lines and tumorgrafts correlates with sensitivity to anti-IGF-IR therapy," *Clinical Cancer Research*, vol. 17, no. 8, pp. 2314–2327, 2011.

[98] Z. Castaño, T. Marsh, R. Tadipatri et al., "Stromal EGF and IGF-I together modulate plasticity of disseminated triple-negative breast tumors," *Cancer Discovery*, vol. 3, no. 8, pp. 922–935, 2013.

[99] C. Kuperwasser, T. Chavarria, M. Wu et al., "Reconstruction of functionally normal and malignant human breast tissues in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 14, pp. 4966–4971, 2004.

[100] D. M. Janzen, M. A. Rosales, D. Y. Paik et al., "Progesterone receptor signaling in the microenvironment of endometrial cancer influences its response to hormonal therapy," *Cancer Research*, vol. 73, no. 15, pp. 4697–4710, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 140196, 13 pages
http://dx.doi.org/10.1155/2015/140196

Review Article

The Role of Ovarian Sex Steroids in Metabolic Homeostasis, Obesity, and Postmenopausal Breast Cancer: Molecular Mechanisms and Therapeutic Implications

Viroj Boonyaratanakornkit[1] and Prangwan Pateetin[2]

[1] *Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, 154 Rama I Road Patumwan, Bangkok 10330, Thailand*
[2] *Graduate Program in Clinical Biochemistry and Molecular Medicine, Thailand*

Correspondence should be addressed to Viroj Boonyaratanakornkit; viroj.b@chula.ac.th

Received 31 July 2014; Accepted 6 October 2014

Academic Editor: Eileen M. McGowan

Obese postmenopausal women have an increased risk of breast cancer and are likely to have a worse prognosis than nonobese postmenopausal women. The cessation of ovarian function after menopause results in withdrawal of ovarian sex steroid hormones, estrogen, and progesterone. Accumulating evidence suggests that the withdrawal of estrogen and progesterone causes homeostasis imbalances, including decreases in insulin sensitivity and leptin secretion and changes in glucose and lipid metabolism, resulting in a total reduction in energy expenditure. Together with a decrease in physical activity and consumption of a high fat diet, these factors significantly contribute to obesity in postmenopausal women. Obesity may contribute to breast cancer development through several mechanisms. Obesity causes localized inflammation, an increase in local estrogen production, and changes in cellular metabolism. In addition, obese women have a higher risk of insulin insensitivity, and an increase in insulin and other growth factor secretion. In this review, we describe our current understanding of the molecular actions of estrogen and progesterone and their contributions to cellular metabolism, obesity, inflammation, and postmenopausal breast cancer. We also discuss how modifications of estrogen and progesterone actions might be used as a therapeutic approach for obesity and postmenopausal breast cancer.

1. Introduction

The prevalence of obesity in many developed and developing countries has been increasing at an alarming rate reaching pandemic proportions over the past decade [1]. By the year 2030, the number of overweight and obese adults is projected to be 1.35 billion and 573 million individuals, respectively, worldwide [2]. A recent study estimated that one in five deaths in the United States is associated with obesity, surpassing smoking as Americans' number one killer [3]. Health problems that can be attributed to obesity include type 2 diabetes, cardiovascular diseases, hypertension, and cancer of several organs. While the relationship between obesity, diabetes, and cardiovascular diseases has been well studied and documented, the relationship between obesity and cancer has only started to receive much attention in recent years (see review [4]).

An increasing number of studies have highlighted the association between obesity and the risk of various cancers. It is estimated that 15–20% of all cancer deaths in the United States can be ascribed to obesity [5]. A recent study estimated that for every 5 kg/m^2 increase in body mass index (BMI), a risk for developing esophageal, thyroid, and colon cancer in males increased by 52%, 33%, and 24%, respectively, whereas the risk for developing endometrial and postmenopausal breast cancer in females increased by 59% and 12%, respectively [6]. Interestingly, the association between obesity and postmenopausal breast cancer was found to be highest in women in the Asia-Pacific region with a 31% increase in postmenopausal breast cancer risk for every additional 5 kg/m^2 increase in BMI [6]. Epidemiological data suggests that women with breast cancer who are obese at the time of their diagnosis are more likely to

have a worse prognosis than nonobese, lean women [7]. Prospective cohort studies showed about a twofold increase in breast cancer risk among postmenopausal women who had higher production of various sex steroid, including dehydroepiandrosterone sulfate (DHEAS), testosterone, estrone, and total estradiol (E2) and breast cancer risk was inversely correlated with the expression of steroid hormone binding globulin (SHBG) [8]. Analysis of several cohort studies indicated that the association of high BMI with increased breast cancer risk could be attributed mostly to elevated bioavailable E2 [9]. Mortality was also higher among obese women with breast cancer than leaner women [10]. Meta-analysis of 43 studies of comorbidity of obesity and breast cancer revealed that obese patients were 33% more likely to die from breast cancer than nonobese patients. Recent evidence suggested that metabolic syndromes such as insulin resistance, hypertension, and hyperlipidemia increased the risk for postmenopausal breast cancer [11], suggesting a central role of ovarian sex steroids, estrogen, and progesterone, in regulating cellular metabolism, proliferation, and differentiation.

Obesity causes dysregulated metabolism and also provokes chronic inflammation in adipose tissue [12, 13]. It is now widely accepted that cancer is involved in the alteration of cellular metabolism [14] and inflammation [15]. Ovarian sex steroids, estrogen, and progesterone and their cognate receptors (estrogen receptor (ER) and progesterone receptor (PR)) have also been shown to influence metabolism and inflammatory responses. Loss of the ovarian function to supply estrogen and progesterone after menopause can cause deregulation of the body's metabolism and inflammatory responses with increased risk of postmenopausal breast cancer. In this review, we will discuss and provide an integrated view of our current understanding of this complex relationship between ovarian sex steroids and their receptors in relation to obesity and inflammation and their contribution to postmenopausal breast cancer. Detailed molecular mechanisms of how ovarian sex steroids affect obesity and inflammation will also be discussed.

2. Cancer and Metabolism

Cancer is often associated with alterations in cellular metabolism. In 1920, Warburg found that cancer cells prefer to metabolize glucose by glycolysis as compared to oxidative phosphorylation even in the presence of ample oxygen [16]. While the Warburg effect is less efficient in producing ATP it is very effective in providing cellular building blocks and macromolecules such as amino acids, lipids, and nucleic acids [17], allowing cancer cells to rapidly proliferate. For example, pyruvate kinase (PK), a rate-limiting step enzyme responsible for changing phoshphoenolypruvate (PEP) to pyruvate, is expressed in most cell types as four different isoforms PKL, PKR, PKM1, and PKM2. Embryo or stem cells express only PKM1 while proliferating and cancer cells express mainly PKM2 [18]. PKM2 is less effective in converting PEP into pyruvate resulting in a shortage of pyruvate available for the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation, making a more favorable metabolic environment for glycolysis. Inhibition of PKM2 expression by short-hairpin RNA (shRNA) resulted in an increase in oxygen consumption and a decrease in pyruvate production [19].

A number of studies have suggested that tumor microenvironment promotes changes in cellular metabolism. Several factors have been shown to shift cellular metabolism toward aerobic glycolysis including ovarian sex steroid signaling, cellular microenvironment, obesity, and inflammation [20–23]. Thus, the combined effect of menopause, obesity, and inflammation could create the right environment to foster development and progression of breast cancer. How these factors alter metabolic pathways and cellular metabolism will be discussed below.

3. Menopause and Obesity

3.1. Role of Estrogen and ER Signaling in Metabolic Control and Homeostasis. Postmenopausal women are more prone to gain weight. However, it is unclear why or how the menopausal transition leads to weight gain and increased breast cancer risk. The physiological withdrawal of ovarian sex steroids, estrogen, and progesterone has been implicated in altered metabolism after menopause. Estrogens have been shown to play an important role in metabolic control and homeostasis. There are three forms of estrogen in women, estrone (E1), estradiol (E2), and estriol (E3). The major and most potent circulating estrogen in women throughout the reproductive years is E2. A key enzyme that aromatizes androgen into estrogen and is required for E2 biosynthesis is a member of P450 enzyme family, aromatase [24]. Meta-analysis of several clinical studies revealed a connection between E2 and control of several key metabolic functions including abdominal obesity, insulin sensitivity, lipid transport, blood pressure, and inflammatory or prothrombotic states [25].

3.2. Role of E2/ER in Insulin-Sensitivity and Glucose Uptake. E2 and estrogen receptors (ERα and ERβ) are involved in blood glucose and lipid homeostasis [26]. ERα knockout (ERαKO) mice as well as aromatase knockout (ArKO) mice are obese and insulin resistant [27, 28]. Mutation of the aromatase gene or males with genetic ERα deficiency developed insulin resistance and glucose intolerance [29, 30]. Microarray analysis revealed that genes involved in hepatic and lipid biosynthesis were upregulated while genes involved in lipid transport were decreased in ERαKO mice. Interestingly, ERβ knockout (ERβKO) mice showed normal glucose tolerance and insulin release, suggesting that ERβ at most plays a minor role in regulating body's metabolism [31]. Deletion of ERα gene in mouse myeloid cells showed adipose tissue inflammation with insulin resistance, acceleration of atherosclerotic lesion, and obesity [32]. Several lines of evidence indicate that ERα is the major form of ER that plays a predominant role in regulating glucose and lipid metabolism [27, 32, 33]. ERα plays a central role in regulating metabolism and physical activities in the brain [34, 35]. Mice lacking ERα in the hypothalamic steroidogenic factor 1 neuron showed a significant decrease in energy expenditure and an increase in abdominal fat [35]. Ovariectomized

(OVX) mice fed with high fat diet showed increased adiposity and insulin insensitivity, and E2 treatment improved insulin sensitivity and reduced liver fat deposit. However, E2 failed to improve insulin resistance and fatty liver induced by high-fat diet in hepatocyte-specific ERαKO mice [33]. In addition, ERα could help induce insulin sensitivity through GLUT4, a major insulin-stimulated glucose transporter in muscle cells and adipocytes. In aging female rats, E2 treatment increased insulin sensitivity and improved glucose homeostasis through increasing GLUT4 expression in muscle cells [36]. The E2 increase of GLUT4 expression is likely mediated through ERα since ERαKO mice are glucose intolerant and insulin resistant [28]. On the other hand, both glucose tolerance and insulin sensitivity in ERβKO mice are normal or better than their wild-type littermates [31]. Together, these data suggest that E2 through ERα regulates insulin sensitivity and lipid biosynthesis and transport.

How E2 and ERα mediate an increase in insulin sensitivity and decrease adiposity is likely to be multifactorial. ERs are expressed in all metabolically important tissues including brain, adipose tissue, skeletal muscle, and pancreas [37]. E2 suppresses accumulation of white adipose tissue (WAT) by decreasing fatty acid and triglyceride synthesis. OVX female mice treated with E2 showed a decrease in adipocyte size by reducing fatty acid uptake through decreasing lipoprotein lipase expression, by reducing lipogenesis through decreasing acetyl-coA carboxylate and fatty acid synthase expression, and by increasing lipolysis through catecholamine activation [38].

3.3. Role of E2/ERα in Cellular Metabolism of Glucose and Lipid Metabolism. At the cellular level, E2 and ERα have been shown to directly regulate glucose and lipid metabolism. E2/ERα affects several key processes of glucose metabolism including glycolysis, the TCA cycle, and oxidative phosphorylation [39]. E2 increases the expression and activities of several key glycolytic enzymes. Glycolysis in MCF-7 breast cancer cells was shown to be induced by E2 and inhibited by tamoxifen (TAM) [40]. E2 induced several key glycolytic enzymes in the cytosol such as hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK) in the female rat brain after 4 hours of treatment [41]. Proteomic analysis of E2-regulated proteins in the bones of female mice found that both glycolytic enzymes, enolase and pyruvate kinase isoform M2 (PKM2), were regulated by E2 [42]. In addition, E2/ERα also regulated several key enzymes of the TCA cycle found in the inner membrane of the mitochondria. Treatment of OVX rats with E2 increased the activity of citrate synthase, a key enzyme condensing an acetyl group from acetyl-CoA with oxaloacetate to form citrate in TCA cycle, in muscle cells and in cerebral blood vessel cells [43, 44]. Proteomic analysis of brain mitochondrial proteins after treatment of OVX rats with E2 for 24 hours showed significant increased expression of aconitase, pyruvate dehydrogenase isoform E1b and E2, and malate dehydrogenase, as well as enzymes coupling TCA with amino acid synthesis such as 2-oxoglutarate dehydrogenase and glutamine dehydrogenase [45]. These data underscore the significance of E2/ER in regulating glucose metabolism in glycolysis and the TCA cycle. Local production of E2 after menopause could create a microenvironment conducive to aerobic glycolysis metabolism to promote cell proliferation and cancer development.

Oxidative phosphorylation occurs in the mitochondrial inner membrane and generates the majority of cellular energy in the form of ATP, NADH, and FADH [39]. Studies have shown the significance of ERs in mitochondria and oxidative phosphorylation. Both forms of ERs, ERα and ERβ, have been shown to localize to the mitochondria in several E2-target cells including uterine cells, neurons, cardiomyocytes, bone cells, hypothalamic cells, endothelial cells, liver cells, and breast cancer cells [46–50]. Thirteen proteins in the mitochondrial respiratory chain complexes are encoded by the mitochondrial DNA (mtDNA) within the mitochondria, whereas the majority of proteins required for mtDNA replication, transcription, and translation are encoded by nuclear DNA. Several studies in various cell types have demonstrated that E2 stimulated mtDNA transcription [44, 48, 51, 52], and ICI182780, a pure ER antagonist, inhibited E2-induced mtRNA gene expression [48], demonstrating the significance of E2 and mitochondrial ER in regulating mtDNA gene transcription. E2 not only activated mtDNA gene transcription, but also induced expression of nuclear DNA of genes encoding MRC proteins leading to E2-induced mitochondrial respiration [39]. Together, these data suggest that E2/ER coordinately induces transcription of mtDNA and nDNA encoding respiratory chain subunits and other regulatory proteins required for mitochondria replication and function. Therefore, E2 withdrawal or defects in ER functions could result in a decrease in mitochondrial respiration and a reduction in energy expenditure. In fact, OVX-induced obesity in mice was associated with decreased oxygen consumption, indicating a reduction in energy expenditure in the absence of E2 [21]. Similar reduction in energy expenditure in the mitochondria could also be observed in women after menopause [53].

E2 also plays an important role in fatty acid β-oxidation. Analysis of liver collected from aromatase knockout mice with undetectable levels of E2 revealed a reduction of mRNA expression and activity of enzymes involved in fatty acid metabolism. E2 treatment of aromatase knockout mice restored mRNA expression and increased activity of fatty acid metabolism enzymes [54]. Analysis of adipose tissue and muscle from female mice at 2–4 weeks after ovariectomy showed that nuclear receptors and proteins required for efficient energy expenditure such as PPARγ, PPARδ, PCG1α, PCG1β, and ERR1 were all reduced as compared to sham-operated control mice [55]. In addition, expression of enzymes involved in fatty acid β-oxidation and transcription factors required for lipolysis were also decreased [55]. Thus, depletion of E2 by ovariectomy or during menopause may lead to a decrease in expression of genes required for efficient energy expenditure and genes for fatty acid or lipid catabolism which may contribute, in part, to obesity after menopause.

3.4. Role of E2/ER in Adipokine Signaling. In addition to directly affecting adipocyte metabolism, E2/ER signaling

interacts with adipokine, leptin, and adiponectin signaling [56]. Leptin regulates energy intake and expenditure, and leptin levels are directly proportional to the amount of adipose tissue in the body. Leptin binds to leptin receptors in the hypothalamus and inhibits appetite and food intake (see review [57]). Studies have shown that E2 may mediate antiobesity effects through ERα in the hypothalamus, in the ventral medial nucleus (VL VMN), and the arcuate (ARC), by increasing expression of leptin receptors, thus increasing leptin sensitivity when E2 levels are higher [58]. Leptin levels correlate with the levels of estrogen in premenopausal women; however, this correlation between leptin and estrogen often disappears in postmenopausal women and could help explain a rise in obesity in the postmenopausal woman population [59].

Adiponectin is an important adipokine that is produced by adipose tissue and regulates peripheral glucose and insulin levels [60]. Studies suggest that ERα is a positive regulator of adiponectin levels in adipose tissue. After menopause, there is a shift in the balance between ERα and ERβ in adipose tissue, with ERβ becoming more dominant [61]. Secretion of adiponectin from adipose tissue is stimulated by peroxisome proliferator activated receptor gamma (PPARγ). ERβ has been shown to reduce PPARγ-expression and activity resulting in a reduction in adiponectin secretion from adipose tissue [62] and may result in decreased insulin levels associated with menopause. More studies will be needed to determine the role of adiponectin in metabolic disorder such as menopause-induced obesity.

Therefore, E2 withdrawal in postmenopausal women could lead to a decrease in insulin and leptin sensitivity and a decrease in mitochondria oxidative phosphorylation and lipid metabolism. These changes in glucose and lipid uptakes along with a decrease in carbohydrate and lipid metabolism render postmenopausal women less efficient in energy expenditure and more susceptible to obesity after E2 withdrawal.

4. Molecular Mechanism of Metabolic Control by ERα: Master Regulator of Glucose and Lipid Metabolism

Different forms of ERs mediate distinct functions of E2. Recent data strongly indicates that the beneficial effects of E2 on metabolic regulation are mediated through ERα. ERα is a member of the nuclear receptor superfamily and comprises six modular domains including the N-terminal domain or the A/B domain, DNA binding domain, hinge region, ligand binding domain, and the C-terminal or the E domain [63, 64]. ERα contains two activation functions (AFs) with AF-1 in the A/B domain and AF-2 in the ligand binding domain [65]. ERα can mediate its biological effects through nuclear and extranuclear signaling [63]. In nuclear signaling, ERα directly binds to estrogen response elements (ERE) or interacts with other transcription factors to regulate gene transcription in the nucleus. In extranuclear signaling, ERα interacts with cytoplasmic signaling molecules or other signal transduction scaffolding proteins and activates many key cytoplasmic signaling pathways (see review [66, 67]). A recent study suggested that the AF2 domain is essential for E2-mediated control of glucose homeostasis [68]. Mice expressing a mutant ERα lacking the AF2 domain rapidly gained weight and became severely obese with insulin resistance and glucose intolerance symptoms, similar to ERα knockout mice, whereas mice expressing a mutant ERα lacking the AF1 domain had similar body weight and metabolic functions to wild-type mice. E2 treatment in mice with wild type or AF-1-deleted ERα showed increased energy expenditure and insulin sensitivity but failed to show these beneficial metabolic effects in mice with AF-2-deleted ERα [68].

However, detailed molecular mechanisms of how ERα mediated its beneficial metabolic effects are not well understood. Mice expressing the ERα mutant with disrupted ERE binding showed a decrease in body weight, improved insulin sensitivity, and increased energy expenditure after E2 treatment. These data suggested that ERα likely mediated its metabolic effects in an ERE-independent manner, possibly through tethering with other transcription factors such as activator protein-1 (AP-1), specificity protein 1 (Sp1), or nuclear factor κB (NF-κB) [69]. More studies will be required to determine the role of AF2 in E2-mediated ERE-independent signaling of ERα in metabolic regulation. ERα activation through AF-1 or through both AF-1 and AF-2 has been shown to promote breast cancer proliferation [70]; however, only AF-2 activation is needed for ERα-mediated metabolic regulation. Therefore, it would be of great interest therapeutically to develop an ERα AF-2 agonist to harness the beneficial metabolic effects of ERα without the harmful effects of ERα AF-1 [71].

5. Role of Progesterone and PR Signaling in Metabolic Control and Homeostasis

After menopause, cessation of ovarian functions leads to the withdrawal of both estrogen and progesterone. While the role of E2/ER signaling in metabolic controls has been well documented, how progestins and PR signaling influences metabolic control and homeostasis is still unclear. Biological effects of progestin are mediated through PR. PR exits as two isoforms: full-length PR-B and 164-amino acid N-terminal truncated, PR-A [72]. The two isoforms of PR play distinct roles both *in vitro* and *in vivo*. In most cases, PR-B acts as a stronger transcriptional activator as compared to PR-A. PR-A can ligand-dependently act as a transcriptional repressor of ER, androgen receptor (AR), or glucocorticoid receptor (GR) [73]. Selective deletion of the PR isoform showed distinct functions *in vivo* [74, 75]. PR mediates its actions by binding ligands, translocating into the nucleus, and directly binding to DNA or nuclear signaling. In addition, PR can also directly interact with cytoplasmic signaling molecules and activate cytoplasmic signaling pathways such as Src/Ras/Raf/MAPK [76], PI3K/Akt, or Src/Stat3 [77] (see review [78]). Progesterone regulates genes that are involved in RNA and protein processing, cell cycle, apoptosis, and cellular metabolism [79]. Microarray gene expression studies showed that PR regulates several important genes involved

in cholesterol or steroid metabolism and trafficking, fatty acid and lipid metabolism, and nucleotide and amino acid metabolism in breast cancer cells [79].

The role of progesterone/PR signaling in metabolic control is not well understood. PRs have been shown to be expressed in adipocytes [80], where they prevent lipolysis in rat adipose tissue [81]. A recent study showed that progesterone inhibits glucose uptake in 3T3-L1 adipocytes through a decrease in IRS-1 expression leading to a decrease in IRS phosphorylation, the association of IRS-1 with p85, and a subsequent decrease in Akt1 and Akt2 phosphorylation [82]. Progesterone activates glycogen phosphorylase (a rate-limiting step in glycogenolysis) or the breakdown of glycogen through an extranuclear signaling pathway independent of cAMP leading to increased blood glucose levels [83]. Rats treated with progesterone for 14 days showed a decrease in GLUT4 in both adipose and skeletal muscle cells [84]. While estrogen appears to increase the capacity of carbohydrate and lipid metabolism, progesterone seems to abolish estrogen-induced metabolic effects in skeletal muscle cells [84]. However, progesterone appears to exert different metabolic influences in cancer cells. In breast cancer cells medroxyprogesterone acetate (MPA) treatment for 6 days increased glucose uptake and fatty acid synthase (FASN) and activation of stearoyl-CoA desaturase-1 (SCD-1). These changes in FASN and SCD-1 activities increased monounsaturated fatty acid production and contributed to an increase in cellular phospholipids, triglycerides, and formation of lipid droplets in breast cancer cells [85].

Effects of progesterone on metabolic control have been extensively studied in gestational diabetes. Progesterone levels increase to support embryo transplantation and inhibit ovulation during pregnancy. Hepatic glucose production increases 15–30% to accommodate fetal needs, which is compensated by a 50–70% decrease in insulin-stimulated glucose uptake in late pregnancy [80]. This decrease in insulin sensitivity or insulin resistance is one of a number of the metabolic changes associated with pregnancy, and 3–7% of pregnant women develop impaired glucose tolerance or diabetes [81]. Pregnancy is an altered metabolic state where there is an increase in the production of insulin from β-cells to facilitate an increase in glucose uptake. However, progesterone could also alter insulin insensitivity. Pregnant rats treated with progesterone are more insulin resistant than placebo-treated control rats [82]. Studies suggest that progesterone-induced insulin resistance may be due to reductions in Glut4 expression and glucose uptake in skeletal muscle cells [83]. In C57BL/6 mice, nonfasting plasma glucose is correlated with progesterone levels [86]. High dose progesterone treatment accelerated the progression of diabetes in female obese diabetic-prone db/db mice and treatment with RU486 increased insulin and reduced blood glucose levels in both wild-type and db/db mice [86].

PR knockout mice showed lower fasting blood sugar and improved glucose tolerance compared to wild-type mice due to enhanced insulin secretion caused by an increase in β-cell proliferation and β-cell mass [86]. Interestingly, PR knockout improved insulin sensitivity only in female mice, not in male mice, suggesting that other factors, other than PR, might play a role in insulin sensitivity in males. A recent study showed that PR activation enhanced the proinflammatory cytokine- PIC-induced cell injury in Min6 mouse pancreatic islet β-cells, possibly through an increase in the mitogen-activated protein kinase (MAPK) signaling and p53 expression and a decrease in Akt signaling. A nonsteroidal PR antagonist or PR siRNA interference protected β-cells from PIC-induced injury [87]. Together, these data suggest that progestin-mediated activation of PR could have adverse effects on insulin sensitivity possibly through a decrease in insulin secretion. In postmenopausal women, the absence of progesterone is likely to enhance insulin production and could promote insulin resistance. In addition, progesterone via PR has been shown to activate expression of insulin-like growth factor binding protein-1 (IGFBP1) that sequesters and inhibits insulin-like-growth factor-I (IGF-I) action [88, 89]. Physiological withdrawal of progesterone after menopause could make IGF-1 more available to promote cell proliferation. Along with the dysregulated metabolic state induced by estrogen withdrawal, the combination of both estrogen and progesterone withdrawal in postmenopausal women could strongly increase the risk for obesity and breast cancer. Together, these data suggest the interplay of obesity, ER PR expression, and breast cancer aggressiveness. However, more studies will be required to examine detailed molecular mechanisms of these interactions. Better understanding of these interactions will help us to develop better and more effective treatments for postmenopausal breast cancer.

6. Obesity and Inflammation Increase Risk for Postmenopausal Breast Cancer

It is well established that chronic inflammation increases cancer risk [90]. Obesity is associated with adipose tissue inflammation, characterized by macrophage infiltration into the adipose deposit [12]. In addition, large adipose deposits in obese individuals could have limited blood supply resulting in adipose tissue hypoxia [91] and induction of hypoxic-inducible factor 1-α (HIF-α) expression in adipocytes. HIF1α in turn stimulates expression of monocyte chemotactic factor 1 (MCP1), promoting macrophage recruitment to adipose deposits. In addition, production of saturated fatty acids by the breakdown of large fat droplets in large adipocytes in obese individuals can lead to activation of caspase-1, interleukin-1 β, and the NF-κB signaling cascade [92]. Together, adipocytes and macrophages in adipose tissue induce expression of inflammatory cytokines such as TNF-α, IL-6, and prostaglandin-E2 (PGE2) [90] (Figure 1).

Both TNFα and PGE2 have been shown to stimulate expression of aromatase in adipose tissues and increase the risk of breast cancer, especially in obese postmenopausal women [93, 94]. Expression of aromatase in fibroblasts or stromal cells surrounding the adipocytes and mammary ducts helps to convert circulating androgen into estrogens in the breast. Examination of aromatase activity and expression of aromatase in breast tissues from different quadrants surrounding breast tumors found that aromatase activity and expression were highest in the quadrant that contained the tumor and decreased with increasing distance away from

the tumor [95, 96]. The increase in the aromatase expression was shown to be largely due to the levels of PGE2 produced by the tumor, suggesting that inflammatory cytokines were produced by the adipocytes and tumors of obese women [96]. TNFα via NFκB signaling stimulates aromatase expression through CYP19A1 (aromatase) promoter I.4 by stimulating the binding of c-Fos and c-Jun transcription factors to the activation protein-1 (AP-1) element upstream of promoter I.4, while PGE2 stimulates aromatase expression through promoter II/I.3 of the human CYP19A1 gene [13]. Recent studies showed the presence of crown-like structures, macrophages surrounding large lipid-filled adipocytes of obese subjects, in the breasts of obese women [97]. The presence of these crown-like structures correlated with an increase in NFκB expression and elevated aromatase activity [97]. Further study indicated that the increase in aromatase expression correlated with an increase in the activity of the CYP19A1 proximal II/I.3 promoter and is associated with an increase in the cyclooxygenase-2 (COX2) expression and PGE2 levels in the breasts of obese women [93].

6.1. Molecular Mechanism of ER and PR Crosstalk with NFκB Inflammatory Signaling Pathway. The NFκB pathways play crucial roles in the inflammation process in several tissues including mammary adipose tissues [98]. The influence of ovarian sex steroids on NFκB signaling has been well documented. Both ER and PR have been shown to regulate cellular inflammation through interacting with NFκB signaling pathways [98]. Constitutive activation of NFκB signaling promotes the development of hormone-independent breast tumors [99]. Obese postmenopausal women have an increased risk for developing steroid hormone receptor positive breast cancer [100]. NFκB activation in ER+ postmenopausal breast cancer is associated with endocrine resistance and a more aggressive phenotype [101]. Studies have demonstrated that ER interacts with RelA/p65, a member of the NFκB family, and synergistically regulates expression of a group of genes that are important in regulating breast cancer cell survival and chemoresistance. ER interacts with RelA and enhances binding of both ER and RelA to ERE, resulting in activation of prostaglandin-E synthase-1 (PGES) gene expression leading to an increase in PGE2 production [102]. High levels of PGE promote breast cancer invasiveness, angiogenesis, and expression of aromatase in stromal and adipose tissue in the breast [103, 104]. In addition, ER can crosstalk with the NFκB pathway through interaction between ER and IκB Kinase α (IκKα) at promoters of ER target genes, important for breast cancer cell-cycle progression including cyclin-D1 and E2F1 [105, 106]. Together these studies demonstrate that obesity could promote inflammation and the development of more aggressive, invasive breast cancer phenotypes and chemo- and endocrine resistant breast cancers.

In some cases, ER has been shown to repress NFκB activity and may be involved in anti-inflammatory effects of E2. ER inhibits NFκB signaling through NFκB DNA binding or NFκB transcriptional activation [107]. ER represses expression of RelB, a member of the NFκB family, by directly or indirectly preventing NFκB and AP-1 interaction at the RelB promoter, resulting in formation of an unfavorable DNA complex for transcriptional activation [108]. In addition, a study by Nettles and colleagues suggested that transrepression of NFκB activity by ER may occur through a competition between ER and NFκB, displacing cAMP response element-binding protein-binding protein (CBP)/p300 coactivator from the promoter of NFκB target genes and resulting in suppression of NFκB target genes [109].

Increasing evidence suggests that PR plays a significant role in anti-inflammatory responses in breast cancer cells [110, 111]. Interestingly, lack of PR in ER+ breast cancer is associated with less differentiated, more aggressive breast tumors that are resistant to endocrine therapies [112]. A recent study demonstrated that PR through progestin-dependent and independent mechanisms decreased expression of aromatase, COX-2, and HER-2/neu [111]. PR expression or progesterone treatment was shown to increase the expression of the nuclear factor-κB inhibitor (IκBα). Expression of IκBα positively correlated with PR expression in 28 breast cancer cell lines [110]. Gene expression analysis in both normal mammary epithelial and breast cancer cells revealed that PR inhibited NFκB target genes through interacting with DNA-bound RelA/p65, resulting in transcription inhibition of NFκB target genes. PR inhibited transcription of NF-κB target genes through two different mechanisms: activation function-2 (AF-2) dependent and AF-2 independent [111]. Agonist-bound PR repressed the AF-2 dependent class of genes while the AF-2 independent class of genes was equally sensitive to both PR agonists and antagonists. Together, these studies suggested that the anti-inflammatory effect of PR is likely mediated through multiple mechanisms. PR agonists may inhibit some NFκB target genes but may not affect others. More studies will be needed to exploit the anti-inflammatory role of PR and to design specific PR ligands to selectively inhibit NFκB target genes that are crucial for the development and progression of breast cancer. Collectively, these data suggest an association between ER PR expression and obesity induced inflammation. Chronic inflammation induced by adipocytes and macrophages in adipose tissue could be further modulated by ER or PR signaling. Thus, interfering with ER PR signaling may help reduce obesity induced inflammation and decrease the incidence of breast cancer in obese postmenopausal women.

6.2. Possible Role of ER, PR in Obesity-Induced Inflammation and Postmenopausal Breast Cancer: Clinical Evidences and In Vivo Models. Systemic analyses of clinical studies have suggested that obese postmenopausal women have almost a twofold increase in hormone receptor positive (ER+/PR+) breast cancer [113]. Data from several *in vitro* and *in vivo* studies indicated that chronic inflammation induced by obesity causes an increase in inflammatory mediators, leading to an increase in aromatase activity and expression in adipocytes and hormone-dependent breast cancer. Extragonadal local E2 production, induced by chronic inflammation, in the breast of obese postmenopausal women supplies breast epithelial cells with E2 and increases the risk for breast cancer by promoting the growth of hormone receptor positive breast cancer in these women.

In vivo models of diet-induced obese animals have provided further support for obesity and development of breast cancer in postmenopausal women. In a recent study in a diet-induced obesity model, Wistar rats were fed with a high fat diet and were classified into three categories: obese, mid-weight, and lean [21]. Mammary tumors were induced by a single injection of 1-methyl-1-nitrourea (MNU) into rats at about age 52 days. MNU-induced rat mammary tumors are similar to breast tumors seen in human breasts in term of percentage of intraductal tumors and pattern of progression and steroid receptor positivity [114]. Rats in the obese group were allowed to gain weight under the obesogenic conditions of high-fat diet and were restricted to limited physical activity; and some rats were also ovariectomized (OVX) at 19 weeks of age (obese-OVX). Tumor numbers were similar in all groups before OVX. After OVX, obese-OVX group showed almost a twofold increase in tumor numbers as compared to pre-OVX values while tumor numbers remained relatively the same in lean-OVX and mid-weight OVX-rats. Seventy percent (70%) of tumors in obese-OVX group expressed high ERα levels as compared to 30% of mammary tumors from non-obese-OVX rats, similar to breast tumors seen in obese postmenopausal women with a high portion of tumors classified as receptor-positive. It is likely that extragonadal local E2 production in adipose tissue of OVX-obese animals fostered the development of ER+ tumors.

An association of obesity and progesterone receptor positivity in mammary tumors has recently been demonstrated in an *in vivo* obesity and overfeeding rat model conducted in the MacLean laboratory [20]. Rats were classified into four groups, including obesity-prone (obese) and obesity-resistant (lean) groups and heaviest and lightest groups. These groups were further subdivided into low and high energy excess group based on energy expenditure within the last 48 hour prior to sacrifice. Mammary tumors from obese animals with high-energy excess had statistically significantly higher glucose uptake than that of tumors from obese with low-energy excess and lean rats. While lean animals preferred to deposit excess nutrients in mammary and peripheral tissues, obese animals deposited their excess nutrients into their mammary tumors. Interestingly, tumors from obese animals showed an increase in PR expression, mainly the PR-A isoform in preference to the PR-B isoform [20]. Furthermore, elevated PR expression was positively correlated with expression of glycolytic and lipogenic enzymes, glucose uptake, and proliferation markers in mammary tumors. Treatment with metformin, an antidiabetic drug, during OVX-induced obesity resulted in tumor regression and a decrease in PR expression [20]. Consistent with *in vivo* findings, analysis of expression microarrays of breast tumors from postmenopausal women revealed that PR+ tumors had an increase in metabolic enzymes similar to that of PR+ tumors from the obese-prone rat model, suggesting that PR+ breast tumors have increased metabolic activities. Interestingly, PR-A has been shown to induce inflammatory processes in the mammary gland by promoting leukocyte recruitment to mammary epithelium [115]. Data obtained from these animal models are consistent with increased risk of developing hormone receptor positive breast cancer among obese postmenopausal women [7]. These studies also suggested that expression of PR-A in breast tumors enhanced metabolic capacity and induced proinflammatory responses in breast cancer. Most clinical studies reported PR positivity in breast tumor specimens without isoform specification. Therefore, future studies will be needed to determine the role of PR isoforms in breast cancer metabolism and aggressiveness.

7. Conclusion and Future Perspectives

How the cessation of ovarian functions and withdrawal of ovarian steroids, E2 and progesterone, promote postmenopausal obesity and contribute to the development of hormone receptor positive breast cancer is summarized in Figure 1. The lack of estrogen and progesterone after menopause can cause an imbalance in a woman's homeostasis. The lack of estrogen can bring about a decrease in insulin sensitivity and glucose uptake and a decrease in glucose metabolism and mitochondria respiration. The lack of ovarian steroids, especially estrogen, could lead to a reduction in cellular metabolism and a reduction in total energy expenditure. Reduction in energy expenditure together with lack of physical activities and high fat diet promote postmenopausal weight gain and obesity [116]. Large fat deposits in obese women could limit blood and oxygen supply to the area and can result in tissue hypoxia. A hypoxic state induces expression of HIF1α and inflammation by promoting macrophage recruitment and secretion of inflammatory cytokines such as IL-6, IL-1β, PGE2, and TNFα. In addition, induction of HIF1α may also cause a shift in cellular metabolism to favor aerobic glycolysis often found in actively proliferating or cancer cells. The breakdown of large lipid droplets in obese women could cause an increase in saturated fatty acid (FAS) production and activation of inflammatory signaling pathways such as NFκB activation. The release of inflammatory cytokines and activation of the NFκB pathway help activate the CYP19A1 promoter and increase aromatase gene expression. Aromatase expression in the adipose tissue helps produce extragonadal estrogen from androgen in the surrounding tissues. Studies, as described in this review, suggest that obese women have lower levels of SHBG. Local extragonadal estrogen production together with low SHBG makes estrogen readily available to enhance the growth of ER+ breast cancer cells. Furthermore, obesity and the lack of ovarian sex steroids could help promote an insulin-resistant state with high circulating insulin and IGF-I. Leptin production from adipocytes is increased in obese individuals and increasing evidence shows a positive correlation between increasing leptin levels and the risk for postmenopausal breast cancer [117]. High levels of circulating IGF-1, insulin, and leptin may help promote the development and growth of breast cancer cells in postmenopausal women. Together, these data demonstrate a link between ovarian sex steroids, obesity, and inflammation with postmenopausal breast cancer.

While several advances have been made in our understanding of the role of ovarian steroids and their receptors on postmenopausal breast cancer, many important factors remain unclear and will need to be further explored to

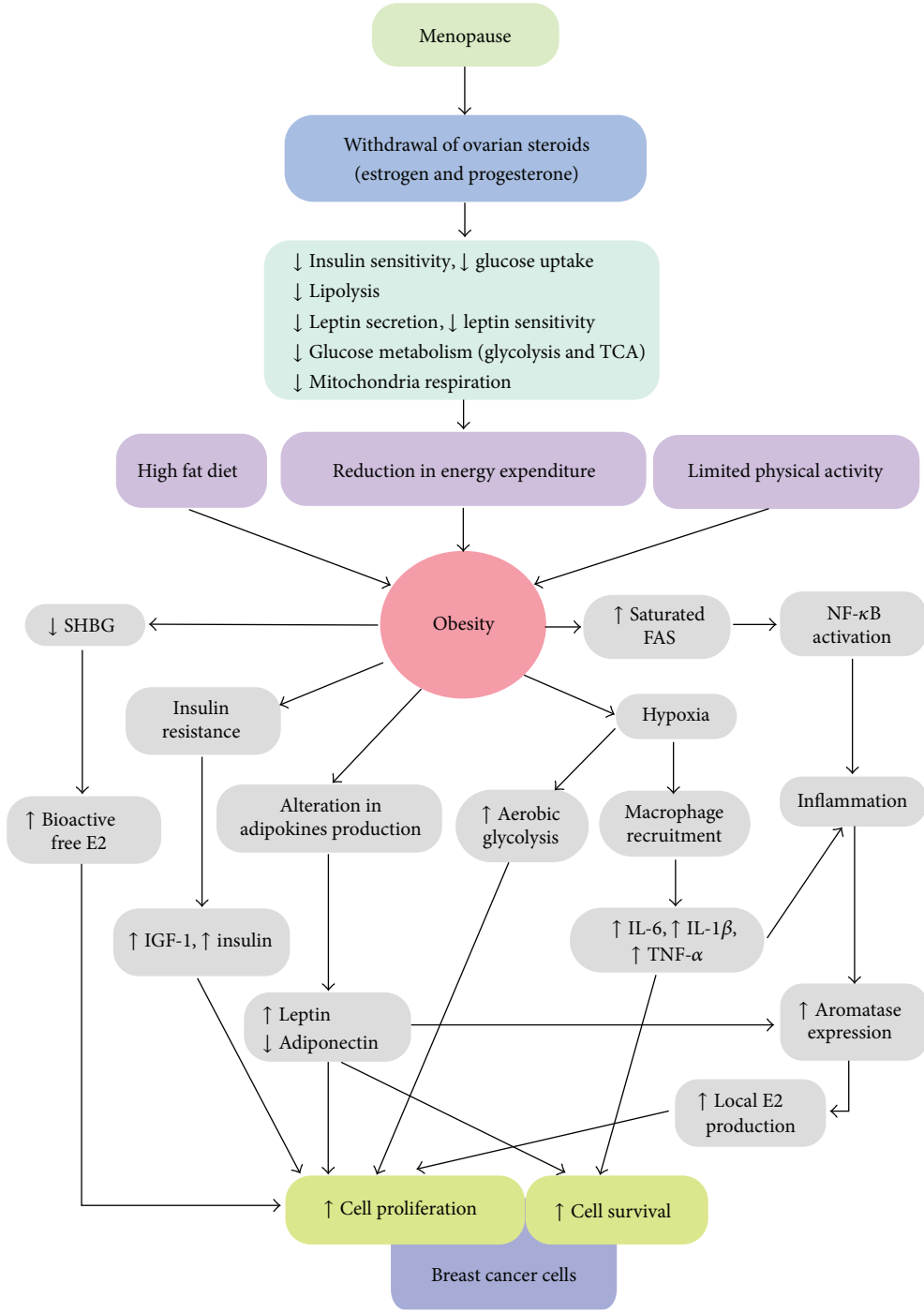


Figure 1: Diagram linking menopause and alterations in cellular metabolism with obesity and breast cancer. FAS: saturated fatty acid; TCA: tricarboxylic acid cycle; TNFα: tumor necrosis factor α; IL-1: interleukin-1; IL-6, interleukin-6; SHBG: sex hormone binding globulin; E2: estradiol; IGF-1: insulin-like-growth-factor-1.

fully exploit their possible beneficial effects after menopause. Studies suggested that AF2 of ERα is required to mediate E2 beneficial metabolic effects such as insulin sensitivity and energy expenditure, in an ERE-independent mechanism [68]. However, a detailed molecular mechanism of how ERα-AF2 is involved in these effects remains unclear. Better understanding of this molecular mechanism could help us develop a specific ligand that could specifically harness beneficial metabolic effects of ER without its harmful side effects.

Leptin plays a significant role in breast cancer development and progression [117]. Leptin stimulates production of proinflammatory cytokines and promotes cell proliferation and angiogenesis in breast cancer [117]. E2/ER stimulates

leptin production both in human subjects and in *in vivo* animal models [118]. However, a detailed molecular mechanism of how E2 induces leptin expression and promotes leptin sensitivity is still to be explored. Better understanding of how E2/ER increases leptin levels could help devise new strategies for breast cancer prevention and reduce breast cancer incidence in obese postmenopausal patients.

How progesterone/PR affects cellular metabolism remains unclear. However, a recent study in an overfeeding OVX obese rat model for postmenopausal obesity showed that mammary tumors from obese rats with high energy excess overexpressed PR, mostly in form of the PR-A isoform [20]. The association between high PR expression and obesity or overfeeding is an interesting area of study. PR+ tumors showed an almost 50% increase in glucose uptake and lower retention of dietary fat as compared to PR− tumors [20]. Whether PR directly or indirectly influences cellular metabolism or whether PR-B could exert similar metabolic effects in breast cancer is under investigation. Future studies will be required to unravel the association between high PR expression and glucose metabolism in breast cancer cells. In addition, it will be interesting to examine how expression of different PR isoforms affects breast cancer cell metabolism.

Progesterone/PR has an anti-inflammatory role in several animal models and in breast cancer cells [110, 111, 119]. Since obesity can induce a state of chronic inflammation [13], it would be interesting if we could take advantage of PR's anti-inflammatory function to reduce inflammation caused by obesity. Additional studies will be necessary to determine detailed molecular mechanisms of how PR exerts its anti-inflammatory function and to enable the design of specific PR ligands that specifically activate PR's anti-inflammatory activity. Metformin, the antidiabetic drug, has been shown to decrease PR expression and induce regression of mammary tumors and endometrial cancer [20, 120]. While a recent study suggested that metformin promotes PR expression by increasing AMP-activated protein kinase (AMPK) phosphorylation and by inhibiting the mTOR pathway [120]. Future studies will be needed to determine the molecular mechanism of how metformin affects breast cancer growth and PR expression.

Depletion of ovarian sex steroids, E2 and progesterone, in combination with limited physical activity and high fat diet after menopause could cause several metabolic changes that facilitate weight gain and obesity. Obesity in E2 and progesterone deprived states in postmenopausal women may cause imbalance in homeostasis and changes in epithelial cells, stromal cells and adipocytes in the breasts that promote the growth and survival of receptor positive breast cancer cells (Figure 1). Better understanding of molecular mechanisms and signaling pathways that mediate these effects will help us to design better cancer prevention and treatment strategies for postmenopausal breast cancer.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank Dr. Gary C. Chamness for his comments and helpful editing of the paper. This work was supported by Ratchadaphisek Somphot Faculty Development Fund (GDNS 55-043-37-003) & Ratchadaphisek Somphot Endowment Fund (R_007_2556) to Viroj Boonyaratanakornkit and Doctoral Degree Chulalongkorn University 100th Year Birthday Anniversary Scholarship (GCUGE1125561006D) to Prangwan Pateetin.

References

[1] T. Kelly, W. Yang, C.-S. Chen, K. Reynolds, and J. He, "Global burden of obesity in 2005 and projections to 2030," *International Journal of Obesity*, vol. 32, no. 9, pp. 1431–1437, 2008.

[2] Y. C. Wang, K. McPherson, T. Marsh, S. L. Gortmaker, and M. Brown, "Health and economic burden of the projected obesity trends in the USA and the UK," *The Lancet*, vol. 378, no. 9793, pp. 815–825, 2011.

[3] R. K. Masters, E. N. Reither, D. A. Powers, Y. C. Yang, A. E. Burger, and B. G. Link, "The impact of obesity on US mortality levels: the importance of age and cohort factors in population estimates," *American Journal of Public Health*, vol. 103, no. 10, pp. 1895–1901, 2013.

[4] E. E. Calle and R. Kaaks, "Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms," *Nature Reviews Cancer*, vol. 4, no. 8, pp. 579–591, 2004.

[5] E. E. Calle, C. Rodriguez, K. Walker-Thurmond, and M. J. Thun, "Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. Adults," *The New England Journal of Medicine*, vol. 348, no. 17, pp. 1625–1638, 2003.

[6] A. G. Renehan, M. Tyson, M. Egger, R. F. Heller, and M. Zwahlen, "Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies," *The Lancet*, vol. 371, no. 9612, pp. 569–578, 2008.

[7] M. Protani, M. Coory, and J. H. Martin, "Effect of obesity on survival of women with breast cancer: systematic review and meta-analysis," *Breast Cancer Research and Treatment*, vol. 123, no. 3, pp. 627–635, 2010.

[8] A. Zeleniuch-Jacquotte, R. E. Shore, K. L. Koenig et al., "Postmenopausal levels of oestrogen, androgen, and SHBG and breast cancer: long-term results of a prospective study," *British Journal of Cancer*, vol. 90, no. 1, pp. 153–159, 2004.

[9] T. J. Key, P. N. Appleby, G. K. Reeves et al., "Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women," *Journal of the National Cancer Institute*, vol. 95, no. 16, pp. 1218–1226, 2003.

[10] S. Tretli, T. Haldorsen, and L. Ottestad, "The effect of premorbid height and weight on the survival of breast cancer patients," *British Journal of Cancer*, vol. 62, no. 2, pp. 299–303, 1990.

[11] V. Rosato, C. Bosetti, R. Talamini et al., "Metabolic syndrome and the risk of breast cancer in postmenopausal women," *Annals of Oncology*, vol. 22, no. 12, pp. 2687–2692, 2011.

[12] C. K. Glass and J. M. Olefsky, "Inflammation and lipid signaling in the etiology of insulin resistance," *Cell Metabolism*, vol. 15, no. 5, pp. 635–645, 2012.

[13] E. R. Simpson and K. A. Brown, "Minireview: obesity and breast cancer: a tale of inflammation and dysregulated metabolism," *Molecular Endocrinology*, vol. 27, no. 5, pp. 715–725, 2013.

[14] R. A. Cairns, I. S. Harris, and T. W. Mak, "Regulation of cancer cell metabolism," *Nature Reviews Cancer*, vol. 11, no. 2, pp. 85–95, 2011.

[15] S. Basu, R. Nachat-Kappes, F. Caldefie-Chézet, and M.-P. Vasson, "Eicosanoids and adipokines in breast cancer: from molecular mechanisms to clinical considerations," *Antioxidants & Redox Signaling*, vol. 18, no. 3, pp. 323–360, 2013.

[16] O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[17] P. P. Hsu and D. M. Sabatini, "Cancer cell metabolism: warburg and beyond," *Cell*, vol. 134, no. 5, pp. 703–707, 2008.

[18] S. Mazurek, "Pyruvate kinase type M2: a key regulator of the metabolic budget system in tumor cells," *The International Journal of Biochemistry & Cell Biology*, vol. 43, no. 7, pp. 969–980, 2011.

[19] H. R. Christofk, M. G. Vander Heiden, M. H. Harris et al., "The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth," *Nature*, vol. 452, no. 7184, pp. 230–233, 2008.

[20] E. D. Giles, E. A. Wellberg, D. P. Astling et al., "Obesity and overfeeding affecting both tumor and systemic metabolism activates the progesterone receptor to contribute to postmenopausal breast cancer," *Cancer Research*, vol. 72, no. 24, pp. 6490–6501, 2012.

[21] P. S. MacLean, E. D. Giles, G. C. Johnson et al., "A surprising link between the energetics of ovariectomy-induced weight gain and mammary tumor progression in obese rats," *Obesity*, vol. 18, no. 4, pp. 696–703, 2010.

[22] S. A. Salama, M. A. Mohammad, C. R. Diaz-Arrastia et al., "Estradiol-17β upregulates Pyruvate kinase M2 expression to co-activate estrogen receptor-α and to integrate metabolic reprogramming with the mitogenic response in endometrial cells," *The Journal of Clinical Endocrinology Metabolism*, vol. 99, no. 10, pp. 3790–3799, 2014.

[23] M. J. Khandekar, P. Cohen, and B. M. Spiegelman, "Molecular mechanisms of cancer development in obesity," *Nature Reviews Cancer*, vol. 11, no. 12, pp. 886–895, 2011.

[24] Y. Zhao, V. R. Agarwal, C. R. Mendelson, and E. R. Simpson, "Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene," *Endocrinology*, vol. 137, no. 12, pp. 5739–5742, 1996.

[25] S. R. Salpeter, J. M. E. Walsh, T. M. Ormiston, E. Greyber, N. S. Buckley, and E. E. Salpeter, "Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women," *Diabetes, Obesity and Metabolism*, vol. 8, no. 5, pp. 538–554, 2006.

[26] A. B. Ropero, P. Alonso-Magdalena, I. Quesada, and A. Nadal, "The role of estrogen receptors in the control of energy and glucose homeostasis," *Steroids*, vol. 73, no. 9-10, pp. 874–879, 2008.

[27] C. Ohlsson, N. Hellberg, P. Parini et al., "Obesity and disturbed lipoprotein profile in estrogen receptor-α-deficient male mice," *Biochemical and Biophysical Research Communications*, vol. 278, no. 3, pp. 640–645, 2000.

[28] P. A. Heine, J. A. Taylor, G. A. Iwamoto, D. B. Lubahn, and P. S. Cooke, "Increased adipose tissue in male and female estrogen receptor-α knockout mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 23, pp. 12729–12734, 2000.

[29] A. Morishima, M. M. Grumbach, E. R. Simpson, C. Fisher, and K. Qin, "Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens," *The Journal of Clinical Endocrinology & Metabolism*, vol. 80, no. 12, pp. 3689–3698, 1995.

[30] E. P. Smith, J. Boyd, G. R. Frank et al., "Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man," *The New England Journal of Medicine*, vol. 331, no. 16, pp. 1056–1061, 1994.

[31] G. Bryzgalova, H. Gao, B. Ahren et al., "Evidence that oestrogen receptor-α plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver," *Diabetologia*, vol. 49, no. 3, pp. 588–597, 2006.

[32] V. Ribas, B. G. Drew, J. A. Le et al., "Myeloid-specific estrogen receptor α deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 39, pp. 16457–16462, 2011.

[33] L. Zhu, W. C. Brown, Q. Cai et al., "Estrogen treatment after ovariectomy protects against fatty liver and may improve pathway-selective insulin resistance," *Diabetes*, vol. 62, no. 2, pp. 424–434, 2013.

[34] Q. Gao, G. Mezei, Y. Nie et al., "Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals," *Nature Medicine*, vol. 13, no. 1, pp. 89–94, 2007.

[35] R. Yonezawa, T. Wada, N. Matsumoto et al., "Central versus peripheral impact of estradiol on the impaired glucose metabolism in ovariectomized mice on a high-fat diet," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 303, no. 4, pp. E445–E456, 2012.

[36] M. Moreno, P. Ordoñez, A. Alonso, F. Díaz, J. Tolivia, and C. González, "Chronic 17β-estradiol treatment improves skeletal muscle insulin signaling pathway components in insulin resistance associated with aging," *Age*, vol. 32, no. 1, pp. 1–13, 2010.

[37] R. P. A. Barros and J.-Å. Gustafsson, "Estrogen receptors and the metabolic network," *Cell Metabolism*, vol. 14, no. 3, pp. 289–299, 2011.

[38] T. M. D'Eon, S. C. Souza, M. Aronovitz, M. S. Obin, S. K. Fried, and A. S. Greenberg, "Estrogen regulation of adiposity and fuel partitioning: evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways," *Journal of Biological Chemistry*, vol. 280, no. 43, pp. 35983–35991, 2005.

[39] J.-Q. Chen, T. R. Brown, and J. Russo, "Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1793, no. 7, pp. 1128–1143, 2009.

[40] E. Furman, E. Rushkin, R. Margalit, P. Bendel, and H. Degani, "Tamoxifen induced changes in MCF7 human breast cancer: *In vitro* and *in vivo* studies using nuclear magnetic resonance spectroscopy and imaging," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 43, no. 1–3, pp. 189–195, 1992.

[41] A. Kostanyan and K. Nazaryan, "Rat brain glycolysis regulation by estradiol-17β," *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research*, vol. 1133, no. 3, pp. 301–306, 1992.

[42] R. Pastorelli, D. Carpi, L. Airoldi et al., "Proteome analysis for the identification of in vivo estrogen-regulated proteins in bone," *Proteomics*, vol. 5, no. 18, pp. 4936–4945, 2005.

[43] T. Beckett, A. Tchernof, and M. J. Toth, "Effect of ovariectomy and estradiol replacement on skeletal muscle enzyme activity in female rats," *Metabolism: Clinical and Experimental*, vol. 51, no. 11, pp. 1397–1401, 2002.

[44] C. Stirone, S. P. Duckles, D. N. Krause, and V. Procaccio, "Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels," *Molecular Pharmacology*, vol. 68, no. 4, pp. 959–965, 2005.

[45] J. Nilsen, R. W. Irwin, T. K. Gallaher, and R. D. Brinton, "Estradiol in vivo regulation of brain mitochondrial proteome," *The Journal of Neuroscience*, vol. 27, no. 51, pp. 14069–14077, 2007.

[46] S.-H. Yang, R. Liu, E. J. Perez et al., "Mitochondrial localization of estrogen receptor β," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 12, pp. 4130–4135, 2004.

[47] T. A. Milner, K. Ayoola, C. T. Drake et al., "Ultrastructural localization of estrogen receptor β immunoreactivity in the rat hippocampal formation," *Journal of Comparative Neurology*, vol. 491, no. 2, pp. 81–95, 2005.

[48] J. Q. Chen, M. Eshete, W. L. Alworth, and J. D. Yager, "Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors α and β to human mitochondrial DNA estrogen response elements," *Journal of Cellular Biochemistry*, vol. 93, no. 2, pp. 358–373, 2004.

[49] A. Pedram, M. Razandi, D. C. Wallace, and E. R. Levin, "Functional estrogen receptors in the mitochondria of breast cancer cells," *Molecular Biology of the Cell*, vol. 17, no. 5, pp. 2125–2137, 2006.

[50] S. Solakidi, A.-M. G. Psarra, and C. E. Sekeris, "Differential subcellular distribution of estrogen receptor isoforms: localization of ERα in the nucleoli and ERβ in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1745, no. 3, pp. 382–392, 2005.

[51] C. M. van Itallie and P. S. Dannies, "Estrogen induces accumulation of the mitochondrial ribonucleic acid for subunit II of cytochrome oxidase in pituitary tumor cells," *Molecular Endocrinology*, vol. 2, no. 4, pp. 332–337, 1988.

[52] J.-Q. Chen, P. A. Russo, C. Cooke, I. H. Russo, and J. Russo, "ERβ shifts from mitochondria to nucleus during estrogen-induced neoplastic transformation of human breast epithelial cells and is involved in estrogen-induced synthesis of mitochondrial respiratory chain proteins," *Biochimica et Biophysica Acta—Molecular Cell Research*, vol. 1773, no. 12, pp. 1732–1746, 2007.

[53] R. D. Brinton, "Estrogen regulation of glucose metabolism and mitochondrial function: therapeutic implications for prevention of Alzheimer's disease," *Advanced Drug Delivery Reviews*, vol. 60, no. 13-14, pp. 1504–1511, 2008.

[54] K. Toda, K. Takeda, S. Akira et al., "Alternations in hepatic expression of fatty-acid metabolizing enzymes in ArKO mice and their reversal by the treatment with 17β-estradiol or a peroxisome proliferator," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 79, no. 1–5, pp. 11–17, 2001.

[55] Y. Kamei, M. Suzuki, H. Miyazaki et al., "Ovariectomy in mice decreases lipid metabolism-related gene expression in adipose tissue and skeletal muscle with increased body fat," *Journal of Nutritional Science and Vitaminology*, vol. 51, no. 2, pp. 110–117, 2005.

[56] J. R. Rettberg, J. Yao, and R. D. Brinton, "Estrogen: a master regulator of bioenergetic systems in the brain and body," *Frontiers in Neuroendocrinology*, vol. 35, no. 1, pp. 8–30, 2014.

[57] Y. Zhou and L. Rui, "Leptin signaling and leptin resistance," *Frontiers of Medicine*, vol. 7, no. 2, pp. 207–222, 2013.

[58] L. M. Brown, L. Gent, K. Davis, and D. J. Clegg, "Metabolic impact of sex hormones on obesity," *Brain Research*, vol. 1350, pp. 77–85, 2010.

[59] S. C. Hong, S. W. Yoo, G. J. Cho et al., "Correlation between estrogens and serum adipocytokines in premenopausal and postmenopausal women," *Menopause*, vol. 14, no. 5, pp. 835–840, 2007.

[60] A. J. G. Hanley, D. Bowden, L. E. Wagenknecht et al., "Associations of adiponectin with body fat distribution and insulin sensitivity in nondiabetic Hispanics and African-Americans," *The Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 7, pp. 2665–2671, 2007.

[61] N. J. Tomicek, T. S. Lancaster, and D. H. Korzick, "Increased estrogen receptor β in adipose tissue is associated with increased intracellular and reduced circulating adiponectin protein levels in aged female rats," *Gender Medicine*, vol. 8, no. 5, pp. 325–333, 2011.

[62] A. Foryst-Ludwig, M. Clemenz, S. Hohmann et al., "Metabolic actions of estrogen receptor beta (ERβ) are mediated by a negative cross-talk with PPARγ," *PLoS Genetics*, vol. 4, no. 6, Article ID e1000108, 2008.

[63] V. Boonyaratanakornkit, "Mechanisms of estrogen receptor extranuclear signaling in human diseases," in *Estrogen Receptors: Mechanisms, Structure and Role in Diseases*, G. Chen, Ed., pp. 11–28, Nova Science Publishers, New York, NY, USA, 2012.

[64] M. Beato and J. Klug, "Steroid hormone receptors: an update," *Human Reproduction Update*, vol. 6, no. 3, pp. 225–236, 2000.

[65] L. Tora, J. White, C. Brou et al., "The human estrogen receptor has two independent nonacidic transcriptional activation functions," *Cell*, vol. 59, no. 3, pp. 477–487, 1989.

[66] V. Boonyaratanakornkit, "Scaffolding proteins mediating membrane-initiated extra-nuclear actions of estrogen receptor," *Steroids*, vol. 76, no. 9, pp. 877–884, 2011.

[67] V. Boonyaratanakornkit and D. P. Edwards, "Receptor mechanisms mediating non-genomic actions of sex steroids," *Seminars in Reproductive Medicine*, vol. 25, no. 3, pp. 139–153, 2007.

[68] S. Handgraaf, E. Riant, A. Fabre et al., "Prevention of obesity and insulin resistance by estrogens requires ERα activation function-2 (ERαAF-2), whereas ERαAF-1 is dispensable," *Diabetes*, vol. 62, no. 12, pp. 4098–4108, 2013.

[69] C. J. Park, Z. Zhao, C. Glidewell-Kenney et al., "Genetic rescue of nonclassical ERα signaling normalizes energy balance in obese Erα-null mutant mice," *The Journal of Clinical Investigation*, vol. 121, no. 2, pp. 604–612, 2011.

[70] T. Fujita, Y. Kobayashi, O. Wada et al., "Full activation of estrogen receptor α activation function-1 induces proliferation of breast cancer cells," *Journal of Biological Chemistry*, vol. 278, no. 29, pp. 26704–26714, 2003.

[71] T. Wada, H. Tsuneki, and T. Sasaoka, "New insights into metabolic regulation via bifurcated function of estrogen receptor a," *Diabetes*, vol. 62, no. 12, pp. 3996–3998, 2013.

[72] P. Kastner, A. Krust, B. Turcotte et al., "Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B," *EMBO Journal*, vol. 9, no. 5, pp. 1603–1614, 1990.

[73] K. M. Scarpin, J. D. Graham, P. A. Mote, and C. L. Clarke, "Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression," *Nuclear Receptor Signaling*, vol. 7, article e009, 2009.

[74] B. Mulac-Jericevic, R. A. Mullinax, F. J. DeMayo, J. P. Lydon, and O. M. Conneely, "Subgroup of reproductive functions of progesterone mediated by progesterone receptor-B isoform," *Science*, vol. 289, no. 5485, pp. 1751–1754, 2000.

[75] B. Mulac-Jericevic, J. P. Lydon, F. J. DeMayo, and O. M. Conneely, "Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 17, pp. 9744–9749, 2003.

[76] V. Boonyaratanakornkit, M. P. Scott, V. Ribon et al., "Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases," *Molecular Cell*, vol. 8, no. 2, pp. 269–280, 2001.

[77] R. P. Carnevale, C. J. Proietti, M. Salatino et al., "Progestin effects on breast cancer cell proliferation, proteases activation, and in vivo development of metastatic phenotype all depend on progesterone receptor capacity to activate cytoplasmic signaling pathways," *Molecular Endocrinology*, vol. 21, no. 6, pp. 1335–1358, 2007.

[78] V. Boonyaratanakornkit, E. McGowan, L. Sherman, M. A. Mancini, B. J. Cheskis, and D. P. Edwards, "The role of extranuclear signaling actions of progesterone receptor in mediating progesterone regulation of gene expression and the cell cycle," *Molecular Endocrinology*, vol. 21, no. 2, pp. 359–375, 2007.

[79] J. K. Richer, B. M. Jacobsen, N. G. Manning, M. G. Abel, D. M. Wolf, and K. B. Horwitz, "Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells," *Journal of Biological Chemistry*, vol. 277, no. 7, pp. 5209–5218, 2002.

[80] N. F. Butte, "Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus," *The American Journal of Clinical Nutrition*, vol. 71, no. 5, pp. 1256s–1261s, 2000.

[81] American Diabetes Association, "Gestational diabetes mellitus," *Diabetes Care*, vol. 27, supplement 1, pp. S88–S90, 2004.

[82] C. Gonzalez, A. Alonso, N. Alvarez et al., "Role of 17β-estradiol and/or progesterone on insulin sensitivity in the rat: Implications during pregnancy," *Journal of Endocrinology*, vol. 166, no. 2, pp. 283–291, 2000.

[83] S. E. Campbell and M. A. Febbraio, "Effect of the ovarian hormones on GLUT4 expression and contraction-stimulated glucose uptake," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 282, no. 5, pp. E1139–E1146, 2002.

[84] S. E. Campbell and M. A. Febbraio, "Effects of ovarian hormones on exercise metabolism," *Current Opinion in Clinical Nutrition & Metabolic Care*, vol. 4, no. 6, pp. 515–520, 2001.

[85] I. R. Schlaepfer, C. A. Hitz, M. A. Gijón, B. C. Bergman, R. H. Eckel, and B. M. Jacobsen, "Progestin modulates the lipid profile and sensitivity of breast cancer cells to docetaxel," *Molecular and Cellular Endocrinology*, vol. 363, no. 1-2, pp. 111–121, 2012.

[86] F. Picard, M. Wanatabe, K. Schoonjans, J. Lydon, B. W. O'Malley, and J. Auwerx, "Progesterone receptor knockout mice have an improved glucose homeostasis secondary to β-cell proliferation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 24, pp. 15644–15648, 2002.

[87] R. Zhou, X. Yao, X. Xu et al., "Blockage of progesterone receptor effectively protects pancreatic islet beta cell viability," *Steroids*, vol. 78, no. 10, pp. 987–995, 2013.

[88] J. Gao, J. Mazella, M. Tang, and L. Tseng, "Ligand-activated progesterone receptor isoform hPR-A is a stronger transactivator than hPR-B for the expression of IGFBP-1 (insulin-like growth factor binding protein-1) in human endometrial stromal cells," *Molecular Endocrinology*, vol. 14, no. 12, pp. 1954–1961, 2000.

[89] J. Gao and L. Tseng, "Progesterone receptor (PR) inhibits expression of insulin-like growth factor-binding protein-1 (IGFBP-1) in human endometrial cell line HEC-1B: characterization of the inhibitory effect of PR on the distal promoter region of the IGFP-1 gene," *Molecular Endocrinology*, vol. 11, no. 7, pp. 973–979, 1997.

[90] S. I. Grivennikov, F. R. Greten, and M. Karin, "Immunity, Inflammation, and Cancer," *Cell*, vol. 140, no. 6, pp. 883–899, 2010.

[91] J. Yin, Z. Gao, Q. He, D. Zhou, Z. Guo, and J. Ye, "Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 296, no. 2, pp. E333–E342, 2009.

[92] H. Wen, D. Gris, Y. Lei et al., "Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling," *Nature Immunology*, vol. 12, no. 5, pp. 408–415, 2011.

[93] K. Subbaramaiah, P. G. Morris, X. K. Zhou et al., "Increased levels of COX-2 and prostaglandin E_2 contribute to elevated aromatase expression in inflamed breast tissue of obese women," *Cancer Discovery*, vol. 2, no. 4, pp. 356–365, 2012.

[94] K. A. Brown and E. R. Simpson, "Obesity and breast cancer: progress to understanding the relationship," *Cancer Research*, vol. 70, no. 1, pp. 4–7, 2010.

[95] S. E. Bulun, T. M. Price, J. Aitken, M. S. Mahendroo, and E. R. Simpson, "A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription," *The Journal of Clinical Endocrinology & Metabolism*, vol. 77, no. 6, pp. 1622–1628, 1993.

[96] J. S. O'Neill, R. A. Elton, and W. R. Miller, "Aromatase activity in adipose tissue from breast quadrants: a link with tumour site," *British Medical Journal*, vol. 296, no. 6624, pp. 741–743, 1988.

[97] P. G. Morris, C. A. Hudis, D. Giri et al., "Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer," *Cancer Prevention Research*, vol. 4, no. 7, pp. 1021–1029, 2011.

[98] S. C. Baumgarten and J. Frasor, "Minireview: inflammation: an instigator of more aggressive estrogen receptor (ER) positive breast cancers," *Molecular Endocrinology*, vol. 26, no. 3, pp. 360–371, 2012.

[99] H. Nakshatri, P. Bhat-Nakshatri, D. A. Martin, R. J. Goulet Jr., and G. W. Sledge Jr., "Constitutive activation of NF-κB during progression of breast cancer to hormone-independent growth," *Molecular and Cellular Biology*, vol. 17, no. 7, pp. 3629–3639, 1997.

[100] L. M. Morimoto, E. White, Z. Chen et al., "Obesity, body size, and risk of postmenopausal breast cancer: the women's health initiative (United States)," *Cancer Causes and Control*, vol. 13, no. 8, pp. 741–751, 2002.

[101] Y. Zhou, S. Eppenberger-Castori, C. Marx et al., "Activation of nuclear factor-κB (NFκB) identifies a high-risk subset of hormone-dependent breast cancers," *The International Journal of Biochemistry & Cell Biology*, vol. 37, no. 5, pp. 1130–1144, 2005.

[102] J. Frasor, A. E. Weaver, M. Pradhan, and K. Mehta, "Synergistic up-regulation of prostaglandin E synthase expression in breast cancer cells by 17β-estradiol and proinflammatory cytokines," *Endocrinology*, vol. 149, no. 12, pp. 6272–6279, 2008.

[103] S.-H. Chang, C. H. Liu, R. Conway et al., "Role of prostaglandin E2-dependent angiogenic switch in cyclooxygenase 2-induced

breast cancer progression," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 2, pp. 591–596, 2004.

[104] T. L. Larkins, M. Nowell, S. Singh, and G. L. Sanford, "Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression," *BMC Cancer*, vol. 6, no. 1, article 181, 2006.

[105] K.-J. Park, V. Krishnan, B. W. O'Malley, Y. Yamamoto, and R. B. Gaynor, "Formation of an IKKα-dependent transcription complex is required for estrogen receptor-mediated gene activation," *Molecular Cell*, vol. 18, no. 1, pp. 71–82, 2005.

[106] Z. Tu, S. Prajapati, K.-J. Park, N. J. Kelly, Y. Yamamoto, and R. B. Gaynor, "IKKα regulates estrogen-induced cell cycle progression by modulating E2F1 expression," *The Journal of Biological Chemistry*, vol. 281, no. 10, pp. 6699–6706, 2006.

[107] R. H. Straub, "The complex role of estrogens in inflammation," *Endocrine Reviews*, vol. 28, no. 5, pp. 521–574, 2007.

[108] D. Kalaitzidis and T. D. Gilmore, "Transcription factor cross-talk: The estrogen receptor and NF-κB," *Trends in Endocrinology & Metabolism*, vol. 16, no. 2, pp. 46–52, 2005.

[109] K. W. Nettles, G. Gil, J. Nowak, R. Métivier, V. B. Sharma, and G. L. Greene, "CBP is a dosage-dependent regulator of nuclear factor-κB suppression by the estrogen receptor," *Molecular Endocrinology*, vol. 22, no. 2, pp. 263–272, 2008.

[110] D. B. Hardy, B. A. Janowski, C.-C. Chen, and C. R. Mendelson, "Progesterone receptor inhibits aromatase and inflammatory response pathways in breast cancer cells via ligand-dependent and ligand-independent mechanisms," *Molecular Endocrinology*, vol. 22, no. 8, pp. 1812–1824, 2008.

[111] S. Kobayashi, J. P. Stice, D. Kazmin et al., "Mechanisms of progesterone receptor inhibition of inflammatory responses in cellular models of breast cancer," *Molecular Endocrinology*, vol. 24, no. 12, pp. 2292–2302, 2010.

[112] C. J. Creighton, C. Kent Osborne, M. J. van de Vijver et al., "Molecular profiles of progesterone receptor loss in human breast tumors," *Breast Cancer Research and Treatment*, vol. 114, no. 2, pp. 287–299, 2009.

[113] M. D. Althuis, J. H. Fergenbaum, M. Garcia-Closas, L. A. Brinton, M. P. Madigan, and M. E. Sherman, "Etiology of hormone receptor-defined breast cancer: a systematic review of the literature," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 13, no. 10, pp. 1558–1568, 2004.

[114] H. J. Thompson, J. N. McGinley, P. Wolfe, M. Singh, V. E. Steele, and G. J. Kelloff, "Temporal sequence of mammary intraductal proliferations, ductal carcinomas in situ and adenocarcinomas induced by 1-methyl-1-nitrosourea in rats," *Carcinogenesis*, vol. 19, no. 12, pp. 2181–2185, 1998.

[115] S. J. Santos, M. D. Aupperlee, J. Xie et al., "Progesterone receptor A-regulated gene expression in mammary organoid cultures," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 115, no. 3, pp. 161–172, 2009.

[116] A. McTiernan, C. Kooperberg, E. White et al., "Recreational Physical Activity and the Risk of Breast Cancer in Postmenopausal Women: The Women's Health Initiative Cohort Study," *Journal of the American Medical Association*, vol. 290, no. 10, pp. 1331–1336, 2003.

[117] G. Newman and R. R. Gonzalez-Perez, "Leptin-cytokine crosstalk in breast cancer," *Molecular and Cellular Endocrinology*, vol. 382, no. 1, pp. 570–582, 2014.

[118] H. Shimizu, Y. Shimomura, Y. Nakanishi et al., "Estrogen increases in vivo leptin production in rats and human subjects," *Journal of Endocrinology*, vol. 154, no. 2, pp. 285–292, 1997.

[119] H. Tan, L. Yi, N. S. Rote, W. W. Hurd, and S. Mesiano, "Progesterone receptor-A and -B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition," *The Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 5, pp. E719–E730, 2012.

[120] Y. Xie, Y.-L. Wang, L. Yu et al., "Metformin promotes progesterone receptor expression via inhibition of mammalian target of rapamycin (mTOR) in endometrial cancer cells," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 126, no. 3, pp. 113–120, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 274585, 17 pages
http://dx.doi.org/10.1155/2015/274585

Review Article

Obesity and Cancer Progression: Is There a Role of Fatty Acid Metabolism?

Seher Balaban,[1,2] Lisa S. Lee,[1,2] Mark Schreuder,[1] and Andrew J. Hoy[1,2,3]

[1]*Discipline of Physiology, School of Medical Sciences and Bosch Institute, The University of Sydney, Sydney, NSW 2006, Australia*
[2]*Charles Perkins Centre, The University of Sydney, Sydney, NSW 2006, Australia*
[3]*Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders, The University of Sydney, Sydney, NSW 2006, Australia*

Correspondence should be addressed to Andrew J. Hoy; andrew.hoy@sydney.edu.au

Received 30 July 2014; Accepted 24 November 2014

Academic Editor: James McManaman

Currently, there is renewed interest in elucidating the metabolic characteristics of cancer and how these characteristics may be exploited as therapeutic targets. Much attention has centered on glucose, glutamine and *de novo* lipogenesis, yet the metabolism of fatty acids that arise from extracellular, as well as intracellular, stores as triacylglycerol has received much less attention. This review focuses on the key pathways of fatty acid metabolism, including uptake, esterification, lipolysis, and mitochondrial oxidation, and how the regulators of these pathways are altered in cancer. Additionally, we discuss the potential link that fatty acid metabolism may serve between obesity and changes in cancer progression.

1. Introduction

Obesity has long been known to be associated with the development of type 2 diabetes and cardiovascular disease [1]. More recently there is a growing acceptance for a link between obesity and cancer [2]. However, the nature of this relationship remains to be fully elucidated. On one hand obesity increases the risk of many types of cancer, including esophageal, endometrial, thyroid, colon, renal, liver, and breast [3, 4]. The other aspect is that obesity is also associated with changes in the progression of many cancers. These include higher grade disease in prostate and breast cancer [5, 6] and poorer outcomes in endometrial, kidney, pancreas, esophageal, and thyroid cancers [7–9].

Obesity is defined by increased adipose mass arising from energy imbalance. The predominant cell type in adipose tissue is the adipocyte, which is the professional lipid storage cell. Alongside the adipocyte there are a number of other cell types in adipose including preadipocytes, endothelial cells, and immune cells such as resident macrophages. This collective results in a highly complex organ that is central to energy homeostasis and its biology is dramatically altered in obesity. These changes include altered adipocyte biology, such as increased efflux of fatty acids and modified adipokine profile, which is often accompanied by low-grade inflammation and hyperinsulinemia [10]. Whilst these changes are common, they are not defining characteristics of the entire obese population. For example, a significant subpopulation is metabolically healthy, retains insulin sensitivity, and has normal lipid and inflammation profiles [11]. Likewise, there are other populations, including the "metabolically obese, normal weight" [12, 13] and those with familial lipodystrophy [14, 15] that have pathogenic metabolic profiles. These other populations highlight that inflammatory mediators and increased growth factor availability (e.g., IGF-1, insulin; see [16]) are not the only mechanisms linking obesity with cancer. In this review, we will highlight the evidence that exists on the role that fatty acid metabolism plays in cancer biology (Table 1), focusing on pathways of fatty acid uptake, storage, mobilization, and oxidation (Figure 1). This focus is based upon the potential link that fatty acid metabolism may play in the obesity/cancer relationship as excessive lipid accumulation, particularly in abdominal regions, is a definitive characteristic of obesity.

Table 1: Summary of regulators of fatty acid metabolism and their effects on cancer cell biology.

Regulator of FA metabolism	Cancer type	Alteration	Associated outcome	Reference(s)
LPL	Prostate	Increased activity	Increased susceptibility	[17]
	Cervical	Enhanced protein expression	Increased invasion capacity	[18]
	Rectal and skin	Increased activity	Increased tumor growth	[19]
	Lung	Increased activity	Lower overall survival	[20, 21]
CD36/FAT	Colon and ovarian	Decreased gene expression	Higher metastatic capacity	[22]
	Breast	Decreased gene expression	Higher metastatic capacity	[23]
FATP	Liver	Increased gene expression	Enhanced progression	[24]
FABP4	Breast	Decreased gene expression	N/A	[25]
	Bladder	Low gene expression	Increased tumor progression and invasion capacity	[26–29]
	Prostate	Increased protein expression	Increased migration and invasion capacities	[30]
	Ovarian	Increased protein expression	Increased migration and invasion capacities	[31]
FABP5	Breast	Increased gene expression	Higher metastatic capacity and lower recurrence-free and overall survival	[32, 33]
	Endometrial	Increased gene expression	No correlated clinical outcome	[34]
	Liver and pancreatic	Increased protein expression	N/A	[35, 36]
	Prostate	Increased gene expression	Increased tumor progression	[37]
		Decreased gene expression	Increased invasion capacity and tumor growth	[26, 38, 39]
FABP7	Breast	Increased gene expression	Lower recurrence rate, improved survival	[40, 41]
		Increased nuclear localization	Increased proliferation, pleomorphism, and tumor stage	[42]
	Primary melanoma	Increased gene expression	N/A	[43]
	Renal	Increased gene expression	No correlated clinical outcome	[44, 45]
ACSL3	Glioblastoma	Increased protein expression	Increased malignant phenotype	[46]
	Colon	Increased gene and protein expression	N/A	[47]
ACSL4	Liver	Increased gene expression	Increased proliferation	[48]
ACSL5	Colon	Increased gene expression	Increased proliferation	[49]
AGPAT2	Ovarian	Increased protein and gene expression	Reduced overall survival and higher tumor grade, mitotic index, and tumor stage	[50–52]
AGPAT11	Breast, cervical, and colon	Increased gene expression	Higher tumor grade	[53]
AGPAT9	Colorectal	Increased gene and protein expression	Increased cell growth	[54]
ATGL	Lung and skin	Increased ATGL activity	Increased tumor growth and cancer-associated cachexia	[55]
HSL	Gastrointestinal	Increased gene and protein expression	Cancer-associated cachexia	[56]
	Colorectal, pancreatic, stomach, and esophageal	Increased gene expression	N/A	[57]
MAGL	Colorectal	Increased gene and protein expression	N/A	[58]
	Ovarian, breast, melanoma, and prostate	Increased gene expression	Aggressiveness	[59]
CPT1A	Ovarian	Increased gene expression	Increased tumor growth	[31]
	Breast	Increased gene and protein expression and activity	N/A	[60]
	Glioblastoma	Increased gene expression	Higher tumor grade	[61]
CPT1C	Lung	Increased gene expression	N/A	[62]
	Glioblastoma	Increased gene expression	Higher tumor grade	[61]

Table 1: Continued.

Regulator of FA metabolism	Cancer type	Alteration	Associated outcome	Reference(s)
ACC1	Breast	Increased protein expression	Increased tumor progression	[63, 64]
	Prostate	Increased gene expression	N/A	[65]
	Lung	Decreased activity	Increased overall survival	[66]
	Liver	Increased gene expression	N/A	[67]

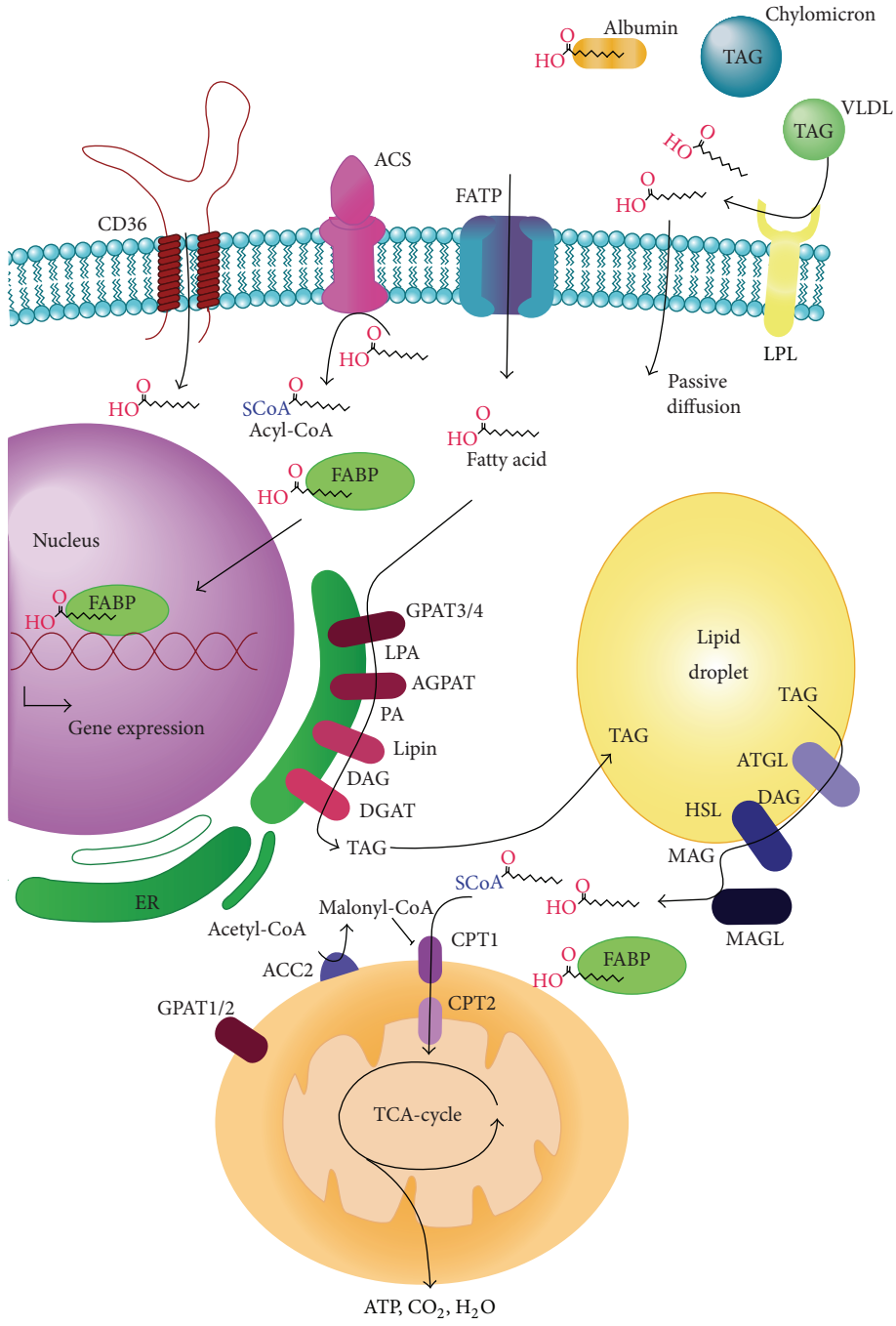


Figure 1: Intracellular fatty acid metabolism. A simplified cartoon of fatty acid metabolism pathways. Fatty acids are transported in the circulation as triacylglycerol (TAG) in lipoproteins and hydrolyzed by lipoprotein lipase (LPL) or they are bound to albumin and are transported across the plasma membrane. A CoA is ligated to fatty acid (FA), and the fatty acyl-CoA (FA-CoA) can enter the glycerolipid synthesis pathway for storage or the mitochondria for oxidation. ACS, acyl-CoA synthase; AGPAT, acyl-CoA: acylglycerol-3-phosphate acyltransferase; ATGL, adipose triglyceride lipase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LPA, lysophosphatidic acid; MAG, monoacylglycerol; MAGL, monoacylglycerol lipase; PA, phosphatidic acid.

2. Lipoprotein Hydrolysis, Fatty Acid Transport, and Trafficking

Long-chain fatty acids travel in the circulation either as free fatty acids that are released from adipocytes bound to albumin or as triacylglycerol (TAG) contained in very low-density lipoproteins and chylomicrons. This circulating TAG is hydrolyzed by lipoprotein lipase (LPL) to free fatty acids [68] and then taken up into cells (Figure 1). There remains some controversy as to whether these fatty acids enter the cell

by passive diffusion or by protein mediated transport. As will be discussed below, it is clear that the latter process does contribute to fatty acid uptake.

2.1. Lipoprotein Hydrolysis. Altered expression of LPL has been reported in many cancers. For example, Narita and colleagues [17] reported a significant association between increased hydrolytic activity of LPL due to the LPL polymorphism (Ser447stop) and the susceptibility to prostate cancer. This association was even stronger in patients with high grade tumors or metastasis. Similarly, this pattern was observed in cervical cancer where LPL is frequently overexpressed in cervical squamous cell carcinomas and associated with an increased invasion capacity [18]. LPL activity has been reported in gastric and rectal cancers, malignant fibrous histiocytomas, and osteosarcomas, with the high proliferating outer area of rectal tumors and fibrous histiocytomas having an enhanced expression of LPL compared with the center [19]. Interestingly, the increased LPL activity in cancer tissue, compared with healthy lung tissue, predicts lower overall survival in non-small-cell lung cancer [20, 21]. The location of tumor LPL is somewhat controversial as a recent study observed that increased LPL expression was in a subgroup of macrophages and not in cancer cells [69]. These studies mostly report gene expression and therefore future studies linking functional changes in cancer cell LPL activity driving FA release from circulating TAG are required, especially as LPL activity is regulated by a variety of physiological stimuli (see review [70]).

2.2. Fatty Acid Transport. Several proteins have been identified to facilitate the uptake of fatty acids into cells. These include CD36/fatty acid translocase, the fatty acid binding protein (FABP) family, and the fatty acid transport proteins (FATP) [71]. Many of these transporters are ubiquitously expressed, while some display tissue-specific expression [72, 73]. Interestingly, most tissues have coexpression of different fatty acid transporters [74]. The reason for this remains unknown. Possibilities may include differences in uptake capacity and substrate specificity, sensitivity to hormonal stimuli such as insulin [75], or preferences in partitioning into downstream pathway, for example, fatty acid esterification (storage) or oxidation [74].

2.2.1. CD36/Fatty Acid Translocase. CD36, also known as fatty acid translocase (FAT), is a multifunctional transmembrane glycoprotein which is abundantly expressed in cell types active in fatty acid metabolism, including adipocytes, skeletal muscles cells, cardiomyocytes, intestinal enterocytes, monocytes, and hepatocytes [76]. It was originally isolated from platelet membranes as a thrombospondin receptor [77] but has also been shown as a receptor for collagen [78], oxidized lipoproteins [79], and, of greatest interest to this review, long-chain fatty acids [80].

CD36 has been implicated in contributing to cancer progression. Low CD36 gene expression correlates with a higher metastasis grade in colon and ovarian cancers and with low recurrence-free survival [22]. Conversely, CD36 mRNA expression in breast cancer is inversely correlated with the metastatic potential of five breast cancer cell lines [23], where its expression is relatively higher in less aggressive cell lines (T47-D and MCF-7) and almost absent in highly aggressive lines (ZR-75 and MDA-MB-231). This inconsistency between cancer types may be explained by the multifunctionality of CD36. While it functions as a fatty acid transporter, CD36 is also involved in collagen adhesion and, therefore, less CD36 may reduce cell adhesion, providing cancer cells with a higher metastatic potential. That said, the above studies have reported gene or protein expression and not the rates of fatty acid uptake.

Fatty acid transporter abundance is not the only factor regulating FA uptake. An aspect that is often overlooked is that FA uptake is increased by insulin stimulation [81, 82]. This is thought to be mediated by translocation of CD36 to the plasma membrane which has been observed in hepatocytes of obese Zucker rats [83], skeletal muscle [84, 85], cardiomyocytes [86, 87], and ovary cells [85]. This is analogous to the translocation of the insulin sensitive glucose transporter GLUT4 [88, 89].

So far, no studies have investigated the influence of obesity on fatty acid transporters in cancer cells. It is clear from studies in other model systems that CD36 expression and fatty acid uptake are influenced by the microenvironment. For example, CD36 gene expression and protein levels are increased in steatotic hepatocytes [90] and liver biopsies of obese patients, correlating with the circulating free fatty acids levels [91]. In subcutaneous adipose tissue, CD36 protein expression is upregulated in both obese patients and type 2 diabetics [92]. Furthermore, CD36 mRNA expression levels are greatly enhanced in liver and adipose tissue of *ob/ob* mice, a monogenic model of obesity [93]. Interestingly, incubation of human skeletal muscle cells with adipocyte conditioned media increased both fatty acid uptake and CD36 protein levels [94]. Similar changes in CD36 expression by adipocyte factors, such as adipokines and fatty acids, have been reported in vascular smooth muscle cells [95], cardiomyocytes [86, 96], and adipocytes [97, 98]. Collectively this suggests that changes in adipocyte biology, especially in the context of obesity, can alter CD36 expression in nonadipose cells such as cancer cells that may influence the inherent role that CD36 plays in cancer biology.

2.2.2. Fatty Acid Transport Protein. Fatty acid transport proteins form a highly conserved family of six transporters named FATP1–6 [99]. FATPs are integral membrane proteins and are differentially expressed in a wide variety of cells [100]. These transporters are unique as they can express fatty acyl-CoA synthetase activity [101] as well as an endoplasmic reticulum localization signal domain, at least for FATP4 [102]. Alongside CD36, FATPs regulate long-chain fatty acid and very long-chain fatty acid uptake [103] although the functional differences between CD36 and FATPs are yet to be resolved. A recent study in Madin-Darby Canine cells reported that CD36 is 30-fold more effective in fatty acid uptake compared with FATP4 or the acyl-CoA synthetase ACSL1 [104]. However, cooverexpression of CD36 with either FATP4 or ACSL1 results in an enhanced fatty acid uptake rate greater than expected from the combined individual capacity

suggesting a synergistic relationship between CD36, FATP4, and ACSL1 to facilitate fatty acid uptake.

To date, only one study has described a possible role for FATPs in tumor metabolism. In this study, FATP mRNA expression is increased in rat hepatomas compared with normal liver tissue which correlated with fatty acid uptake rates [24]. Similar to CD36, FATP expression is influenced by the microenvironment, especially in obesity. FATP expression is elevated in adipose tissue of obese patients [105, 106] and in heart [107], skeletal muscle [108], and adipose tissue [109] of rodent models of obesity. Overall, FATPs are important players in lipid uptake and metabolism. However, their role in cancer, especially in the context of obesity-sensitive cancers, is far from understood and further research is needed to elucidate this role.

2.3. Intracellular Trafficking. Fatty acid binding proteins (FABPs) are a family of transport proteins with high affinity for long-chain fatty acids, bile acids, and retinoids [110]. Twelve FABP isoforms have been identified, each with its own tissue and substrate specificity [111]. Although their physiological functions are not fully understood, they appear to facilitate the transport of fatty acids intracellularly and thereby regulate substrate availability for complex lipid synthesis (esterification) and oxidation [112, 113]. Changes in FABP expression have been associated with various diseases including several forms of cancer [113] with FABP5 being the most well characterized FABP isoform in cancer cell biology. For example, prostate [32], endometrial [34], liver [35], pancreatic [36], and breast [32] cancers have increased FABP5 gene or protein expression. However, the observations in prostate are controversial as other studies report reduced expression in multiple prostate cancer lines [26, 38]. Despite this, increased expression of FABP5 in prostate cancer cells increased fatty acid uptake and peroxisome proliferator-activated receptor gamma (PPARγ) expression which enhanced tumor progression [37]. Additionally, overexpression of FABP5 in the benign breast cancer cell line, Rama 37, increased metastatic capacity in rats [32]. Interestingly, expression is higher in estrogen and progesterone negative breast cancer cells, with the highest expression found in triple-negative breast cancer [33]. Furthermore, patients with higher FABP5 mRNA levels had lower recurrence-free and overall survival probabilities [33]. Conversely, invasion capacity and tumor growth were significantly reduced in prostate cancer cells with reduced FABP5 expression [39].

FABP7 has emerged as another participant of intracellular FA metabolism that may contribute to cancer cell biology. Its gene expression is elevated in triple-negative breast cancer cells [40], primary melanomas [43], and renal cell carcinomas [44, 45]. Interestingly and in contrast to FABP5, FABP7 positive basal-like breast tumors had a significant lower recurrence rate and improved survival rate [41]. In cell culture studies, siRNA knockdown of FABP7 reduced proliferation and invasion in melanoma cells whilst the contraobservation was reported with overexpression enhancing proliferation and invasion [114]. Furthermore, investigations of the organelle-specific roles of FABP7 demonstrated that increased nuclear, but not cytoplasmic, FABP7 is associated with increased proliferation, pleomorphism, and tumor stage in breast cancer suggesting that nuclear FABP7 drives a more aggressive phenotype [42]. However, the mechanism by which FABP7 influences gene expression is yet to be resolved. FABPs may act as coactivators for transcription factors like PPARs [115] or simply function as transporters to carry FA into the nucleus to modulate gene expression [116] via the many intranuclear targets including sterol regulatory binding protein, PPARs, and liver X receptors [117].

FABP4 has also been implicated in cancer biology. FABP4 mRNA levels are downregulated in breast cancer cells [25]. Conversely, FABP4 expression is inversely correlated with tumor progression and invasiveness in bladder cancer [26–29]. FAPB4 is also susceptible to the extracellular milieu as there is growing evidence that adipocytes increase FABP4 mRNA and protein expression in cancer cells. An elegant study in ovarian cancer demonstrated that coculture with adipocytes increases FABP4 protein expression and promotes migration and invasion of ovarian cancer cells, while FABP4 deficiency ameliorated the adipocyte-derived metastatic potential [31]. A similar observation of adipocyte-induced increase in FABP4 expression has been reported in PC3 prostate cancer cells [30]. The same study also reported an increased expression of FABP4 in prostate cancer bone metastasis from high-fat diet mice and prostate cancer patients [30]. The fact that bone marrow is adipocyte-rich [118] suggests a role for adipocytes in enhancing FABP4 expression and thereby playing an important role in cancer progression. Overall, FABPs are emerging as important factors in cancer cell lipid metabolism but more research is needed to fully elucidate the roles of FABPs in healthy tissue and tumor cells and how these are altered by obesity.

3. Fatty Acid Activation, Esterification, and Mobilization

Once FAs are taken up by cells, they are activated by the addition of coenzyme A (CoA) to the fatty acid molecule by the actions of long-chain acyl-CoA synthetase (ACSL). From here, evidence suggests that fatty acyl-CoAs can be partitioned into the esterification pathway in the endoplasmic reticulum or the mitochondria for oxidation [119]. Recently, this notion has been challenged by studies in human skeletal muscle [120] and isolated hepatocytes from mice lacking adipose triglyceride lipase (ATGL) [121]. These studies suggest that extracellular FAs enter the esterification pathway to be stored in lipid droplets prior to mitochondrial oxidation. Irrespective of the precise pathways, fatty acids have a multitude of intracellular fates, but at the most basic level FAs can be either oxidized or stored as complex lipids.

3.1. Fatty Acid Activation. ACSLs are a family of enzymes that catalyze the addition of a CoA to a free fatty acid and differ in their preference to the chain length of their fatty acids substrates (short, medium, long, and very long). ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 are members of the long-chain family that vary in both subcellular localization and substrate specificity [122]. Along with FABPs, individual

ACSL isoforms have been proposed to channel fatty acids to specific metabolic pathways.

Significant evidence suggests an important role for ACSLs in cancer biology including increased expression of ACSLs in many types of cancer such as colon, liver, lung, brain, and colorectal cancers and estrogen receptor negative breast tumors and androgen receptor negative prostate tumors [46, 47, 49, 123–125]. More specifically, ACSL5 gene expression is consistently elevated in the colon cancer tissue compared to normal colon tissue [49], so are ACSL4 gene expression and protein levels in colon adenocarcinoma compared with adjacent normal tissue [47] and in hepatocellular carcinoma tissues compared to the adjacent noncancerous liver tissue [48]. Finally, ACSL3 expression is elevated in the highly tumorigenic U87 human glioblastoma cell line and cells derived from tumorigenic primary glioblastoma xenografts (Mayo 22) compared with the less tumorigenic U373 glioma cells [46].

Collectively, the results from these studies suggest that expression of ACSLs is related to tumorigenesis and tumor progression. Cell culture loss and gain of function studies provide insight into the relationship between altered intracellular fatty acid metabolism and cancer cell biology. In terms of fatty acid metabolism, both ACSL3 and ACSL5 overexpression in HepG2 cells increase fatty acid oxidation and reduce TAG levels [126]. Supporting the gene expression observations, altered ASCL expression in cancer cells is linked with survival, proliferation, and chemoresistance. For example, overexpressed ACSL4 in human epithelial cells reduced the level of arachidonic acid-induced apoptosis [127], whereas siRNA-mediated ACSL3 knockdown reduced growth rates of lung cancer cell lines and colony formation [124]. Similarly, ACSL4 knockdown inhibited growth rates of the human hepatocellular carcinoma cell line Hep3B [48]. Additionally, pharmacological inhibition of ACS activity by triacsin-C induced apoptosis in HEK293 cells [127] and glioma cells, which was completely suppressed by overexpression of ACSL5 [128].

The impact of ASCL expression and function in cancer biology in the obese setting has not been reported. Interestingly, ACSL activity and *Acsl1* gene expression are upregulated in liver and adipose tissues in genetic obese models, including ob/ob mice and Zucker fatty rat (fa/fa) [93, 129] and high-fat fed rats [130]. This suggests that the elevated expression reported may be exacerbated in obesity and therefore may accelerate cancer progression. How changes in ASCL-mediated fatty acid metabolism are linked to altered cancer progression is yet to be fully elucidated. However, Cao and colleagues [127] proposed that changes in proapoptotic arachidonic acid levels may play a role yet other bioactive lipids such as sphingolipids, including ceramides, or changes in fatty acyl-CoA availability for mitochondrial oxidation are potential contributors.

3.2. Fatty Acid Esterification. FAs are the building blocks for many complex lipids including phospholipids, sphingolipids, and glycerolipids. We will focus on the synthesis of glycerolipids, such as TAG, as this is a major pool that is susceptible to the obese environment. The storage of fatty acids as TAG involves several condensation reactions. The first step involves esterifying a fatty acyl-CoA with glycerol-3-phosphate to generate lysophosphatidic acid (LPA) by the enzyme glycerol-3-phosphate acyltransferase (GPAT). LPA is then condensed into phosphatidic acid (PA) by 1-acylglycerol-3-phosphate-O-acyltransferase (AGPAT). The subsequent reaction is catalyzed by lipin, which dephosphorylates PA to produce diacylglycerol (DAG). The final step involves the addition of a third fatty acyl-CoA to DAG by diacylglycerol acyltransferase (DGAT) to generate TAG. This process occurs in the endoplasmic reticulum where TAG is packaged into lipid droplets [131]. Alongside the endoplasmic reticulum pathway, there is evidence that DGAT can also catalyze the conversion of DAG to TAG at the lipid droplet [132–134].

The lipid intermediates of the esterification pathway are substrates for the generation of other complex lipids, such as phospholipids in membrane synthesis, and can also act as lipid signals that modify membrane structures and promote gene transcription for cell growth, proliferation, and differentiation [135].

To date, gene or protein expression profiling of GPAT in cancer cells has not been reported. However, it is known that four isoforms of GPATs are expressed in mammals; GPAT1 and GPAT2 are localized in the mitochondria and GPAT3 and GPAT4 in the endoplasmic reticulum [136]. As rate-limiting enzymes of fatty acid esterification, GPATs are key regulators of TAG synthesis [137, 138].

Similarly, little is known about the expression of lipin and DGAT in cancer patients. Mammals have three lipin proteins and two isoforms of DGAT that regulate phospholipid synthesis and lipid storage [139, 140]. Consequently, these proteins modulate the availability of fatty acid substrates for lipid signaling and metabolism, which may influence cancer progression [141].

The most studied enzyme of lipid esterification in relation to cancer is 1-acylglycerol-3-phosphate-O-acyltransferase (AGPAT). There are 11 known isoforms of AGPAT, which differ by tissue expression and enzymatic activity [137]. There is consistent evidence suggesting a role for AGPATs in cancer cells. For example, AGPAT2 expression is elevated in ovarian cancer patients with aggressive ovarian cancers and associated with reduced overall survival [50–52]. Gene expression of AGPAT11 is also increased in breast and cervical cancers, as well as colorectal cancer [53]. Interestingly, transcriptional expression of AGPAT9, which is highly homologous with AGPAT11, is upregulated in colorectal cancer, but not in breast and cervical cancers [54].

Obesity is characterized by increased levels of TAG stored in tissues such as skeletal muscle, liver, and cardiac muscle [142], which is a consequence of increased esterification rates [130, 143–145]. Loss and gain of function studies in various tissues provide an insight into the complex regulation of the intracellular lipid environment. For example, AGPAT6 knockout mice have reduced TAG content in brown and white adipose tissue and interestingly altered fatty acid profile of complex lipids, such as DAG and phospholipids with a shift towards the polyunsaturated more than the monounsaturated fatty acids [146]. Similarly, adipose tissue TAG levels are decreased in mice lacking DGAT [147] and both lipin1 and

lipin3 [148] and protection from high-fat diet induced obesity and associated metabolic perturbations [139, 149]. Finally, GPAT-deficient mice have lower levels of liver and plasma TAG [150]. On the other hand, overexpressions of GPAT1 [151], GPAT4 [152], AGPAT1 [153], lipin1 [154], and DGAT1 [155] all result in increased TAG levels. From this, it is evident that enzymes involved in esterification significantly influence intracellular and extracellular lipid homeostasis. How this translates to pathogenic changes in cancer cells is yet to be described.

3.3. Lipolysis. TAGs, along with cholesterol esters, are stored in lipid droplets to serve as a readily available source of energy for ATP generation in the mitochondria, as well as providing building blocks for phospholipids and other complex lipids. In terms of metabolic energy capacity, an average nonobese person stores up to 2,500 kJ of metabolic energy in glycogen, but >500,000 kJ as TAGs [156]. Whilst most of this TAG is stored in adipocytes, all cells have the capacity to synthesize and breakdown TAGs. Interestingly, intracellular lipid stores, or lipid droplet size and/or number, are elevated in various malignant cells, such as breast [157], prostate [158], cervical [159], liver [160], and colon cancer cells [161]. Furthermore, biochemical assessment of lipid droplets in breast cancer cells has shown that the TAG content is increased [162]. Not only that, TAG levels are higher in more aggressive breast cancer cells and are associated with long-term breast cancer cell survival [157, 162]. These findings suggest that intracellular TAG may play a critical, yet unexplored, role in supporting both substrates for complex lipid synthesis [163] as well as energy production in cancer cells that collectively promote cell growth and proliferation. To do this, TAGs need to be broken down to FAs and glycerol by a process called lipolysis.

ATGL, otherwise known as desnutrin [164], is the predominant TAG lipase that is thought to be rate-limiting [165]. It catalyzes the conversion of TAG to DAG and releases a free fatty acid from the sn-2 position [166]. Hormone-sensitive lipase (HSL) catalyzes the hydrolysis of DAG into monoacylglycerol (MAG) and a fatty acid [167]. HSL has broad substrate specificity, including TAG, DAG, MAG, and cholesterol ester lipid classes, but has the highest affinity for DAG [168]. MAG is then broken down by monoacylglycerol lipase (MAGL) resulting in the metabolic end-product, glycerol, and the liberation of the final fatty acid. This process is highly conserved across species and highly regulated with most insight arising from studies in adipocytes (see review [169]).

Adipose neutral lipase expression in various cancer patients has been reported. Compared to normal individuals without cancer, HSL mRNA expression is elevated in adipose tissue of colorectal, pancreatic, esophageal, and stomach cancer patients [57]. This was also observed in ovarian cancer patients, where adipocyte lipid depots which contain TAG were reduced, while the lipolytic products, MAG and free fatty acids, were increased, collectively suggesting elevated lipolytic activity [170]. Similarly, transcriptional and protein expression of HSL are increased in the adipose tissue of late-stage cancer patients exhibiting uncontrolled loss of adipose and muscle tissue, known as cachexia [55]. Interestingly, upregulated ATGL activity in adipose tissue was found to be responsible for this tissue-wasting syndrome [56].

MAGL is currently the most well-documented neutral lipase and its transcriptional expression is altered in several different cancers. For example, high mRNA expression of MAGL has been reported in ovarian [59], colorectal [58], breast, and melanoma cancer cells and particularly in aggressive prostate cancer cell lines [59]. Interestingly, *in vitro* studies overexpressing MAGL in nonaggressive ovarian cancer cells raised free FA levels and increased tumor growth rate, migration, and invasion [171]. Alternatively, pharmacological inhibition attenuated MAGL-induced aggressiveness of prostate cancer cells, even in a high-lipid environment [59, 171]. Similar observations have been made in colorectal cancer cells [58].

There are conflicting observations regarding the expression patterns of lipolytic enzymes in obesity [172]. HSL and ATGL gene expression are reduced in the adipose tissue of obese humans [173–175] and insulin resistant high-fat fed rats [176]. Conversely, a study by de Naeyer and colleagues [172] has reported that HSL and ATGL mRNA expression are increased in visceral adipose tissue of morbidly obese men; however, this pattern did not translate to changes in protein or lipase activity. This is not surprising considering that these neutral lipases are predominantly regulated by posttranslational modifications, translocation, and protein-protein interactions [135]. Interestingly, lipolytic enzyme expression, particularly ATGL, appears to be more associated with insulin sensitivity rather than obesity [177]. In order to elucidate the role lipolysis plays in cancer cell biology, future studies need to assess pathway of lipid metabolism and fatty acid flux, rather than gene expression, and investigate how these are altered with obesity.

4. Mitochondrial Fatty Acid Oxidation

4.1. Fatty Acid Entry into the Mitochondria. The other major fate for extracellular fatty acids is oxidation for the generation of ATP in the mitochondria. Alongside glucose and glutamine, fatty acids are a major energy source catabolized through the β-oxidation pathway to generate acetyl-CoA for entry into the TCA cycle as well as $FADH_2$ and NADH reducing equivalents for use by the electron transport chain (ETC).

Changes in cancer cell fatty acid oxidation have been reported. The primary example is observed in prostate cancer. Rather than being secreted as it is in normal prostate cells, citrate is catabolized in the TCA cycle resulting in fatty acid oxidation being the dominant bioenergetic pathway [178]. Interestingly, high-fat feeding of the p48-Kras mouse model of pancreatic cancer accelerated tumor growth and increased energy expenditure and whole body fatty acid oxidation through increased gene expression of CPT1A, ACC, and AOX enzymes, key regulators of fatty acid oxidation [179].

Few other studies have investigated the effect of obesity on cancer fatty acid oxidation. Although there is significant controversy as to the effect that obesity has on fatty acid oxidation in type 2 diabetes, the increased availability of circulating and intracellular fatty acids is thought to drive an increased

oxidative capacity. Evidence for this arises from studies in rodents fed a high-fat diet [124, 180] and obese type II diabetic patients [181]. Conversely, a number of studies reported a reduced capacity to oxidize fatty acids in overweight/obese humans [182, 183]. Considering the high metabolic flexibility of cancer cells, it is conceivable that cancer cells benefit from high lipid availability that characterizes obesity through beta-oxidation either to fulfill increased energy demand or to prevent the lipotoxic effects of high level of fatty acids.

4.2. Carnitine Palmitoyltransferase 1. Unlike short-chain fatty acids, which can freely diffuse into mitochondria, long-chain fatty acids enter the mitochondria by the carnitine shuttle system. First, carnitine palmitoyltransferase 1 (CPT1) catalyzes the transfer of the fatty acid moiety from acyl-coenzyme A (CoA) to a long-chain acyl-carnitine. This is then transported into the mitochondrial matrix by the carnitine acyl-carnitine translocase (CACT) [184]. CPT2 then catalyzes the conversion of acyl-carnitine to carnitine and fatty acyl-CoA which then enters the β-oxidation pathway. CPT1 is regulated by a cytosolic pool of malonyl-CoA produced by acetyl-CoA carboxylase 2 (ACC2) at the mitochondrial membrane [185].

The rate of mitochondrial fatty acid oxidation is regulated by CPT1, which is an integral membrane protein located on the mitochondrial outer membrane [186]. CPT1 has three isoforms with tissue-specific expressions and sensitivity to the allosteric-inhibitory action of malonyl-CoA: CPT1A (liver), CPT1B (muscle), and CPT1C (brain) [187, 188]. Changes in CPT1 expression have been observed in several types of cancer including breast, lung, brain, and liver cancers [60, 62, 189, 190]. A study by Linher-Melville and colleagues [60] reported that *CPT1A* mRNA levels are significantly elevated in both MCF-7 and MDA-MB-231 cells compared to 184B5 human mammary epithelial cells. In another study, *CPT1C* gene expression is upregulated in non-small-cell lung carcinoma tumor tissue compared with matched normal lung tissue [62]. Furthermore, high grade glioblastoma is associated with increased mRNA levels of both *CPT1A* and *CPT1C* [61]. These studies clearly show that CPT1 expression levels are related to not only tumorigenesis but also tumor progression. In contrast, CPT1 expression has been reported to be higher in the low metastatic potential, androgen receptor negative LNCaP prostate cancer cell line compared to the high metastatic potential, androgen receptor positive PC3 and DU145 prostate cancer cell lines [59]. Overexpression of CPT1C in MCF-7 cell line elevated fatty acid oxidation and ATP production to support resistance to glucose deprivation and siRNA-mediated CPT1C knockdown suppressed xenograft tumor growth [62]. Further evidence for a role for CPT1 in cancer biology has been generated from pharmacological studies. Inhibition of CPT1 with either genetic or pharmacological manipulation has been shown to reduce total ATP levels and the rate of ATP production in PC3 prostate cancer cells [62], Burkitt's lymphoma cells [191], and human glioblastoma cells [192] to impair proliferation. Additionally, etomoxir sensitizes human leukemia cells to apoptosis [193]. Collectively, these studies suggest a role for altered CPT1 expression in various cancers but interestingly CPT1 expression is sensitive to the microenvironment. For example, *CPT1A* mRNA expression and fatty acid oxidation are increased in SKOV3ip1 ovarian cancer cells cocultured with adipocytes [31]. However, it must be highlighted that fatty acid oxidation is regulated at a number of levels including CPT1 gene expression, allosterically by malonyl-CoA and fatty acid availability.

4.3. Acetyl-CoA Carboxylase. Acetyl-CoA carboxylase is a biotin-dependent enzyme that catalyzes the conversion of acetyl-CoA into malonyl-CoA. In mammals, two isoforms of ACC are expressed: ACC1 (also known as ACCα) and ACC2 (also known as ACCβ) [194]. ACC1 is primarily expressed in the cytosol of hepatocytes, adipocytes, and other lipogenic cells, while ACC2 is an enzyme associated with the outer mitochondrial membrane and is mainly expressed in cardiomyocytes, skeletal muscles, and hepatocytes [195–198]. Whereas the malonyl-CoA generated by ACC1 is primarily used for *de novo* lipogenesis, the malonyl-CoA product of ACC2 is a potent regulator of fatty acid oxidation by inhibiting CPT1 [199, 200]. Upstream of ACC2 is AMP-activated protein kinase (AMPK), which phosphorylates and inactivates ACC2 to reduce malonyl-CoA levels and thereby increase fatty acid oxidation.

Upregulation of ACC1 and increased *de novo* lipogenesis are observed in several types of cancer including breast [63, 64], prostate [65], lung [66], and liver cancers [67]. Chemical and genetic inhibition studies have identified a role for ACC1 in cell survival. For example, apoptotic cell death results from chemical inhibition of ACC1 by TOFA (5-tetradecyloxy-2-furoic acid) in lung and colon cancer cells [201] and by soraphen A in prostate cancer cells [202]. In addition, RNA interference- (RNAi-) mediated knockdown of ACC1 induces apoptosis in breast [203] and prostate cancer cells [204].

To date, studies have focused on ACC1, yet few studies have been conducted into the role of ACC2 in cancer development or progression. One of these studies demonstrated that knockdown of ACC2 increased fatty acid oxidation and inhibited cell death in A549 human lung carcinoma cells [205]. Similarly, pharmacological inhibition of malonyl-CoA decarboxylase (MCD), which increased the malonyl-CoA pool, suppresses human breast cancer cell proliferation [206]. Therefore, decreasing fatty acid oxidation rates by the modulation of the malonyl-CoA pool by ACC2 and MCD suggests a potential role for these enzymes in cancer metabolism. However, ACC2 functions in other cancer types remain to be elucidated.

The role of ACC2 in obesity is more established. Skeletal muscle ACC2 phosphorylation and activity are reduced in obese patients, as a consequence of reduced AMPK activity [207, 208]. Additionally, the mRNA levels of ACC2 in white adipose tissue are lower in Zucker fatty rats than in lean rats [209]. Interestingly, the AMPK-ACC2-CPT1 axis is modulated by several adipokines, whose levels are altered in obesity. These include leptin [210], adiponectin [211], and CTRP1 [212]. Moreover, recent evidence in liver demonstrates that metformin's actions to suppress *de novo* lipogenesis and increase fatty acid oxidation require AMPK-mediated phosphorylation of ACC1 and ACC2. Thus, the significant interest in the clinical use of metformin as the therapeutic in

many cancers will further contribute to the understanding of the role that ACC1/2 plays in cancer biology [213].

5. Conclusions

The current interest in cancer metabolism has the potential to identify common perturbations arising from differing genetic origins that may serve as therapeutic targets. As the current obesity epidemic continues to grow, there is a need to not only define cancer metabolism but also investigate how it is influenced by the obese microenvironment. It is clear that cancer fatty acid metabolism plays a significant role in cancer biology and that opportunities exist to further define this role, especially in the context of obesity-induced changes in cancer progression.

Abbreviations

CD36/FAT:	Cluster of differentiation 36/fatty acid translocation
LPL:	Lipoprotein lipase
VLDL:	Very-low density lipoprotein
FABP:	Fatty acid binding protein
PPAR:	Peroxisome proliferator-activated receptor
FATPs:	Fatty acid transport proteins
TAG:	Triacylglycerol
LPA:	Lysophosphatidic acid
GPAT:	Glycerol-3-phosphate acyltransferase
AGPAT:	1-Acylglycerol-3-phosphate-O-acyltransferase
DAG:	Diacylglycerol
HSL:	Hormone-sensitive lipase
MAG:	Monoacylglycerol
MAGL:	Monoacylglycerol lipase
ATGL:	Adipose triglyceride lipase
ACS:	Acyl-CoA synthetase
CPT1:	Carnitine palmitoyltransferase 1
CoA:	Coenzyme A
ETC:	Electron transport chain
ACSL:	Acyl-CoA synthetase long-chain family.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Andrew J. Hoy is supported by Helen and Robert Ellis Postdoctoral Research Fellowship and funding from the University of Sydney. Seher Balaban is a recipient of a University of Sydney Australian Postgraduate Award. Mark Schreuder is supported by funding from the Dutch Cancer Institute KWF.

References

[1] S. M. Grundy, "Obesity, metabolic syndrome, and cardiovascular disease," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2595–2600, 2004.

[2] S. M. Louie, L. S. Roberts, and D. K. Nomura, "Mechanisms linking obesity and cancer," *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids*, vol. 1831, no. 10, pp. 1499–1508, 2013.

[3] K. Y. Wolin, K. Carson, and G. A. Colditz, "Obesity and cancer," *Oncologist*, vol. 15, no. 6, pp. 556–565, 2010.

[4] G. de Pergola and F. Silvestris, "Obesity as a major risk factor for cancer," *Journal of Obesity*, vol. 2013, Article ID 291546, 11 pages, 2013.

[5] W. C. Buschemeyer III and S. J. Freedland, "Obesity and prostate cancer: epidemiology and clinical implications," *European Urology*, vol. 52, no. 2, pp. 331–343, 2007.

[6] B. Majed, T. Moreau, K. Senouci, R. J. Salmon, A. Fourquet, and B. Asselain, "Is obesity an independent prognosis factor in woman breast cancer?" *Breast Cancer Research and Treatment*, vol. 111, no. 2, pp. 329–342, 2008.

[7] Y. Zhu, H.-K. Wang, H.-L. Zhang et al., "Visceral obesity and risk of high grade disease in clinical T1a renal cell carcinoma," *The Journal of Urology*, vol. 189, no. 2, pp. 447–453, 2013.

[8] A. Ansary-Moghaddam, R. Huxley, F. Barzi et al., "The effect of modifiable risk factors on pancreatic cancer mortality in populations of the Asia-Pacific region," *Cancer Epidemiology Biomarkers and Prevention*, vol. 15, no. 12, pp. 2435–2440, 2006.

[9] C. A. Gilbert and J. M. Slingerland, "Cytokines, obesity, and cancer: new insights on mechanisms linking obesity to cancer risk and progression," *Annual Review of Medicine*, vol. 64, pp. 45–57, 2013.

[10] J. K. Sethi and A. J. Vidal-Puig, "Thematic review series: adipocyte Biology. Adipose tissue function and plasticity orchestrate nutritional adaptation," *Journal of Lipid Research*, vol. 48, no. 6, pp. 1253–1262, 2007.

[11] D. Samocha-Bonet, D. J. Chisholm, K. Tonks, L. V. Campbell, and J. R. Greenfield, "Insulin-sensitive obesity in humans—a "favorable fat" phenotype?" *Trends in Endocrinology & Metabolism*, vol. 23, no. 3, pp. 116–124, 2012.

[12] N. Ruderman, D. Chisholm, X. Pi-Sunyer, and S. Schneider, "The metabolically obese, normal-weight individual revisited," *Diabetes*, vol. 47, no. 5, pp. 699–713, 1998.

[13] R. Kelishadi, S. R. Cook, M. E. Motlagh et al., "Metabolically obese normal weight and phenotypically obese metabolically normal youths: the CASPIAN Study," *Journal of the American Dietetic Association*, vol. 108, no. 1, pp. 82–90, 2008.

[14] T. Nolis, "Exploring the pathophysiology behind the more common genetic and acquired lipodystrophies," *Journal of Human Genetics*, vol. 59, no. 1, pp. 16–23, 2014.

[15] J. Capeau, J. Magré, M. Caron-Debarle et al., "Human lipodystrophies: genetic and acquired diseases of adipose tissue," *Endocrine Development*, vol. 19, pp. 1–20, 2010.

[16] M. J. Khandekar, P. Cohen, and B. M. Spiegelman, "Molecular mechanisms of cancer development in obesity," *Nature Reviews Cancer*, vol. 11, no. 12, pp. 886–895, 2011.

[17] S. Narita, N. Tsuchiya, L. Wang et al., "Association of lipoprotein lipase gene polymorphism with risk of prostate cancer in a Japanese population," *International Journal of Cancer*, vol. 112, no. 5, pp. 872–876, 2004.

[18] J. C. Carter and F. C. Church, "Mature breast adipocytes promote breast cancer cell motility," *Experimental and Molecular Pathology*, vol. 92, no. 3, pp. 312–317, 2012.

[19] K. Sakayama, H. Masuno, T. Miyazaki, H. Okumura, T. Shibata, and H. Okuda, "Existence of lipoprotein lipase in human sarcomas and carcinomas," *Japanese Journal of Cancer Research*, vol. 85, no. 5, pp. 515–521, 1994.

[20] D. Cerne, I. P. Zitnik, and M. Sok, "Increased fatty acid synthase activity in non-small cell lung cancer tissue is a weaker predictor of shorter patient survival than increased lipoprotein lipase activity," *Archives of Medical Research*, vol. 41, no. 6, pp. 405–409, 2010.

[21] Z. Trost, M. Sok, J. Marc, and D. Cerne, "Increased lipoprotein lipase activity in non-small cell lung cancer tissue predicts shorter patient survival," *Archives of Medical Research*, vol. 40, no. 5, pp. 364–368, 2009.

[22] S. M. Rachidi, T. Qin, S. Sun, W. J. Zheng, and Z. Li, "Molecular profiling of multiple human cancers defines an inflammatory cancer-associated molecular pattern and uncovers KPNA2 as a uniform poor prognostic cancer marker," *PLoS ONE*, vol. 8, no. 3, Article ID e57911, 2013.

[23] I. P. Uray, Y. Liang, and S. M. Hyder, "Estradiol down-regulates CD36 expression in human breast cancer cells," *Cancer Letters*, vol. 207, no. 1, pp. 101–107, 2004.

[24] D. E. Blask, L. A. Sauer, R. T. Dauchy, E. W. Holowachuk, M. S. Ruhoff, and H. S. Kopff, "Melatonin inhibition of cancer growth in vivo involves suppression of tumor fatty acid metabolism via melatonin receptor-mediated signal transduction events," *Cancer Research*, vol. 59, no. 18, pp. 4693–4701, 1999.

[25] R. Hammamieh, N. Chakraborty, M. Barmada, R. Das, and M. Jett, "Expression patterns of fatty acid binding proteins in breast cancer cells," *Journal of Experimental Therapeutics and Oncology*, vol. 5, no. 2, pp. 133–143, 2005.

[26] A. Tölle, S. Suhail, M. Jung, K. Jung, and C. Stephan, "Fatty acid binding proteins (FABPs) in prostate, bladder and kidney cancer cell lines and the use of IL-FABP as survival predictor in patients with renal cell carcinoma," *BMC cancer*, vol. 11, article 302, 2011.

[27] J. E. Celis, M. Østergaard, B. Basse et al., "Loss of adipocyte-type fatty acid binding protein and other protein biomarkers is associated with progression of human bladder transitional cell carcinomas," *Cancer Research*, vol. 56, no. 20, pp. 4782–4790, 1996.

[28] G. Ohlsson, J. M. A. Moreira, P. Gromov, G. Sauter, and J. E. Celis, "Loss of expression of the adipocyte-type fatty acid-binding protein (A-FABP) is associated with progression of human urothelial carcinomas," *Molecular & Cellular Proteomics*, vol. 4, no. 4, pp. 570–581, 2005.

[29] P. J. Wild, A. Herr, C. Wissmann et al., "Gene expression profiling of progressive papillary noninvasive carcinomas of the urinary bladder," *Clinical Cancer Research*, vol. 11, no. 12, pp. 4415–4429, 2005.

[30] M. K. Herroon, E. Rajagurubandara, A. L. Hardaway et al., "Bone marrow adipocytes promote tumor growth in bone via FABP4-dependent mechanisms," *Oncotarget*, vol. 4, no. 11, pp. 2108–2123, 2013.

[31] K. M. Nieman, H. A. Kenny, C. V. Penicka et al., "Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth," *Nature Medicine*, vol. 17, no. 11, pp. 1498–1503, 2011.

[32] C. Jing, C. Beesley, C. S. Foster et al., "Identification of the messenger RNA for human cutaneous fatty acid-binding protein as a metastasis inducer," *Cancer Research*, vol. 60, no. 9, pp. 2390–2398, 2000.

[33] R.-Z. Liu, K. Graham, D. D. Glubrecht, D. R. Germain, J. R. Mackey, and R. Godbout, "Association of FABP5 expression with poor survival in triple-negative breast cancer: implication for retinoic acid therapy," *The American Journal of Pathology*, vol. 178, no. 3, pp. 997–1008, 2011.

[34] Z. Li, C. Huang, S. Bai et al., "Prognostic evaluation of epidermal fatty acid-binding protein and calcyphosine, two proteins implicated in endometrial cancer using a proteomic approach," *International Journal of Cancer*, vol. 123, no. 10, pp. 2377–2383, 2008.

[35] K. Fujii, T. Kondo, H. Yokoo, T. Yamada, K. Iwatsuki, and S. Hirohashi, "Proteomic study of human hepatocellular carcinoma using two-dimensional difference gel electrophoresis with saturation cysteine dye," *Proteomics*, vol. 5, no. 5, pp. 1411–1422, 2005.

[36] P. Sinha, G. Hütter, E. Köttgen, M. Dietel, D. Schadendorf, and H. Lage, "Increased expression of epidermal fatty acid binding protein, cofilin, and 14-3-3-sigma (stratifin) detected by two-dimensional gel electrophoresis, mass spectrometry and microsequencing of drug-resistant human adenocarcinoma of the pancreas," *Electrophoresis*, vol. 20, no. 14, pp. 2952–2960, 1999.

[37] Z. Bao, M. I. Malki, S. S. Forootan et al., "A novel cutaneous Fatty Acid-binding protein-related signaling pathway leading to malignant progression in prostate cancer cells," *Genes and Cancer*, vol. 4, no. 7-8, pp. 297–314, 2013.

[38] R. Das, R. Hammamieh, R. Neill, M. Melhem, and M. Jett, "Expression pattern of fatty acid-binding proteins in human normal and cancer prostate cells and tissues," *Clinical Cancer Research*, vol. 7, no. 6, pp. 1706–1715, 2001.

[39] J. Adamson, E. A. Morgan, C. Beesley et al., "High-level expression of cutaneous fatty acid-binding protein in prostatic carcinomas and its effect on tumorigenicity," *Oncogene*, vol. 22, no. 18, pp. 2739–2749, 2003.

[40] X. Y. Tang, S. Umemura, H. Tsukamoto, N. Kumaki, Y. Tokuda, and R. Y. Osamura, "Overexpression of fatty acid binding protein-7 correlates with basal-like subtype of breast cancer," *Pathology Research and Practice*, vol. 206, no. 2, pp. 98–101, 2010.

[41] H. Zhang, E. A. Rakha, G. R. Ball et al., "The proteins FABP7 and OATP2 are associated with the basal phenotype and patient outcome in human breast cancer," *Breast Cancer Research and Treatment*, vol. 121, no. 1, pp. 41–51, 2010.

[42] A. T. Alshareeda, E. A. Rakha, C. C. Nolan, I. O. Ellis, and A. R. Green, "Fatty acid binding protein 7 expression and its subcellular localization in breast cancer," *Breast Cancer Research and Treatment*, vol. 134, no. 2, pp. 519–529, 2012.

[43] Y. Goto, K. Koyanagi, N. Narita et al., "Aberrant fatty acid-binding protein-7 gene expression in cutaneous malignant melanoma," *Journal of Investigative Dermatology*, vol. 130, no. 1, pp. 221–229, 2010.

[44] C. Tan, T. Takayama, N. Takaoka et al., "Impact of gender in renal cell carcinoma: the relationship of FABP7 and BRN2 expression with overall survival," *Clinical Medicine Insights: Oncology*, vol. 8, pp. 21–27, 2014.

[45] T. Teratani, T. Domoto, K. Kuriki et al., "Detection of transcript for brain-type fatty acid-binding protein in tumor and urine of patients with renal cell carcinoma," *Urology*, vol. 69, no. 2, pp. 236–240, 2007.

[46] Z. Pei, P. Sun, P. Huang, B. Lal, J. Laterra, and P. A. Watkins, "Acyl-CoA synthetase VL3 knockdown inhibits human glioma cell proliferation and tumorigenicity," *Cancer Research*, vol. 69, no. 24, pp. 9175–9182, 2009.

[47] Y. Cao, K. B. Dave, T. P. Doan, and S. M. Prescott, "Fatty acid CoA ligase 4 is up-regulated in colon adenocarcinoma," *Cancer Research*, vol. 61, no. 23, pp. 8429–8434, 2001.

[48] Y.-C. Liang, C.-H. Wu, J.-S. Chu et al., "Involvement of fatty acid-CoA ligase 4 in hepatocellular carcinoma growth: roles of

cyclic AMP and p38 mitogen-activated protein kinase," *World Journal of Gastroenterology*, vol. 11, no. 17, pp. 2557–2563, 2005.

[49] C.-S. Yeh, J.-Y. Wang, T.-L. Cheng, C.-H. Juan, C.-H. Wu, and S.-R. Lin, "Fatty acid metabolism pathway play an important role in carcinogenesis of human colorectal cancers by Microarray-Bioinformatics analysis," *Cancer Letters*, vol. 233, no. 2, pp. 297–308, 2006.

[50] C. S. M. Diefenbach, R. A. Soslow, A. Iasonos et al., "Lysophosphatidic acid acyltransferase-β (LPAAT-β) is highly expressed in advanced ovarian cancer and is associated with aggressive histology and poor survival," *Cancer*, vol. 107, no. 7, pp. 1511–1519, 2006.

[51] S. Niesporek, C. Denkert, W. Weichert et al., "Expression of lysophosphatidic acid acyltransferase beta (LPAAT-beta) in ovarian carcinoma: correlation with tumour grading and prognosis," *British Journal of Cancer*, vol. 92, no. 9, pp. 1729–1736, 2005.

[52] G. M. Springett, L. Bonham, A. Hummer et al., "Lysophosphatidic acid acyltransferase-beta is a prognostic marker and therapeutic target in gynecologic malignancies," *Cancer Research*, vol. 65, no. 20, pp. 9415–9425, 2005.

[53] A. K. Agarwal and A. Garg, "Enzymatic activity of the human 1-acylglycerol-3-phosphate-O-acyltransferase isoform 11: upregulated in breast and cervical cancers," *Journal of Lipid Research*, vol. 51, no. 8, pp. 2143–2152, 2010.

[54] F. Mansilla, K.-A. Da Costa, S. Wang et al., "Lysophosphatidylcholine acyltransferase 1 (LPCAT1) overexpression in human colorectal cancer," *Journal of Molecular Medicine*, vol. 87, no. 1, pp. 85–97, 2009.

[55] T. Agustsson, M. Rydén, J. Hoffstedt et al., "Mechanism of increased lipolysis in cancer cachexia," *Cancer Research*, vol. 67, no. 11, pp. 5531–5537, 2007.

[56] S. K. Das, S. Eder, S. Schauer et al., "Adipose triglyceride lipase contributes to cancer-associated cachexia," *Science*, vol. 333, no. 6039, pp. 233–238, 2011.

[57] M. P. Thompson, S. T. Cooper, B. R. Parry, and J. A. Tuckey, "Increased expression of the mRNA for hormone-sensitive lipase in adipose tissue of cancer patients," *Biochimica et Biophysica Acta*, vol. 1180, no. 3, pp. 236–242, 1993.

[58] L. Ye, B. Zhang, E. G. Seviour et al., "Monoacylglycerol lipase (MAGL) knockdown inhibits tumor cells growth in colorectal cancer," *Cancer Letters*, vol. 307, no. 1, pp. 6–17, 2011.

[59] D. K. Nomura, D. P. Lombardi, J. W. Chang et al., "Monoacylglycerol lipase exerts dual control over endocannabinoid and fatty acid pathways to support prostate cancer," *Chemistry and Biology*, vol. 18, no. 7, pp. 846–856, 2011.

[60] K. Linher-Melville, S. Zantinge, T. Sanli, H. Gerstein, T. Tsakiridis, and G. Singh, "Establishing a relationship between prolactin and altered fatty acid β-oxidation via carnitine palmitoyl transferase 1 in breast cancer cells," *BMC Cancer*, vol. 11, article 56, 2011.

[61] A. Cirillo, A. Di Salle, O. Petillo et al., "High grade glioblastoma is associated with aberrant expression of ZFP57, a protein involved in gene imprinting, and of CPT1A and CPT1C that regulate fatty acid metabolism," *Cancer Biology and Therapy*, vol. 15, no. 6, pp. 735–741, 2014.

[62] K. Zaugg, Y. Yao, P. T. Reilly et al., "Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress," *Genes & Development*, vol. 25, no. 10, pp. 1041–1051, 2011.

[63] L. Z. Milgraum, L. A. Witters, G. R. Pasternack, and F. P. Kuhajda, "Enzymes of the fatty acid synthesis pathway are highly expressed in in situ breast carcinoma," *Clinical Cancer Research*, vol. 3, no. 11, pp. 2115–2120, 1997.

[64] J. T. Moncur, J. P. Park, V. A. Memoli, T. K. Mohandas, and W. B. Kinlaw, "The "Spot 14" gene resides on the telomeric end of the 11q13 amplicon and is expressed in lipogenic breast cancers: implications for control of tumor metabolism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 12, pp. 6989–6994, 1998.

[65] J. V. Swinnen, F. Vanderhoydonc, A. A. Elgamal et al., "Selective activation of the fatty acid synthesis pathway in human prostate cancer," *International Journal of Cancer*, vol. 88, no. 2, pp. 176–179, 2000.

[66] E. Conde, A. Suarez-Gauthier, E. García-García et al., "Specific pattern of LKB1 and phospho-acetyl-CoA carboxylase protein immunostaining in human normal tissues and lung carcinomas," *Human Pathology*, vol. 38, no. 9, pp. 1351–1360, 2007.

[67] N. Yahagi, H. Shimano, K. Hasegawa et al., "Co-ordinate activation of lipogenic enzymes in hepatocellular carcinoma," *European Journal of Cancer*, vol. 41, no. 9, pp. 1316–1322, 2005.

[68] H. Wong and M. C. Schotz, "The lipase gene family," *Journal of Lipid Research*, vol. 43, no. 7, pp. 993–999, 2002.

[69] H. Podgornik, M. Sok, I. Kern, J. Marc, and D. Cerne, "Lipoprotein lipase in non-small cell lung cancer tissue is highly expressed in a subpopulation of tumor-associated macrophages," *Pathology—Research and Practice*, vol. 209, no. 8, pp. 516–520, 2013.

[70] S. Kersten, "Physiological regulation of lipoprotein lipase," *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids*, vol. 1841, no. 7, pp. 919–933, 2014.

[71] J. F. F. Brinkmann, N. A. Abumrad, A. Ibrahimi, G. J. Van der Vusse, and J. F. C. Glatz, "New insights into long-chain fatty acid uptake by heart muscle: a crucial role for fatty acid translocase/CD36," *Biochemical Journal*, vol. 367, no. 3, pp. 561–570, 2002.

[72] A. Bonen, A. Chabowski, J. J. F. P. Luiken, and J. F. C. Glatz, "Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence," *Physiology*, vol. 22, pp. 15–29, 2007.

[73] D. Hirsch, A. Stahl, and H. F. Lodish, "A family of fatty acid transporters conserved from mycobacterium to man," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 15, pp. 8625–8629, 1998.

[74] J. G. Nickerson, H. Alkhateeb, C. R. Benton et al., "Greater transport efficiencies of the membrane fatty acid transporters FAT/CD36 and FATP4 compared with FABPpm and FATP1 and differential effects on fatty acid esterification and oxidation in rat skeletal muscle," *The Journal of Biological Chemistry*, vol. 284, no. 24, pp. 16522–16530, 2009.

[75] Q. Wu, A. M. Ortegon, B. Tsang, H. Doege, K. R. Feingold, and A. Stahl, "FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity," *Molecular and Cellular Biology*, vol. 26, no. 9, pp. 3455–3467, 2006.

[76] L. Love-Gregory and N. A. Abumrad, "CD36 genetics and the metabolic complications of obesity," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 14, no. 6, pp. 527–534, 2011.

[77] A. S. Asch, J. Barnwell, R. L. Silverstein, and R. L. Nachman, "Isolation of the thrombospondin membrane receptor," *The Journal of Clinical Investigation*, vol. 79, no. 4, pp. 1054–1061, 1987.

[78] N. N. Tandon, U. Kralisz, and G. A. Jamieson, "Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion," *The Journal of Biological Chemistry*, vol. 264, no. 13, pp. 7576–7583, 1989.

[79] G. Endemann, L. W. Stanton, K. S. Madden, C. M. Bryant, R. T. White, and A. A. Protter, "CD36 is a receptor for oxidized low density lipoprotein," *The Journal of Biological Chemistry*, vol. 268, no. 16, pp. 11811–11816, 1993.

[80] A. G. S. Baillie, C. T. Coburn, and N. A. Abumrad, "Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog," *The Journal of Membrane Biology*, vol. 153, no. 1, pp. 75–81, 1996.

[81] D. M. Muoio, G. L. Dohm, E. B. Tapscott, and R. A. Coleman, "Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 276, no. 5, part 1, pp. E913–E921, 1999.

[82] J. F. C. Glatz, A. Bonen, and J. J. F. P. Luiken, "Exercise and insulin increase muscle fatty acid uptake by recruiting putative fatty acid transporters to the sarcolemma," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 5, no. 4, pp. 365–370, 2002.

[83] X. Buqué, M. J. Martínez, A. Cano et al., "A subset of dysregulated metabolic and survival genes is associated with severity of hepatic steatosis in obese Zucker rats," *Journal of Lipid Research*, vol. 51, no. 3, pp. 500–513, 2010.

[84] A. Bonen, C. R. Benton, S. E. Campbell et al., "Plasmalemmal fatty acid transport is regulated in heart and skeletal muscle by contraction, insulin and leptin, and in obesity and diabetes," *Acta Physiologica Scandinavica*, vol. 178, no. 4, pp. 347–356, 2003.

[85] J. Smith, X. Su, R. El-Maghrabi, P. D. Stahl, and N. A. Abumrad, "Opposite regulation of CD36 ubiquitination by fatty acids and insulin: effects on fatty acid uptake," *The Journal of Biological Chemistry*, vol. 283, no. 20, pp. 13578–13585, 2008.

[86] Y. Angin, L. K. M. Steinbusch, P. J. Simons et al., "CD36 inhibition prevents lipid accumulation and contractile dysfunction in rat cardiomyocytes," *Biochemical Journal*, vol. 448, no. 1, pp. 43–53, 2012.

[87] A. Chabowski, S. L. M. Coort, J. Calles-Escandon et al., "Insulin stimulates fatty acid transport by regulating expression of FAT/CD36 but not FABPpm," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 287, no. 4, pp. E781–E789, 2004.

[88] N. J. Bryant, R. Govers, and D. E. James, "Regulated transport of the glucose transporter GLUT4," *Nature Reviews Molecular Cell Biology*, vol. 3, no. 4, pp. 267–277, 2002.

[89] J. W. Slot, H. J. Geuze, S. Gigengack, D. E. James, and G. E. Lienhard, "Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 17, pp. 7815–7819, 1991.

[90] I. N. Hines, H. J. Hartwell, Y. Feng et al., "Insulin resistance and metabolic hepatocarcinogenesis with parent-of-origin effects in A×B mice," *The American Journal of Pathology*, vol. 179, no. 6, pp. 2855–2865, 2011.

[91] L. P. Bechmann, R. K. Gieseler, J.-P. Sowa et al., "Apoptosis is associated with CD36/fatty acid translocase upregulation in non-alcoholic steatohepatitis," *Liver International*, vol. 30, no. 6, pp. 850–859, 2010.

[92] A. Bonen, N. N. Tandon, J. F. C. Glatz, J. J. F. P. Luiken, and G. J. F. Heigenhauser, "The fatty acid transporter FAT/CD36 is upregulated in subcutaneous and visceral adipose tissues in human obesity and type 2 diabetes," *International Journal of Obesity*, vol. 30, no. 6, pp. 877–883, 2006.

[93] R. A. Memon, J. Fuller, A. H. Moser, P. J. Smith, C. Grunfeld, and K. R. Feingold, "Regulation of putative fatty acid transporters and Acyl-CoA synthetase in liver and adipose tissue in ob/ob mice," *Diabetes*, vol. 48, no. 1, pp. 121–127, 1999.

[94] A. Taube, S. Lambernd, G. van Echten-Deckert, K. Eckardt, and J. Eckel, "Adipokines promote lipotoxicity in human skeletal muscle cells," *Archives of Physiology and Biochemistry*, vol. 118, no. 3, pp. 92–101, 2012.

[95] S. El Akoum, I. Cloutier, and J.-F. Tanguay, "Vascular smooth muscle cell alterations triggered by mice adipocytes: role of high-fat diet," *Journal of Atherosclerosis and Thrombosis*, vol. 19, no. 12, pp. 1128–1141, 2012.

[96] M. Anan, K. Uchihashi, S. Aoki et al., "A promising culture model for analyzing the interaction between adipose tissue and cardiomyocytes," *Endocrinology*, vol. 152, no. 4, pp. 1599–1605, 2011.

[97] R. Gandhi, M. Takahashi, C. Virtanen, K. Syed, J. R. Davey, and N. N. Mahomed, "Microarray analysis of the infrapatellar fat pad in knee osteoarthritis: relationship with joint inflammation," *Journal of Rheumatology*, vol. 38, no. 9, pp. 1966–1972, 2011.

[98] C. W. Wu, E. S. H. Chu, C. N. Y. Lam et al., "PPARγ is essential for protection against nonalcoholic steatohepatitis," *Gene Therapy*, vol. 17, no. 6, pp. 790–798, 2010.

[99] A. Stahl, "A current review of fatty acid transport proteins (SLC27)," *Pflugers Archiv European Journal of Physiology*, vol. 447, no. 5, pp. 722–727, 2004.

[100] J. Pohl, A. Ring, T. Hermann, and W. Stremmel, "Role of FATP in parenchymal cell fatty acid uptake," *Biochimica et Biophysica Acta*, vol. 1686, no. 1-2, pp. 1–6, 2004.

[101] R. E. Gimeno, "Fatty acid transport proteins," *Current Opinion in Lipidology*, vol. 18, no. 3, pp. 271–266, 2007.

[102] C. M. Anderson and A. Stahl, "SLC27 fatty acid transport proteins," *Molecular Aspects of Medicine*, vol. 34, no. 2-3, pp. 516–528, 2013.

[103] H. Doege and A. Stah, "Protein-mediated fatty acid uptake: novel insights from in vivo models," *Physiology*, vol. 21, no. 4, pp. 259–268, 2006.

[104] H. Schneider, S. Staudacher, M. Poppelreuther, W. Stremmel, R. Ehehalt, and J. Füllekrug, "Protein mediated fatty acid uptake: synergy between CD36/FAT-facilitated transport and acyl-CoA synthetase-driven metabolism," *Archives of Biochemistry and Biophysics*, vol. 546, pp. 8–18, 2014.

[105] J. F. Bower, J. M. Davis, E. Hao, and H. A. Barakat, "Differences in transport of fatty acids and expression of fatty acid transporting proteins in adipose tissue of obese black and white women," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 290, no. 1, pp. E87–E91, 2006.

[106] K. Gertow, K. H. Pietiläinen, H. Yki-Järvinen et al., "Expression of fatty-acid-handling proteins in human adipose tissue in relation to obesity and insulin resistance," *Diabetologia*, vol. 47, no. 6, pp. 1118–1125, 2004.

[107] H.-C. Chiu, A. Kovacs, R. M. Blanton et al., "Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy," *Circulation Research*, vol. 96, no. 2, pp. 225–233, 2005.

[108] M. Marotta, A. Ferrer-Martínez, J. Parnau, M. Turini, K. MacÉ, and A. M. Gómez Foix, "Fiber type- and fatty acid

composition-dependent effects of high-fat diets on rat muscle triacylglyceride and fatty acid transporter protein-1 content," *Metabolism: Clinical and Experimental*, vol. 53, no. 8, pp. 1032–1036, 2004.

[109] P. D. Berk, S.-L. Zhou, C.-L. Kiang, D. Stump, M. Bradbury, and L. M. Isola, "Uptake of long chain free fatty acids is selectively up-regulated in adipocytes of zucker rats with genetic obesity and non-insulin-dependent diabetes mellitus," *The Journal of Biological Chemistry*, vol. 272, no. 13, pp. 8830–8835, 1997.

[110] P. Besnard, I. Niot, H. Poirier, L. Clément, and A. Bernard, "New insights into the fatty acid-binding protein (FABP) family in the small intestine," *Molecular and Cellular Biochemistry*, vol. 239, no. 1-2, pp. 139–147, 2002.

[111] A. Chmurzyńska, "The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism," *Journal of Applied Genetics*, vol. 47, no. 1, pp. 39–48, 2006.

[112] R. M. Kaikaus, N. M. Bass, and R. K. Ockner, "Functions of fatty acid binding proteins," *Experientia*, vol. 46, no. 6, pp. 617–630, 1990.

[113] A. W. Zimmerman and J. H. Veerkamp, "New insights into the structure and function of fatty acid-binding proteins," *Cellular and Molecular Life Sciences*, vol. 59, no. 7, pp. 1096–1116, 2002.

[114] Y. Goto, Y. Matsuzaki, S. Kurihara et al., "A new melanoma antigen fatty acid-binding protein 7, involved in proliferation and invasion, is a potential target for immunotherapy and molecular target therapy," *Cancer Research*, vol. 66, no. 8, pp. 4443–4449, 2006.

[115] C. Wolfrum, C. M. Borrmann, T. Börchers, and F. Spener, "Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors α- and γ-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 5, pp. 2323–2328, 2001.

[116] N. H. Haunerland and F. Spener, "Fatty acid-binding proteins—insights from genetic manipulations," *Progress in Lipid Research*, vol. 43, no. 4, pp. 328–349, 2004.

[117] L. A. Afman and M. Müller, "Human nutrigenomics of gene regulation by dietary fatty acids," *Progress in Lipid Research*, vol. 51, no. 1, pp. 63–70, 2012.

[118] J. Justesen, K. Stenderup, E. N. Ebbesen, L. Mosekilde, T. Steiniche, and M. Kassem, "Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis," *Biogerontology*, vol. 2, no. 3, pp. 165–171, 2001.

[119] D. G. Mashek, L. O. Li, and R. A. Coleman, "Long-chain acyl-CoA synthetases and fatty acid channeling," *Future Lipidology*, vol. 2, no. 4, pp. 465–476, 2007.

[120] J. A. Kanaley, S. Shadid, M. T. Sheehan, Z. Guo, and M. D. Jensen, "Relationship between plasma free fatty acid, intramyocellular triglycerides and long-chain acylcarnitines in resting humans," *Journal of Physiology*, vol. 587, part 24, pp. 5939–5950, 2009.

[121] S. M. Turpin, A. J. Hoy, R. D. Brown et al., "Adipose triacylglycerol lipase is a major regulator of hepatic lipid metabolism but not insulin sensitivity in mice," *Diabetologia*, vol. 54, no. 1, pp. 146–156, 2011.

[122] R. A. Coleman and D. P. Lee, "Enzymes of triacylglycerol synthesis and their regulation," *Progress in Lipid Research*, vol. 43, no. 2, pp. 134–176, 2004.

[123] M. E. Monaco, C. J. Creighton, P. Lee, X. Zou, M. K. Topham, and D. M. Stafforini, "Expression of long-chain fatty Acyl-CoA synthetase 4 in breast and prostate cancers is associated with sex steroid hormone receptor negativity," *Translational Oncology*, vol. 3, no. 2, pp. 91–98, 2010.

[124] Z. Pei, P. Fraisl, X. Shi et al., "Very long-chain acyl-CoA synthetase 3: overexpression and growth dependence in lung cancer," *PLoS ONE*, vol. 8, no. 7, Article ID e69392, 2013.

[125] K. S. Young, K. P. Mi, H. H. Su et al., "Regulation of cell growth by fatty acid-CoA ligase 4 in human hepatocellular carcinoma cells," *Experimental and Molecular Medicine*, vol. 39, no. 4, pp. 477–482, 2007.

[126] Y. Zhou, P. Abidi, A. Kim et al., "Transcriptional activation of hepatic ACSL3 and ACSL5 by oncostatin M reduces hypertriglyceridemia through enhanced β-oxidation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 10, pp. 2198–2205, 2007.

[127] Y. Cao, A. T. Pearman, G. A. Zimmerman, T. M. McIntyre, and S. M. Prescott, "Intracellular unesterified arachidonic acid signals apoptosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 21, pp. 11280–11285, 2000.

[128] T. Mashima, S. Sato, S. Okabe et al., "Acyl-CoA synthetase as a cancer survival factor: its inhibition enhances the efficacy of etoposide," *Cancer Science*, vol. 100, no. 8, pp. 1556–1562, 2009.

[129] I. Shimomura, K. Tokunaga, S. Jiao et al., "Marked enhancement of acyl-CoA synthetase activity and mRNA, paralleled to lipoprotein lipase mRNA, in adipose tissues of Zucker obese rats (fa/fa)," *Biochimica et Biophysica Acta*, vol. 1124, no. 2, pp. 112–118, 1992.

[130] B. D. Hegarty, G. J. Cooney, E. W. Kraegen, and S. M. Furler, "Increased efficiency of fatty acid uptake contributes to lipid accumulation in skeletal muscle of high fat-fed insulin-resistant rats," *Diabetes*, vol. 51, no. 5, pp. 1477–1484, 2002.

[131] V. K. Khor, W. J. Shen, and F. B. Kraemer, "Lipid droplet metabolism," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 16, no. 6, pp. 632–637, 2013.

[132] S. Cases, S. J. Smith, Y.-W. Zheng et al., "Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 13018–13023, 1998.

[133] R. Coleman and R. M. Bell, "Triacylglycerol synthesis in isolated fat cells. Studies on the microsomal diacylglycerol acyltransferase activity using ethanol-dispersed diacylglycerols," *The Journal of Biological Chemistry*, vol. 251, no. 15, pp. 4537–4543, 1976.

[134] R. A. Coleman, T. M. Lewin, and D. M. Muoio, "Physiological and nutritional regulation of enzymes of triacylglycerol synthesis," *Annual Review of Nutrition*, vol. 20, pp. 77–103, 2000.

[135] R. Zechner, R. Zimmermann, T. O. Eichmann et al., "FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling," *Cell Metabolism*, vol. 15, no. 3, pp. 279–291, 2012.

[136] R. E. Gimeno and J. Cao, "*Thematic review series*: *glycerolipids*. Mammalian glycerol-3-phosphate acyltransferases: new genes for an old activity," *The Journal of Lipid Research*, vol. 49, no. 10, pp. 2079–2088, 2008.

[137] K. Takeuchi and K. Reue, "Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 296, no. 6, pp. E1195–E1209, 2009.

[138] A. A. Wendel, T. M. Lewin, and R. A. Coleman, "Glycerol-3-phosphate acyltransferases: rate limiting enzymes of triacylglycerol biosynthesis," *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids*, vol. 1791, no. 6, pp. 501–506, 2009.

[139] L. S. Csaki and K. Reue, "Lipins: multifunctional lipid metabolism proteins," *Annual Review of Nutrition*, vol. 30, pp. 257–272, 2010.

[140] R. Stienstra and S. Kersten, "Fight fat with DGAT," *Journal of Lipid Research*, vol. 52, no. 4, pp. 591–592, 2011.

[141] E. Currie, A. Schulze, R. Zechner, T. C. Walther, and R. V. Farese Jr., "Cellular fatty acid metabolism and cancer," *Cell Metabolism*, vol. 18, no. 2, pp. 153–161, 2013.

[142] G. P. Holloway, L. A. Snook, R. J. Harris, J. F. C. Glatz, J. J. F. P. Luiken, and A. Bonen, "In obese Zucker rats, lipids accumulate in the heart despite normal mitochondrial content, morphology and long-chain fatty acid oxidation," *The Journal of Physiology*, vol. 589, no. 1, pp. 169–180, 2011.

[143] Z. K. Guo and M. D. Jensen, "Accelerated intramyocellular triglyceride synthesis in skeletal muscle of high-fat-induced obese rats," *International Journal of Obesity*, vol. 27, no. 9, pp. 1014–1019, 2003.

[144] X.-J. Zhang, D. L. Chinkes, Z. Wu, D. N. Herndon, and R. R. Wolfe, "The synthetic rate of muscle triglyceride but not phospholipid is increased in obese rabbits," *Metabolism: Clinical and Experimental*, vol. 58, no. 11, pp. 1649–1656, 2009.

[145] A. Bonen, M. L. Parolin, G. R. Steinberg et al., "Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport increased sarcolemmal FAT/CD36," *FASEB Journal*, vol. 18, no. 10, pp. 1144–1146, 2004.

[146] L. Vergnes, A. P. Beigneux, R. Davis, S. M. Watkins, S. G. Young, and K. Reue, "Agpat6 deficiency causes subdermal lipodystrophy and resistance to obesity," *Journal of Lipid Research*, vol. 47, no. 4, pp. 745–754, 2006.

[147] S. J. Smith, S. Cases, D. R. Jensen et al., "Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat," *Nature Genetics*, vol. 25, no. 1, pp. 87–90, 2000.

[148] L. S. Csaki, J. R. Dwyer, X. Li et al., "Lipin-1 and lipin-3 together determine adiposity *in vivo*," *Molecular Metabolism*, vol. 3, no. 2, pp. 145–154, 2014.

[149] H. C. Chen and R. V. Farese Jr., "Inhibition of triglyceride synthesis as a treatment strategy for obesity: lessons from DGAT1-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 3, pp. 482–486, 2005.

[150] L. E. Hammond, S. Neschen, A. J. Romanelli et al., "Mitochondrial glycerol-3-phosphate acyltransferase-1 is essential in liver for the metabolism of excess acyl-CoAs," *The Journal of Biological Chemistry*, vol. 280, no. 27, pp. 25629–25636, 2005.

[151] D. Lindén, L. William-Olsson, M. Rhedin, A.-K. Asztély, J. C. Clapham, and S. Schreyer, "Overexpression of mitochondrial GPAT in rat hepatocytes leads to decreased fatty acid oxidation and increased glycerolipid biosynthesis," *The Journal of Lipid Research*, vol. 45, no. 7, pp. 1279–1288, 2004.

[152] C. A. Nagle, L. Vergnes, H. Dejong et al., "Identification of a novel *sn*-glycerol-3-phosphate acyltransferase isoform, GPAT4, as the enzyme deficient in *Agpat6*$^{-/-}$ mice," *Journal of Lipid Research*, vol. 49, no. 4, pp. 823–831, 2008.

[153] H. Ruan and H. J. Pownall, "Overexpression of 1-acyl-glycerol-3-phosphate acyltransferase-alpha enhances lipid storage in cellular models of adipose tissue and skeletal muscle," *Diabetes*, vol. 50, no. 2, pp. 233–240, 2001.

[154] J. Phan and K. Reue, "Lipin, a lipodystrophy and obesity gene," *Cell Metabolism*, vol. 1, no. 1, pp. 73–83, 2005.

[155] C. Bagnato and R. A. Igal, "Overexpression of diacylglycerol acyltransferase-1 reduces phospholipid synthesis, proliferation, and invasiveness in simian virus 40-transformed human lung fibroblasts," *The Journal of Biological Chemistry*, vol. 278, no. 52, pp. 52203–52211, 2003.

[156] T. C. Walther and R. V. Farese, "Lipid droplets and cellular lipid metabolism," *Annual Review of Biochemistry*, vol. 81, pp. 687–714, 2012.

[157] C. J. Antalis, T. Arnold, T. Rasool, B. Lee, K. K. Buhman, and R. A. Siddiqui, "High ACAT1 expression in estrogen receptor negative basal-like breast cancer cells is associated with LDL-induced proliferation," *Breast Cancer Research and Treatment*, vol. 122, no. 3, pp. 661–670, 2010.

[158] J. V. Swinnen, P. P. van Veldhoven, M. Esquenet, W. Heyns, and G. Verhoeven, "Androgens markedly stimulate the accumulation of neutral lipids in the human prostatic adenocarcinoma cell line LNCaP," *Endocrinology*, vol. 137, no. 10, pp. 4468–4474, 1996.

[159] N. G. Than, B. Sumegi, S. Bellyei et al., "Lipid droplet and milk lipid globule membrane associated placental protein 17b (PP17b) is involved in apoptotic and differentiation processes of human epithelial cervical carcinoma cells," *European Journal of Biochemistry*, vol. 270, no. 6, pp. 1176–1188, 2003.

[160] A. M. Dvorak, P. F. Weller, V. S. Harvey, E. S. Morgan, and H. F. Dvorak, "Ultrastructural localization of prostaglandin endoperoxide synthase (cyclooxygenase) to isolated, purified fractions of guinea pig peritoneal macrophage and line 10 hepatocarcinoma cell lipid bodies," *International Archives of Allergy and Immunology*, vol. 101, no. 2, pp. 136–142, 1993.

[161] M. T. Accioly, P. Pacheco, C. M. Maya-Monteiro et al., "Lipid bodies are reservoirs of cyclooxygenase-2 and sites of prostaglandin-E2 synthesis in colon cancer cells," *Cancer Research*, vol. 68, no. 6, pp. 1732–1740, 2008.

[162] E. Przybytkowski, É. Joly, C. J. Nolan et al., "Upregulation of cellular triacylglycerol—free fatty acid cycling by oleate is associated with long-term serum-free survival of human breast cancer cells," *Biochemistry and Cell Biology*, vol. 85, no. 3, pp. 301–310, 2007.

[163] R. J. DeBerardinis, J. J. Lum, G. Hatzivassiliou, and C. B. Thompson, "The biology of cancer: metabolic reprogramming fuels cell growth and proliferation," *Cell Metabolism*, vol. 7, no. 1, pp. 11–20, 2008.

[164] J. A. Villena, B. Viollet, F. Andreelli, A. Kahn, S. Vaulont, and H. S. Sul, "Induced adiposity and adipocyte hypertrophy in mice lacking the AMP-activated protein kinase-alpha2 subunit," *Diabetes*, vol. 53, no. 9, pp. 2242–2249, 2004.

[165] R. Zimmermann, J. G. Strauss, G. Haemmerle et al., "Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase," *Science*, vol. 306, no. 5700, pp. 1383–1386, 2004.

[166] T. O. Eichmann, M. Kumari, J. T. Haas et al., "Studies on the substrate and stereo/regioselectivity of adipose triglyceride lipase, hormone-sensitive lipase, and diacylglycerol-*O*-acyltransferases," *The Journal of Biological Chemistry*, vol. 287, no. 49, pp. 41446–41457, 2012.

[167] G. Haemmerle, R. Zimmermann, M. Hayn et al., "Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis," *Journal of Biological Chemistry*, vol. 277, no. 7, pp. 4806–4815, 2002.

[168] G. Fredrikson, P. Stralfors, N. O. Nilsson, and P. Belfrage, "Hormone-sensitive lipase of rat adipose tissue. Purification and some properties," *The Journal of Biological Chemistry*, vol. 256, no. 12, pp. 6311–6320, 1981.

[169] R. Zechner, P. C. Kienesberger, G. Haemmerle, R. Zimmermann, and A. Lass, "Adipose triglyceride lipase and the lipolytic

catabolism of cellular fat stores," *Journal of Lipid Research*, vol. 50, no. 1, pp. 3–21, 2009.

[170] C. Gercel-Taylor, D. L. Doering, F. B. Kraemer, and D. D. Taylor, "Aberrations in normal systemic lipid metabolism in ovarian cancer patients," *Gynecologic Oncology*, vol. 60, no. 1, pp. 35–41, 1996.

[171] D. K. Nomura, J. Z. Long, S. Niessen, H. S. Hoover, S.-W. Ng, and B. F. Cravatt, "Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis," *Cell*, vol. 140, no. 1, pp. 49–61, 2010.

[172] H. de Naeyer, D. M. Ouwens, Y. van Nieuwenhove et al., "Combined gene and protein expression of hormone-sensitive lipase and adipose triglyceride lipase, mitochondrial content, and adipocyte size in subcutaneous and visceral adipose tissue of morbidly obese men," *Obesity Facts*, vol. 4, no. 5, pp. 407–416, 2011.

[173] D. Langin, A. Dicker, G. Tavernier et al., "Adipocyte lipases and defect of lipolysis in human obesity," *Diabetes*, vol. 54, no. 11, pp. 3190–3197, 2005.

[174] G. R. Steinberg, B. E. Kemp, and M. J. Watt, "Adipocyte triglyceride lipase expression in human obesity," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 293, no. 4, pp. E958–E964, 2007.

[175] J. W. E. Jocken, D. Langin, E. Smit et al., "Adipose triglyceride lipase and hormone-sensitive lipase protein expression is decreased in the obese insulin-resistant state," *The Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 6, pp. 2292–2299, 2007.

[176] P. Oliver, A. Caimari, R. Díaz-Rúa, and A. Palou, "Diet-induced obesity affects expression of adiponutrin/PNPLA3 and adipose triglyceride lipase, two members of the same family," *International Journal of Obesity*, vol. 36, no. 2, pp. 225–232, 2012.

[177] R. A. Coleman and D. G. Mashek, "Mammalian triacylglycerol metabolism: synthesis, lipolysis, and signaling," *Chemical Reviews*, vol. 111, no. 10, pp. 6359–6386, 2011.

[178] Y. Liu, "Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer," *Prostate Cancer and Prostatic Diseases*, vol. 9, no. 3, pp. 230–234, 2006.

[179] J. Khasawneh, M. D. Schulz, A. Walch et al., "Inflammation and mitochondrial fatty acid β-oxidation link obesity to early tumor promotion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 9, pp. 3354–3359, 2009.

[180] N. Turner, C. R. Bruce, S. M. Beale et al., "Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle: evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents," *Diabetes*, vol. 56, no. 8, pp. 2085–2092, 2007.

[181] G. P. Holloway, A. Bonen, and L. L. Spriet, "Regulation of skeletal muscle mitochondrial fatty acid metabolism in lean and obese individuals," *American Journal of Clinical Nutrition*, vol. 89, no. 1, pp. 455S–462S, 2009.

[182] M. W. Hulver, J. R. Berggren, R. N. Cortright et al., "Skeletal muscle lipid metabolism with obesity," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 284, no. 4, pp. E741–E747, 2003.

[183] J.-Y. Kim, R. C. Hickner, R. L. Cortright, G. L. Dohm, and J. A. Houmard, "Lipid oxidation is reduced in obese human skeletal muscle," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 279, no. 5, pp. E1039–E1044, 2000.

[184] J. D. McGarry and N. F. Brown, "The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis," *European Journal of Biochemistry*, vol. 244, no. 1, pp. 1–14, 1997.

[185] L. Abu-Elheiga, M. M. Matzuk, K. A. H. Abo-Hashema, and S. J. Wakil, "Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2," *Science*, vol. 291, no. 5513, pp. 2613–2616, 2001.

[186] G. Peluso, R. Nicolai, E. Reda, P. Benatti, A. Barbarisi, and M. Calvani, "Cancer and anticancer therapy-induced modifications on metabolism mediated by carnitine system," *Journal of Cellular Physiology*, vol. 182, no. 3, pp. 339–350, 2000.

[187] N. T. Price, F. R. van der Leij, V. N. Jackson et al., "A novel brain-expressed protein related to carnitine palmitoyltransferase I," *Genomics*, vol. 80, no. 4, pp. 433–442, 2002.

[188] F. R. van der Leij, A. M. Kram, B. Bartelds et al., "Cytological evidence that the C-terminus of carnitine palmitoyltransferase I is on the cytosolic face of the mitochondrial outer membrane," *Biochemical Journal*, vol. 341, no. 3, pp. 777–784, 1999.

[189] P. Mazzarelli, S. Pucci, E. Bonanno, F. Sesti, M. Calvani, and L. G. Spagnoli, "Carnitine palmitoyltransferase I in human carcinomas: a novel role in histone deacetylation?" *Cancer Biology and Therapy*, vol. 6, no. 10, pp. 1606–1613, 2007.

[190] P. T. Reilly and T. W. Mak, "Molecular pathways: tumor cells Co-opt the brain-specific metabolism gene *CPT1C* to promote survival," *Clinical Cancer Research*, vol. 18, no. 21, pp. 5850–5855, 2012.

[191] A. Pacilli, M. Calienni, S. Margarucci et al., "Carnitine-acyltransferase system inhibition, cancer cell death, and prevention of myc-induced lymphomagenesis," *Journal of the National Cancer Institute*, vol. 105, no. 7, pp. 489–498, 2013.

[192] L. S. Pike, A. L. Smift, N. J. Croteau, D. A. Ferrick, and M. Wu, "Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells," *Biochimica et Biophysica Acta*, vol. 1807, no. 6, pp. 726–734, 2011.

[193] I. Samudio, R. Harmancey, M. Fiegl et al., "Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction," *The Journal of Clinical Investigation*, vol. 120, no. 1, pp. 142–156, 2010.

[194] S. J. Wakil, E. B. Titchener, and D. M. Gibson, "Evidence for the participation of biotin in the enzymic synthesis of fatty acids," *Biochimica et Biophysica Acta*, vol. 29, no. 1, pp. 225–226, 1958.

[195] L. Abu-Elheiga, W. Oh, P. Kordari, and S. J. Wakil, "Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 18, pp. 10207–10212, 2003.

[196] A. J. Iverson, A. Bianchi, A.-C. Nordlund, and L. A. Witters, "Immunological analysis of acetyl-CoA carboxylase mass, tissue distribution and subunit composition," *Biochemical Journal*, vol. 269, no. 2, pp. 365–371, 1990.

[197] K. G. Thampy, "Formation of malonyl coenzyme A in rat heart. Identification and purification of an isozyme of acetyl coenzyme A carboxylase from rat heart," *The Journal of Biological Chemistry*, vol. 264, no. 30, pp. 17631–17634, 1989.

[198] K. G. Thampy and S. J. Wakil, "Regulation of acetyl-coenzyme A carboxylase. I. Purification and properties of two forms of acetyl-coenzyme A carboxylase from rat liver," *The Journal of Biological Chemistry*, vol. 263, no. 13, pp. 6447–6453, 1988.

[199] H. J. Harwood Jr., "Treating the meytabolic syndrome: acetyl-CoA carboxylase inhibition," *Expert Opinion on Therapeutic Targets*, vol. 9, no. 2, pp. 267–281, 2005.

[200] L. Tong, "Structure and function of biotin-dependent carboxylases," *Cellular and Molecular Life Sciences*, vol. 70, no. 5, pp. 863–891, 2013.

[201] C. Wang, C. Xu, M. Sun, D. Luo, D.-F. Liao, and D. Cao, "Acetyl-CoA carboxylase-alpha inhibitor TOFA induces human cancer cell apoptosis," *Biochemical and Biophysical Research Communications*, vol. 385, no. 3, pp. 302–306, 2009.

[202] A. Beckers, S. Organe, L. Timmermans et al., "Chemical inhibition of acetyl-CoA carboxylase induces growth arrest and cytotoxicity selectively in cancer cells," *Cancer Research*, vol. 67, no. 17, pp. 8180–8187, 2007.

[203] V. Chajès, M. Cambot, K. Moreau, G. M. Lenoir, and V. Joulin, "Acetyl-CoA carboxylase α is essential to breast cancer cell survival," *Cancer Research*, vol. 66, no. 10, pp. 5287–5294, 2006.

[204] K. Brusselmans, E. de Schrijver, G. Verhoeven, and J. V. Swinnen, "RNA interference—mediated silencing of the acetyl-Coa-carboxylase-α gene induces growth inhibition and apoptosis of prostate cancer cells," *Cancer Research*, vol. 65, no. 15, pp. 6719–6725, 2005.

[205] S.-M. Jeon, N. S. Chandel, and N. Hay, "AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress," *Nature*, vol. 485, no. 7400, pp. 661–665, 2012.

[206] W. Zhou, Y. Tu, P. J. Simpson, and F. P. Kuhajda, "Malonyl-CoA decarboxylase inhibition is selectively cytotoxic to human breast cancer cells," *Oncogene*, vol. 28, no. 33, pp. 2979–2987, 2009.

[207] G. K. Bandyopadhyay, J. G. Yu, J. Ofrecio, and J. M. Olefsky, "Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects," *Diabetes*, vol. 55, no. 8, pp. 2277–2285, 2006.

[208] G. R. Steinberg, B. J. Michell, B. J. W. van Denderen et al., "Tumor necrosis factor α-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling," *Cell Metabolism*, vol. 4, no. 6, pp. 465–474, 2006.

[209] M. Yamasaki, S. Hasegawa, T. Kitani, K. Hidai, and T. Fukui, "Differential effects of obesity on acetoacetyl-CoA synthetase gene in rat adipose tissues," *European Journal of Lipid Science and Technology*, vol. 109, no. 6, pp. 617–622, 2007.

[210] A. Janovská, G. Hatzinikolas, V. Staikopoulos, J. McInerney, M. Mano, and G. A. Wittert, "AMPK and ACC phosphorylation: effect of leptin, muscle fibre type and obesity," *Molecular and Cellular Endocrinology*, vol. 284, no. 1-2, pp. 1–10, 2008.

[211] K. L. Mullen, J. Pritchard, I. Ritchie et al., "Adiponectin resistance precedes the accumulation of skeletal muscle lipids and insulin resistance in high-fat-fed rats," *American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 296, no. 2, pp. R243–R251, 2009.

[212] J. M. Peterson, S. Aja, Z. Wei, and G. W. Wong, "CTRP1 protein enhances fatty acid oxidation via AMP-activated protein kinase (AMPK) activation and acetyl-CoA carboxylase (ACC) inhibition," *Journal of Biological Chemistry*, vol. 287, no. 2, pp. 1576–1587, 2012.

[213] M. D. Fullerton, S. Galic, K. Marcinko et al., "Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin," *Nature Medicine*, vol. 19, no. 12, pp. 1649–1654, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 538019, 24 pages
http://dx.doi.org/10.1155/2015/538019

Review Article

Insulin-Like Growth Factor System in Cancer: Novel Targeted Therapies

Varsha P. Brahmkhatri,[1] Chinmayi Prasanna,[1] and Hanudatta S. Atreya[1,2]

[1]*NMR Research Centre, Indian Institute of Science, Bangalore 560012, India*
[2]*Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore 560012, India*

Correspondence should be addressed to Hanudatta S. Atreya; hsatreya@gmail.com

Received 7 July 2014; Revised 13 October 2014; Accepted 20 October 2014

Academic Editor: Eileen M. McGowan

Insulin-like growth factors (IGFs) are essential for growth and survival that suppress apoptosis and promote cell cycle progression, angiogenesis, and metastatic activities in various cancers. The IGFs actions are mediated through the IGF-1 receptor that is involved in cell transformation induced by tumour. These effects depend on the bioavailability of IGFs, which is regulated by IGF binding proteins (IGFBPs). We describe here the role of the IGF system in cancer, proposing new strategies targeting this system. We have attempted to expand the general viewpoint on IGF-1R, its inhibitors, potential limitations of IGF-1R, antibodies and tyrosine kinase inhibitors, and IGFBP actions. This review discusses the emerging view that blocking IGF via IGFBP is a better option than blocking IGF receptors. This can lead to the development of novel cancer therapies.

1. Introduction

Insulin-like growth factor (IGF) is a natural growth hormone and plays crucial role in normal growth and development. The IGF family is comprised of insulin and two factors similar to insulin termed IGF-1 and IGF-2. These factors directly regulate cellular functions by interacting with specific cell surface receptors and activating various intracellular signalling cascades. The cellular responses to the IGFs are mediated primarily by the IGF-1 receptor. The IGF-1 receptor is a member of the family of tyrosine kinase growth factor receptors.

IGFs actions are regulated by six soluble IGF binding proteins (IGFBPs) and IGFBP proteases. The IGFBPs comprise a superfamily of six proteins (IGFBP-1-6) that bind to IGFs with high affinity and specificity and a family of IGFBP-related proteins (IGFBP-rPs), which are structurally similar to the IGFBPs but bind IGFs with much lower affinity.

IGF-1 circulates in relatively high concentrations in plasma, approximately 150–400 ng per mL, where it mostly exists as the protein-bound form. The free ligand concentration is very little that is less than 1% [1]. IGFs in circulation are protected from degradation by forming a complex with a family of high affinity IGF binding proteins (IGFBPs) [2]. IGFBP-3 is the most abundant IGF binding protein in the blood stream followed by IGFBP-2, which is produced in the liver. Most of the circulating IGF-1 and IGF-2 are associated with a high molecular weight complex ~150 kDa consisting of IGFBP-3 and the acid labile subunit (ALS) [2]. Once the ternary complex dissociates, the binary complexes of IGFBP-IGF are removed from the circulation and cross the endothelium to reach the target tissues and to interact with cell surface receptors (Figure 1). In the tissues, IGFBPs may inhibit the interaction of the IGFs with their receptors, as the IGFBPs have a higher affinity for the IGFs than the receptors. In some cases, IGFBPs can enhance IGF action in the local microenvironment by acting as a reservoir that can slowly release the ligands. In addition, some IGFBPs can have IGF-independent effects on cells [2].

The IGFs are signalling proteins (~7.5 kDa) whose actions are mediated by the IGF-1R, and access to the receptor is regulated by the IGFBPs, which vary in size (~22–31 kDa) and share overall sequence and structural homology with each other. The IGFBPs bind strongly to IGFs (K_D ~ 300–700 pM) and inhibit the action of IGFs by blocking their access to

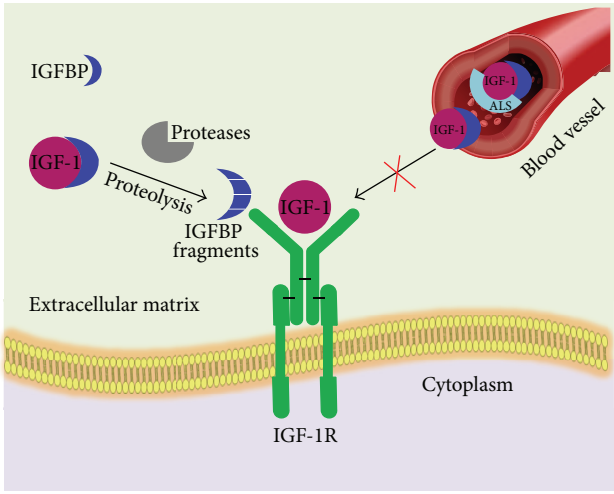


Figure 1: The IGF axis: circulating IGFs are protected from degradation by forming complex with IGFBPs. IGFs, apart from their local functioning in an autocrine or a paracrine manner, enter the bloodstream, where they exist as binary complexes with each IGFBP. In addition, ternary complex also exists when the binary complexes with IGFBP-3 or IGFBP-5 interact with the acid labile subunit (ALS). After dissociation of ternary complex, the binary complexes of IGFBP-IGF are removed from the circulation and cross the endothelium to reach the target tissues and to interact with cell surface receptors.

the receptors. Proteolysis of the IGFBPs dissociates IGFs from the complex, enabling them to bind and activate the cell surface receptors. Deregulation of IGF-1R signalling has been noted to contribute to a variety of diseases including diabetic retinopathy [3], diabetic nephropathy [4], age-related macular degeneration [5], cardiovascular disease, and aging and in a variety of cancers [5].

IGF system is gaining tremendous interest over the last decade because it plays an important role in cancer. The current treatment options for cancer have shifted more towards the targeted therapies [6, 7] rather than the traditional chemotherapy. Many strategies have been exploited to target tumours. The most commonly used strategy is engineered antibodies or antibody fragments [8]. Though monoclonal antibodies are very selective, poor penetration inside the tumours and high production cost hinder their usage as therapeutic agents [9]. Current therapeutics targeting the IGF-signalling pathways focus on blocking IGF-1R, directly, and/or its downstream effectors [10]. However, a potential drawback of such approaches is the resulting adverse side effects or toxicities due to its interference with the insulin pathway. As a more efficacious alternative, we propose that IGFBPs can be developed as IGF-antagonist based cancer therapeutics serving to block the IGF-1R, mediated tumour progression. Notably, the IGFBPs do not bind insulin and thus do not interfere with insulin-insulin receptor interactions.

In the current paper, we will provide a brief overview on IGF system and discuss some literature and experimental data reported to demonstrate the role of IGF system in cancer and development of new targeted anticancer therapies. Because it is not possible to provide a complete coverage of all published papers dealing with IGF system, we have mainly focused on different strategies targeting IGF system in cancer and attempted to provide an overview on IGF system including IGF-1R, its inhibitors and potential limitations of IGF-1R, antibodies and tyrosine kinase inhibitors, IGFBP actions, and blocking IGF via IGFBP (which is better option than blocking IGF receptors) leading to development of novel cancer therapies.

2. Discovery/History of IGF System

The first member of IGF family to be identified was insulin, with subsequent investigation resulting in the elucidation of its role in glucose metabolism and its implication in the aetiology of diabetes mellitus. This discovery effected an explosion in the investigation of the structure, function, and mechanisms of action of insulin. The enormous interest in this molecule resulted in the concession of three Nobel Prizes for the investigation of insulin: in 1923 for the discovery of its capacity to treat diabetes by Frederick Banting and J. J. Macleod [11], in 1958 for the first sequence of a protein by Frederick Sanger [12], and in 1963 for the first determination of the three-dimensional structure of a protein by Dorothy Hodgkin [13]. Hence, the investigation of insulin has been a pioneer in many scientific fields. Later, the IGFs were discovered and found to be intricately involved in embryonic development and postnatal growth.

The existence of the IGFs was first proposed by Salmon and Daughaday in 1957, on the basis of studies indicating that growth hormone (GH) did not directly stimulate the incorporation of sulfate into cartilage but rather acted through a serum factor [14]. In the original study by Salmon and Daughaday, ^{35}S-labeled amino acid was incorporated into cartilage explants and was used as a surrogate for growth. The serum of normal rats induced ^{35}S-amino acid incorporation into cartilage, but not serum from hypophysectomized rats. However, serum from hypophysectomized rats treated with GH yielded serum that allowed for ^{35}S-amino acid incorporation, indicating that a second messenger was necessary for GH signalling. This factor was originally termed sulfation factor, then somatomedin, and, ultimately, insulin-like growth factor-1 and insulin-like growth factor-2. IGF-1 was not purified and characterized until more than two decades later [15]. The terminology "insulin-like" was used because these factors are able to stimulate glucose uptake into fat cells and muscle, and, indeed, both IGF-1 and IGF-2 show approximately 50% homology with insulin [15, 16].

Subsequent investigation demonstrated that GH, after binding to its transmembrane receptor, initiates a signalling cascade leading to transcriptional regulation of IGF-1 and related genes. It was originally thought that systemic growth was promoted by GH acting mainly on the liver to stimulate IGF-1 production, which then reached target tissues via the circulation to activate mechanisms involved in tissue proliferation, growth, and metabolism. It is now evident that not only does GH have independent actions that do not involve IGF-1 production [17], but IGF-1 synthesis occurs

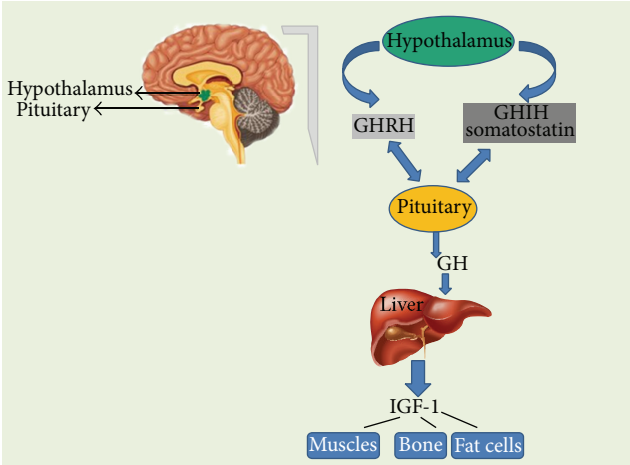


Figure 2: Growth hormone-releasing hormone (GHRH) is a hormone, produced by the hypothalamus which stimulates the pituitary gland to produce GH. Somatostatin secreted by the cells of hypothalamus and also by the cells of stomach, intestine, and pancreas that inhibits GH production. When pituitary secretes GH into the bloodstream, it results in the production of IGF-1 in the liver. IGF-1 is the factor that actually causes the growth of bones and other tissues of the body. It also plays an important role in signalling the pituitary to reduce GH production.

in many tissues under the control of a variety of local and circulating factors, which may or may not include GH [18–21]. Furthermore, this local production of IGF-1 may be directly responsible for the growth promoting effects of GH, rather than the circulating growth factor [22].

2.1. IGF-1 Synthesis and Secretion. IGF functions as both a circulating hormone and as a tissue growth factor. Liver is the production house for the most circulating IGFs that are subject to both hormonal and nutritional factors. Growth hormone (GH), which is produced in the pituitary gland under the control of the hypothalamic factors, stimulates IGF-1 production (Figure 2).

The insulin like growth factor binding proteins (IGFBPs) are also synthesized in the liver. The IGF ligands in addition to the IGFBPs are delivered in an endocrine manner through the circulation from the liver to act in IGF-responsive tissues. IGFs and IGFBPs are also produced in other organs where autocrine or paracrine mechanisms take place, frequently involving interactions between stromal and epithelial cell populations [23].

2.2. Autocrine and Paracrine Actions of IGF. The insulin-like growth factors play a major role in regulating cell proliferation and inhibiting apoptosis. The IGFs are expressed ubiquitously and act in an autocrine/paracrine manner through binding to the IGF-1 receptor (IGF-1R). The bioavailability of IGF in tissues is determined by both local and systemic factors. The local factors include the levels of receptors that are expressed, various IGF binding proteins (IGFBPs), and IGFBP proteases. The systemic factors involved are mainly those that regulate the circulating levels of IGFs, such as growth hormone (GH) and various nutritional factors. Studies in cultured cells have demonstrated that the IGF-1R is frequently overexpressed in cancer cell lines.

The IGFs are not stored within cells of a specific tissue but are present at very high levels throughout the body. They circulate at total concentrations approximately 1000 times higher than those of most peptide hormones and although tissue levels are somewhat lower, they are still present in vast excess compared to that required for maximal cellular stimulation. These high levels are maintained due to their association with the IGFBPs, which dramatically slow their clearance. The IGFBPs bind the IGFs with greater affinity than their cell surface receptors, enabling them to tightly control tissue activity. The IGFBP proteases modify the IGFBPs, lowering the affinity with which they bind IGFs. In the tissues, the IGFs are regulators of cell survival, growth, metabolism, and differentiated function; the complex system confers specificity on these actions.

2.3. Evidence for Paracrine/Autocrine IGF-1 Actions from Studies of Transgenic Mice. The most convincing evidence of local IGF-1 actions comes from lines of transgenic (Tg) mice made to overexpress IGF-1 in specific tissues, for example, brain, mammary gland, and muscle. Each of these Tg mouse models exhibits specific overgrowth in the organ or tissue of IGF-1 overexpression, and none has an alteration in circulating IGF-1 levels. Reports of such mouse models are summarized in Table 1. In every model studied biologic actions in the organ of IGF-1 transgene expression have been demonstrated. IGF-1, therefore, can exert local in vivo actions.

Other experiments that address IGF-1 local actions are the generation of Tg mice that overexpress IGFBPs in specific tissues. Here, the expectation is that these IGFBPs will inhibit the actions of locally expressed IGF-I. Such studies have yielded results consistent with those obtained from studies of site-specific IGF-1 overexpression. An example is the overexpression of rat IGFBP-4 in smooth muscle driven by the regulatory region of the α-actin gene [24]. Transgene IGFBP-4 expression results in smooth muscle hypoplasia. The lack of any change in circulating IGFBP-4 or IGF-1 and the restriction of hypoplasia to smooth muscle argue for the inhibition of IGF-1 growth promoting effects on smooth muscle. Alternative, but unlikely, interpretations are that IGFBP-4 inhibited the actions of IGF-1 derived from the circulation and/or that IGFBP-4 inhibits growth by mechanisms independent of IGF-I. Other Tg mouse models have yielded consistent results. For example, a number of lines of IGFBP-1 Tg mice exhibit organ growth retardation that appears due to the capacity of IGFBP-1 to inhibit IGF activity in specific tissues, for example, in brain [25–27].

3. IGF Receptors (IGF-Rs)

The IGF system comprises two main receptors (IGF-1R and IGF-2R). Both IGFIR and IGF-2R are transmembrane glycoproteins that differ completely in their structure and function [19–21, 28–31]. IGF-1R is a tetramer which comprised two equal α-subunits and two equal β-subunits [28, 29, 32]. IGF-1R resembles the insulin receptor at structural level, with 60%

Table 1: IGF-1 transgenic mice with tissue-specific IGF-1 overexpression.

Organs	IGF-1 action	Promoter	Reference
Brain	Increased brain size, characterized by increased neuron number.	M IGF-2 5′ flanking region	[35, 36, 181, 182]
Bone	Increased trabecular bone.	Bovine osteocalcin	[37]
Heart	Increased myocyte proliferation.	r α myosin heavy chain	[38]
Muscle: skeletal	Stimulates differentiation and myofibril hypertrophy.	Avian skeletal α actin	[39]
Muscle: smooth	Smooth muscle hyperplasia in many flanking fragments organs/tissues. Increased vascular contractility. Enhanced neointimal formation after injury.	r smooth muscle α actin (mSMA)	[183–185]
Ovary	Increased testosterone and cyst.	m LH receptor	[186]
Prostate	Epithelial neoplasia.	Bovine keratin-5	[187]
Thyroid	When the IGF-1R is also overexpressed, there is a decreased TSH requirement and goiter.	Bovine thyroglobulin	[188]

homology. IGFs and insulin are proficient to cross-bind to each other's receptor, although with much weaker binding affinity than that for the preferred ligand [33, 34]. IGF-1R and IR can hybridize to form a heterodimer composed of one α-subunit and one β-subunit of each receptor [28, 30] as shown in Figure 3. The amount of insulin/IGF-1 hybrid receptor varies significantly from tissue to tissue. Since its binding affinity for IGF-1 is higher than that for insulin, the receptor is thought to function principally as an IGF-1 receptor, but its biologic significance remains mostly unidentified.

The postreceptor signal transduction events include phosphorylation of insulin receptor substrate (IRS) family of proteins and activation of phosphatidylinositol-3 (PI-3) and mitogen-activated protein kinases (MAPK) [19, 35]. This will result in a myriad of events, including the upregulation of cyclin D1 leading to the phosphorylation of retinoblastoma protein and expression of downstream target genes such as cyclin E [36, 37]. Moreover, IGF-1R activation downregulates the cell-cycle suppressors like PTEN [38, 39], indicating that multiple pathways are involved in producing its mitogenic effect. Activated IRSs trigger the activation of two intracellular signaling networks: Ras/Raf/Mek/Erk and PI3K pathways. The first one is mainly involved in mediating the mitogenic effect of insulin and IGFs, while the PI3K pathway, via Akt, mediates both metabolic and cell growth responses. The Akt-mediated metabolic effects are induced by the activation of enzymes involved in gluconeogenesis, glucose uptake, protein synthesis, and lipogenesis, whereas the cell growth responses are mainly induced by the mTOR pathway.

IGF-2R is monomeric [29, 40–42], the largest transmembrane receptor that is completely unrelated to the IGF-1R, and insulin receptor (IR). In the extracellular domain of the receptor, three ligand-binding regions are found one for IGF-2 binding and two for proteins containing mannose-6-phosphate (M6P) and the dormant form of transforming growth factor- (TGF-) β [30]. Binding of IGF-2R, to TGF-β, activates the latter [40, 43]. IGF-2R is also called the IGF-II/M6P receptor as it can bind both IGF-2 and M6P-containing Molecules. The expression of IGF-1R is regulated by steroid hormones and growth factors [29, 32]. Since high IGF-1 levels result in a low levels of IGF-1R, IGFs may act as negative feedback signals to suppress expression of IGF-1R [44, 45]. In contradiction of the effect of IGFs, other growth factors, including basic FGF, PDGF, and EGF, stimulate IGF-1R expression [32, 46, 47]. The expression of IGF-1R is also stimulated by estrogens, glucocorticoids, GH, FSH, luteinizing hormone, and thyroid hormones [28, 32]. On the other hand, tumour suppressor gene products, such as wild type p53 protein and WT1 (Wilms' tumour protein), inhibit expression of IGF-1R [11, 48–50]. IGF-1R levels are also affected by nutrition [13, 51, 52]. Not much is known about the regulation of IGF-2R expression, although some studies [29, 30, 53, 54] have suggested that insulin, IGFs, EGF, and M6P may increase the level of IGF-2R, in the cell membrane. Binding of IGFs to IGF-1R activates the receptor's tyrosine kinase activity, which starts a cascade of reactions among a number of molecules involved in the signal transduction pathway (Figure 3).

IGF-2R acts as a scavenger for circulating IGF-2 uniquely. The extracellular domain of the receptor disassociates upon proteolytic cleavage, from the cell membrane as a soluble fragment, circulating in the blood with the ability to bind to IGF-2 and facilitate its degradation [55–60]. These receptors, additionally to the IGFBPs, provide an extra control on the circulating levels of IGF-II.

4. Insulin-Like Growth Factor Binding Proteins (IGFBPs)

The insulin-like growth factor binding proteins (IGFBPs) were originally discovered while purifying IGF-1 from serum [61, 62]. The insulin-like growth factors (IGFs) are present in extracellular fluids bound to high affinity carrier proteins (Table 2). Six forms of IGF binding proteins (IGFBPs) have been cloned and their complete sequences have been obtained [63].

IGFBPs have three domains. Human IGFBPs 1–6 each contain 216–289 amino acids organized into three domains of approximately equal size, with the conserved N- and C-domains being joined by a "linker" L-domain [2, 64]. IGFBPs 1–5 have 18 conserved cysteines, whereas IGFBP-6 has 16

Table 2: Human insulin-like growth factor binding proteins.

IGFBPs	Mass (kDa)	Source of purification	Relative binding affinity for IGFs
IGFBP1	25.0	Amniotic fluid, placenta	IGFI = IGFII
IGFBP-2	31.3	BRL-3A and MDBK cells, human serum	IGFII > IGFI
IGFBP-3	28.7	Plasma	IGFI = IGFII
IGFBP-4	25.9	Human osteosarcomas, prostatic carcinoma, colon carcinoma, and glioblastoma	IGFI = IGFII
IGFBP-5	28.5	C2 myoblasts conditioned media, human bone	IGFI = IGFII
IGFBP-6	22.8	Cerebrospinal fluid, human serum	IGFII > IGFI

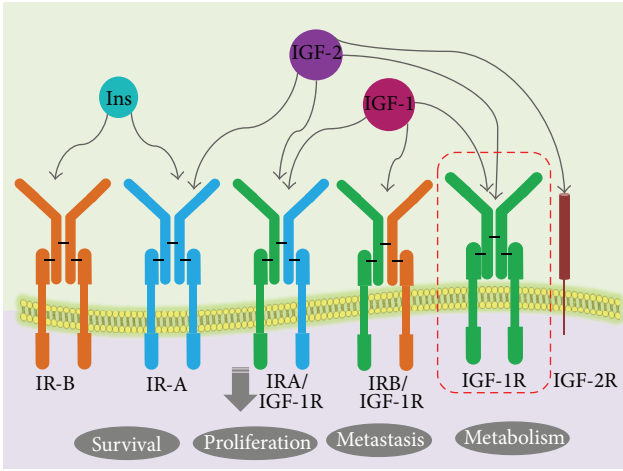


Figure 3: IGF receptor signalling: IGF-1R is a tetramer of two identical α-subunits and two identical β-subunits. IGF-1R and IR can hybridize to form a heterodimer composed of one α-subunit and one β-subunit of each receptor. Formation of hybrid receptors is explained with different colour code scheme. IGF-IIR, the mannose-6-phosphate (M6P) receptor, has high affinity for binding the IGF-II ligand but is a nonsignalling receptor. The biological activities of the IGF ligands are mediated by IGF-IR, but the IGF-IIR is considered to function as a "sink" that controls the local bioavailability of IGF ligands for binding to the IGF-IR.

[2, 65]. The N-domains of IGFBPs 1–5 contain six disulfides and share a conserved GCGCC motif; IGFBP-6 shares all of these except the two adjacent cysteines in this motif. Therefore, the first three N-terminal disulfide linkages of IGFBP-6 differ from those of IGFBP-1 and, by implication, the other IGFBPs [65]. By contrast, the remaining N-domain disulfides and all three C-domain disulfides are probably conserved in all IGFBPs.

The sequence alignment of IGFBPs 1–6 is depicted in Figure 4, where the N-domains of IGFBP 1–5 contain six disulfides and share a conserved GCGCC motif; IGFBP-6 shares all of these except the two adjacent cysteines in this motif. The C-domains are known to share the highly conserved CWCV motif. But the central domains do not contain any cysteines and exhibit little homology.

The six IGF binding proteins are unrelated to the cell surface receptors but are structurally very closely related to each other, although they are each products of distinct genes and they all have very distinct functional properties. Table 3 summarizes the results of inhibiting IGFBPs activity and their role in cancer.

4.1. IGFBP Proteases. Ever since the discovery of IGFBP-3 protease in seminal plasma [66] and human pregnancy serum [67], IGFBP proteases have been known to be present in various body fluids [68]. IGFBP proteases belong to a superfamily of proteases with specificity towards IGFBPs, thereby regulating the action of IGFBPs. These proteases are prime factors in modulating the levels of IGFBPs and ultimately the bioactivity and downstream actions of IGFs [69].

IGFBP proteases broadly fall into three major super families—serine proteinases (kallikrein-like serine protease), matrix metalloproteinases (MMPs), and cathepsins [70, 71]. The work of Cohen et al. demonstrating the significance of IGFBP proteases and a descriptive review by Fowlkes talk miles about their classification [70, 71]. Table 4 summarizes different IGFBP proteases and their target substrates with target sequence specificity.

Prostate specific antigen (PSA), the first IGFBP protease to be discovered in seminal plasma [66] and later on in pregnancy serum [72], is a serine proteinase produced by the prostate gland and is known to degrade IGFBP-3 [66]. γ-nerve growth factor (NGF), homologous to PSA, is also known to degrade IGFBP-3 and IGFBPs 4, 5, and 6, thereby enhancing IGF actions. Epidermal growth factor binding protein (EGFBP), human plasma kallikrein (hPK), and renin are relatively poor IGFBP proteases [71].

Matrix metalloproteinases are calcium-dependent zinc-containing endopeptidases, with the capability of degrading several extracellular matrix molecules including collagens, elastins, gelatin, matrix glycoproteins, and proteoglycan [71, 73–75]. These extracellular degrading enzymes are also known to be active against IGFBPs [74]. They were first discovered as IGFBP-3 proteinases in human dermal fibroblasts [74, 76]. These MMPs are known to contribute to the degradation of IGFBPs 1, 2, 3, 4, and 5 known from various scientific studies including a study showing the proteolytic cleavage of IGFPB-1 and IGFBP-2 by MMP-1 in smooth muscle airway cells. [71, 73, 76–78]. Research has shown that MMP-3 and MMP-9 can cleave IGFBP-1. MMP-1 and MMP-3 degraded rhIGFBP-3 to much greater extent than MMP-2 in vitro [74]. ADAM-12, a disintegrin metalloproteinase, is also known to have proteolytic activity against IGFBP-3 [77].

Cathepsins belong to a family of lysosomal proteinases with optimal activity in acidic conditions discovered by their

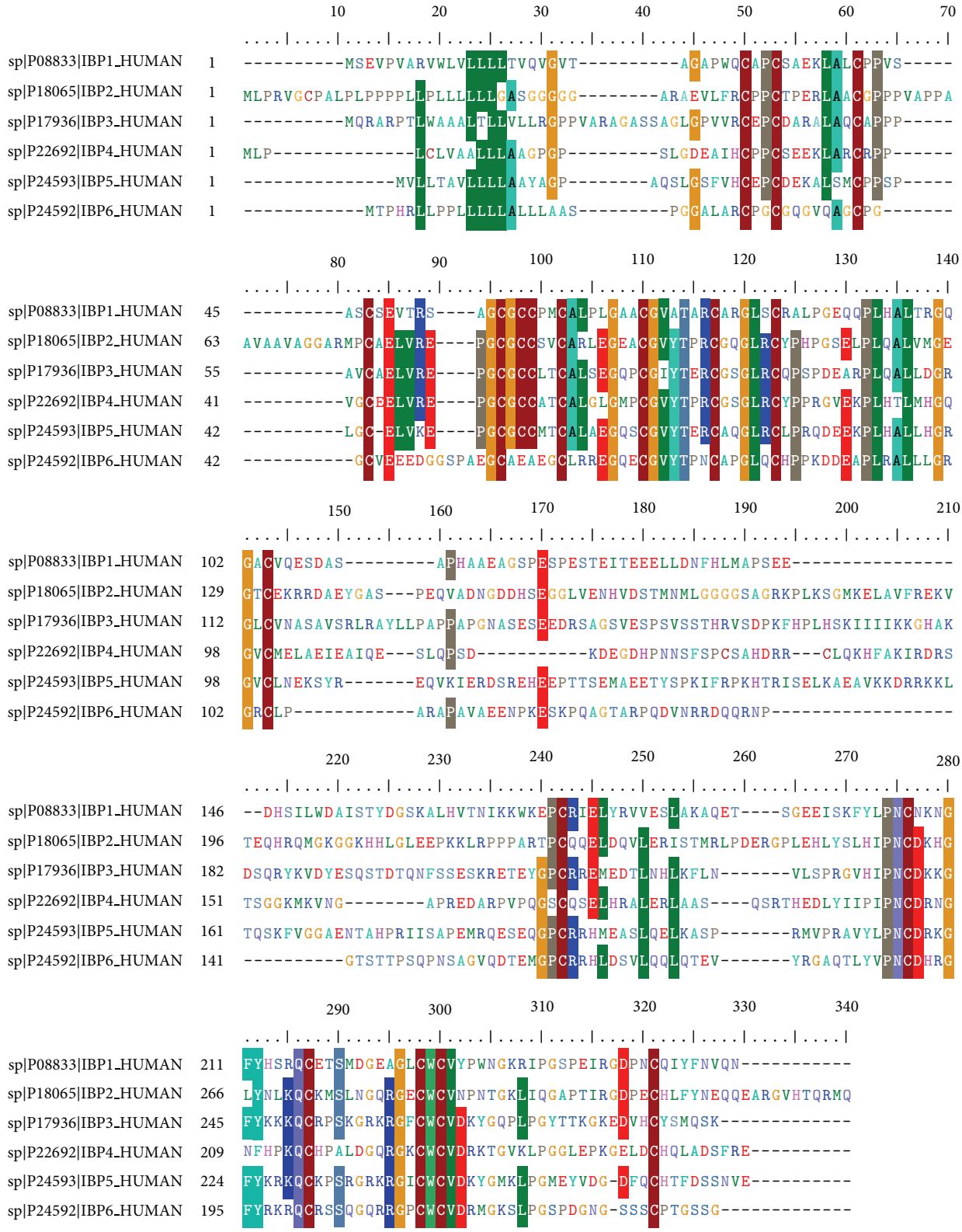


Figure 4: Amino acid sequence alignment of human IGFBP-1 to IGFBP-6. Alignment was performed using the ClustalW program. Small gaps were introduced to optimize alignment.

Table 3: Consequences of inhibiting IGFBP activity and cancer.

IGFBPs	Expression	Results of inhibiting IGFBP activity	Reference
IGFBP-1	Liver	It can induce or inhibit the IGF actions in many types of cells. As an example of the inhibiting activity of IGFBP-1, it inhibited IGF-I-induced growth in MCF-7 breast cancer cells.	[30, 157]
IGFBP-2	Liver, adipocytes, reproductive system, and central nervous system	IGFBP-2 level changes were associated with the development of different types of cancer including breast and prostate cancer. In prostate cancer, high level of serum IGFBP-2 was associated with low grade prostate cancer.	[189, 190]
IGFBP-3	Circulating carrier protein, expressed in many tissues	IGFBP-3 plays important role in different types of human cancers. IGFBP-3 can induce apoptosis by increasing the ratio of proapoptotic to antiapoptotic proteins in breast cancer cells.	[191]
IGFBP-4	Liver, bone tissue, and muscles	IGFBP-4 showed a strong inhibitory effect on IGF-1 by preventing the activation of the IGF-1R, when the IGFBP-4 is found in the tissue. Conversely, intravenous administration of IGFBP-4, in the presence of a protease, will promote cellular proliferation.	[79, 192–195]
IGFBP-5	Mammary glands	In breast cancer, IGFBP-5 induced apoptosis and inhibited cellular differentiation in an IGF-dependent manner.	[196, 197]
IGFBP-6	Epithelial layer of human bronchial organ	It can inhibit IGF-2 activity mediated through the IGF-1R, including proliferation, differentiation, migration, and survival in different cell lines.	[198, 199]

Table 4: Summary of IGFBP proteases and their proteolytic cleavage sites.

Proteolytic cleavage sites	IGFBP protease	Reference
IGFBP-2		
Met166-Gly167, Lys168-Gly169, Tyr103-Gly104, Leu152-Ala153, Arg156-Glu157, Gln165-Met166, Thr205-Met206, Arg287-Met288	Unknown protease in hemofiltrate	[80]
Leu3-Phe4, Lys168-Gly169, Lys181-Leu182	Unknown in milk	[200]
Arg164-Gln165	Human kallikrein-2	[201]
Leu152, Gly175-Leu176, Lys181-Leu182	Matrix metalloproteinase-7	[202]
Gln165-Met166	PAPP-A	[203]
His165-Arg166	Calpain	[204]
IGFBP-3		
Arg97-Ala98, Lys160-Val161	Plasmin	[205]
Arg95-Leu96, Lys160-Val16	Plasmin	[206]
Arg97-Ala98, Arg206-Gly207	Thrombin	[205]
Arg97-Ala98, Lys149-Lys150, Lys150-Gly151, Lys154-Asp155	Serum	[205]
Arg97-Ala98, Arg132-Val133, Tyr159-Lys160, Phe173-Ser174, Arg179-Glu180	Seminal plasma PSA	[157]
Arg97-Ala98, His131-Arg132, Tyr159-Lys160	Urinary PSA	[207]
Arg97-Ala98	Cysteine protease from MCF-7 cells	[208]
Tyr99-Leu100, Leu96-Arg97, Leu141-His142	MMP-1, MMP-2	[176]
Tyr99-Leu100, Asn109-Ala110, Glu176-Ser177	MMP-3	[176]
IGFBP-4		
Lys120-His121	Calcium-dependent serine protease from smooth muscle cells	[193, 209, 210]
Met135-Lys136	PAPP-A	[211, 212]
IGFBP-5		
Arg138-Arg139	Serine protease from smooth muscle cells	[213]
Ser143-Lys144 (secondary cleavage site), Ser143-Lys144	PAPP-A2	[214]
Gln142-Ser-143	PAPPA	[214]
Lys120-His121, Arg156-Ile157, Arg192-Ala193	Thrombin	[215]

proteolytic activity on IGFBP-3 [70, 71]. Cathepsin D is a well-known IGFBP protease shown to have proteolytic activity against IGFBPs 1–5 in acidified condition [70, 79]. In neutral conditions, their proteolytic activity seems to be unclear.

The central linker domain which is the least conserved region has not been cited to be a part of the IGF binding site for any IGFBPs but is reported to have four major protease cleavage sites in IGFBP-2, determined to be between Tyr103 and Gly104, Leu152 and Ala153, Arg156 and Glu157, and Gln165 and Met166 [80]. A study involving mutation of selected residues of the linker domain of IGFBP-4 led to protease resistivity of IGFBP-4 [81]. This leads to the conclusion that the proteolysis of IGFBPs occurs at specific sites by proteases in unstimulated, homeostatic conditions (e.g., PAPP-A activity in normal cell lines). As the reports suggest the linker domain to be most proteolysis susceptible among the N-, C-, and the linker domain, it acts as the determinant in the release of IGF from IGFBPs. Thus, a detailed understanding of the interaction of L-IGFBP-2 with IGF at atomic level is important. This may help to determine the changes which can be brought about in the linker domain for careful modulation of IGF release, which could in turn prevent unwanted IGF-1R, signalling controlling abnormal cellular growth and proliferation. Alternatively, in conditions where cellular proliferation is desired (e.g., wound healing), control on release of IGF may facilitate IGF mediated cellular growth and proliferation. Thus, a study of the structure of linker domain (L-IGFBP-2) and its interaction with IGF-1 together with the change in dynamics in presence of IGF-1 was studied in our laboratory.

4.2. Significance of IGFBP Proteases in Cancer. IGFBP proteases are known to target and degrade IGFBPs to smaller fragments and thus bring down the affinity of IGFBPs to IGFs. This results in IGFs binding to their respective IGF receptors resulting in signalling cell proliferation, growth, and cell migration. Kallikreins have also been employed as biomarkers in cancer [82]. Apart from the significance of proteolysis in regulating the bioavailability of IGFs in tissues and increasing the affinities of IGFs to IGF receptors, this seems to play a significant role in tumour progression and tumour cell survival considering the autocrine-paracrine actions in the IGF axis. Thus, IGFBP proteases have potential clinical implications in cancer research.

A novel approach in this regard is development of mutant IGFBPs lacking the IGFBP protease cleavage sites, rendering them protease resistant. This serves as a potential therapeutic agent as it inhibits IGF signally through IGF receptors. Such studies reported a decade ago where a protease resistant IGFBP-4 was designed and in vivo studies of this protease resistant IGFBP-4 [81] were explored confirming the complete resistance to IGFBP-4 protease indicating that the mutant IGFBP-4 resulted in greater growth inhibition than equivalent levels of native IGFBP-4 demonstrating a role for IGFBP-4 proteolysis in the regulation of IGF-1 action and a potential implication in cancer [81]. In yet another similar in vivo study, protease resistant IGFBP-4 has been shown to block IGF activity, tumour growth, and angiogenesis [83].

In another such recent study, a novel approach has been used to develop protease resistant (PR) and protease resistant/non-matrix-binding (PR/NMB) variants of IGFBP-2 as potential tumour growth inhibitors [84]. They hypothesized that lack of protease and matrix-binding sites render the IGFBP-2 devoid of the ability to promote IGF-dependent action (through release of IGFs to the receptors) and IGF-independent action (through ECM binding). The in vitro and in vivo studies indicate that the mutant IGFBP-2 (lacking a large portion of the central linker domain) is able to inhibit tumour growth possibly by inhibition of angiogenesis. Their studies promise to open up new avenues for better targeting strategies for the effectiveness of cancer treatment in the near future.

4.3. IGFBP-Related Proteins (IGFBP-rPs). The IGFBP superfamily includes 6 members (IGFBP-1 to IGFBP-6) with high affinity for IGF-1 and IGF-2 and 10 IGFBP-related proteins (IGFBP-rP1 to IGFBP-rP10) with low affinity for these ligands. Remarkably, IGFBP-related protein 1 (IGFBP-rP1), also known as insulin-like growth factor binding protein-7 (IGFBP-7) [85], is identified as a secretory and low-affinity IGFBPs. It is distinct from other low-affinity IGFBP-rPs in that it can bind strongly to insulin [86], suggesting that IGFBP-7 is likely to have distinct biological functions from other IGFBPs. IGFBP-related protein 1 (or IGFBP-7) has been found to have an important role in the female reproductive system. It was implicated in human endometrial receptivity, folliculogenesis as well as growth, development, and regression of the corpus luteum in higher mammals [87–89]. Other studies showed that it could induce apoptosis in M12 prostate cancer cell line [90].

Rupp et al. demonstrated that, adding to IGFBP-7 tumour suppressor function, it can promote anchorage-independent growth of malignant mesenchymal cells and of epithelial cells with an EMT-phenotype when IGFBP-7 is expressed by the tumour cells themselves [91]. Expression of IGFBP-7 in tumour-associated fibroblasts can also promote colony formation when epithelial tumour cells are cocultured with IGFBP-7-expressing cancer-associated fibroblasts (CAFs) by secondary paracrine tumour-stroma interactions. Zhu et al. recently reviewed role of insulin-like growth factor binding protein-related protein 1, IGFBP-rP1, in cancer [92]. In many cancers, IGFBP-rP1 acts as a tumour suppressor gene by suppressing proliferation and inducing apoptosis and senescence. However, there are some contradictory data and different opinions; for example, IGFBP-rP1 has been reported as promoting glioma cell growth and migration [93]. It has been recently reported that IGFBP-rP1 could bind to the IGF-1R and block its activation [94].

4.4. IGFBP Structure. The structural features of IGFBPs, which carry IGFs in the circulation, are very important for understanding their role in normal growth and development as well as in diseases. The insulin-like growth factor binding protein-2, the second most abundant IGFBP in circulation and known to form binary complexes with IGF, is 32 kDa (289 amino acid residues) in size with three distinct

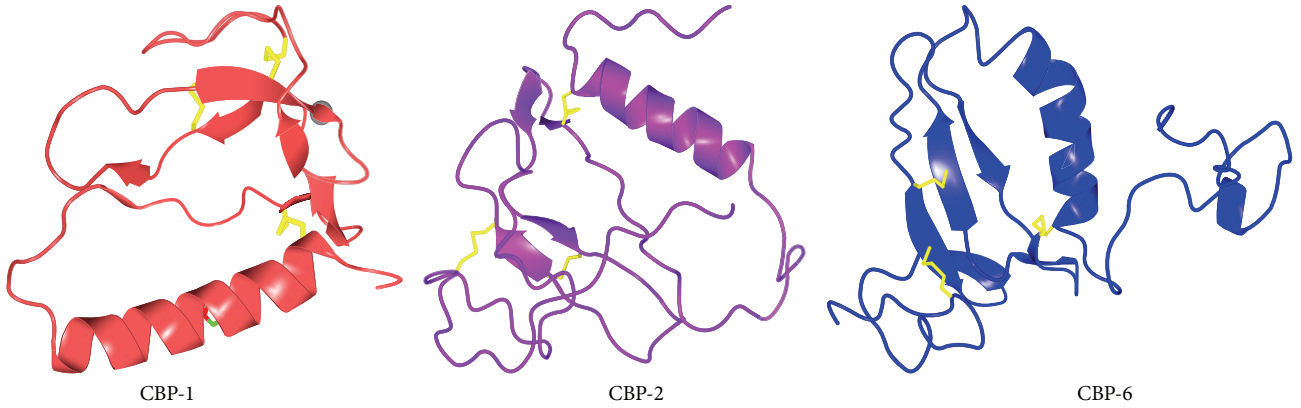


Figure 5: Structures of C-terminal domains of IGFBP-1, IGFBP-2, and IGFBP-6 represented as CBP-1 (**1ZT5**), CBP-2 (**2H7T**), and CBP-6 (**1RMJ**), respectively.

regions: the highly conserved N-terminal region (IGFBP homolog domain), the highly conserved C-terminal region with thyroglobulin type 1 repeat [95], and the mid-region known as the linker domain of IGFBP-2 with multiple cleavage sites. The structures of C-terminal domains of IGFBP-1, IGFBP-2, and IGFBP-6 are shown in Figure 5.

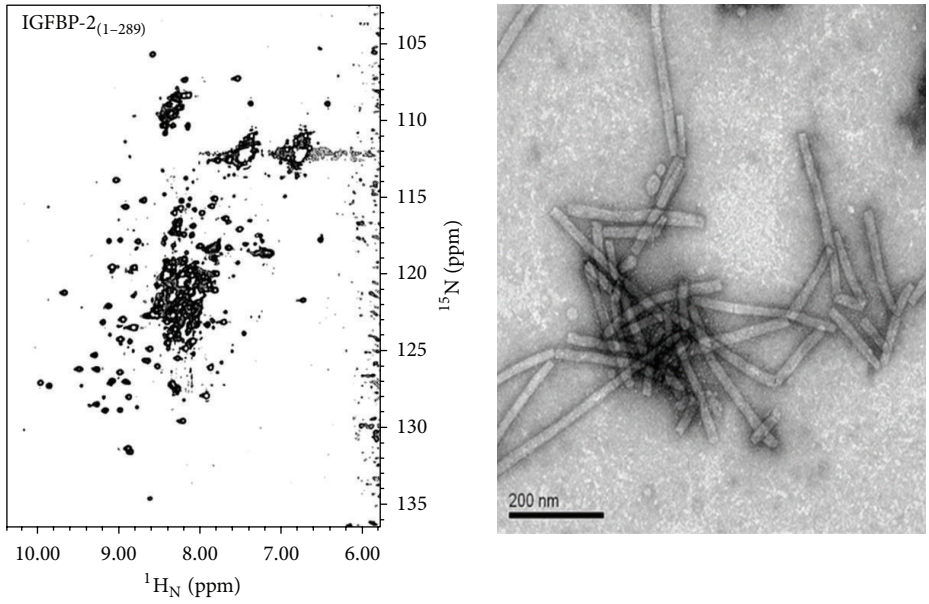
Notably, the C-terminal domain contains an arginine-glycine-aspartic acid (RGD) motif which can bind to integrins and take part in cell mediated signaling. The N- and C-terminal domains are cysteine rich and are structured, with both of them having IGF binding properties capable of modulating the IGF/IGF receptor interactions [96]. While some reports have emphasized the importance of the binding of N-terminal domain to IGF by mutagenesis experiments [97] and by iodination protection study [98], others have described the C-terminal region of IGFBP-2 as playing important role in the binding to IGFs by mutagenesis experiments [99, 100] and by nuclear magnetic resonance spectroscopy [101]. Some others emphasize the cooperative role which the N-terminal and the C-terminal domain play in the binding to IGF-1 [102]. The structural aspects of IGFBPs have been recently reviewed by Forbes et al. [103]. The important structural features for interaction of IGFBPs with extracellular matrix and integrins were described. Further, they highlighted the important structural features for binding with IGFs and other partners also.

4.5. Structural Studies of Human IGFBP-2 Binding by NMR. While the biological actions of IGF-1-IGFBP-IGF-1R axis have been extensively studied, a complete understanding of IGF-IGFBP interactions on a structural level is lacking. Our objective was to elucidate the mechanistic aspects of IGF-IGFBP interactions at the atomic level in order to develop IGFBPs as cancer therapeutics.

A critical challenge in the structural characterization of full-length IGFBPs has been the difficulty in expressing large amounts of these proteins for NMR/X-ray crystallography analysis. We have developed a method for high-yield expression of full-length recombinant human IGFBP-2 (hIGFBP-2) in *E. coli* [104]. Using a single step purification protocol, we obtain hIGFBP-2 with >95% purity. The protein exists as a monomer at the high concentrations (up to 30 mg/mL) required for structural studies in a single conformation exhibiting a unique intramolecular disulfide-bonding pattern. We have thus, for the first time, obtained high-yield expression of wild type recombinant human IGFBP-2 in *E. coli* and initiated structural characterization of a full-length IGFBP. We are currently studying the molecular interactions of the different domains of hIGFBP-2 with IGF-1, in particular the central flexible domain which is known to play a pivotal role in the protein function and regulation. These are described in the proceeding section.

4.5.1. Study of Nanotubular Structures Formed by a Fragment of IGFBP-2. We recently discovered that the C-terminal fragment of hIGFBP-2 (residues 249–289) self-assembles spontaneously and reversibly into nanotubular structures under nonreducing conditions and remains as a monomer under reducing condition. These nanotubular structures were studied extensively by transmission electron microscopy (TEM), NMR spectroscopy (Figures 6(a) and 6(b)), and circular dichroism (CD) and a mechanism for their formation has been worked out [105].

4.5.2. Biomedical Applications of IGFBP-2 Nanotubes. The presence of an RGD motif in this polypeptide fragment offers avenues for novel biomedical applications. The RGD motif is known to be recognized by integrins. The design of such self-assembling polypeptide fragments containing an RGD motif can be utilized to enhance the efficacy of cancer therapeutics. We have explored the possibility of using these nanotubes for cancer cell imaging. This is based on the idea that, in many cancers, integrins are expressed in large quantities on the cell surface. Thus, IGFBP-$2_{249-289}$ nanotubes can be developed to identify the location of cancer cells through their binding to integrins via the RGD motif. Towards this end, we have carried out cell-adhesion and cell-proliferation assays which have helped to characterize the binding of the nanotubes to integrin via the RGD motif.



(a) (b)

Figure 6: (a) 2D [^{15}N-^{1}H] HSQC spectrum of purified full-length hIGFBP-2 (1.0 mM; nondeuterated) recorded at a ^{1}H resonance frequency of 800 MHz at 285 K. (b) TEM images of (hollow) nanotubular structures formed by the C-terminal fragment of human IGFBP-2.

5. Therapeutic Strategies Targeting IGF System in Cancer

Therapeutic strategies targeting various components of the IGF system, with varying degree of success, have been developed for treatment of different types of cancer. Description and challenges of each targeting strategy will be enlightened in this section.

5.1. Targeting IGF-R: Therapeutic Potential of IGF-Rs in Cancer. IGF-1R activation by tyrosine phosphorylation of β subunit results in activation of PI3K/AKT and RAS/MAPK pathways [106, 107] which in turn regulate cell survival and proliferation. IGF axis is tightly regulated under normal physiological conditions maintaining cell homeostasis and growth. Genetic alterations of IGF-1R leading to varying levels of their expression are found to have a link in cancer [108]. These receptors maybe activated in the tumour cells in an unregulated manner. (mutation, chromosomal translocation, abnormal stimulation, and loss of genomic imprinting).

IGF-1R does not solely drive tumour cell proliferation; however, most oncogenes are required in mediating anchorage independent growth given its property to mediate proliferation and cell survival. This is one of the key processes to achieve metastasis among tumour cells [107, 109].

High levels of IGF-1 have been reported in several cases of breast and prostate cancers [110] and since IGF-2 is maternally imprinted [111, 112], loss of this imprinting results in biallelic expression, resulting in increased IGF-2 production and a suspected mechanism of cancer development and progression in many conditions [111, 113–115]. These higher levels of IGF-1 and IGF-2 promote IGF-1R signalling and the consequently activated downstream pathways. Increases in IGF-1R have been shown in different types of cancer, melanoma, and carcinomas [116–118]. Considering disease prognosis, therapeutic approaches based on targeting IGFRs seem to be promising in cancer research.

Another aspect of IGF-R is the formation of IGF-1R/IR hybrids by random association of insulin half-receptor (IR-A) with an IGF half-receptor adding further complexity in receptor targeting strategy [119]. IR isoform (IR-A) is overexpressed in cancer and it is the fetal isoform of IR (while other half is IR-B involved in regulating glucose uptake) and IGF-1R is also overexpressed in cancer. With the overexpression of these receptors, formation of IGF-1R/IR hybrid receptors is expected. These have broad binding specificity as they bind IGF-1, IGF-2, and also insulin [119]. Targeting these hybrid receptors becomes one of the several strategies.

There are several approaches of targeting IGF-R till date, namely, small molecule tyrosine kinase inhibitors (TKIs), anti-IGF-1R antibodies, and molecular agents such as antisense and small interfering RNAs (si-RNAs) [107, 120] (Figure 7).

While a lot is known on targeting IGF-Rs through TKIs and anti-IGF-1R antibodies and there are detailed multiple reviews on their targeting strategies [108, 120–129], little is known on targeting the former using antisense technology and si-RNAs. Tables 5 and 6 summarize few of the several different TKIs and anti-IGF-1Rs studied.

Recent advancements in this approach show us that it is possible to genetically target IGF-Rs. Adenoviruses expressing antisense IGF-1R and truncated IGF-1R, nonviral vectors expressing truncated IGF-1R, were used to successfully block

Table 5: Few examples of small molecule TKIs (tyrosine kinase inhibitors) directed against IGF receptors.

Small molecule inhibitor	Mode of action	Effects	Reference
NVP-AEW541 NVP-AEW54 in combination with gemcitabine	Kinase inhibition	Antineoplastic, tumour regression and inhibition of metastasis	[216, 217]
Picropodophyllin (PPP)	Against autophosphorylation at the substrate level	Inhibition and downregulation of IGF-1R	[218–221]
BMS-554417	ATP-competitive, dual kinase inhibition	Antiproliferative activity	[222]
INSM-18	Reversible ATP-competitive	Inhibitor of transcription (blocking also cdc2, survivin, and VEGF)	[223]
OSI-906	Reversible ATP-competitive	Derived from compound-1, also known as PQIP	[223]
XL-228 (XL-2280)		Inhibits bcr-abl, scr, and IGF-1R	[224]
BVP-51004 Biovitrum (Cyclolignan PPP)	Non-ATP-competitive	Causes IGF-1R downregulation, probably through the induction of ubiquitination.	[223]

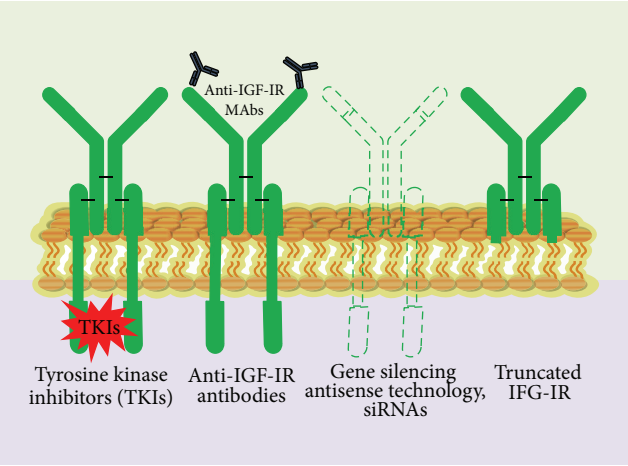


Figure 7: Various strategic approaches to targeting IGF-1R receptors. Small-molecule TKIs, inactivating anti-IGF-1R antibodies, reduction or elimination of IGF-1R, protein expression by blocking IGF-1R, transcription (with triple helix) or translation (antisense technology and siRNA), IGF-1R, and mutants lacking beta-subunits (dominant-negative receptors).

IGF-1R, thereby suppressing tumorigenicity in vitro and in vivo, and also effectively blocked both IGF-1- and IGF-2-induced activation of Akt-1.

Studies in which small interfering RNAs (siRNAs) induce potent IGF-1R gene silencing without affecting the insulin receptor demonstrate that siRNAs block IGF signalling, thereby enhancing radio and chemosensitivity and paving yet another way of therapeutic potential, and may in future generate nucleic-acid-based therapeutics [125, 130]. The efficacy of IGF-1R targeting in the clinics depends on major factors such as the role of IFGR in itself in the tumours, inhibition potential of siRNAs and antisense therapies in vivo, and compensation of other signalling pathways due to IGFR loss [130].

These studies also prove the potential genetic blockade studies of IGF-1R and its efficacy and prognosis in several malignancies, lung, colon, and pancreatic carcinoma [131, 132]. Such antisense and dominant negative strategies (truncated) also enhance tumour cell chemosensitivity (effective chemo- and radiotherapy induced apoptosis). One more prominent feature is the immune protection induced by tumour cells killed in vivo by IGF-1R-antisense technique. Major drawback is that antisense agents cause adequate IGF-1R downregulation and also affect insulin receptor.

Cotargeting IGF-Rs along with other tumour promoting pathways is yet another way to effectively overcome the limitations of resistance to conventional chemo- and endocrine therapy to single agent targets discussed in previous sections as cross talk between IFG-R and RTKs/steroid hormones is known to promote tumorigenesis. IGF-1R is known to interact with several pathways and molecules, receptor tyrosine kinases (RTKs), including insulin receptor (IR), epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), mesenchymal-epithelial transition factor (MET), platelet-derived growth factor receptor (PDGFR), and fibroblast growth factor receptor (FGFR), and steroid hormones, including estrogen receptors alpha and beta, androgen receptor (AR), and progesterone receptor (PR). This novel approach pertains to cross talk cotargeting [133]. Examples of such a targeting strategy include monoclonal antibodies and small molecule tyrosine kinase inhibitors, in combination or cotargeting IGF-1R and EGFR receptors [123, 134, 135], where simultaneously both receptors are targeted making it a promising novel approach. In a recent study, cotargeting the IGF system and HIF-1 (hypoxia-inducible factor-1) has been shown to inhibit the migration and invasion by breast cancer cells [136], indicating that ligand-targeting compounds, or coinhibition of the IGF and HIF-1 systems, may prevent activation of compensatory signalling (due to cross talks), thereby providing a valuable and novel addition to IGF-1R inhibitor-based therapies [136].

IGF-2R deserves a mention since studies implicate that the mannose 6-phosphate/insulin-like growth factor-II receptor (M6P/IGF-2R) functions in the intracellular trafficking of lysosomal enzymes, the activation of the potent growth inhibition transforming growth factor beta 2, and the

Table 6: Few examples of anti-IGF-RI monoclonal antibodies (MAbs) [223].

Monoclonal antibody	Class	Clinical information
CP-751,871	Fully human IgG2 mab	Ewing's sarcoma family of tumours, breast cancer, single agent in metastatic CRC
IMC-A12	Fully human IgG1 mab	Ewing's sarcoma family of tumours CRC and H&N cancer
R1507	Fully human IgG1 mab previously known as RO4858696	Pediatric patients and sarcomas.
AMG-479	Fully human mab	Ewing's sarcoma family of tumours, pancreatic cancer
SCH-717454	Fully human mab previously known as 19D12 (Medarex)	Colorectal cancer (CRC)
AVE-1642	Humanized mab	Previously known as EM164 (ImmunoGen)
MK-0646	Fabre Humanized mab Previously known as A2CHM, F50035, 7C10, or 7H2HM	Colorectal cancer (CRC)
BIIB022	Fully human nonglycosylated IgG4.P antibody	Devoid of Fc-effector function to eliminate potential Fc mediated toxicity to the normal vital organs.

degradation of IGF2 (which are overexpressed in tumours). Studies have shown that M6P/IGF-2R gene functions as a tumour suppressor in human liver carcinogenesis [137].

5.2. Targeting IGFs: Therapeutic Potential of IGFs in Cancer. The insulin-like growth factors (IGFs), IGF-1 and IGF-2, are ligands that bind to IGF receptor (IGF-1R,) and regulate cancer cell proliferation, survival, and metastasis. Since IGF axis is involved in regulating cell metastasis, the pathway plays a significant role in cancer cell metastasis and proliferation and many studies over a couple of decades have tried to establish the relationship between serum IGF levels and cancer risk.

Many experiments demonstrate the increase in neoplastic cell proliferation with increasing IGF-1 concentration [138]. Various human epidemiological studies describe the correlation between circulating levels of IGF-1 coupled with IGFBPs and the risk of developing various cancers, lung, colon, breast, and prostrate [139–143]. Circulating IGF-1 levels play a significant role as a risk factor in the onset and development of mammary tumours in breast cancer [144]. In vivo studies suggest that carcinogenesis and cancer progression are influenced by germ line variation of genes encoding signalling molecules in the GH-IGF-1 axis and these mutations are often associated with genetic manipulations [144] and low IGF-1 levels; thus, tumour growth is influenced by IGF-1 physiology [145]. Yet the connection between circulating IGF-1 levels and cancer risk remains inadequately hidden. Two contradictory hypotheses on relationship between IGF-1 and cancer risk are underlined by Pollak [146].

Firstly, if a cell at risk is considered (e.g., somatic cell mutations lead to accumulating DNA damage), IGF bioactivity in the cellular microenvironment influences the fate of the cell survival and evolves to malignant cell lineage or apoptosis in early carcinogenesis. To balance apoptotic cell death and survival of damaged cells might be slightly inclined towards survival in an environment with high IGF levels, and this would favour the appearance of a malignant clone. The fate of such millions of DNA damaged cells is determined every hour, and even a modest influence of higher IGF-1 level on survival probability might lead to an association of circulating level with cancer risk [146]. Secondly, the influence of IGF-1 level on cancer risk is somewhat related to early carcinogenesis. Higher IGF-1 levels facilitate the more rapid proliferation of early cancers to the stage at which they can be clinically detected. Such lesions would be common in all adults, and cancer diagnosis would reflect the probability of these lesions progressing toward a detectable and clinically significant size, with this latter process being influenced by IGF-1 level [146].

Findings in the case of prostate cancer may be consistent with this second hypothesis. This is consistent with the view that the IGF-1 level is more related to the probability of progression of early lesions than to the actual process of early carcinogenesis. According to Pollak, both hypotheses are plausible and are not mutually exclusive; also there is no definitive mechanistic evidence to support either of them [146].

IGF-2 is also a ligand for the IGF-1 receptor and is present in serum at concentrations that are generally higher than IGF-I. IGF-2R serves as a sink to IGF-2R and does not allow the signal transduction of the latter and has the characteristics of a tumour suppressor which is discussed in previous section on targeting IGF-Rs [137].

Several drug candidates that target IGF-1 signalling were found to have antineoplastic activity by using in vitro studies and in vivo models, both as single agents and in combination with currently approved drugs. Several high-affinity antibodies are developed which cross-react with both IGF-1 and IGF-2 and these are at their early developmental stage. MEDI-573 is one such human antibody (fully human) that neutralizes both IGF-1 and IGF-2, thus inhibiting IGF signalling through

both the IGF-1R and IR-A pathways. Studies also show that MEDI-573 inhibited the in vivo growth of IGF-I- or IGF-II-driven tumours [147]. Hypophysectomy is also thought to be one of the IGF-1 ligand lowering strategies which was also successfully employed in patients with hormone-responsive breast cancer [148]. Advantage of antiligand approach is that it has the potential to block the action of IGF-2 at the insulin isoform A, without interfering with insulin action. This finding is in view of various cancers where IGF-2 production is autocrine [126].

5.3. Targeting IGFBPs: Therapeutic Potential of IGFBPs in Cancer. There is accumulating evidence in the literature stating that IGFBPs can also cause apoptosis in an IGF-independent manner [149] and they can show inhibitory effects towards tumour growth and cancer [150].

Although IGFBPs can prevent IGF from binding to IGF-1R, because of their higher affinity to IGF than the IGF-1R, it can also induce tumour growth and progression in situations where the IGFBP proteases levels are high and/or when IGFBPs interact with ECM. Thus, modifying IGFBP depends on the targeted tissue and the disease state. For example, IGFBP-3 has shown proapoptotic, antiproliferative, and antiangiogenic functions in in vitro tumour models [69, 151]. On the other side, IGFBPs can promote tumour progression in the presence of proteases. IGFBP-2 and IGFBP-5 upregulation in CRPC are a good example of that. In the presence of PSA and other factors affecting the IGF-I/IGFBP-2 and IGFBP-5 binding, it will result in the delivery of the IGFs to the IGF-1R and activation of the downstream signalling 21 pathway, thus helping the progression to castration resistant disease [152, 153]. Recently, Baxter et al. reviewed IGFBPs and their cellular actions beyond their endocrine role in IGF transport [154]. They suggest that IGFBPs can also function in their pericellular and intracellular sections to regulate cell growth and survival. Further they interact with many other proteins including their canonical ligands IGF-1 and IGF-II. Also they have shown that the intracellular functions of IGFBPs in transcriptional regulation, induction of apoptosis, and DNA damage repair which also point to their friendly participation in tumour development, progression, and resistance to treatment.

5.3.1. Cancer Stimulatory/Inhibitory Effects of IGFBPs

IGFBP-1. IGFBP-1 has higher IGF-1 binding affinity in various phosphorylated forms than the unphosphorylated protein and is inhibitory to IGF action [155]. An interesting study using IGFBP-1 deficient mice demonstrated that IGFBP-1 can function as a cell survival factor by repressing TGFβ activation [156], but the relevance of this effort for cancer cell survival is not understood. On the whole there is no specific confirmation that IGFBP-1 stimulates tumour growth or it is extensively a tumour growth inhibitor [157].

IGFBP-2. IGFBP-2 overexpression in mice is found to inhibit development of colorectal adenomas by reducing the tumor growth by inhibition of cell proliferation [158]. Further there is significant evidence for a growth promoting effect of IGFBP-2 in many tumour systems, by sequestering IGFs [159]. IGFBP-2 contains an Arg-Gly-Asp motif, but substitution of these amino acid residues did not affect the cell binding of IGFBP-2 [160]. Additionally, this motif interacts with α5 integrin and is found to be involved in regulating the effect of IGFBP-2 on glioma cell migration and invasion [161, 162].

IGFBP-3. IGFBP-3 can function as a cancer suppressor and is downregulated in some cancer tissues. However, growth promotion by IGFBP-3 has been described by several mechanisms, which involve its overlap with other cell signaling systems. Potentiation of IGF-I dependent proliferation by IGFBP-3 that was first described in human skin fibroblasts in 1988 [163], has also been revealed in breast cancer and some other cell types [68, 164–166]. Further in some cases, IGFBP-3 was shown to stimulate IGF-1 action, even for IGF derivatives that have negligible interaction with the binding protein [167], so the consequence is unlikely to involve IGFBP-3 somehow presenting IGFs to their receptor.

In patients with NSCLC, the greatest activation of IGF-1R was observed in tumours that expressed high levels of IGFBP-3 [168], although it is not clear whether this activation was ligand dependent. The high expression levels of both EGFR and IGFBP-3 are seen in tumour tissue compared with normal tissue in case of oesophageal cancer [72].

IGFBP-4. Cancer inhibitory effects of IGFBP-4 are generally accepted. IGFBP-4 is found to inhibit tumour progression by sequestering IGFs [66], but some reports demonstrate that, in some circumstances, it might suppress cell death [72] or stimulate cell migration. In epithelial ovarian cancer, IGFBP-4 mRNA is found to be highly expressed [170] but has not been shown to be significant for prognosis.

IGFBP-5. In breast cancer models, IGFBP-5 overexpression was strongly tumour inhibitory in vitro and in vivo [171], whereas the opposite effects were observed in some other cancer models, in which IGFBP-5 can stimulate IGF-dependent and IGF-independent cell survival and proliferation [172–175]. In noncancer cell lines, similar effects have been reported [176, 177]. In prostate cancer cells, down regulation of IGFBP-5 inhibited IGF-dependent growth in vitro and in vivo and castration induced upregulation of IGFBP-5 in mice accelerated the development of androgen independence [178].

IGFBP-6. As recently reviewed [82], IGFBP-6 is also known to have inhibitory effects in cancer by blocking IGF signalling, extraordinarily IGFII, but there is evidence where in some circumstances it may have oncogenic actions stimulating migration [179] and proliferation [70] which is mechanistically stronger than for IGFBP-4. The IGFBP-6 was shown to be involved in cell surface interaction with prohibitin 2, a protein found in the mitochondria and nucleus, as well as in the plasma membrane; thus, it stimulates rhabdomyosarcoma cell migration. IGFBP-6 ligation results in tyrosine-phosphorylation of Prohibitin 2 [180]. Primarily, IGFBP-6 is tumour suppressive [82], but an ultimate link between its activity in vivo remains to be established.

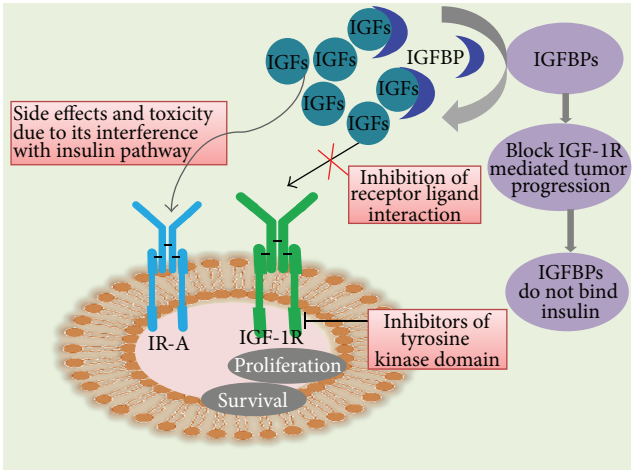


Figure 8: Targeting IGFBPs, a novel strategy in cancer therapeutics. The cancer therapeutics targeting the IGF-signalling pathway focus on blocking IGF-1R, directly, and/or its downstream effect. Drawback of such approaches is the adverse side effects or toxicities due to its interference with the insulin pathway. The more efficacious alternatives, IGFBPs, as IGF-antagonist based cancer therapeutics also contribute to block the IGF-1R, mediated tumour progression. As IGFBPs do not bind insulin, they do not interfere with insulin-insulin receptor interactions.

It is now clear that the IGFBPs have many effects on cell death, via both IGF-dependent and IGF-independent actions. Although the mechanisms underlying these latter actions are only beginning to be understood, it is already clear that they may provide very specific strategies for fine-tuning therapeutic interventions. Current therapeutics targeting the IGF-signalling pathway focus on blocking IGF-1R, directly, and/or its downstream effect. Potential drawback of such approaches is the resulting adverse side effects or toxicities due to its interference with the insulin pathway. As a more efficacious alternative, we propose that IGFBPs can be developed as IGF-antagonist based cancer therapeutics serving to block the IGF-1R mediated tumour progression (Figure 8). The IGFBPs do not bind insulin and thus do not interfere with insulin-insulin receptor interactions.

6. Natural Products: Targeting IGF Signalling Pathways

Natural products are known to have medicinal benefits from ancient history. They have been used for the treatment of various diseases and are gaining tremendous importance in the area of drug discovery. These natural product derived phytochemicals have been extensively studied and have exhibited anticarcinogenic activities by interfering at various stages of cancer through various mechanisms including cellular proliferation, differentiation, apoptosis, angiogenesis, and metastasis [230]. We have a rich historical record from ancient physicians about the use of natural product medicines alone and in combination, which might provide important hints for inventing new drugs. Nowadays, many anticancer drugs available in the market are natural product phytochemicals or their derivatives [231] and some are under clinical trials [232].

The natural products including curcumin (3,3′-diindolylmethane (DIM)), isoflavone genistein (indole-3-carbinol (I3C)), epigallocatechin-3-gallate (EGCG), resveratrol, lycopene, and apigenin have been recognized as cancer chemopreventive agents (Figure 9) because of their anticarcinogenic activity [233, 234]. The in vitro and in vivo studies have demonstrated that these natural products have inhibitory effects on various human and animal cancers [235–239]; therefore, many researchers have focused on interpreting the molecular mechanisms and identifying the targets of action of these natural products. The various natural products perturbing IGF signalling pathways and their mechanism of actions have been summarised in Table 7. The understanding of molecular mechanism of natural product derived phytochemical against a specific cancer type will lead to the development of novel anticancer drugs.

7. Future Perspectives: Challenges and Opportunities for Novel IGF Therapies

The success of targeted therapies for cancer is undisputed; strong preclinical evidence and on-going clinical trials of some of the drugs chemical molecules, antibodies, antisense technology, si-RNA therapy against members of the IGF-axis-IGF ligands, IGFBPs, and IGF-Rs have resulted in the approval of several new agents for cancer treatment. Not only targeting of these by single substances but also the approaches of cotargeting strategies seem to be a very promising avenue with more and more studies directed in this approach to solve the complications which come across while targeting specific molecules involved in cancer pathways.

Targeting IGF ligands seems to be problematic since the IGF mediated signalling has important roles in regulating cellular proliferation and apoptosis (role as circulating hormone and a tissue growth factor) apart from their increased levels in various cancers. Another important factor to bear in mind is that higher levels of IGFBPs might increase IGF-1 concentration by increasing its circulating half-life, and this may not possibly lead to increase in receptor activation at the tissue level and the link between higher IGF levels and neoplasm seems to be unclear here.

Another approach is to target IGFBPs in a way which sequesters more and more IGFs, thereby downregulating the IGF mediated signalling in cancer pathway. Since IGFBPs are further regulated by IGFBP proteases, developing mutants which lack proteolytic cleavage sites for these proteases can pave a way for strong interaction between IGF and IGFBPs. A recent study in this regard showed that novel, modified IGFBP-2 proteins (protease resistant alone or also lacked the ability to bind extracellular matrix) sequestered both the IGFs and thereby was able to inhibit tumour growth. These modified IGFBPs were found to do so by inhibition of angiogenesis both in vitro and in vivo [84]. Apart from IGF-dependent (proteolysis) activities, IGFBPs also have IGF-independent activities in relation to cancer; mutants

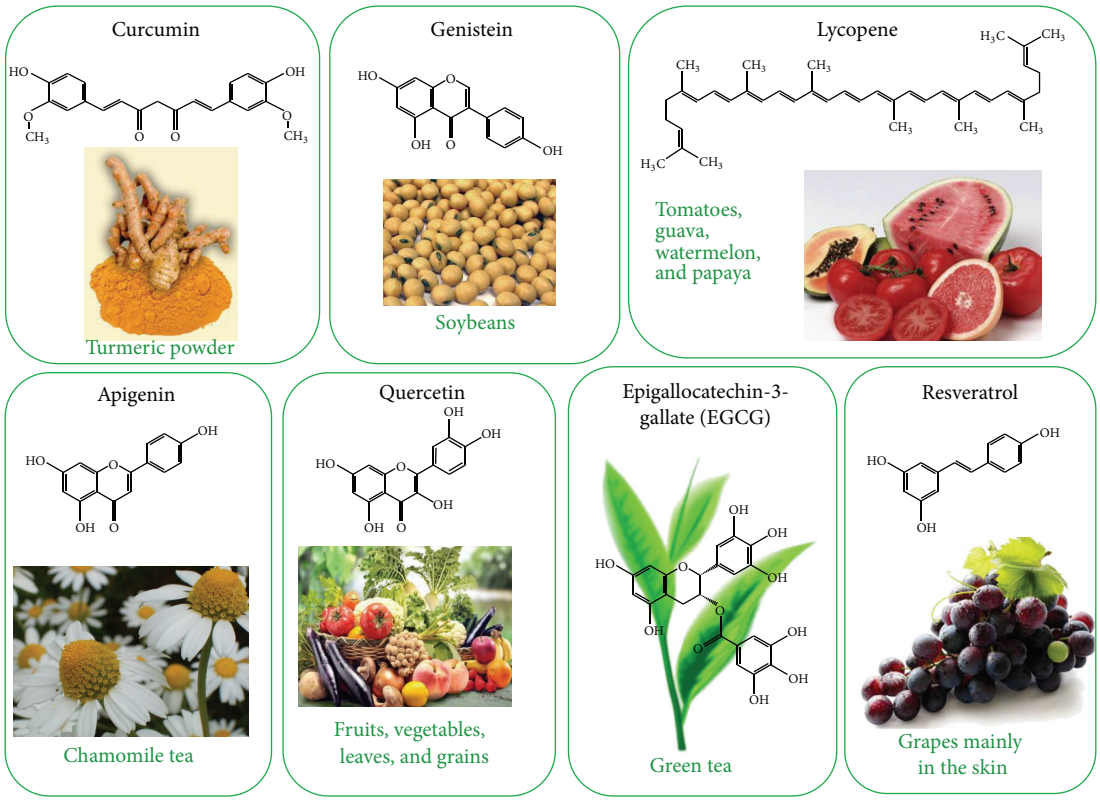


Figure 9: Natural product derived phytochemicals with anticancer activity perturbing IGF signalling pathways.

Table 7: Natural products perturbing IGF signalling pathways.

Active phytochemicals	Natural source	Mode of action	Molecular target
Curcumin [225, 226]	*Curcuma longa (turmeric powder)*	Antiproliferation, anticarcinogenesis, cell cycle arrest, apoptosis, and antiangiogenesis	IGF-1R
Genistein [226]	Soybeans and soy products, red clover (*Trifolium pratense*), and sicilian pistachio (*Pistacia vera*)	Antioxidant, antiproliferation, antiproliferation, anticarcinogenesis, cell cycle arrest, apoptosis, antiangiogenesis, and anti-inflammation	IGF-1R
Lycopene [226]	Tomatoes, guava, rosehip, watermelon, papaya, apricot, and pink grapefruit; most abundant in red tomatoes	Antioxidant, antiproliferation (growth inhibition, cell cycle arrest, and apoptosis), antiangiogenesis, anti-inflammation, and immunomodulator	IGFBP-3
Apigenin [227]	Fruits and vegetables, including oranges, grapefruits, parsley, celery, onions, wheat sprouts, cereals of millet and wheat, and in some seasonings, such as coriander, marjoram, oregano, rosemary, tarragon, and chamomile tea	Inhibit cellular proliferation, suppress tumorigenesis and angiogenesis, and induce apoptosis	IGF axis and its intracellular signalling in prostate cancer
Quercetin [228]	Fruits, vegetables, leaves, and grains	Inhibits the proliferation and induces apoptosis of cancer cells	IGFIR
Epigallocatechin-3-gallate [229]	Green tea	Inhibits angiogenesis	Inhibitory effects on IGF-I-induced VEGF expression
Resveratrol [225]	Grapes (mainly in the skin), mulberries, peanuts, vines, and pines	Antioxidant, antiproliferation, anticarcinogenesis, cell cycle arrest, apoptosis, antiangiogenesis, and anti-inflammation	Suppression of IGF-1R/Akt/Wnt signalling pathways

lacking both proteolysis and matrix-binding activities may be effective for the treatment of cancers in the future.

While IGF receptors seem to be the most favourite targets in the IGF-axis in relation to cancer, the drawbacks and challenges in achieving this seem to add further complexity because of the cross talks between IGF-R mediated pathways and other growth mediated pathways in cells. Though various TKIs against IGF-1Rs seem to be in clinical trial, specificity and concentrations can be well documented in vitro while their extent of in vivo roles seems to be a question mark considering the variation in concentration among different tissues and toxicity could be another issue. Anti-IGFR antibodies are advantageous over TKIs in this regard while blockage of IGFRs may pressurize the tumour cells to compensate for blockade by increased signalling through alternate receptors (e.g., EGFRs). In some instances, IGF-2 action via the IR-A also promotes resistance to anti-IGF-1R inhibitors. Thus, specific therapeutic combinations can be an answer to this problem.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] D. R. Clemmons, "Value of insulin-like growth factor system markers in the assessment of growth hormone status," *Endocrinology and Metabolism Clinics of North America*, vol. 36, no. 1, pp. 109–129, 2007.

[2] S. M. Firth and R. C. Baxter, "Cellular actions of the insulin-like growth factor binding proteins," *Endocrine Reviews*, vol. 23, no. 6, pp. 824–854, 2002.

[3] L. E. H. Smith, W. Shen, C. Perruzzi et al., "Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor," *Nature Medicine*, vol. 5, no. 12, pp. 1390–1395, 1999.

[4] M. J. Horney, D. W. Shirley, D. T. Kurtz, and S. A. Rosenzweig, "Elevated glucose increases mesangial cell sensitivity to insulin-like growth factor I," *American Journal of Physiology: Renal Physiology*, vol. 274, no. 6, part 2, pp. F1045–F1053, 1998.

[5] M. G. Slomiany and S. A. Rosenzweig, "IGF-1-induced VEGF and IGFBP-3 secretion correlates with increased HIF-1α expression and activity in retinal pigment epithelial cell line D407," *Investigative Ophthalmology & Visual Science*, vol. 45, no. 8, pp. 2838–2847, 2004.

[6] K. T. Flaherty, F. S. Hodi, and D. E. Fisher, "From genes to drugs: targeted strategies for melanoma," *Nature Reviews Cancer*, vol. 12, no. 5, pp. 349–361, 2012.

[7] M. J. Higgins and J. Baselga, "Targeted therapies for breast cancer," *Journal of Clinical Investigation*, vol. 121, no. 10, pp. 3797–3803, 2011.

[8] A. M. Scott, J. D. Wolchok, and L. J. Old, "Antibody therapy of cancer," *Nature Reviews Cancer*, vol. 12, no. 4, pp. 278–287, 2012.

[9] P. Chames, M. van Regenmortel, E. Weiss, and D. Baty, "Therapeutic antibodies: successes, limitations and hopes for the future," *British Journal of Pharmacology*, vol. 157, no. 2, pp. 220–233, 2009.

[10] S. A. Rosenzweig and H. S. Atreya, "Defining the pathway to insulin-like growth factor system targeting in cancer," *Biochemical Pharmacology*, vol. 80, no. 8, pp. 1115–1124, 2010.

[11] H. Werner, G. G. Re, I. A. Drummond et al., "Increased expression of the insulin-like growth factor I receptor gene, IGF1R, in Wilms tumor is correlated with modulation of IGF1R promoter activity by the WT1 Wilms tumor gene product," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 12, pp. 5828–5832, 1993.

[12] A. O. W. Stretton, "The first sequence: fred Sanger and insulin," *Genetics*, vol. 162, no. 2, pp. 527–532, 2002.

[13] Z. Qu, J. C. Chow, P.-R. Ling, T. R. Ziegler, B. R. Bistrian, and R. J. Smith, "Tissue-specific effects of chronic dietary protein restriction and gastrostomy on the insulin-like growth factor-I pathway in the liver and colon of adult rats," *Metabolism: Clinical and Experimental*, vol. 46, no. 6, pp. 691–697, 1997.

[14] W. D. Salmon Jr. and W. H. Daughaday, "A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro," *The Journal of Laboratory and Clinical Medicine*, vol. 49, no. 6, pp. 825–836, 1957.

[15] E. Rinderknecht and R. E. Humbel, "The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin," *The Journal of Biological Chemistry*, vol. 253, no. 8, pp. 2769–2776, 1978.

[16] T. L. Blundell, S. Bedarkar, and R. E. Humbel, "Tertiary structures, receptor binding, and antigenicity of insulinlike growth factors," *Federation Proceedings*, vol. 42, no. 9, pp. 2592–2597, 1983.

[17] J. Wang, J. Zhou, and C. A. Bondy, "Igf1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy," *The FASEB Journal*, vol. 13, no. 14, pp. 1985–1990, 1999.

[18] A. J. D'Ercole, G. T. Applewhite, and L. E. Underwood, "Evidence that somatomedin is synthesized by multiple tissues in the fetus," *Developmental Biology*, vol. 75, no. 2, pp. 315–328, 1980.

[19] C. T. Roberts Jr., S. R. Lasky, W. L. Lowe Jr., W. T. Seaman, and D. LeRoith, "Molecular cloning of rat insulin-like growth factor I complementary deoxyribonucleic acids: differential messenger ribonucleic acid processing and regulation by growth hormone in extrahepatic tissues," *Molecular Endocrinology*, vol. 1, no. 3, pp. 243–248, 1987.

[20] V. K. M. Han, P. K. Lund, D. C. Lee, and A. J. D'Ercole, "Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution," *The Journal of Clinical Endocrinology & Metabolism*, vol. 66, no. 2, pp. 422–429, 1988.

[21] W. L. Lowe Jr., S. R. Lasky, D. LeRoith, and C. T. Roberts Jr., "Distribution and regulation of rat insulin-like growth factor I messenger ribonucleic acids encoding alternative carboxyterminal E-peptides: evidence for differential processing and regulation in liver," *Molecular Endocrinology*, vol. 2, no. 6, pp. 528–535, 1988.

[22] O. G. P. Isaksson, J. O. Jansson, and I. A. M. Gause, "Growth hormone stimulates longitudinal bone growth directly," *Science*, vol. 216, no. 4551, pp. 1237–1239, 1982.

[23] M. N. Pollak, E. S. Schernhammer, and S. E. Hankinson, "Insulin-like growth factors and neoplasia," *Nature Reviews Cancer*, vol. 4, no. 7, pp. 505–518, 2004.

[24] J. Wang, W. Niu, D. P. Witte et al., "Overexpression of insulin-like growth factor-binding protein-4 (IGFBP- 4) in smooth

muscle cells of transgenic mice through a smooth muscle α-actin-IGFBP-4 fusion gene induces smooth muscle hypoplasia," *Endocrinology*, vol. 139, no. 5, pp. 2605–2614, 1998.

[25] P. Ye, J. Carson, and A. J. D'Ercole, "In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice," *The Journal of Neuroscience*, vol. 15, no. 11, pp. 7344–7356, 1995.

[26] A. J. D'Ercole, Z. Dai, Y. Xing et al., "Brain growth retardation due to the expression of human insulin like growth factor binding protein-1 in transgenic mice: an in vivo model for the analysis of igf function in the brain," *Developmental Brain Research*, vol. 82, no. 1-2, pp. 213–222, 1994.

[27] W. Ni, K. Rajkumar, J. I. Nagy, and L. J. Murphy, "Impaired brain development and reduced astrocyte response to injury in transgenic mice expressing IGF binding protein-1," *Brain Research*, vol. 769, no. 1, pp. 97–107, 1997.

[28] D. LeRoith, H. Werner, D. Beitner-Johnson, and C. T. Roberts Jr., "Molecular and cellular aspects of the insulin-like growth factor I receptor," *Endocrine Reviews*, vol. 16, no. 2, pp. 143–163, 1995.

[29] C. E. H. Stewart and P. Rotwein, "Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors," *Physiological Reviews*, vol. 76, no. 4, pp. 1005–1026, 1996.

[30] J. I. Jones and D. R. Clemmons, "Insulin-like growth factors and their binding proteins: biological actions," *Endocrine Reviews*, vol. 16, no. 1, pp. 3–34, 1995.

[31] H. Yu and T. Rohan, "Role of the insulin-like growth factor family in cancer development and progression," *Journal of the National Cancer Institute*, vol. 92, no. 18, pp. 1472–1489, 2000.

[32] L. Sepp-Lorenzino, "Structure and function of the insulin-like growth factor I receptor," *Breast Cancer Research and Treatment*, vol. 47, no. 3, pp. 235–253, 1998.

[33] G. Steele-Perkins, J. Turner, J. C. Edman et al., "Expression and characterization of a functional human insulin-like growth factor I receptor," *The Journal of Biological Chemistry*, vol. 263, no. 23, pp. 11486–11492, 1988.

[34] A. L. Frattali and J. E. Pessin, "Relationship between α subunit ligand occupancy and β subunit autophosphorylation in insulin/insulin-like growth factor-1 hybrid receptors," *The Journal of Biological Chemistry*, vol. 268, no. 10, pp. 7393–7400, 1993.

[35] D. Chrysis, A. S. Calikoglu, P. Ye, and A. J. D'Ercole, "Insulin-like growth factor-I overexpression attenuates cerebellar apoptosis by altering the expression of Bcl family proteins in a developmentally specific manner," *The Journal of Neuroscience*, vol. 21, no. 5, pp. 1481–1489, 2001.

[36] K. D. Dentremont, P. Ye, A. J. D'Ercole, and J. R. O'Kusky, "Increased insulin-like growth factor-I (IGF-I)expression during early postnatal development differentially increases neuron number and growth in medullary nuclei of the mouse," *Developmental Brain Research*, vol. 114, no. 1, pp. 135–141, 1999.

[37] G. Zhao, M.-C. Monier-Faugere, M. C. Langub et al., "Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation," *Endocrinology*, vol. 141, no. 7, pp. 2674–2682, 2000.

[38] K. Reiss, W. Cheng, A. Ferber et al., "Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 16, pp. 8630–8635, 1996.

[39] M. E. Coleman, F. Demayo, R. Geske, C. Montgomery, and R. J. Schwartz, "Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice," *The Journal of Biological Chemistry*, vol. 270, no. 20, pp. 12109–12116, 1995.

[40] A. J. Oates, L. M. Schumaker, S. B. Jenkins et al., "The mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R), a putative breast tumor suppressor gene," *Breast Cancer Research and Treatment*, vol. 47, no. 3, pp. 269–281, 1998.

[41] D. O. Morgan, J. C. Edman, D. N. Standring et al., "Insulin-like growth factor II receptor as a multifunctional binding protein," *Nature*, vol. 329, no. 6137, pp. 301–307, 1987.

[42] A. Oshima, C. M. Nolan, J. W. Kyle, J. H. Grubb, and W. S. Sly, "The human cation-independent mannose 6-phosphate receptor. Cloning and sequence of the full-length cDNA and expression of functional receptor in cos cells," *The Journal of Biological Chemistry*, vol. 263, no. 5, pp. 2553–2562, 1988.

[43] T. Braulke, "Type-2 IGF receptor: a multi-ligand binding protein," *Hormone and Metabolic Research*, vol. 31, no. 2-3, pp. 242–246, 1999.

[44] Y. Yang, A. Hoeflich, O. Butenandt, and W. Kiess, "Opposite regulation of IGF-I and IGF-I receptor mRNA and concomitant changes of GH receptor and IGF-II/M6P receptor mRNA in human IM-9 lymphoblasts," *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research*, vol. 1310, no. 3, pp. 317–324, 1996.

[45] C. Hernández-Sánchez, H. Werner, C. T. Roberts Jr. et al., "Differential regulation of insulin-like growth factor-I (IGF-I) receptor gene expression by IGF-I and basic fibroblastic growth factor," *The Journal of Biological Chemistry*, vol. 272, no. 8, pp. 4663–4670, 1997.

[46] S. M. Rosenthal, E. J. Brown, A. Brunetti, and I. D. Goldfine, "Fibroblast growth factor inhibits insulin-like growth factor-II (IGF-II) gene expression and increases IGF-I receptor abundance in BC3H-1 muscle cells," *Molecular Endocrinology*, vol. 5, no. 5, pp. 678–684, 1991.

[47] M. Rubini, H. Werner, E. Gandini, C. T. Roberts Jr., D. Leroith, and R. Baserga, "Platelet-derived growth factor increases the activity of the promoter of the insulin-like growth factor-1 (IGF-1) receptor gene," *Experimental Cell Research*, vol. 211, no. 2, pp. 374–379, 1994.

[48] H. Werner, "Dysregulation of the Type 1 IGF receptor as a paradigm in tumor progression," *Molecular and Cellular Endocrinology*, vol. 141, no. 1-2, pp. 1–5, 1998.

[49] H. Werner, E. Karnieli, F. J. Rauscher III, and D. LeRoith, "Wild-type and mutant p53 differentially regulate transcription of the insulin-like growth factor I receptor gene," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 16, pp. 8318–8323, 1996.

[50] M. Prisco, A. Hongo, M. G. Rizzo, A. Sacchi, and R. Baserga, "The insulin-like growth factor I receptor as a physiologically relevant target of p53 in apoptosis caused by interleukin-3 withdrawal," *Molecular and Cellular Biology*, vol. 17, no. 3, pp. 1084–1092, 1997.

[51] J. P. Thissen, J. M. Ketelslegers, and L. E. Underwood, "Nutritional regulation of the insulin-like growth factors," *Endocrine Reviews*, vol. 15, no. 1, pp. 80–101, 1994.

[52] W. Zhang, W. H. Thornton Jr., and R. S. MacDonald, "Insulin-like growth factor-I and II receptor expression in rat colon mucosa are affected by dietary lipid intake," *Journal of Nutrition*, vol. 128, no. 2, pp. 158–165, 1998.

[53] A. Hoeflich, Y. Yang, W. Rascher et al., "Coordinate expression of insulin-like growth factor II (IGF-II) and IGP-II/mannose-6-phosphate receptor mRNA and stable expression of IGF-I receptor mRNA during differentiation of human colon carcinoma cells (Caco-2)," *European Journal of Endocrinology*, vol. 135, no. 1, pp. 49–59, 1996.

[54] M. Takigawa, T. Okawa, H.-O. Pan et al., "Insulin-like growth factors I and II are autocrine factors in stimulating proteoglycan synthesis, a marker of differentiated chondrocytes, acting through their respective receptors on a clonal human chondrosarcoma-derived chondrocyte cell line, HCS-2/8," *Endocrinology*, vol. 138, no. 10, pp. 4390–4400, 1997.

[55] W. Kiess, L. A. Greenstein, R. M. White, L. Lee, M. M. Rechler, and S. P. Nissley, "Type II insulin-like growth factor receptor is present in rat serum," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 21, pp. 7720–7724, 1987.

[56] R. G. MacDonald, M. A. Tepper, K. B. Clairmont, S. B. Perregaux, and M. P. Czech, "Serum form of the rat insulin-like growth factor II/mannose 6-phosphate receptor is truncated in the carboxyl-terminal domain," *The Journal of Biological Chemistry*, vol. 264, no. 6, pp. 3256–3261, 1989.

[57] K. J. Valenzano, J. Remmler, and P. Lobel, "Soluble insulin-like growth factor II/mannose 6-phosphate receptor carries multiple high molecular weight forms of insulin-like growth factor II in fetal bovine serum," *The Journal of Biological Chemistry*, vol. 270, no. 27, pp. 16441–16448, 1995.

[58] S. Zaina and S. Squire, "The soluble type 2 insulin-like growth factor (IGF-II) receptor reduces organ size by IGF-II-mediated and IGF-II-independent mechanisms," *The Journal of Biological Chemistry*, vol. 273, no. 44, pp. 28610–28616, 1998.

[59] Y. Xu, A. Papageorgiou, and C. Polychronakos, "Developmental regulation of the soluble form of insulin-like growth factor-II/mannose 6-phosphate receptor in human serum and amniotic fluid," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 2, pp. 437–442, 1998.

[60] M. Costello, R. C. Baxter, and C. D. Scott, "Regulation of soluble insulin-like growth factor II/mannose 6-phosphate receptor in human serum: measurement by enzyme-linked immunosorbent assay," *The Journal of Clinical Endocrinology & Metabolism*, vol. 84, no. 2, pp. 611–617, 1999.

[61] J. Zapf, M. Waldvogel, and E. R. Froesch, "Binding of nonsuppressible insulinlike activity to human serum: evidence for a carrier protein," *Archives of Biochemistry and Biophysics*, vol. 168, no. 2, pp. 638–645, 1975.

[62] R. L. Hintz and F. Liu, "Demonstration of specific plasma protein binding sites for somatomedin," *The Journal of Clinical Endocrinology and Metabolism*, vol. 45, no. 5, pp. 988–995, 1977.

[63] M. M. Rechler and A. L. Brown, "Insulin-like growth factor binding proteins: gene structure and expression," *Growth Regulation*, vol. 2, no. 2, pp. 55–68, 1992.

[64] L. A. Bach and M. M. Rechler, "Insulin-like growth factor binding proteins," *Diabetes Reviews*, vol. 3, no. 1, pp. 38–61, 1995.

[65] G. M. Neumann and L. A. Bach, "The N-terminal disulfide linkages of human insulin-like growth factor- binding protein-6 (hIGFBP-6) and hIGFBP-1 are different as determined by mass spectrometry," *The Journal of Biological Chemistry*, vol. 274, no. 21, pp. 14587–14594, 1999.

[66] R. Durai, M. Davies, W. Yang et al., "Biology of insulin-like growth factor binding protein-4 and its role in cancer (review)," *International Journal of Oncology*, vol. 28, no. 6, pp. 1317–1325, 2006.

[67] B. Kübler, S. Cowell, J. Zapf, and T. Braulke, "Proteolysis of insulin-like growth factor binding proteins by a novel 50-kilodalton metalloproteinase in human pregnancy serum," *Endocrinology*, vol. 139, no. 4, pp. 1556–1563, 1998.

[68] J. C. Chen, Z. M. Shao, M. S. Sheikh et al., "Insulin-like growth factor-binding protein enhancement of insulin-like growth factor-I (IGF-I)-mediated DNA synthesis and IGF-I binding in a human breast carcinoma cell line," *Journal of Cellular Physiology*, vol. 158, no. 1, pp. 69–78, 1994.

[69] P. M. Yamada and K.-W. Lee, "Perspectives in mammalian IGFBP-3 biology: local vs. systemic action," *American Journal of Physiology—Cell Physiology*, vol. 296, no. 5, pp. C954–C976, 2009.

[70] D. Romero, C. O'Neill, A. Terzic et al., "Endoglin regulates cancer-stromal cell interactions in prostate tumors," *Cancer Research*, vol. 71, no. 10, pp. 3482–3493, 2011.

[71] R. Rajah, L. Katz, S. Nunn, P. Solberg, T. Beers, and P. Cohen, "Insulin-like growth factor binding protein (IGFBP) proteases: functional regulators of cell growth," *Cytokine and Growth Factor Reviews*, vol. 6, no. 2–4, pp. 273–284, 1995.

[72] M. Takaoka, H. Harada, C. D. Andl et al., "Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3," *Cancer Research*, vol. 64, no. 21, pp. 7711–7723, 2004.

[73] J. L. Fowlkes, "Insulinlike growth factor-binding protein proteolysis: an emerging paradigm in insulinlike growth factor physiology," *Trends in Endocrinology and Metabolism*, vol. 8, no. 8, pp. 299–306, 1997.

[74] J. L. Fowlkes, J. J. Enghild, K. Suzuki, and H. Nagase, "Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures," *Journal of Biological Chemistry*, vol. 269, no. 41, pp. 25742–25746, 1994.

[75] R. P. Verma and C. Hansch, "Matrix metalloproteinases (MMPs): chemical-biological functions and (Q)SARs," *Bioorganic & Medicinal Chemistry*, vol. 15, no. 6, pp. 2223–2268, 2007.

[76] J. L. Fowlkes, K. M. Thrailkill, D. M. Serra, K. Suzuki, and H. Nagase, "Matrix metalloproteinases as insulin-like growth factor binding protein-degrading proteinases," *Cytokine and Growth Factor Reviews*, vol. 6, no. 2–4, pp. 255–263, 1995.

[77] H. A. Coppock, A. White, J. D. Aplin, and M. Westwood, "Matrix metalloprotease-3 and -9 proteolyze insulin-like growth factor-binding protein-1," *Biology of Reproduction*, vol. 71, no. 2, pp. 438–443, 2004.

[78] R. Rajah, R. V. Nachajon, M. H. Collins, H. Hakonarson, M. M. Grunstein, and P. Cohen, "Elevated levels of the IGF-binding protein protease MMP-1 in asthmatic airway smooth muscle," *The American Journal of Respiratory Cell and Molecular Biology*, vol. 20, no. 2, pp. 199–208, 1999.

[79] C. A. Conover, G. F. Faessen, K. E. Ilg et al., "Pregnancy-associated plasma protein-A is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum," *Endocrinology*, vol. 142, no. 5, pp. 2155–2158, 2001.

[80] S. Mark, B. Kübler, S. Höning et al., "Diversity of human insulin-like growth factor (IGF) binding protein-2 fragments in plasma: primary structure, IGF-binding properties, and disulfide bonding pattern," *Biochemistry*, vol. 44, no. 9, pp. 3644–3652, 2005.

[81] M. Zhang, E. P. Smith, H. Kuroda, W. Banach, S. D. Chernausek, and J. A. Fagin, "Targeted expression of a protease-resistant

IGFBP-4 mutant in smooth muscle of transgenic mice results in IGFBP-4 stabilization and smooth muscle hypotrophy," *The Journal of Biological Chemistry*, vol. 277, no. 24, pp. 21285–21290, 2002.

[82] L. A. Bach, P. Fu, and Z. Yang, "Insulin-like growth factor-binding protein-6 and cancer," *Clinical Science*, vol. 124, no. 4, pp. 215–229, 2013.

[83] A. J. Ryan, S. Napoletano, P. A. Fitzpatrick, C. A. Currid, N. C. O'Sullivan, and J. H. Harmey, "Expression of a protease-resistant insulin-like growth factor-binding protein-4 inhibits tumour growth in a murine model of breast cancer," *British Journal of Cancer*, vol. 101, no. 2, pp. 278–286, 2009.

[84] C.-L. Soh, K. McNeil, C. M. Owczarek et al., "Exogenous administration of protease-resistant, non-matrix-binding IGFBP-2 inhibits tumour growth in a murine model of breast cancer," *British Journal of Cancer*, vol. 110, no. 12, pp. 2855–2864, 2014.

[85] Y. Oh, S. R. Nagalla, Y. Yamanaka, H.-S. Kim, E. Wilson, and R. G. Rosenfeld, "Synthesis and characterization of insulin-like growth factor-binding protein (IGFBP)-7: recombinant human mac25 protein specifically binds IGF-I and -II," *The Journal of Biological Chemistry*, vol. 271, no. 48, pp. 30322–30325, 1996.

[86] M. R. Corkins, J. A. Vanderhoof, D. H. Slentz, R. G. MacDonald, and J. H. Y. Park, "Growth stimulation by transfection of intestinal epithelial cells with an antisense insulin-like growth factor binding protein-2 construct," *Biochemical and Biophysical Research Communications*, vol. 211, no. 3, pp. 707–713, 1995.

[87] O. M. Casey, R. Fitzpatrick, J. O. McInerney, D. G. Morris, R. Powell, and J. M. Sreenan, "Analysis of gene expression in the bovine corpus luteum through generation and characterisation of 960 ESTs," *Biochimica et Biophysica Acta: Gene Structure and Expression*, vol. 1679, no. 1, pp. 10–17, 2004.

[88] F. Domínguez, S. Avila, A. Cervero et al., "A combined approach for gene discovery identifies insulin-like growth factor-binding protein-related protein I as a new gene implicated in human endometrial receptivity," *The Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 4, pp. 1849–1857, 2003.

[89] S.-A. Wandji, J. E. Gadsby, J. A. Barber, and J. M. Hammond, "Messenger ribonucleic acids for mac25 and connective tissue growth factor (CTGF) are inversely regulated during folliculogenesis and early luteogenesis," *Endocrinology*, vol. 141, no. 7, pp. 2648–2657, 2000.

[90] C. C. Sprenger, M. E. Vail, K. Evans, J. Simurdak, and S. R. Plymate, "Over-expression of insulin-like growth factor binding protein-related protein-1(IGFBP-rP1/mac25) in the M12 prostate cancer cell line alters tumor growth by a delay in G1 and cyclin A associated apoptosis," *Oncogene*, vol. 21, no. 1, pp. 140–147, 2002.

[91] C. Rupp, M. Scherzer, A. Rudisch et al., "IGFBP7, a novel tumor stroma marker, with growth-promoting effects in colon cancer through a paracrine tumor-stroma interaction," *Oncogene*, 2014.

[92] S. Zhu, F. Xu, J. Zhang, W. Ruan, and M. Lai, "Insulin-like growth factor binding protein-related protein 1 and cancer," *Clinica Chimica Acta*, vol. 431, pp. 23–32, 2014.

[93] W. Jiang, C. Xiang, S. Cazacu, C. Brodie, and T. Mikkelsen, "Insulin-like growth factor binding protein 7 mediates glioma cell growth and migration," *Neoplasia*, vol. 10, no. 12, pp. 1335–1342, 2008.

[94] V. Evdokimova, C. E. Tognon, T. Benatar et al., "IGFBP7 binds to the IGF-1 receptor and blocks its activation by insulin-like growth factors," *Science Signaling*, vol. 5, no. 255, article ra92, 2012.

[95] T. Fukushima and H. Kataoka, "Roles of insulin-like growth factor binding protein-2 (IGFBP-2) in glioblastoma," *Anticancer Research*, vol. 27, no. 6, pp. 3685–3692, 2007.

[96] F. E. Carrick, B. E. Forbes, and J. C. Wallace, "BIAcore analysis of bovine insulin-like growth factor (IGF)-binding protein-2 identifies major IGF binding site determinants in both the amino- and carboxyl-terminal domains," *The Journal of Biological Chemistry*, vol. 276, no. 29, pp. 27120–27128, 2001.

[97] G. D. Hobba, A. Löthgren, E. Holmberg, B. E. Forbes, G. L. Francis, and J. C. Wallace, "Alanine screening mutagenesis establishes tyrosine 60 of bovine insulin-like growth factor binding protein-2 as a determinant of insulin-like growth factor binding," *The Journal of Biological Chemistry*, vol. 273, no. 31, pp. 19691–19698, 1998.

[98] G. D. Hobba, B. E. Forbes, E. J. Parkinson, G. L. Francis, and J. C. Wallace, "The insulin-like growth factor (IGF) binding site of bovine insulin-like growth factor binding protein-2 (biGFBP-2) probed by iodination," *The Journal of Biological Chemistry*, vol. 271, no. 48, pp. 30529–30536, 1996.

[99] B. E. Forbes, D. Turner, S. J. Hodge, K. A. McNeil, G. Forsberg, and J. C. Wallace, "Localization of an insulin-like growth factor (IGF) binding site of bovine IGF binding protein-2 using disulfide mapping and deletion mutation analysis of the C-terminal domain," *The Journal of Biological Chemistry*, vol. 273, no. 8, pp. 4647–4652, 1998.

[100] M. M. Kibbey, M. J. Jameson, E. M. Eaton, and S. A. Rosenzweig, "Insulin-like growth factor binding protein-2: contributions of the C-terminal domain to insulin-like growth factor-1 binding," *Molecular Pharmacology*, vol. 69, no. 3, pp. 833–845, 2006.

[101] F. E. Carrick, M. G. Hinds, K. A. McNeil, J. C. Wallace, B. E. Forbes, and R. S. Norton, "Interaction of insulin-like growth factor (IGF)-I and -II with IGF binding protein-2: mapping the binding surfaces by nuclear magnetic resonance," *Journal of Molecular Endocrinology*, vol. 34, no. 3, pp. 685–698, 2005.

[102] Z. Kuang, S. Yao, K. A. McNeil et al., "Cooperativity of the N- and C-terminal domains of insulin-like growth factor (IGF) binding protein 2 in IGF binding," *Biochemistry*, vol. 46, no. 48, pp. 13720–13732, 2007.

[103] B. E. Forbes, P. McCarthy, and R. S. Norton, "Insulin-like growth factor binding proteins: a structural perspective," *Frontiers in Endocrinology*, vol. 3, article 38, 2012.

[104] M. Swain, M. G. Slomiany, S. A. Rosenzweig, and H. S. Atreya, "High-yield bacterial expression and structural characterization of recombinant human insulin-like growth factor binding protein-2," *Archives of Biochemistry and Biophysics*, vol. 501, no. 2, pp. 195–200, 2010.

[105] M. Swain, R. Thirupathi, B. Krishnarjuna et al., "Spontaneous and reversible self-assembly of a polypeptide fragment of insulin-like growth factor binding protein-2 into fluorescent nanotubular structures," *Chemical Communications*, vol. 46, no. 2, pp. 216–218, 2010.

[106] S. Y. Kim, J. A. Toretsky, D. Scher, and L. J. Helman, "The role of IGF-1R in pediatric malignancies," *The Oncologist*, vol. 14, no. 1, pp. 83–91, 2009.

[107] M. M. Chitnis, J. S. P. Yuen, A. S. Protheroe, M. Pollak, and V. M. Macaulay, "The type 1 insulin-like growth factor receptor pathway," *Clinical Cancer Research*, vol. 14, no. 20, pp. 6364–6370, 2008.

[108] K. Takeuchi and F. Ito, "Receptor tyrosine kinases and targeted cancer therapeutics," *Biological and Pharmaceutical Bulletin*, vol. 34, no. 12, pp. 1774–1780, 2011.

[109] R. Baserga, F. Peruzzi, and K. Reiss, "The IGF-1 receptor in cancer biology," *International Journal of Cancer*, vol. 107, no. 6, pp. 873–877, 2003.

[110] A. G. Renehan, M. Zwahlen, C. Minder, S. T. O'Dwyer, S. M. Shalet, and M. Egger, "Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis," *The Lancet*, vol. 363, no. 9418, pp. 1346–1353, 2004.

[111] H. Cui, M. Cruz-Correa, F. M. Giardiello et al., "Loss of IGF2 imprinting: a potential marker of colorectal cancer risk," *Science*, vol. 299, no. 5613, pp. 1753–1755, 2003.

[112] G. E. Krassas, N. Pontikides, T. Kaltsas et al., "Free and total insulin-like growth factor (IGF)-I, -II, and IGF binding protein-1, -2, and -3 serum levels in patients with active thyroid eye disease," *The Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 1, pp. 132–135, 2003.

[113] P. Vorwerk, H. Wex, C. Bessert, B. Hohmann, U. Schmidt, and U. Mittler, "Loss of imprinting of IGF-II gene in children with acute lymphoblastic leukemia," *Leukemia Research*, vol. 27, no. 9, pp. 807–812, 2003.

[114] C. M. Coutinho-Camillo, M. M. Brentani, O. Butugan, H. Torloni, and M. A. Nagai, "Relaxation of imprinting of IGFII gene in juvenile nasopharyngeal angiofibromas," *Diagnostic Molecular Pathology*, vol. 12, no. 1, pp. 57–62, 2003.

[115] O. Ogawa, D. M. Becroft, I. M. Morison et al., "Constitutional relaxation of insulin-like growth factor II gene imprinting associated with Wilms' tumour and gigantism," *Nature Genetics*, vol. 5, no. 4, pp. 408–412, 1993.

[116] J. Zhang, J. M. Trent, and P. S. Meltzer, "Rapid isolation and characterization of amplified DNA by chromosome microdissection: Identification of IGF1R amplification in malignant melanoma," *Oncogene*, vol. 8, no. 10, pp. 2827–2831, 1993.

[117] G. Armengol, S. Knuutila, F. Lluís, G. Capellà, R. Miró, and M. R. Caballín, "DNA copy number changes and evaluation of MYC, IGF1R, and FES amplification in xenografts of pancreatic adenocarcinoma," *Cancer Genetics and Cytogenetics*, vol. 116, no. 2, pp. 133–141, 2000.

[118] A. Almeida, M. Muleris, B. Dutrillaux, and B. Malfoy, "The insulin-like growth factor I receptor gene is the target for the 15q26 amplicon in breast cancer," *Genes Chromosomes and Cancer*, vol. 11, no. 1, pp. 63–65, 1994.

[119] A. Belfiore, "The role of insulin receptor isoforms and hybrid insulin/IGF-I receptors in human cancer," *Current Pharmaceutical Design*, vol. 13, no. 7, pp. 671–686, 2007.

[120] E. A. Bohula, M. P. Playford, and V. M. Macaulay, "Targeting the type 1 insulin-like growth factor receptor as anti-cancer treatment," *Anti-Cancer Drugs*, vol. 14, no. 9, pp. 669–682, 2003.

[121] J. S. P. Yuen and V. M. Macaulay, "Targeting the type 1 insulin-like growth factor receptor as a treatment for cancer," *Expert Opinion on Therapeutic Targets*, vol. 12, no. 5, pp. 589–603, 2008.

[122] E. Surmacz, "Growth factor receptors as therapeutic targets: Strategies to inhibit the insulin-like growth factor I receptor," *Oncogene*, vol. 22, no. 42, pp. 6589–6597, 2003.

[123] I. Singh, H. Amin, B. Rah, and A. Goswami, "Targeting EGFR and IGF 1R: a promising combination therapy for metastatic cancer," *Frontiers in Bioscience—Scholar*, vol. 5, no. 1, pp. 231–246, 2013.

[124] L. Mohanraj and Y. Oh, "Targeting IGF-I, IGFBPs and IGF-I receptor system in cancer: the current and future in breast cancer therapy," *Recent Patents on Anti-Cancer Drug Discovery*, vol. 6, no. 2, pp. 166–177, 2011.

[125] J. Riedemann and V. M. Macaulay, "IGF1R signalling and its inhibition," *Endocrine-Related Cancer*, vol. 13, no. 1, pp. S33–S43, 2006.

[126] M. Pollak, "The insulin receptor/insulin-like growth factor receptor family as a therapeutic target in oncology," *Clinical Cancer Research*, vol. 18, no. 1, pp. 40–50, 2012.

[127] F. I. Arnaldez and L. J. Helman, "Targeting the insulin growth factor receptor 1," *Hematology/Oncology Clinics of North America*, vol. 26, no. 3, pp. 527–542, 2012.

[128] A. Arcaro, "Targeting the insulin-like growth factor-1 receptor in human cancer," *Frontiers in Pharmacology*, vol. 4, article 30, 2013.

[129] D. Yee, "Insulin-like growth factor receptor inhibitors: baby or the bathwater?" *Journal of the National Cancer Institute*, vol. 104, no. 13, pp. 975–981, 2012.

[130] V. M. Macaulay, "The IGF receptor as anticancer treatment target," *Novartis Foundation Symposium*, vol. 262, pp. 235–246, 2004.

[131] Y. Adachi, C. T. Lee, and D. P. Carbone, "Genetic blockade of the insulin-like growth factor 1 receptor for human malignancy," *Novartis Foundation Symposium*, vol. 262, pp. 177–268, 2004.

[132] Y. Adachi, C. Lee, K. Coffee et al., "Effects of genetic blockade of the insulin-like growth factor receptor in human colon cancer cell lines," *Gastroenterology*, vol. 123, no. 4, pp. 1191–1204, 2002.

[133] C. Liu, Z. Zhang, H. Tang, Z. Jiang, L. You, and Y. Liao, "Crosstalk between IGF-1R and other Tumor Promoting Pathways," *Current Pharmaceutical Design*, vol. 20, no. 17, pp. 2912–2921, 2014.

[134] M. Xue, X. Cao, Y. Zhong et al., "Insulin-like growth factor-1 receptor (IGF-1R) kinase inhibitors in cancer therapy: advances and perspectives," *Current Pharmaceutical Design*, vol. 18, no. 20, pp. 2901–2913, 2012.

[135] J. van der Veeken, S. Oliveira, R. M. Schiffelers, G. Storm, P. M. P. Van Bergen En Henegouwen, and R. C. Roovers, "Crosstalk between epidermal growth factor receptor- and insulin-like growth factor-1 receptor signaling: Implications for cancer therapy," *Current Cancer Drug Targets*, vol. 9, no. 6, pp. 748–760, 2009.

[136] M. Mancini, M. B. Gariboldi, E. Taiana et al., "Co-targeting the IGF system and HIF-1 inhibits migration and invasion by (triple-negative) breast cancer cells," *British Journal of Cancer*, vol. 110, no. 12, pp. 2865–2873, 2014.

[137] A. T. de Souza, G. R. Hankins, M. K. Washington, T. C. Orton, and R. L. Jirtle, "M6P/IGF2R gene is mutated in human hepatocellular carcinomas with loss of heterozygosity," *Nature Genetics*, vol. 11, no. 4, pp. 447–449, 1995.

[138] Y. Myal, R. P. C. Shiu, B. Bhaumick, and M. Bala, "Receptor binding and growth-promoting activity of insulin-like growth factors in human breast cancer cells (T-47D) in culture," *Cancer Research*, vol. 44, no. 12, part 1, pp. 5486–5490, 1984.

[139] S. E. Hankinson, W. C. Willett, G. A. Colditz et al., "Circulating concentrations of insulin-like growth factor-I and risk of breast cancer," *The Lancet*, vol. 351, no. 9113, pp. 1393–1396, 1998.

[140] H. Yu, M. R. Spitz, J. Mistry, J. Gu, W. K. Hong, and X. Wu, "Plasma levels of insulin-like growth factor-I and lung cancer risk: a case-control analysis," *Journal of the National Cancer Institute*, vol. 91, no. 2, pp. 151–156, 1999.

[141] J. Ma, M. N. Pollak, E. Giovannucci et al., "Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3," *Journal of the National Cancer Institute*, vol. 91, no. 7, pp. 620–625, 1999.

[142] R. Shi, H. J. Berkel, and H. Yu, "Insulin-like growth factor-I and prostate cancer: a meta-analysis," *British Journal of Cancer*, vol. 85, no. 7, pp. 991–996, 2001.

[143] J. M. Chan, M. J. Stampfer, E. Giovannucci et al., "Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study," *Science*, vol. 279, no. 5350, pp. 563–566, 1998.

[144] Y. Wu, K. Cui, K. Miyoshi et al., "Reduced circulating insulin-like growth factor I levels delay the onset of chemically and genetically induced mammary tumors," *Cancer Research*, vol. 63, no. 15, pp. 4384–4388, 2003.

[145] N. Majeed, M.-J. Blouin, P. J. Kaplan-Lefko et al., "A germ line mutation that delays prostate cancer progression and prolongs survival in a murine prostate cancer model," *Oncogene*, vol. 24, no. 29, pp. 4736–4740, 2005.

[146] M. Pollak, "Insulin, insulin-like growth factors and neoplasia," *Best Practice and Research: Clinical Endocrinology and Metabolism*, vol. 22, no. 4, pp. 625–638, 2008.

[147] J. Gao, J. W. Chesebrough, S. A. Cartlidge et al., "Dual IGF-I/II-neutralizing antibody MEDI-573 potently inhibits IGF signaling and tumor growth," *Cancer Research*, vol. 71, no. 3, pp. 1029–1040, 2011.

[148] G. T. Tindall, S. S. Ambrose, J. H. Christy, and J. M. Patton, "Hypophysectomy in the treatment of disseminated carcinoma of the breast and prostate gland," *Southern Medical Journal*, vol. 69, no. 5, pp. 579–587, 1976.

[149] A. Grimberg, "p53 and IGFBP-3: apoptosis and cancer protection," *Molecular Genetics and Metabolism*, vol. 70, no. 2, pp. 85–98, 2000.

[150] M. J. Moreno, M. Ball, M. Rukhlova et al., "IGFBP-4 anti-angiogenic and anti-tumorigenic effects are associated with anti-cathepsin B activity," *Neoplasia*, vol. 15, no. 5, pp. 554–567, 2013.

[151] A. Grimberg and P. Cohen, "Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis," *Journal of Cellular Physiology*, vol. 183, no. 1, pp. 1–9, 2000.

[152] T. Nickerson, H. Miyake, M. E. Gleave, and M. Pollak, "Castration-induced apoptosis of androgen-dependent Shionogi carcinoma is associated with increased expression of genes encoding insulin-like growth factor-binding proteins," *Cancer Research*, vol. 59, no. 14, pp. 3392–3395, 1999.

[153] T. Nickerson and M. Pollak, "Bicalutamide (Casodex)-induced prostate regression involves increased expression of genes encoding insulin-like growth factor binding proteins," *Urology*, vol. 54, no. 6, pp. 1120–1125, 1999.

[154] R. C. Baxter, "IGF binding proteins in cancer: mechanistic and clinical insights," *Nature Reviews Cancer*, vol. 14, no. 5, pp. 329–341, 2014.

[155] J. I. Jones, A. Joseph D'Ercole, C. Camacho-Hubner, and D. R. Clemmons, "Phosphorylation of insulin-like growth factor (IGF)-binding protein 1 in cell culture and in vivo: effects on affinity for IGF-I," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 17, pp. 7481–7485, 1991.

[156] J. I. Leu, M. A. S. Crissey, and R. Taub, "Massive hepatic apoptosis associated with TGF-β1 activation after Fas ligand treatment of IGF binding protein-1-deficient mice," *The Journal of Clinical Investigation*, vol. 111, no. 1, pp. 129–139, 2003.

[157] D. Yee, J. G. Jackson, T. W. Kozelsky, and J. A. Figueroa, "Insulin-like growth factor binding protein 1 expression inhibits insulin-like growth factor I action in MCF-7 breast cancer cells," *Cell Growth & Differentiation*, vol. 5, no. 1, pp. 73–77, 1994.

[158] D. Diehl, E. Hessel, D. Oesterle et al., "IGFBP-2 overexpression reduces the appearance of dysplastic aberrant crypt foci and inhibits growth of adenomas in chemically induced colorectal carcinogenesis," *International Journal of Cancer*, vol. 124, no. 9, pp. 2220–2225, 2009.

[159] A. Hoeflich, R. Reisinger, H. Lahm et al., "Insulin-like growth factor-binding protein 2 in tumorigenesis: protector or promoter?" *Cancer Research*, vol. 61, no. 24, pp. 8601–8610, 2001.

[160] V. C. Russo, B. S. Schütt, E. Andaloro et al., "Insulin-like growth factor binding protein-2 binding to extracellular matrix plays a critical role in neuroblastoma cell proliferation, migration, and invasion," *Endocrinology*, vol. 146, no. 10, pp. 4445–4455, 2005.

[161] S. W. Song, G. N. Fuller, A. Khan et al., "IIp45, an insulin-like growth factor binding protein 2 (IGFBP-2) binding protein, antagonizes IGFBP-2 stimulation of glioma cell invasion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 2, pp. 13970–13975, 2003.

[162] G. K. Wang, L. Hu, G. N. Fuller, and W. Zhang, "An interaction between insulin-like growth factor-binding protein 2 (IGFBP2) and integrin α5 is essential for IGFBP2-induced cell mobility," *The Journal of Biological Chemistry*, vol. 281, no. 20, pp. 14085–14091, 2006.

[163] J. S. M. de Mellow and R. C. Baxter, "Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-I-stimulated DNA synthesis in human skin fibroblasts," *Biochemical and Biophysical Research Communications*, vol. 156, no. 1, pp. 199–204, 1988.

[164] W. F. Blum, E. W. Jenne, F. Reppin, K. Kietzmann, M. B. Ranke, and J. R. Bierich, "Insulin-like growth factor I (IGF-I)-binding protein complex is a better mitogen than free IGF-I," *Endocrinology*, vol. 125, no. 2, pp. 766–772, 1989.

[165] J. L. Martin, M. Z. Lin, E. M. McGowan, and R. C. Baxter, "Potentiation of growth factor signaling by insulin-like growth factor-binding protein-3 in breast epithelial cells requires sphingosine kinase activity," *The Journal of Biological Chemistry*, vol. 284, no. 38, pp. 25542–25552, 2009.

[166] S. Y. Heazlewood, R. J. Neaves, B. Williams, D. N. Haylock, T. E. Adams, and S. K. Nilsson, "Megakaryocytes co-localise with hemopoietic stem cells and release cytokines that up-regulate stem cell proliferation," *Stem Cell Research*, vol. 11, no. 2, pp. 782–792, 2013.

[167] C. A. Conover, "Potentiation of insulin-like growth factor (IGF) action by IGF-binding protein-3: studies of underlying mechanism," *Endocrinology*, vol. 130, no. 6, pp. 3191–3199, 1992.

[168] W.-Y. Kim, M.-J. Kim, H. Moon et al., "Differential impacts of insulin-like growth factor-binding protein-3 (IGFBP-3) in epithelial IGF-induced lung cancer development," *Endocrinology*, vol. 152, no. 6, pp. 2164–2173, 2011.

[169] B. Bartling, A. Koch, A. Simm, R. Scheubel, R. E. Silber, and A. N. Santos, "Insulin-like growth factor binding proteins-2 and -4 enhance the migration of human CD34-/CD133+ hematopoietic stem and progenitor cells," *International Journal of Molecular Medicine*, vol. 25, no. 1, pp. 89–96, 2010.

[170] R. A. Mosig, M. Lobl, E. Senturk et al., "IGFBP-4 tumor and serum levels are increased across all stages of epithelial ovarian cancer," *Journal of Ovarian Research*, vol. 5, no. 1, article 3, 2012.

[171] A. J. Butt, K. A. Dickson, F. McDougall, and R. C. Baxter, "Insulin-like growth factor-binding protein-5 inhibits the growth of human breast cancer cells *in vitro* and *in vivo*," *The Journal of Biological Chemistry*, vol. 278, no. 32, pp. 29676–29685, 2003.

[172] B. Tanno, V. Cesi, R. Vitali et al., "Silencing of endogenous IGFBP-5 by micro RNA interference affects proliferation, apoptosis and differentiation of neuroblastoma cells," *Cell Death and Differentiation*, vol. 12, no. 3, pp. 213–223, 2005.

[173] C. McCaig, C. M. Perks, and J. M. P. Holly, "Intrinsic actions of IGFBP-3 and IGFBP-5 on Hs578T breast cancer epithelial cells: inhibition or accentuation of attachment and survival is dependent upon the presence of fibronectin," *Journal of Cell Science*, vol. 115, no. 22, pp. 4293–4303, 2002.

[174] S. K. Johnson and R. S. Haun, "Insulin-like growth factor binding protein-5 influences pancreatic cancer cell growth," *World Journal of Gastroenterology*, vol. 15, no. 27, pp. 3355–3366, 2009.

[175] X. L. Xu, T. C. Lee, N. Offor et al., "Tumor-associated retinal astrocytes promote retinoblastoma cell proliferation through production of IGFBP-5," *The American Journal of Pathology*, vol. 177, no. 1, pp. 424–435, 2010.

[176] A. Sokolović, M. Sokolović, W. Boers, R. P. O. Elferink, and P. J. Bosma, "Insulin-like growth factor binding protein 5 enhances survival of LX2 human hepatic stellate cells," *Fibrogenesis and Tissue Repair*, vol. 3, article 3, 2010.

[177] L. J. Cobb, D. A. M. Salih, I. Gonzalez et al., "Partitioning of IGFBP-5 actions in myogenesis: IGF-independent anti-apoptotic function," *Journal of Cell Science*, vol. 117, part 9, pp. 1737–1746, 2004.

[178] H. Miyake, M. Pollak, and M. E. Gleave, "Castration-induced up-regulation of insulin-like growth factor binding protein-5 potentiates insulin-like growth factor-I activity and accelerates progression to androgen independence in prostate cancer models," *Cancer Research*, vol. 60, no. 11, pp. 3058–3064, 2000.

[179] P. Fu, J. A. Thompson, and L. A. Bach, "Promotion of cancer cell migration: An insulin-like growth factor (IGF)-independent action of IGF-binding protein-6," *Journal of Biological Chemistry*, vol. 282, no. 31, pp. 22298–22306, 2007.

[180] P. Fu, Z. Yang, and L. A. Bach, "Prohibitin-2 binding modulates insulin-like growth factor-binding protein-6 (IGFBP-6)-induced rhabdomyosarcoma cell migration," *The Journal of Biological Chemistry*, vol. 288, no. 41, pp. 29890–29900, 2013.

[181] P. Ye, Y. Xing, Z. Dai, and A. J. D'Ercole, "In vivo actions of insulin-like growth factor-I (IGF-I) on cerebellum development in transgenic mice: evidence that IGF-I increases proliferation of granule cell progenitors," *Developmental Brain Research*, vol. 95, no. 1, pp. 44–54, 1996.

[182] P. Ye and J. D'Ercole, "Insulin-like growth factor I (IGF-I) regulates IGF binding protein-5 gene expression in the brain," *Endocrinology*, vol. 139, no. 1, pp. 65–71, 1998.

[183] J. Wang, W. Niu, Y. Nikiforov et al., "Targeted overexpression of IGF-I evokes distinct patterns of organ remodeling in smooth muscle cell tissue beds of transgenic mice," *The Journal of Clinical Investigation*, vol. 100, no. 6, pp. 1425–1439, 1997.

[184] G. Zhao, R. L. Sutliff, C. S. Weber et al., "Smooth muscle-targeted overexpression of insulin-like growth factor I results in enhanced vascular contractility," *Endocrinology*, vol. 142, no. 2, pp. 623–632, 2001.

[185] B. Zhu, G. Zhao, D. P. Witte, D. Y. Hui, and J. A. Fagin, "Targeted overexpression of IGF-I in smooth muscle cells of transgenic mice enhances neointimal formation through increased proliferation and cell migration after intraarterial injury," *Endocrinology*, vol. 142, no. 8, pp. 3598–3606, 2001.

[186] M. K. Dyck, A. F. Parlow, J.-F. Sénéchal, M.-A. Sirard, and F. Pothier, "Ovarian expression of human insulin-like growth factor-I in transgenic mice results in cyst formation," *Molecular Reproduction and Development*, vol. 59, no. 2, pp. 178–185, 2001.

[187] J. DiGiovanni, K. Kiguchi, A. Frijhoff et al., "Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 7, pp. 3455–3460, 2000.

[188] S. Clément, S. Refetoff, B. Robaye, J. E. Dumont, and S. Schurmans, "Low TSH requirement and goiter in transgenic mice overexpressing IGF-I and IGF-I receptor in the thyroid gland," *Endocrinology*, vol. 142, no. 12, pp. 5131–5139, 2001.

[189] L.-T. Busund, E. Richardsen, R. Busund et al., "Significant expression of IGFBP2 in breast cancer compared with benign lesions," *Journal of Clinical Pathology*, vol. 58, no. 4, pp. 361–366, 2005.

[190] M. L. Neuhouser, E. A. Platz, C. Till et al., "Insulin-like growth factors and insulin-like growth factor-binding proteins and prostate cancer risk: results from the prostate cancer prevention trial," *Cancer Prevention Research*, vol. 6, no. 2, pp. 91–99, 2013.

[191] A. J. Butt, S. M. Firth, M. A. King, and R. C. Baxter, "Insulin-like growth factor-binding protein-3 modulates expression of Bax and Bcl-2 and potentiates p53-independent radiation-induced apoptosis in human breast cancer cells," *The Journal of Biological Chemistry*, vol. 275, no. 50, pp. 39174–39181, 2000.

[192] N. Miyakoshi, X. Qin, Y. Kasukawa et al., "Systemic administration of insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) increases bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism," *Endocrinology*, vol. 142, no. 6, pp. 2641–2648, 2001.

[193] A. Parker, A. Gockerman, W. H. Busby, and D. R. Clemmons, "Properties of an insulin-like growth factor-binding protein-4 protease that is secreted by smooth muscle cells," *Endocrinology*, vol. 136, no. 6, pp. 2470–2476, 1995.

[194] M. Iwashita, Y. Kudo, and Y. Takeda, "Effect of follicle stimulating hormone and insulin-like growth factors on proteolysis of insulin-like growth factor binding protein-4 in human granulosa cells," *Molecular Human Reproduction*, vol. 4, no. 4, pp. 401–405, 1998.

[195] S. Mazerbourg, M. T. Overgaard, C. Oxvig et al., "Pregnancy-associated plasma protein-A (PAPP-A) in ovine, bovine, porcine, and equine ovarian follicles: involvement in IGF binding protein-4 proteolytic degradation and mRNA expression during follicular development," *Endocrinology*, vol. 142, no. 12, pp. 5243–5253, 2001.

[196] S. Swami, N. Raghavachari, U. R. Muller, Y. P. Bao, and D. Feldman, "Vitamin D growth inhibition of breast cancer cells: gene expression patterns assessed by cDNA microarray," *Breast Cancer Research and Treatment*, vol. 80, no. 1, pp. 49–62, 2003.

[197] H. Huynh, X. F. Yang, and M. Pollak, "A role for insulin-like growth factor binding protein 5 in the antiproliferative action of the antiestrogen ICI 182780," *Cell Growth and Differentiation*, vol. 7, no. 11, pp. 1501–1506, 1996.

[198] L. A. Bach, "IGFBP-6 five years on; not so "forgotten"?" *Growth Hormone and IGF Research*, vol. 15, no. 3, pp. 185–192, 2005.

[199] L. A. Bach, "Insulin-like growth factor binding protein-6: the "forgotten" binding protein?" *Hormone and Metabolic Research*, vol. 31, no. 2-3, pp. 226–234, 1999.

[200] P. Jean Ho and R. C. Baxter, "Characterization of truncated insulin-like growth factor-binding protein-2 in human milk," *Endocrinology*, vol. 138, no. 9, pp. 3811–3818, 1997.

[201] S. Réhault, P. Monget, S. Mazerbourg et al., "Insulin-like growth factor binding proteins (IGFBPs) as potential physiological substrates for human kallikreins hK2 and hK3," *European Journal of Biochemistry*, vol. 268, no. 10, pp. 2960–2968, 2001.

[202] S. Miyamoto, M. Nakamura, K. Yano et al., "Matrix metalloproteinase-7 triggers the matricrine action of insulin-like growth factor-II via proteinase activity on insulin-like growth factor binding protein 2 in the extracellular matrix," *Cancer Science*, vol. 98, no. 5, pp. 685–691, 2007.

[203] P. Monget, S. Mazerbourg, T. Delpuech et al., "Pregnancy-associated plasma protein-A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: identification of cleavage site and characterization of IGFBP-2 degradation," *Biology of Reproduction*, vol. 68, no. 1, pp. 77–86, 2003.

[204] U. Berg, P. Bang, and C. Carlsson-Skwirut, "Calpain proteolysis of insulin-like growth factor binding protein (IGFBP) -2 and -3, but not of IGFBP-1," *Biological Chemistry*, vol. 388, no. 8, pp. 859–863, 2007.

[205] B. A. Booth, M. Boes, and R. S. Bar, "IGFBP-3 proteolysis by plasmin, thrombin, serum: heparin binding, IGF binding, and structure of fragments," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 271, no. 3, pp. E465–E470, 1996.

[206] C. Lalou, S. Sawamura, B. Segovia, Y. Ogawa, and M. Binoux, "Proteolytic fragments of insulin-like growth factor binding protein-3: N-terminal sequences and relationships between structure and biological activity," *Comptes Rendus de l'Academie des Sciences—Serie III*, vol. 320, no. 8, pp. 621–628, 1997.

[207] E. Okabe, J.-I. Kajihara, Y. Usami, and K. Hirano, "The cleavage site specificity of human prostate specific antigen for insulin-like growth factor binding protein-3," *FEBS Letters*, vol. 447, no. 1, pp. 87–90, 1999.

[208] H. Salahifar, S. M. Firth, R. C. Baxter, and J. L. Martin, "Characterization of an amino-terminal fragment of insulin-like growth factor binding protein-3 and its effects in MCF-7 breast cancer cells," *Growth Hormone and IGF Research*, vol. 10, no. 6, pp. 367–377, 2000.

[209] S. D. Chernausek, C. E. Smith, K. L. Duffin, W. H. Busby, G. Wright, and D. R. Clemmons, "Proteolytic cleavage of insulin-like growth factor binding protein 4 (IGFBP-4). Localization of cleavage site to non-homologous region of native IGFBP-4," *The Journal of Biological Chemistry*, vol. 270, no. 19, pp. 11377–11382, 1995.

[210] C. Rees, D. R. Clemmons, G. D. Horvitz, J. B. Clarke, and W. H. Busby, "A protease-resistant form of insulin-like growth factor (IGF) binding protein 4 inhibits IGF-1 actions," *Endocrinology*, vol. 139, no. 10, pp. 4182–4188, 1998.

[211] C. A. Conover, S. K. Durham, J. Zapf, F. R. Masiarz, and M. C. Kiefer, "Cleavage analysis of insulin-like growth factor (IGF)-dependent IGF-binding protein-4 proteolysis and expression of protease-resistant IGF-binding protein-4 mutants," *Journal of Biological Chemistry*, vol. 270, no. 9, pp. 4395–4400, 1995.

[212] J. B. Lawrence, C. Oxvig, M. T. Overgaard et al., "The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 6, pp. 3149–3153, 1999.

[213] Y. Imai, W. H. Busby Jr., C. E. Smith et al., "Protease-resistant form of insulin-like growth factor-binding protein 5 is an inhibitor of insulin-like growth factor-I actions on porcine smooth muscle cells in culture," *Journal of Clinical Investigation*, vol. 100, no. 10, pp. 2596–2605, 1997.

[214] M. T. Overgaard, H. B. Boldt, L. S. Laursen, L. Sottrup-Jensen, C. A. Conover, and C. Oxvig, "Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase," *The Journal of Biological Chemistry*, vol. 276, no. 24, pp. 21849–21853, 2001.

[215] B. Zheng, J. B. Clarke, W. H. Busby, C. Duan, and D. R. Clemmons, "Insulin-like growth factor-binding protein-5 is cleaved by physiological concentrations of thrombin," *Endocrinology*, vol. 139, no. 4, pp. 1708–1714, 1998.

[216] C. García-Echeverría, M. A. Pearson, A. Marti et al., "In vivo antitumor activity of NVP-AEW541—A novel, potent, and selective inhibitor of the IGF-IR kinase," *Cancer Cell*, vol. 5, no. 3, pp. 231–239, 2004.

[217] S. Wolf, J. Lorenz, J. Mössner, and M. Wiedmann, "Treatment of biliary tract cancer with NVP-AEW541: mechanisms of action and resistance," *World Journal of Gastroenterology*, vol. 16, no. 2, pp. 156–166, 2010.

[218] A. Girnita, L. Girnita, F. Del Prete, A. Bartolazzi, O. Larsson, and M. Axelson, "Cyclolignans as inhibitors of the insulin-like growth factor-1 receptor and malignant cell growth," *Cancer Research*, vol. 64, no. 1, pp. 236–242, 2004.

[219] D. Vasilcanu, A. Girnita, L. Girnita, R. Vasilcanu, M. Axelson, and O. Larsson, "The cyclolignan PPP induces activation loop-specific inhibition of tyrosine phosphorylation of the insulin-like growth factor-1 receptor. Link to the phosphatidyl inositol-3 kinase/Akt apoptotic pathway," *Oncogene*, vol. 23, no. 47, pp. 7854–7862, 2004.

[220] R. Vasilcanu, D. Vasilcanu, L. Rosengren et al., "Picropodophyllin induces downregulation of the insulin-like growth factor 1 receptor: potential mechanistic involvement of Mdm2 and β-arrestin1," *Oncogene*, vol. 27, no. 11, pp. 1629–1638, 2008.

[221] E. Menu, H. Jernberg-Wiklund, H. de Raeve et al., "Targeting the IGF-1R using picropodophyllin in the therapeutical 5T2MM mouse model of multiple myeloma: beneficial effects on tumor growth, angiogenesis, bone disease and survival," *International Journal of Cancer*, vol. 121, no. 8, pp. 1857–1861, 2007.

[222] M. A. Economou, S. Andersson, D. Vasilcanu et al., "Oral picropodophyllin (PPP) is well tolerated in vivo and inhibits IGF-1R expression and growth of uveal melanoma," *Investigative Ophthalmology and Visual Science*, vol. 49, no. 6, pp. 2337–2342, 2008.

[223] J. Rodon, V. DeSantos, R. J. Ferry Jr., and R. Kurzrock, "Early drug development of inhibitors of the insulin-like growth factor-I receptor pathway: lessons from the first clinical trials," *Molecular Cancer Therapeutics*, vol. 7, no. 9, pp. 2575–2588, 2008.

[224] J. A. Toretsky, S. M. Steinberg, M. Thakar et al., "Insulin-like growth factor type 1 (IGF-1) and IGF binding protein-3 in patients with Ewing sarcoma family of tumors," *Cancer*, vol. 92, no. 11, pp. 2941–2947, 2001.

[225] A. P. Majumdar, S. Banerjee, J. Nautiyal et al., "Curcumin synergizes with resveratrol to inhibit colon cancer," *Nutrition and Cancer*, vol. 61, no. 4, pp. 544–553, 2009.

[226] Y. Li and T. Zhang, "Targeting cancer stem cells by curcumin and clinical applications," *Cancer Letters*, vol. 346, no. 2, pp. 197–205, 2014.

[227] M. A. Babcook and S. Gupta, "Apigenin: a promising anticancer agent for the modulation of the Insulin-like Growth Factor (IGF) axis in prostate cancer," *Biomedical Research*, vol. 23, supplement 1, pp. 55–68, 2012.

[228] M. Jung, S. Y. Bu, K.-H. Tak, J.-E. Park, and E. Kim, "Anti-carcinogenic effect of quercetin by inhibition of insulin-like growth factor (IGF)-1 signaling in mouse skin cancer," *Nutrition Research and Practice*, vol. 7, no. 6, pp. 439–445, 2013.

[229] X. Li, Y. Feng, J. Liu, X. Feng, K. Zhou, and X. Tang, "Epigallocatechin-3-gallate inhibits IGF-I-stimulated lung cancer angiogenesis through downregulation of HIF-1alpha and VEGF expression," *Journal of Nutrigenetics and Nutrigenomics*, vol. 6, no. 3, pp. 169–178, 2013.

[230] D. Chen and Q. P. Dou, "Tea polyphenols and their roles in cancer prevention and chemotherapy," *International Journal of Molecular Sciences*, vol. 9, no. 7, pp. 1196–1206, 2008.

[231] S. Mondal, S. Bandyopadhyay, M. K. Ghosh, S. Mukhopadhyay, S. Roy, and C. Mandal, "Natural products: promising resources for cancer drug discovery," *Anti-Cancer Agents in Medicinal Chemistry*, vol. 12, no. 1, pp. 49–75, 2012.

[232] N. Sultana, "Clinically useful anticancer, antitumor, and anti-wrinkle agent, ursolic acid and related derivatives as medicinally important natural product," *Journal of Enzyme Inhibition and Medicinal Chemistry*, vol. 26, no. 5, pp. 616–642, 2011.

[233] Y.-J. Surh, "Cancer chemoprevention with dietary phytochemicals," *Nature Reviews Cancer*, vol. 3, no. 10, pp. 768–780, 2003.

[234] N. Khan, N. Hadi, F. Afaq, D. N. Syed, M.-H. Kweon, and H. Mukhtar, "Pomegranate fruit extract inhibits prosurvival pathways in human A549 lung carcinoma cells and tumor growth in athymic nude mice," *Carcinogenesis*, vol. 28, no. 1, pp. 163–173, 2007.

[235] C. A. Lamartiniere, M. S. Cotroneo, W. A. Fritz, J. Wang, R. Mentor-Marcel, and A. Elgavish, "Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate," *The Journal of Nutrition*, vol. 132, no. 3, pp. 552s–558s, 2002.

[236] Y. Li and F. H. Sarkar, "Gene expression profiles of genistein-treated PC3 prostate cancer cells," *The Journal of Nutrition*, vol. 132, no. 12, pp. 3623–3631, 2002.

[237] S. R. Chinni, Y. Li, S. Upadhyay, P. K. Koppolu, and F. H. Sarkar, "Indole-3-carbinol (I3C) induced cell growth inhibition, G1 cell cycle arrest and apoptosis in prostate cancer cells," *Oncogene*, vol. 20, no. 23, pp. 2927–2936, 2001.

[238] Y. Li, X. Li, and F. H. Sarkar, "Gene expression profiles of 13C- and DIM-treated PC3 human prostate cancer cells determined by cDNA microarray analysis," *Journal of Nutrition*, vol. 133, no. 4, pp. 1011–1019, 2003.

[239] K. Hastak, S. Gupta, N. Ahmad, M. K. Agarwal, M. L. Agarwal, and H. Mukhtar, "Role of p53 and NF-κB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells," *Oncogene*, vol. 22, no. 31, pp. 4851–4859, 2003.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 845340, 14 pages
http://dx.doi.org/10.1155/2015/845340

Review Article

Chemotherapy and Chemoprevention by Thiazolidinediones

Eleonore Fröhlich[1,2] and Richard Wahl[1]

[1] *Internal Medicine, Department of Endocrinology, Metabolism, Nephrology and Clinical Chemistry, Department IV, University of Tuebingen, Otfried-Muellerstrasse 10, 72076 Tuebingen, Germany*
[2] *Center for Medical Research, Medical University of Graz, Stiftingtalstraße 24, 8010 Graz, Austria*

Correspondence should be addressed to Richard Wahl; richard.wahl@med.uni-tuebingen.de

Received 31 May 2014; Revised 29 July 2014; Accepted 27 August 2014

Academic Editor: Viroj Boonyaratanakornkit

Thiazolidinediones (TZDs) are synthetic ligands of Peroxisome-Proliferator-Activated Receptor gamma (PPARγ). Troglitazone, rosiglitazone, and pioglitazone have been approved for treatment of diabetes mellitus type II. All three compounds, together with the first TZD ciglitazone, also showed an antitumor effect in preclinical studies and a beneficial effect in some clinical trials. This review summarizes hypotheses on the role of PPARγ in tumors, on cellular targets of TZDs, antitumor effects of monotherapy and of TZDs in combination with other compounds, with a focus on their role in the treatment of differentiated thyroid carcinoma. The results of chemopreventive effects of TZDs are also considered. Existing data suggest that the action of TZDs is highly complex and that actions do not correlate with cellular PPARγ expression status. Effects are cell-, species-, and compound-specific and concentration-dependent. Data from human trials suggest the efficacy of TZDs as monotherapy in prostate cancer and glioma and as chemopreventive agent in colon, lung, and breast cancer. TZDs in combination with other therapies might increase antitumor effects in thyroid cancer, soft tissue sarcoma, and melanoma.

1. Introduction

Glitazones, also called thiazolidinediones (TZDs), are five-membered carbon ring molecules containing two heteroatoms (nitrogen and sulfur). One carbonyl group in the thiazole at position 4 and another at position 2 make the heterocyclic compound a thiazolidine-2,4-dione [1]. TZDs are ligands of the Peroxisome Proliferator Activated Receptor gamma (PPARγ), a nuclear receptor inducing upregulation of specific genes that decrease insulin resistance, inflammation, VEGF-induced angiogenesis, proliferation, and leptin levels, inducing differentiation of adipocytes, and increasing adiponectin levels. This spectrum of actions led to the approval of TZDs for treatment of diabetes mellitus type II. TZDs differ according to the substitution at C5 (Figure 1).

Ciglitazone (CIGLI) is the prototype of all TZDs but has never been approved for medication of diabetes mellitus because its clinical activity was too weak. Troglitazone (TRO) was the first TZD approved for treatment of diabetes mellitus in 1997 [2]. The compound showed beneficial effects on glucose levels, insulin sensitivity, and free fatty acid concentration but was withdrawn from the market in 2000 due to severe hepatotoxicity. The second TZD, rosiglitazone (ROSI), has been banned in Europe and restricted in the USA because of increased cardiovascular morbidity. Also the use of pioglitazone (PIO) as the third TZD with antidiabetic action is restricted due to concerns about a potential facilitation of bladder cancer development. The fourth substance with an antidiabetic profile, rivoglitazone, is still under investigation [3]. Reasons for the troubled history of antidiabetic TZDs are manifold and appear to be due to the highly pleiotropic action of these PPARγ agonists and crosstalk of PPARγ with other signaling pathways.

In addition to diabetes mellitus treatment, ligands to PPARγ could also be exploited for treating other diseases, for instance, in cancer treatment. This idea originated from the finding that PPARγ is involved in cell proliferation and PPARγ expression levels change from normal to transformed tissues. Effects of PPARγ activation are ligand-specific. TZDs with potent PPARγ agonist activity can display, like

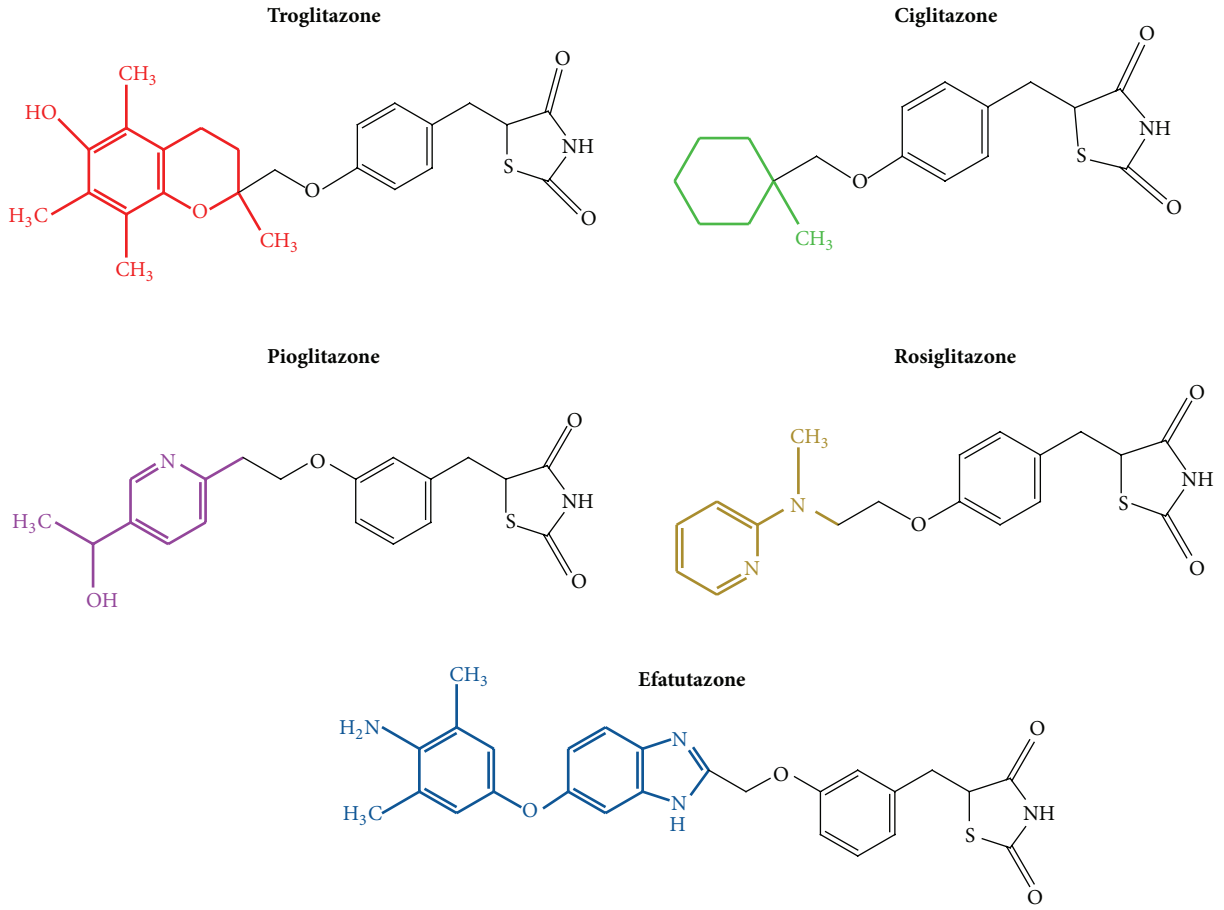


Figure 1: Chemical formulae of the most common TZDs with antitumor action.

rivoglitazone, strong antidiabetic activity, or, like efatutazone (EFA), predominantly antitumor effects. TZDs, such as netoglitazone, can also activate other PPARs and cause antitumor effects [4]. This review will focus on the effects of selective PPARγ TZDs in tumors.

2. Role of PPARγ Expression in Neoplasms

PPARγ expression compared to normal tissue tends to be increased in precursor lesions and differentiated tumors and decreased in the poorly differentiated cancers. This pattern has been reported for instance for gastric, ureteric, and breast cancer [5–7]. In ovarian cancer, however, PPARγ levels independent from tumor differentiation are increased [8]. Upregulation of PPARγ may be an early event in tumorigenesis and a marker for differentiated cancer lesions [9]. Methylation (silencing) of the PPARγ promotor, which is detected in 30% of colorectal tumors, however, correlated with poor prognosis [10]. Studies linking tumor prognosis and PPARγ expression were mainly based on immunohistochemical detection of the PPARγ antigen in paraffin-embedded tissue. Since antigenicity is low and may decrease during storage of the paraffin samples, the absence of PPARγ staining in archival tissues may be a false negative due to methodological problems [11].

Identification of the contribution of PPARγ to tumor development and progression is further complicated by crosstalk with other pathways. Akt phosphorylation in the endometrium, for instance, is directly regulated by PPARγ and indirectly through induction of PTEN by PPARγ, where PTEN decreases p-Akt via inhibition of PI3K [12].

3. Mechanism of Antitumor Action by TZDs

Although all TZDs are PPARγ ligands, the observed antitumor effects can only be explained in part by genomic PPARγ activation. Genomic activation is defined as the binding of a nuclear receptor to a response element, which activates the transcription of certain genes. The process is also termed transactivation. Another DNA-mediated effect is transrepression, which describes the binding of receptors to transcription factors (e.g., nuclear factor kappa B (NFκB) or activator protein 1 (AP-1)).

PPARγ ligands trigger a conformational change of the PPARγ receptor that attracts transcriptional coactivators of the steroid receptor coactivator family. Once activated by ligand binding, the PPARγ receptor forms heterodimers with the retinoid X-receptor and transcription is initiated. Transcriptional activation may result in decreased proliferation, migration and inflammation and increased differentiation

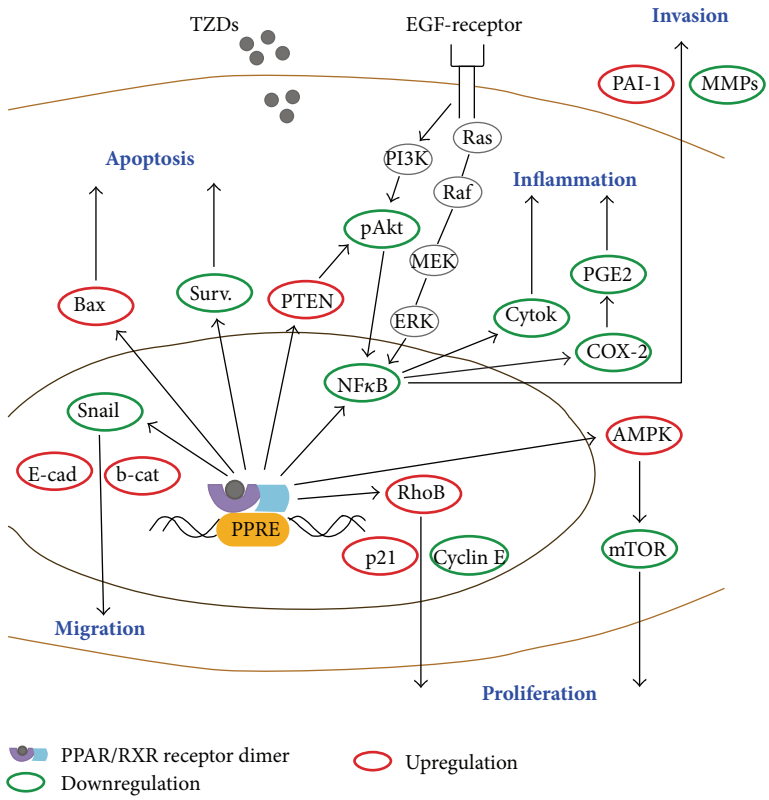


FIGURE 2: Effects of TZDs on apoptosis, migration, invasion, and proliferation of cancer cells and on inflammation. In some ellipses, only one representative is listed; Bax and p53 react similarly, as well as p27 and p21. MMPs represents MMP-2 and MMP-9 and Cyclin E represents cyclin D1, cyclin B1, CDK2, and CDK4. Abbreviations: EGF: epithelial growth factor receptor; PPRE: PPARγ response element, Surv: survivin, E-cad: E-cadherin, b-cat: β-catenin, Cytok: cytokines.

and apoptosis (Figure 2). Inflammatory effects are usually mediated by transrepression [13].

Figure 2 illustrates the variety of pathways influenced by genomic activation of PPARγ by TZDs, resulting in downregulation of migration, proliferation, inflammation, and invasion and in upregulation of apoptosis. Common mechanisms involve influence on EGF signaling, cyclins, Ki-67, c-myc, cyclin-dependent kinases, p53 and PTEN expression, adhesion proteins, metalloproteinases, and cytokines [14–19].

Hormone-dependent cancers react through different mechanisms to TZDs depending on the hormone receptor status. In androgen-dependent prostate carcinoma, for instance, CIGLI downregulated aromatase activity, while in androgen-independent tumors proliferation was reduced [20].

Different TZDs may act by different mechanisms; while CIGLI downregulated cyclin D1 and upregulated p21 by PPARγ independent pathways, ROSI used PPARγ signaling to induce these effects in androgen-independent prostate carcinoma cells [21].

The description of all mechanisms of TZDs is beyond the scope of this review but one important signaling pathway for tumor cells and for surrounding tissue (tumor microenvironment) each illustrates the variety of PPARγ effects. Tumor biology is not only determined by tumor cells but to a high extent by properties of stromal cells in the tumor microenvironment. Among the diverse cells in the tumor stroma (endothelial cells, cancer-associated fibroblasts, leukocytes, myofibroblasts, and mesenchymal stem cells), tumor-associated macrophages play the most decisive role in tumor progression [22].

For tumor cells, signaling by Epidermal Growth Factor receptor (EGF-receptor, Figure 2) is highly relevant. The signaling cascade of the EGF-receptor involves the ERK cascade, consisting of Ras-Raf-MEK1/MEK2-ERK1/ERK2 and is seen in several cancer types [23]. ERK may phosphorylate PPARγ and reduce its genomic activity. This effect occurs in cancer cell lines and a variety of normal cells alike [24]. TRO, for example, was reported to bind to the EGF receptor and trigger its internalization in EGF-receptor transfected endothelial cells [25]. This action is an example of nongenomic effects of TZDs since no ligand binding to response element occurred.

Normal macrophages can transform into tumor-associated macrophages under stimulation of PPARγ ligands [26]. ROSI decreased activation of macrophages and thereby reduced inflammation in nondiabetic patients with symptomatic carotid artery stenosis [27]. In murine macrophages, these effects are mediated by interaction of PPARγ with NfκB [28]. In these effects, transrepression appears to be the main mechanism.

Finally, MEK1 action by ROSI may lead to nuclear export and cytoplasmic retention of PPARγ and off-DNA interaction with proteins in MEK1-GFP and PPARγ (wild-type and mutant) cotransfected HEK-293 cells [29]. In this effect no genomic action of TZDs was involved.

4. Therapeutic Efficacy of TZDs in Specific Cancers

Decrease of cell proliferation, cytotoxicity, and proapoptotic effects induced by CIGLI, TRO, ROSI, and PIO has been reported in a variety of cell lines (sarcoma, melanoma, glioblastoma, breast carcinoma, colorectal cancer, gastric cancer, pancreatic cancer, prostate, bladder cancer, hepatic cancer, thyroid cancer, ovarian cancer, endometrial cancer, and lung cancer cells), which will not be listed in detail. Based on promising cellular action, animal experiments and clinical trials have been conducted in several common cancers.

EFA, which was developed as a chemostatic rather than an antidiabetic drug, has also been studied in some of these cancers. EFA is 500x more potent an activator of PPARγ than TRO and 50x stronger than ROSI. EFA was studied in a preclinical murine model for breast cancer based on BRCA1 (BReast CAncer 1) deficiency. In the MMTV-Cr $BRCA1^{flox/flox}$ $p53^{+/-}$ model, exon 11 of the BRCA1 gene is deleted by Mouse Mammary Tumor Virus (MMTV)-Cre transgene. The deletion is accompanied by loss of one germline copy of TP53. EFA reduced the incidence of non-invasive and well-differentiated tumors in this model [30].

Cell proliferation and xenograft size of pancreatic, anaplastic thyroid, and colorectal cancer were reduced by EFA administration [31].

Based on these promising preclinical effects, phase I trials were initiated either as monotherapy or in combination with other compounds. After monotherapy with EFA, stable disease was induced in 10/22 patients with advanced liposarcoma [14]. A phase 1 study evaluating the combination of bexarotene with EFA in solid tumors is currently recruiting patients (NCT01504490).

The first trial of antitumor effects of the antidiabetic TZDs was conducted in three liposarcoma patients, where decrease of proliferation with TRO has been reported [32]. No beneficial effects, however, were obtained in a trial with ROSI in 9 liposarcoma patients [33]. Despite the negative outcome of this trial, another phase II trial on ROSI is ongoing (NCT00004180; http://www.cancer.gov/clinicaltrials/).

TZDs showed variable efficacy in studies of common cancers using xenograft and transgenic mouse models, in case studies and clinical trials (an overview is provided in Table 1).

4.1. Colorectal Cancer. Studies on human tumor samples support the hypothesis that PPARγ expression has protective effects in colorectal cancer [34]; patients with PPARγ expression usually showed a better prognosis [11]. Accordingly, reduction of β-catenin and PPARγ was associated with high numbers of tumor-associated macrophages, increased metastasis, and poor survival [35]. On the other hand, loss of function point mutations of the PPARγ gene and polymorphisms in PPARγ genes were encountered in 8% of colorectal carcinoma patients, but some studies on PPARγ expression in colorectal samples did not find any relation of PPARγ immunoreactivity and tumor parameters [36, 37]. The role of PPARγ activation in the progression of malignant lesions is questioned by the fact that heterozygous and homozygous intestinal-specific PPARγ deficiency promoted tumor formation [38]. This suggests that murine models might not be representative for the study of TZDs in colorectal cancer.

Consistent with the unclear role of PPARγ in tumor samples, TZDs showed variable effects *in vivo*. PPARγ activation inhibited xenograft growth in mice and PPARγ agonists reduced the number of aberrant cryptal foci in chemically induced inflammatory bowel disease in mice [39, 40]. On the other hand, PIO induced increased polyp numbers in mice with APC mutation, prone to developing colon adenoma (APC^{min}), not in wild-type mice, suggesting that, under certain genetic conditions, TZDs could also promote colon cancer development [41]. The disparate results might be explained by *in vitro* studies in colon cancer cell lines showing that the level of PPARγ expression correlated to cells' sensitivity to proliferation inhibition [42].

A phase II trial with TRO did not increase progression-free survival in 25 colorectal cancer patients [43].

4.2. Lung Cancer. PPARγ expression in well-differentiated lung adenocarcinoma was higher than in poorly differentiated tumors, suggesting that it promotes tumor formation but is not a marker for aggressive growth [44]. In another study, expression was linked to poor prognosis, showing the opposite trend [45]. ROSI decreased progression of chemically induced murine cancer model [46].

4.3. Breast Cancer. In breast cancer PPARγ mRNA levels did not correlate with nodal involvement and tumor grade but significantly lower PPARγ levels were seen in large metastatic tumors, patients with local recurrence and poor survival [47]. Despite the fact that samples of aggressive tumors showed increased PPARγ expression, TZDs displayed moderate positive effects in breast cancer models. ROSI reduced tumor growth in a chemically induced rat and in a syngenic murine tumor model [48, 49]. Both in patients with advanced breast carcinoma and in patients with early mammary cancer treatment with TZDs did not cause therapeutic effects [50, 51].

4.4. Prostate Cancer. In the majority of prostate cancers (73%), immunoreactivity and expression of PPARγ correlated inversely with tumor size and PSA levels [52]. Data obtained in prostate cancer xenografts as well as results from a phase II trial and a case report showed efficacy of PIO and TRO [53–55].

4.5. Glioblastoma. No correlation of PPARγ expression has been established with glioma [56]. Diabetes mellitus patients under TZD medication, however, showed lower incidence of high-grade glioma than the control group (patients with hip fractures), while survival of patients with glioma was similar

Table 1: Relationship between protective role of PPARγ expression and efficacy of TZDs in therapy.

Cancer type	Role of PPARγ	TZD	Experimental model	Result	Reference
Colon	⇓/⇑	PIO	Xenograft (HT-29) in mice with APC mutation, sc	Increased tumor growth	[41]
			Azoxymethane-induced murine tumors	Reduced tumor growth	[39]
		TRO	HT-29 xenografts, sc	Reduced tumor growth and metastasis	[40]
			Metastatic colon cancer, 25 patients	All progressive disease	[43]
Lung	⇓/⇑	ROSI	Chemically-induced mouse model	Decrease in adenoma formation	[46]
Breast	⇓	ROSI	LMM3 injection into mice, sc	Decreased tumor growth	[48]
			Chemically induced rat model	Decreased tumor growth and incidence	[49]
		TRO	Advanced chemotherapy breast refractory cancer, 22 patients	No CR or PR, 3 SD	[50]
		ROSI	Early stage breast cancer, 38 patients	No decrease in proliferation	[51]
Prostate	⇓	PIO	PC3 xenografts, sc.	Decrease of bone-invasive potential	[53]
		TRO	Advanced prostate carcinoma, 41 patients	Stabilization of PSA levels	[54]
		ROSI	Recurrent prostate carcinoma, 1 patient	Delayed increase of PSA levels	[55]
Glioma	⟺	PIO	LN229 orthotopic xenografts	Reduced tumor volume, invasion	[58]
			Chemorefractory glioma, 14 patients	Disease stabilization (29%)	[59]
Melanoma	⟺	CIGLI	A375 xenografts, sc.	Growth inhibition, pro-apoptotic effects	[62]
Thyroid	⇓	PIO	Transgenic mouse model (PPAR fusion protein/PTEN deletion)	Decreased tumor growth and metastasis	[106]
		ROSI	Transgenic mouse model (Thyroid hormone receptor-β negative)	Delayed progression	[107]
			Metastatic thyroid cancer, 1 patient	Decrease in metastasis size	[109]

PPARγ expression on tumor progression: promotion: ⇑; protection: ⇓; no effect: ⟺; CR: complete response; PR: partial response; SD: stable disease; sc: subcutaneous implantation of tumor cells.

in both groups [57]. Efficacy of PIO has been shown in glioma xenografts and in a phase II trial [58, 59].

4.6. Melanoma. No correlation of PPARγ expression and melanoma prognosis was seen [60]. In a cohort study of diabetes mellitus patients under PIO medication, an increased hazard ratio for melanoma (1.3) was reported [61]. It is not clear whether these data represent an increased incidence of tumors because the maximum duration of follow-up was <6 years after the initiation of PIO. Studies on monotherapy with TZDs in melanoma are limited: only CIGLI was reported to inhibit growth of melanoma xenografts [62].

Higher mRNA or protein expression in well-differentiated tumors compared to poorly differentiated tumors and tumors with poor prognosis is interpreted as protective effect of PPARγ in tumor development. In prostate cancer patients, protective effects of PPARγ and therapeutic effect of TZDs were in line (Table 1). In glioma samples, PPARγ expression was not linked to good prognosis but TZDs showed therapeutic efficacy.

5. Role of TZDs in Chemoprevention

While therapeutic efficacy of monotherapy with TZDs was relatively low, data obtained from meta-analysis of diabetes studies as well as *in vitro* data suggested that TZDs could be efficient in chemoprevention (Table 2).

5.1. Data from Diabetes Trials. Medication with TZDs for >1 year decreased the incidence of head and neck cancers by 40% and lung cancer by 33% in diabetes mellitus patients [63]. The reduction of lung cancer reached 75% in the African-American population. The reduction was specific for lung cancer, as prostate and colorectal cancer incidence was not changed. Of note, in this study, patients with preexisting malignancies were excluded. The largest meta-analysis on cancer incidence and cancer mortality included data of 46 trials. The number of malignancies was disclosed in 28/33 trials with ROSI and in 18/33 trials with PIO [64]. This meta-analysis reported less cancer cases (342 versus 457) in patients treated with TZDs compared to other medications. Overall, treatment with TZDs was associated with a significantly lower

Table 2: Summary of data on chemopreventive effects of TZDs in animal and human epidemiological studies.

Cancer type	Role of PPARγ expression	TZD	Experimental model	Result	Reference
Colon	⇓/⇑	PIO	Chemically-induced rat cancer model	Reduction of tumor incidence	[121]
			Transgenic murine cancer model (nonsense mutation in the adenomatous polyposis coli)	Increase of tumor incidence	[122]
		TRO	Chemically-induced rat cancer model	Reduction of tumor incidence	[123]
		ROSI	Meta-analysis of diabetes trials	Reduced colon cancer incidence	[64]
Lung	⇓/⇑	PIO	Chemically induced murine cancer model	Reduction of tumor incidence	[72]
		PIO	Observational study	Reduced lung cancer incidence	[63]
Breast	⇓	PIO	Meta-analysis of diabetes trials	Reduced breast cancer incidence	[64]
Liver	⇓	PIO	Chemically induced rat cancer model	Reduced tumor incidence	[73]
Endometrium	⇓	ROSI	Transgenic murine cancer model	Reduced tumor incidence	[12]
Oral (squamous cancer)	⇓	PIO	Transgenic rat cancer model	Reduced tumor incidence	[77]
		TRO	Chemically induced rat cancer model	Reduced tumor incidence	[78]

PPARγ expression on tumor progression: promotion: ⇑; protection: ⇓.

incidence of cancer cases (Mantel-Haenszel odds ratio (MH-OR) 0.85; $P = 0.027$). For ROSI this effect was significant for colorectal cancer (MH-OR 0.63; $P = 0.03$). PIO treatment significantly reduced the incidence of breast cancer (MH-OR 0.28; $P = 0.004$). An increase in the incidence of bladder cancer by PIO treatment was not seen (MH-OR 2.05; $P = 0.12$), but cancer mortality was increased upon TZD treatment. Since this mortality most probably is due to preexisting cancers, the question remains whether treatment with TZDs could promote the growth of already existing malignant lesions.

5.2. In Vitro Differentiation Studies. Morphological differentiation (duct formation in collagen gels) increased in pancreatic carcinoma cells treated with TRO [65] and increases of villin and mucin mRNA were observed in colon cancer cell lines [66]. ROSI induced PTEN expression in Caco-2 cells and restored glandular morphogenesis [67]. It increased tyrosinase expression, an indication for differentiation, in a melanoma cell line [68]. ROSI also caused reversal of epithelial-mesenchymal transition in anaplastic thyroid cancer cell lines and increased expression of thyroglobulin, TSH receptor, sodium-iodide symporter, and thyroperoxidase mRNA [69]. CIGLI induced brain tumor stem cell differentiation [70]. In cultures of metaplastic urothelial cells, differentiation markers were increased after treatment with TRO [71].

5.3. TZD Effects in Animal Studies. PIO prevented lung tumor development in carcinogen-induced mouse models [72]. In a similar manner, PIO protected rats against chemically-induced (diethylnitrosamine and acetylaminofluorene) hepatocarcinogenesis [73]. PPARγ could play a tumor-promoting role in hepatoma, because expression is significantly reduced in hepatocellular carcinoma with poor prognosis [74]. A similar situation is seen in endometrium carcinoma, where benign lesions show strong PPARγ immunoreactivity but malignant lesions low to absent PPARγ expression [12]. Chemoprevention of endometrial cancer by ROSI was observed in PTEN heterozygous mice [75]. Increased PPARγ expression was predominantly seen in less invasive oral squamous cancer [76]. Chemically-induced oral squamous carcinoma in rats was reduced by 40% through administration of PIO [77] and tongue carcinoma formation was reduced by 40% by TRO [78].

On the other hand, tumor-promoting effects of PIO were observed in the APC^{min} murine colon cancer model [41]. Because tumor-promoting effects were not seen in all cancer models, a model-specific effect cannot be excluded. The complex and, in part, opposing effects of TZDs on cancer development and progression can be explained by their cell-specific and species-specific action (tumor cells versus tumor environment). Effects of TZDs on immune cells may be the reason for the tumor-promoting effect of PIO in the APC^{min} mouse model and the reduced tumor growth in immune-compromised mice and in the azoxymethane-induced tumor model [79]. While PPARγ activation may decrease proliferation of tumor cells, it may increase macrophage polarization towards the M2 phenotype (TAM) and induce anti-inflammatory effects, also mediated by PPARγ activation (see Section 3)

5.4. Human Data. One phase II trial on prevention of lung, head, and neck carcinoma in 21 patients with oral leukoplakia using PIO has been completed. Fifteen patients showed partial responses, 2 stable disease and 4 patients had progressive disease (NCT00099021; http://www.cancer.gov/clinicaltrials/). Based on these promising results, another trial on prevention of lung cancer is recruiting patients (NCT00780234; http://www.cancer.gov/clinicaltrials/).

In human trials, no general correlation of the protective effect of PPARγ expression against tumor progression and

chemopreventive effects of TZDs was obvious. While a protective role of PPARγ expression was postulated in breast tumors and TZDs also acted preventive on the development of breast cancer in humans, the chemopreventive effect on colon cancer was not consistent with a protective role of PPARγ expression in tumor samples.

6. Combined Treatments of TZDs with Other Drug Compounds

6.1. In Vitro Studies. Several studies evaluated the effect of combined therapies with TZDs and other agents. A large variety of combinations of TZDs have been evaluated *in vitro*. The observed antitumor effects include cytotoxicity/decrease of cell viability, growth inhibition, and apoptosis (for overview see Table 3).

In combination treatment with RXR-α ligands, increased cellular differentiation was reported [80, 81]. Some combined therapies take advantage of the cross-talk of PPARγ with other signaling pathways. For instance, the upregulation of PTEN by ROSI rendered hepatoma cells more sensitive to the action of 5-fluorouracil [82]. Based on the idea of cross-talk between the ERK and PPARγ pathways, combinations of ERK inhibitors and PPARγ agonists could be useful in tumors with deleterious elevation of PPARγ. Experimental data corroborate such an idea: gefitinib and ROSI increased growth inhibition of lung cancer cells and increased PPARγ and PTEN expression [83]. Herceptin, an antibody against the EGF-receptor HER2, sensitized breast cancer cells for the differentiating action of TRO [84].

6.2. Animal Studies. The following examples show that improved antitumor responses were also obtained *in vivo*: growth of lung carcinoma xenografts and of chemically-induced breast tumors was inhibited by a combination of ROSI and platinum-based compounds [85, 86]. ROSI in combination with suberoylanilidehydroxamic acid (SAHA) decreased progression of preinvasive lung cancer in a murine model by 77% [46]. Similarly, a combination of TRO and platinum-based compounds increased survival of mesothelioma-xenografted mice [87]. The combination of EFA and paclitaxel reduced the size of anaplasic thyroid carcinoma xenografts [88]. Progression of ovarian carcinoma xenografts was slower when a combination of CIGLI and cisplatin was administered. Synergistic effects were reduction of angiogenesis and increased proapoptotic effects [89]. Aerosolized budesonide and oral PIO decreased lung cancer mass by 90% in a benz(a)pyrene-induced murine lung cancer model [90].

6.3. Human Data. Phase II trials of combination with the COX-2 inhibitor rofecoxib and PIO were able to induce complete response, partial responses, or stable disease in 5/5 angiosarcoma, 1/1 hemangioendothelioma, 4/19 metastatic melanoma, 10/40 soft tissue sarcoma, and 4/14 glioma patients [59, 91, 92]. Combination of PIO with other chemostatic drugs induced one complete response and prolonged disease-free survival in 2 of 19 patients with advanced melanoma enrolled in this phase II trial [91]. These data suggest potential efficacy of TZDs combined with other compounds in melanoma. For further evaluation of comedication with TZDs in patients, a prospective phase I/II trial of PIO combined with lenalidomide, dexamethasone, and treosulfan (NCT01614301) is currently recruiting patients (http://www.cancer.gov/clinicaltrials/).

According to human trials, only soft tissue sarcoma and melanoma might be sensitive to combinations of TZDs and COX-2 inhibitors and TZDs in polytherapy, respectively.

7. Specific Role of TZDs in Differentiated Thyroid Carcinoma (DTC)

PPARγ has a specific role in thyroid cancer because follicular thyroid cancer is the only known neoplasm to be associated with a PPARγ fusion gene product [93]. PAX8/PPARγ is expressed in 30–35% of follicular thyroid carcinoma and 2–13% of follicular adenomas [94]. This chimeric protein is the result of a genetic translocation between chromosomes 2 and 3 and can activate the PPARγ response element and induce proliferation. The mutation acts both as a gain and loss of function mutant in thyroid cancer and determines thyroid tumor differentiation; in more aggressive tumors gain of function predominates [93].

Thyroid cancer incidence in the United States has increased in the last thirty years not only apparently because of enhanced detection but probably also as a true increase [95]. DTC is the most common type of thyroid carcinoma, mainly in the form of papillary thyroid carcinoma, accounting for 80–90% of all thyroid cancer cases. The second-most common form of DTC is follicular thyroid cancer with 10–15% incidence. The prognosis of DTC is generally good, with a 10-year survival rate of 85% [96]. A total of 10–20% of patients develops distant metastases [97]. In this group, the 10-year survival rate drops to 40%. Recurrence in DTC, however, occurs in up to a third of patients and only 30% of patients with distant metastases respond to radioiodine (RAI) therapy with complete remission [98, 99]. First-line treatment of DTC is by total or near total removal of the thyroid and if necessary lymph node dissection (Figure 3). This is generally followed by RAI treatment for thyroid remnant ablation and elimination of metastases. In case of insufficient efficacy of this treatment, doxorubicin is initiated [100]. Because doxorubicin treatment is not highly efficient, it is expected that, in the future, differentiating therapies will play a prominent role in cancer treatment. Redifferentiating compounds include retinoids, histone deacetylase inhibitors, DNA methyltransferase inhibitors, and TZDs. Somatostatin analogues such as ^{68}Ga-DOTATOC are additional options for RAI-negative thyroid cancer [101].

PIO and CIGLI did not increase differentiation in a study on the human papillary carcinoma cell line NPA [102]. In another, TRO, ROSI, and PIO showed antiproliferative, proapoptotic, and differentiating effects on DTC cells [103]; TRO could increase expression of sodium-iodide symporter in DTC lines [104] and restore radioiodine-uptake *in vitro* [105].

Table 3: Results of therapies combining TZDs with other antitumor treatments.

TZD	Additional compound	Model	Effect	Reference
CIGLI	Gamma-radiation	Lung carcinoma cell lines (A549, H460)	DNA damage, apoptosis	[124]
	RXR-α ligands (SR11237, 6-OH-11-O-hydroxyphenanthrene)	Breast carcinoma cell line (MDA-MB231), lung carcinoma cell line (Calu-3), glioblastoma cell line (U87MG), melanoma cell line (G361)	Growth inhibition; apoptosis	[125–127]
	TNF-α-related apoptosis inducing ligand	Ovarian cancer cell line (HEY)	Decrease of proliferation	[128]
	Lovastatin	Pancreatic carcinoma cell lines (Panc02, MIA, PACa-2), breast carcinoma cell lines (EMT6, MDA-MB-316), colon cancer cell line (C26)	Decrease of cell viability; decrease of proliferation	[129]
	Phenylbutyrate	Lung carcinoma cell lines (A549, H157)	Growth inhibition	[130]
TRO	9-cis retinoic acid	Gastric carcinoma cell line (SGC7901)	Apoptosis	[131]
	Cisplatin	Lung cancer cell lines (A549, H522); mesotheloma cell line (EHMES-10)	Growth inhibition	[87, 132]
	Paclitaxel	Lung carcinoma cell lines (A549, H522)	Growth inhibition	[132]
	RXR-α ligands (bexarotene, all-trans retinoic acid)	Breast cancer cell lines (MCF-7, T-47D, ZR-75-1)	Growth inhibition	[133]
	Cell signalling molecules (TRAIL, heregulin)	Ovarian cancer cell line (HEY); breast cancer cell lines (MCF-7, SKBR-3, MDA-MB-453)	Decrease of cell number; apoptosis	[128, 134]
	Lovastatin	Glioblastoma cell line (DBTRG05MG), lung cancer cell line (CL1-0)	Cell cycle inhibitor expression	[135]
	Aspirin	Lung cancer cell lines (CL1-0, A549)	Decrease of proliferation	[136]
	Tamoxifen	Breast cancer cell line (MCF-7)	Growth inhibition	[137]
	X-rays	Cervix cancer cell lines (HeLa, Me180)	Decrease of cell viability	[138]
ROSI	Platinium-based compounds (cisplatin, carboplatin)	Ovarian cancer cell lines (OVCA420, OVCA429, ES), lung cancer cell lines (A549, Calu-1, H23, H596, H1650)	Growth inhibition	[85]
	5-Fluorouracil	Hepatoma cell lines (BEL7402, Huh-7); colon cancer cell line (HT-29)	Decrease of cell viability, apoptosis	[82, 139]
	RXR-α ligands (bexarotene, 9-cis retinoic acid)	Breast cancer cell lines (MCF-7TR1, SKBR-3, T47D), colon cancer cell line (Moser)	Increase of differentiation, growth inhibition; decrease of cell viability	[80, 140]
	Cell signalling molecules (TNF-α, anti-Fas IgM, Seliciclib)	Breast cancer cell line (MDA-MB-231)	Growth inhibition	[141]
	Gemcitabine	Pancreas cancer cell lines (PANC-1, Panc02)	Decrease of cell viability, growth inhibition	[142]
	Gefitinib	Lung cancer cell line (A549)	Growth inhibition	[83]
	Herceptin	Breast cancer cell line (MCF-7)	Growth inhibition	[84]
	Bortezomib	Melanoma cell lines (MV3, FemX-1, G361)	Growth inhibition	[143]
PIO	Paclitaxel	Lung cancer cell lines (A549, H522)	Growth inhibition	[132]
	RXR-α ligands (LG268)	Liposarcoma cells (primary)	Increase of differentiation	[81]
	Statins (Simvastin, lovastatin)	Glioblastoma cell lines (U87, U138, LN405, RGII); meningeoma cell lines (IOMM-Lee, KT21-MG1)	Decrease of cell viability	[144, 145]
	Gemcitabine	Pancreas cancer cell line (PANC-1)	Decrease of cell viability	[142]
	2-Deoxyglucose	Prostate cancer cell lines (PC-3, LNCaP)	Decrease in tumor spheroid formation	[146]
EFA	Paclitaxel	Anaplastic thyroid carcinoma cell lines (DRO, BHT-101, ARO)	Growth inhibition	[88]

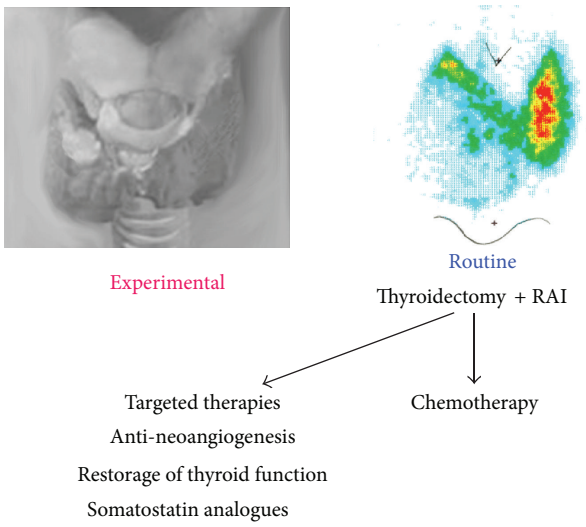


Figure 3: Overview of treatment options for DTC. Scheme of thyroid tumor (upper left) and scintigraphy with ^{123}Iodide showing lack of uptake in the lower part of the right lobe (upper right).

7.1. Animal Studies. PIO was effective in reducing metastatic disease in a tumor model where the effect of PAX8/PPARγ fusion protein is mimicked [106]. ROSI was also able to reduce thyrocyte growth by 40% in a murine knock-in model of thyroid hormone receptor β [107].

7.2. Human Data. In a small cohort of 5 patients treated with PIO for 6 months, no increase in RAI-uptake was seen [108]. Two case reports described successful induction of RAI-uptake after treatment with ROSI in a patient with noniodide avid metastases of DTC [109, 110]. Decreased thyroglobulin levels and tumor size indicated partial success of this treatment. Evidence for increased RAI-uptake upon treatment with ROSI was obtained in one of five patients enrolled in a pilot study [111]. In another pilot study, ROSI treatment resulted in positive RAI scans in 4/10 patients and a clinical trial showed increased RAI-uptake in therapeutic ^{131}I scans in 5/23 patients [112, 113]. Despite reinduction of RAI-uptake in 5/20 patients of another phase II trial, none had a complete or partial response to ROSI after 3 months [114] by RECIST criteria [115]. The status of a current trial (NCT00098852) with ROSI for reinduction of radioiodine-uptake is not yet known (http://www.clinicaltrial.gov/). Also the redifferentiating action of PIO is being reassessed in a trial focused on follicular variants of PTC (NCT01655719; http://www.clinicaltrial.gov/). Interpretation of the results is complicated by limited accuracy of the technique of ^{131}I scans and unknown status of receptor expression of the treated tumors, too low levels of expression by the target cells, inhomogeneity of RAI-uptake into the tumor, and the generally poor correlation between RAI-uptake and clinical remission, all of which may be reasons for lack of efficacy. In addition, observation time of less than one year may not be enough to monitor effects in slow-growing DTC.

8. Conclusion

Current data do not suggest a correlation of clinical efficacy and high PPARγ expression according to mRNA and protein expression in tumor samples. This lack of relation could be due to methodical problems of PPARγ detection in archived tumor samples and in the complexity of TZD action. First, TZDs show a variety of genomic and nongenomic effects and several antitumor effects occur independent of PPARγ. This is particularly obvious in experiments where combination of PPARγ agonists and antagonists act synergistically on inhibition of proliferation [116]. Cell specific effects of TZDs are particularly important in cancer because their action on immune cells may antagonize their effects on tumor cells. This suggests that administration of TZDs after tumor initiation may be inefficient or even deleterious and could explain why cancer mortality was increased in the meta-analysis of cancer incidence in patients with TZD treatment. Species-specific action was reported between human and murine endothelial cells where increase of proliferation was seen in the mouse cells and an antiproliferative effect in human cells [117]. Furthermore, TZDs show compound-specificity. TRO and CIGLI acted as antiproliferatives on ovarian cancer cell lines, while ROSI and PIO did not. This could be due to additional targets and/or PPARγ independent effects; TRO for instance has stronger Akt/mTOR activity than the other TZDs. Finally, the effect of TZDs is concentration-dependent. Low concentrations of TZDs induced cell cycle arrest, while higher doses (>100 μM) caused apoptosis. Effects at higher concentrations can be explained by transactivation of PPARγ by cross-talk between signaling pathways where one receptor activates a receptor for a different ligand. Alternatively, TZDs may activate a specific subunit within a receptor oligomer [118]. As to the concentration, other coactivators may be involved in the effect and different downstream processes may be activated. PPARγ agonists can also change the cell's expression of PPARγ to different extents.

Against the background of limitations of traditional as well as new (transgenic) mouse models [119, 120] for human cancer, only efficacy in human trials is included in our final assessment. Use of TZDs in cancer might be therapeutic in prostate cancer and glioma, chemopreventive in colon, lung, and breast cancer, and increase therapeutic efficacy combined with other therapies in thyroid cancer, soft tissue sarcoma, and melanoma.

Conflict of Interests

The authors declare that there is no conflict of interests.

References

[1] S. Malik, P. K. Upadhyaya, and S. Miglani, "Thiazolidinediones: a plethro of biological load," *International Journal of PharmTech Research*, vol. 3, no. 1, pp. 62–75, 2011.

[2] J. Youssed and M. Badr, "PPAR ligands," in *Peroxisome Proliferator-Activated Receptors: Discovery and Recent Advances*, J. Youssed and M. Badr, Eds., Springer Science+Business Media, New York, NY, USA, 2013.

[3] R. L. Koffarnus, K. A. Wargo, and H. M. Phillippe, "Rivoglitazone: a new thiazolidinedione for the treatment of type 2 diabetes mellitus," *Annals of Pharmacotherapy*, vol. 47, no. 6, pp. 877–885, 2013.

[4] T. Imchen, J. Manasse, K.-W. Min, and S. J. Baek, "Characterization of PPAR dual ligand MCC-555 in AOM-induced colorectal tumorigenesis," *Experimental and Toxicologic Pathology*, vol. 65, no. 6, pp. 919–924, 2013.

[5] E. Mylona, I. Giannopoulou, K. Diamantopoulou et al., "Peroxisome proliferator-activated receptor gamma expression in urothelial carcinomas of the bladder: association with differentiation, proliferation and clinical outcome," *European Journal of Surgical Oncology*, vol. 35, no. 2, pp. 197–201, 2009.

[6] S. Nomura, A. Nakajima, S. Ishimine, N. Matsuhashi, T. Kadowaki, and M. Kaminishi, "Differential expression of peroxisome proliferator-activated receptor in histologically different human gastric cancer tissues," *Journal of Experimental and Clinical Cancer Research*, vol. 25, no. 3, pp. 443–448, 2006.

[7] I. Papadaki, E. Mylona, I. Giannopoulou, S. Markaki, A. Keramopoulos, and L. Nakopoulou, "PPARγ expression in breast cancer: clinical value and correlation with ERβ," *Histopathology*, vol. 46, no. 1, pp. 37–42, 2005.

[8] G. Y. Zhang, N. Ahmed, C. Riley et al., "Enhanced expression of peroxisome proliferator-activated receptor gamma in epithelial ovarian carcinoma," *British Journal of Cancer*, vol. 92, no. 1, pp. 113–119, 2005.

[9] L. Yao, F. Liu, L. Sun et al., "Upregulation of PPARγ in tissue with gastric carcinoma," *Hybridoma*, vol. 29, no. 4, pp. 341–343, 2010.

[10] M. Pancione, L. Sabatino, A. Fucci et al., "Epigenetic silencing of peroxisome proliferator- activated receptor γ is a biomarker for colorectal cancer progression and adverse patients' outcome," *PLoS ONE*, vol. 5, no. 12, Article ID e14229, 2010.

[11] S. Ogino, K. Shima, Y. Baba et al., "Colorectal cancer expression of peroxisome proliferator-activated receptor gamma (PPARG, PPARgamma) is associated with good prognosis," *Gastroenterology*, vol. 136, no. 4, pp. 1242–1250, 2009.

[12] M. Nickkho-Amiry, R. McVey, and C. Holland, "Peroxisome proliferator-activated receptors modulate proliferation and angiogenesis in human endometrial carcinoma," *Molecular Cancer Research*, vol. 10, no. 3, pp. 441–453, 2012.

[13] C. K. Glass and K. Saijo, "Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells," *Nature Reviews Immunology*, vol. 10, no. 5, pp. 365–376, 2010.

[14] M. J. Pishvaian, J. L. Marshall, A. J. Wagner et al., "A phase 1 study of efatutazone, an oral peroxisome proliferator-activated receptor gamma agonist, administered to patients with advanced malignancies," *Cancer*, vol. 118, no. 21, pp. 5403–5413, 2012.

[15] X. Sun, J. D. Ritzenthaler, Y. Zheng, J. Roman, and S. Han, "Rosiglitazone inhibits α4 nicotinic acetylcholine receptor expression in human lung carcinoma cells through peroxisome proliferator-activated receptor γ-independent signals," *Molecular Cancer Therapeutics*, vol. 8, no. 1, pp. 110–118, 2009.

[16] F. Chen and L. E. Harrison, "Ciglitazone-induced cellular antiproliferation increases p27 kip1 protein levels through both increased transcriptional activity and inhibition of proteasome degradation," *Cellular Signalling*, vol. 17, no. 7, pp. 809–816, 2005.

[17] D. Panigrahy, S. Singer, L. Q. Shen et al., "PPARγ ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis," *The Journal of Clinical Investigation*, vol. 110, no. 7, pp. 923–932, 2002.

[18] B. Shen, E. S. H. Chu, G. Zhao et al., "PPARgamma inhibits hepatocellular carcinoma metastases in vitro and in mice," *British Journal of Cancer*, vol. 106, no. 9, pp. 1486–1494, 2012.

[19] A. Galli, E. Ceni, D. W. Crabb et al., "Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARγ independent mechanisms," *Gut*, vol. 53, no. 11, pp. 1688–1697, 2004.

[20] P. E. Moss, B. E. Lyles, and L. V. Stewart, "The PPARγ ligand ciglitazone regulates androgen receptor activation differently in androgen-dependent versus androgen-independent human prostate cancer cells," *Experimental Cell Research*, vol. 316, no. 20, pp. 3478–3488, 2010.

[21] B. E. Lyles, T. O. Akinyeke, P. E. Moss, and L. V. Stewart, "Thiazolidinediones regulate expression of cell cycle proteins in human prostate cancer cells via PPARγ-dependent and PPARγ-independent pathways," *Cell Cycle*, vol. 8, no. 2, pp. 268–277, 2009.

[22] A. Mantovani and A. Sica, "Macrophages, innate immunity and cancer: balance, tolerance, and diversity," *Current Opinion in Immunology*, vol. 22, no. 2, pp. 231–237, 2010.

[23] R. Seger and E. G. Krebs, "The MAPK signaling cascade," *FASEB Journal*, vol. 9, no. 9, pp. 726–735, 1995.

[24] E. Burgermeister and R. Seger, "PPARγ and MEK interactions in cancer," *PPAR Research*, vol. 2008, Article ID 309469, 16 pages, 2008.

[25] X. Li, X. Yang, Y. Xu, X. Jiang, F. Nan, and H. Tang, "Troglitazone inhibits cell proliferation by attenuation of epidermal growth factor receptor signaling independent of peroxisome proliferator-activated receptor γ," *Cell Research*, vol. 19, no. 6, pp. 720–732, 2009.

[26] M. Dall'Asta, E. Derlindati, D. Ardigò, I. Zavaroni, F. Brighenti, and D. Del Rio, "Macrophage polarization: the answer to the diet/inflammation conundrum?" *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 22, no. 5, pp. 387–392, 2012.

[27] F. Meisner, D. Walcher, F. Gizard et al., "Effect of rosiglitazone treatment on plaque inflammation and collagen content in nondiabetic patients: data from a randomized placebo-controlled trial," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 4, pp. 845–850, 2006.

[28] C. Ao, Y. Huo, L. Qi, Z. Xiong, L. Xue, and Y. Qi, "Pioglitazone suppresses the lipopolysaccharide-induced production of inflammatory factors in mouse macrophages by inactivating NF-κB," *Cell Biology International*, vol. 34, no. 7, pp. 723–730, 2010.

[29] E. Burgermeister, D. Chuderland, T. Hanoch, M. Meyer, M. Liscovitch, and R. Seger, "Interaction with MEK causes nuclear export and downregulation of peroxisome proliferator-activated receptor γ," *Molecular and Cellular Biology*, vol. 27, no. 3, pp. 803–817, 2007.

[30] R. E. Nakles, B. V. S. Kallakury, and P. A. Furth, "The PPARγ agonist efatutazone increases the spectrum of well-differentiated mammary cancer subtypes initiated by loss of full-length BRCA1 in association with TP53 haploinsufficiency," *The American Journal of Pathology*, vol. 182, no. 6, pp. 1976–1985, 2013.

[31] N. Shimazaki, N. Togashi, M. Hanai et al., "Anti-tumour activity of CS-7017, a selective peroxisome proliferator-activated receptor gamma agonist of thiazolidinedione class, in human tumour xenografts and a syngeneic tumour implant model," *European Journal of Cancer*, vol. 44, no. 12, pp. 1734–1743, 2008.

[32] G. D. Demetri, C. D. Fletcher, E. Mueller et al., "Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-γ ligand troglitazone in patients with liposarcoma," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 7, pp. 3951–3956, 1999.

[33] G. Debrock, V. Vanhentenrijk, R. Sciot, M. Debiec-Rychter, R. Oyen, and A. van Oosterom, "A phase II trial with rosiglitazone in liposarcoma patients," *British Journal of Cancer*, vol. 89, no. 8, pp. 1409–1412, 2003.

[34] Y. Dai and W. H. Wang, "Peroxisome proliferator-activated receptor gamma and colorectal cancer," *World Journal of Gastrointestinal Oncology*, vol. 2, no. 3, pp. 159–164, 2010.

[35] M. Pancione, N. Forte, L. Sabatino et al., "Reduced β-catenin and peroxisome proliferator-activated receptor-γ expression levels are associated with colorectal cancer metastatic progression: correlation with tumor-associated macrophages, cyclooxygenase 2, and patient outcome," *Human Pathology*, vol. 40, no. 5, pp. 714–725, 2009.

[36] S. Theocharis, C. Giaginis, A. Parasi et al., "Expression of peroxisome proliferator-activated receptor-γ in colon cancer: correlation with histopathological parameters, cell cycle-related molecules, and patients' survival," *Digestive Diseases and Sciences*, vol. 52, no. 9, pp. 2305–2311, 2007.

[37] A. Gustafsson, E. Hansson, U. Kressner et al., "EP1-4 subtype, COX and PPARγ receptor expression in colorectal cancer in prediction of disease-specific mortality," *International Journal of Cancer*, vol. 121, no. 2, pp. 232–240, 2007.

[38] C. A. McAlpine, Y. Barak, I. Matise, and R. T. Cormier, "Intestinal-specific PPARγ deficiency enhances tumorigenesis in ApcMin/+ mice," *International Journal of Cancer*, vol. 119, no. 10, pp. 2339–2346, 2006.

[39] E. Osawa, A. Nakajima, K. Wada et al., "Peroxisome proliferator-activated receptor γ ligands suppress colon carcinogenesis induced by azoxymethane in mice," *Gastroenterology*, vol. 124, no. 2, pp. 361–367, 2003.

[40] T. Yoshizumi, T. Ohta, I. Ninomiya et al., "Thiazolidinedione, a peroxisome proliferator-activated receptor-gamma ligand, inhibits growth and metastasis of HT-29 human colon cancer cells through differentiation-promoting effects," *International Journal of Oncology*, vol. 25, no. 3, pp. 631–639, 2004.

[41] I. K. Choi, Y. H. Kim, J. S. Kim, and J. H. Seo, "PPAR-γ ligand promotes the growth of APC-mutated HT-29 human colon cancer cells in vitro and in vivo," *Investigational New Drugs*, vol. 26, no. 3, pp. 283–288, 2008.

[42] T. Tsukahara and H. Haniu, "Peroxisome proliferator-activated receptor gamma overexpression suppresses proliferation of human colon cancer cells," *Biochemical and Biophysical Research Communications*, vol. 424, no. 3, pp. 524–529, 2012.

[43] M. H. Kulke, G. D. Demetri, N. E. Sharpless et al., "A phase II study of troglitazone, an activator of the PPARγ receptor, in patients with chemotherapy-resistant metastatic colorectal cancer," *Cancer Journal*, vol. 8, no. 5, pp. 395–399, 2002.

[44] S. Theocharis, H. Kanelli, E. Politi et al., "Expression of peroxisome proliferator activated receptor-gamma in non-small cell lung carcinoma: correlation with histological type and grade," *Lung Cancer*, vol. 36, no. 3, pp. 249–255, 2002.

[45] H. Sasaki, M. Tanahashi, H. Yukiue et al., "Decreased perioxisome proliferator-activated receptor gamma gene expression was correlated with poor prognosis in patients with lung cancer," *Lung Cancer*, vol. 36, no. 1, pp. 71–76, 2002.

[46] C. M. Lyon, D. M. Klinge, K. C. Do et al., "Rosiglitazone prevents the progression of preinvasive lung cancer in a murine model," *Carcinogenesis*, vol. 30, no. 12, pp. 2095–2099, 2009.

[47] W. G. Jiang, A. Douglas-Jones, and R. E. Mansel, "Expression of peroxisome-proliferator activated receptor-gamma (PPARγ) and the PPARγ co-activator, PGC-1, in human breast cancer correlates with clinical outcomes," *International Journal of Cancer*, vol. 106, no. 5, pp. 752–757, 2003.

[48] G. Magenta, X. Borenstein, R. Rolando, and M. A. Jasnis, "Rosiglitazone inhibits metastasis development of a murine mammary tumor cell line LMM3," *BMC Cancer*, vol. 8, article 47, 2008.

[49] B. Bojková, M. Garajová, K. Kajo et al., "Pioglitazone in chemically induced mammary carcinogenesis in rats," *European Journal of Cancer Prevention*, vol. 19, no. 5, pp. 379–384, 2010.

[50] H. J. Burstein, G. D. Demetri, E. Mueller, P. Sarraf, B. M. Spiegelman, and E. P. Winer, "Use of the peroxisome proliferator-activated receptor (PPAR) γ ligand troglitazone as treatment for refractory breast cancer: a phase II study," *Breast Cancer Research and Treatment*, vol. 79, no. 3, pp. 391–397, 2003.

[51] L. D. Yee, N. Williams, P. Wen et al., "Pilot study of rosiglitazone therapy in women with breast cancer: effects of short-term therapy on tumor tissue and serum markers," *Clinical Cancer Research*, vol. 13, no. 1, pp. 246–252, 2007.

[52] Y. Nakamura, T. Suzuki, A. Sugawara, Y. Arai, and H. Sasano, "Peroxisome proliferator-activated receptor gamma in human prostate carcinoma," *Pathology International*, vol. 59, no. 5, pp. 288–293, 2009.

[53] J.-S. Annicotte, I. Iankova, S. Miard et al., "Peroxisome proliferator-activated receptor γ regulates E-cadherin expression and inhibits growth and invasion of prostate cancer," *Molecular and Cellular Biology*, vol. 26, no. 20, pp. 7561–7574, 2006.

[54] E. Mueller, M. Smith, P. Sarraf et al., "Effects of ligand activation of peroxisome proliferator-activated receptor γ in human prostate cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 20, pp. 10990–10995, 2000.

[55] J. I. Hisatake, T. Ikezoe, M. Carey, S. Holden, S. Tomoyasu, and H. P. Koeffler, "Down-regulation of prostate-specific antigen expression by ligands for peroxisome proliferator-activated receptor γ in human prostate cancer," *Cancer Research*, vol. 60, no. 19, pp. 5494–5498, 2000.

[56] M. Kato, T. Nagaya, M. Fujieda, K. Saito, J. Yoshida, and H. Seo, "Expression of PPARγ and its ligand-dependent growth inhibition in human brain tumor cell lines," *Japanese Journal of Cancer Research*, vol. 93, no. 6, pp. 660–666, 2002.

[57] C. Grommes, D. S. Conway, A. Alshekhlee, and J. S. Barnholtz-Sloan, "Inverse association of PPARγ agonists use and high grade glioma development," *Journal of Neuro-Oncology*, vol. 100, no. 2, pp. 233–239, 2010.

[58] C. Grommes, J. C. Karlo, A. Caprariello, D. Blankenship, A. Dechant, and G. E. Landreth, "The PPARγ agonist pioglitazone crosses the blood-brain barrier and reduces tumor growth in a human xenograft model," *Cancer Chemotherapy and Pharmacology*, vol. 71, no. 4, pp. 929–936, 2013.

[59] P. Hau, L. Kunz-Schughart, U. Bogdahn et al., "Low-dose chemotherapy in combination with COX-2 inhibitors and PPAR-gamma agonists in recurrent high-grade gliomas—a phase II study," *Oncology*, vol. 73, no. 1-2, pp. 21–25, 2007.

[60] S. Meyer, T. Vogt, M. Landthaler et al., "Research article: Cyclooxygenase 2 (COX2) and peroxisome proliferator-activated receptor gamma (PPARG) are stage-dependent prognostic markers of malignant melanoma," *PPAR Research*, vol. 2010, Article ID 848645, 10 pages, 2010.

[61] A. Ferrara, J. D. Lewis, C. P. Quesenberry Jr. et al., "Cohort study of pioglitazone and cancer incidence in patients with diabetes," *Diabetes Care*, vol. 34, no. 4, pp. 923–929, 2011.

[62] T. Botton, A. Puissant, P. Bahadoran et al., "In vitro and in vivo anti-melanoma effects of ciglitazone," *Journal of Investigative Dermatology*, vol. 129, no. 5, pp. 1208–1218, 2009.

[63] R. Govindarajan, L. Ratnasinghe, D. L. Simmons et al., "Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes," *Journal of Clinical Oncology*, vol. 25, no. 12, pp. 1476–1481, 2007.

[64] M. Monami, I. Dicembrini, and E. Mannucci, "Thiazolidinediones and cancer: results of a meta-analysis of randomized clinical trials," *Acta Diabetologica*, vol. 51, no. 1, pp. 91–101, 2014.

[65] S. Kawa, T. Nikaido, H. Unno, N. Usuda, K. Nakayama, and K. Kiyosawa, "Growth inhibition and differentiation of pancreatic cancer cell lines by PPARγ ligand troglitazone," *Pancreas*, vol. 24, no. 1, pp. 1–7, 2002.

[66] M. Kato, T. Kusumi, S. Tsuchida, M. Tanaka, M. Sasaki, and H. Kudo, "Induction of differentiation and peroxisome proliferator-activated receptor γ expression in colon cancer cell lines by troglitazone," *Journal of Cancer Research and Clinical Oncology*, vol. 130, no. 2, pp. 73–79, 2004.

[67] I. Jagan, A. Fatehullah, R. K. Deevi, V. Bingham, and F. C. Campbell, "Rescue of glandular dysmorphogenesis in PTEN-deficient colorectal cancer epithelium by PPARγ-targeted therapy," *Oncogene*, vol. 32, no. 10, pp. 1305–1315, 2013.

[68] Y. Li, Y. Meng, H. Li et al., "Growth inhibition and differentiation induced by peroxisome proliferator activated receptor gamma ligand rosiglitazone in human melanoma cell line A375," *Medical Oncology*, vol. 23, no. 3, pp. 393–402, 2006.

[69] A. Aiello, G. Pandini, F. Frasca et al., "Peroxisomal proliferator-activated receptor-γ agonists induce partial reversion of epithelial-mesenchymal transition in anaplastic thyroid cancer cells," *Endocrinology*, vol. 147, no. 9, pp. 4463–4475, 2006.

[70] E. Pestereva, S. Kanakasabai, and J. J. Bright, "PPARγ agonists regulate the expression of stemness and differentiation genes in brain tumour stem cells," *British Journal of Cancer*, vol. 106, no. 10, pp. 1702–1712, 2012.

[71] C. L. Varley, J. Stahlschmidt, B. Smith, M. Stower, and J. Southgate, "Activation of peroxisome proliferator-activated receptor-gamma reverses squamous metaplasia and induces transitional differentiation in normal human urothelial cells," *The American Journal of Pathology*, vol. 164, no. 5, pp. 1789–1798, 2004.

[72] M.-Y. Li, A. W. Y. Kong, H. Yuan et al., "Pioglitazone prevents smoking carcinogen-induced lung tumor development in mice," *Current Cancer Drug Targets*, vol. 12, no. 6, pp. 597–606, 2012.

[73] I. Borbath, I. Leclercq, P. Moulin, C. Sempoux, and Y. Horsmans, "The PPARgamma agonist pioglitazone inhibits early neoplastic occurrence in the rat liver," *European Journal of Cancer*, vol. 43, no. 11, pp. 1755–1763, 2007.

[74] J. Yu, L. Qiao, L. Zimmermann et al., "Troglitazone inhibits tumor growth in hepatocellular carcinoma in vitro and in vivo," *Hepatology*, vol. 43, no. 1, pp. 134–143, 2006.

[75] W. Wu, J. Celestino, M. R. Milam et al., "Primary chemoprevention of endometrial hyperplasia with the peroxisome proliferator-activated receptor gamma agonist rosiglitazone in the PTEN heterozygote murine model," *International Journal of Gynecological Cancer*, vol. 18, no. 2, pp. 329–338, 2008.

[76] S. Theocharis, J. Klijanienko, C. Giaginis et al., "Peroxisome proliferator-activated receptor-γ in mobile tongue squamous cell carcinoma: associations with clinicopathological parameters and patients survival," *Journal of Cancer Research and Clinical Oncology*, vol. 137, no. 2, pp. 251–259, 2011.

[77] R. Suzuki, H. Kohno, M. Suzui et al., "An animal model for the rapid induction of tongue neoplasms in human c-Ha-ras proto-oncogene transgenic rats by 4-nitroquinoline 1-oxide: its potential use for preclinical chemoprevention studies," *Carcinogenesis*, vol. 27, no. 3, pp. 619–630, 2006.

[78] K. Yoshida, Y. Hirose, T. Tanaka et al., "Inhibitory effects of troglitazone, a peroxisome proliferator-activated receptor γ ligand, in rat tongue carcinogenesis initiated with 4-nitroquinoline 1-oxide," *Cancer Science*, vol. 94, no. 4, pp. 365–371, 2003.

[79] H. Li, A. L. Sorenson, J. Poczobutt et al., "Activation of PPARγ in myeloid cells promotes lung cancer progression and metastasis," *PLoS ONE*, vol. 6, no. 12, Article ID e28133, 2011.

[80] D. Bonofiglio, E. Cione, H. Qi et al., "Combined low doses of PPARγ and RXR ligands trigger an intrinsic apoptotic pathway in human breast cancer cells," *The American Journal of Pathology*, vol. 175, no. 3, pp. 1270–1280, 2009.

[81] P. Tontonoz, S. Singer, B. M. Forman et al., "Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 1, pp. 237–241, 1997.

[82] L. Q. Cao, X. L. Wang, Q. Wang et al., "Rosiglitazone sensitizes hepatocellular carcinoma cell lines to 5-fluorouracil antitumor activity through activation of the PPARγ signaling pathway," *Acta Pharmacologica Sinica*, vol. 30, no. 9, pp. 1316–1322, 2009.

[83] S. Y. Lee, G. Y. Hur, K. H. Jung et al., "PPAR-γ agonist increase gefitinib's antitumor activity through PTEN expression," *Lung Cancer*, vol. 51, no. 3, pp. 297–301, 2006.

[84] Z. Yang, R. Bagheri-Yarmand, S. Balasenthil et al., "HER2 regulation of peroxisome proliferator-activated receptor γ (PPARγ) expression and sensitivity of breast cancer cells to PPARγ ligand therapy," *Clinical Cancer Research*, vol. 9, no. 8, pp. 3198–3203, 2003.

[85] G. D. Girnun, E. Naseri, S. B. Vafai et al., "Synergy between PPARγ ligands and platinum-based drugs in cancer," *Cancer Cell*, vol. 11, no. 5, pp. 395–406, 2007.

[86] K. Tikoo, P. Kumar, and J. Gupta, "Rosiglitazone synergizes anticancer activity of cisplatin and reduces its nephrotoxicity in 7, 12-dimethyl benzaanthracene (DMBA) induced breast cancer rats," *BMC Cancer*, vol. 9, article 107, 2009.

[87] N. Hamaguchi, H. Hamada, S. Miyoshi et al., "In vitro and in vivo therapeutic efficacy of the PPAR-γ agonist troglitazone in combination with cisplatin against malignant pleural mesothelioma cell growth," *Cancer Science*, vol. 101, no. 9, pp. 1955–1964, 2010.

[88] J. A. Copland, L. A. Marlow, S. Kurakata et al., "Novel high-affinity PPARγ agonist alone and in combination with paclitaxel inhibits human anaplastic thyroid carcinoma tumor growth via p21WAF1/CIP1," *Oncogene*, vol. 25, no. 16, pp. 2304–2317, 2006.

[89] Y. Yokoyama, B. Xin, T. Shigeto, and H. Mizunuma, "Combination of ciglitazone, a peroxisome proliferator-activated receptor gamma ligand, and cisplatin enhances the inhibition of growth

of human ovarian cancers," *Journal of Cancer Research and Clinical Oncology*, vol. 137, no. 8, pp. 1219–1228, 2011.

[90] H. Fu, J. Zhang, J. Pan et al., "Chemoprevention of lung carcinogenesis by the combination of aerosolized budesonide and oral pioglitazone in A/J mice," *Molecular Carcinogenesis*, vol. 50, no. 12, pp. 913–921, 2011.

[91] A. Reichle, K. Bross, T. Vogt et al., "Pioglitazone and rofecoxib combined with angiostatically scheduled trofosfamide in the treatment of far-advanced melanoma and soft tissue sarcoma," *Cancer*, vol. 101, no. 10, pp. 2247–2256, 2004.

[92] T. Vogt, C. Hafner, K. Bross et al., "Antiangiogenetic therapy with pioglitazone, rofecoxib, and metronomic trofosfamide in patients with advanced malignant vascular tumors," *Cancer*, vol. 98, no. 10, pp. 2251–2256, 2003.

[93] F. Ondrey, "Peroxisome proliferator-activated receptor γ pathway targeting in carcinogenesis: implications For Chemoprevention," *Clinical Cancer Research*, vol. 15, no. 1, pp. 2–8, 2009.

[94] O. Omur and Y. Baran, "An update on molecular biology of thyroid cancers," *Critical Reviews in Oncology/Hematology*, vol. 90, no. 3, pp. 233–252, 2014.

[95] N. Li, X. L. Du, L. R. Reitzel, L. Xu, and E. M. Sturgis, "Impact of enhanced detection on the increase in thyroid cancer incidence in the United States: review of incidence trends by socioeconomic status within the surveillance, epidemiology, and end results registry, 1980–2008," *Thyroid*, vol. 23, no. 1, pp. 103–110, 2013.

[96] C. F. A. Eustatia-Rutten, E. P. M. Corssmit, N. R. Biermasz, A. M. Pereira, J. A. Romijn, and J. W. Smit, "Survival and death causes in differentiated thyroid carcinoma," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 1, pp. 313–319, 2006.

[97] C. Durante, N. Haddy, E. Baudin et al., "Long-term outcome of 444 patients with distant metastases from papillary and follicular thyroid carcinoma: Benefits and limits of radioiodine therapy," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 8, pp. 2892–2899, 2006.

[98] O. Dohán, A. De La Vieja, V. Paroder et al., "The sodium/iodide symporter (NIS): characterization, regulation, and medical significance," *Endocrine Reviews*, vol. 24, no. 1, pp. 48–77, 2003.

[99] M. J. Schlumberger, "Diagnostic follow-up of well-differentiated thyroid carcinoma: historical perspective and current status," *Journal of Endocrinological Investigation*, vol. 22, no. 11, pp. 3–7, 1999.

[100] T. Carling and R. Udelsman, Eds., *Thyroid Tumors*, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 9th edition, 2011.

[101] A. Versari, M. Sollini, A. Frasoldati et al., "Differentiated thyroid cancer: a new perspective with radiolabeled somatostatin analogues for imaging and treatment of patients," *Thyroid*, vol. 24, no. 4, pp. 715–726, 2014.

[102] M. L. Martelli, R. Iuliano, I. Le Pera et al., "Inhibitory effects of peroxisome proliferator-activated receptor γ on thyroid carcinoma cell growth," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 10, pp. 4728–4735, 2002.

[103] E. Fröhlich, P. Brossart, and R. Wahl, "Induction of iodide uptake in transformed thyrocytes: a compound screening in cell lines," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 36, no. 5, pp. 780–790, 2009.

[104] J.-W. Park, R. Zarnegar, H. Kanauchi et al., "Troglitazone, the peroxisome proliferator-activated receptor-γ agonist, induces antiproliferation and redifferentiation in human thyroid cancer cell lines," *Thyroid*, vol. 15, no. 3, pp. 222–231, 2005.

[105] E. Fröhlich, F. Machicao, and R. Wahl, "Action of thiazolidinediones on differentiation, proliferation and apoptosis of normal and transformed thyrocytes in culture," *Endocrine-Related Cancer*, vol. 12, no. 2, pp. 291–303, 2005.

[106] M. E. Dobson, E. Diallo-Krou, V. Grachtchouk et al., "Pioglitazone induces a proadipogenic antitumor response in mice with PAX8-PPARγ fusion protein thyroid carcinoma," *Endocrinology*, vol. 152, no. 11, pp. 4455–4465, 2011.

[107] Y. Kato, H. Ying, L. Zhao et al., "PPARγ insufficiency promotes follicular thyroid carcinogenesis via activation of the nuclear factor-κB signaling pathway," *Oncogene*, vol. 25, no. 19, pp. 2736–2747, 2006.

[108] S. J. Rosenbaum-Krumme, A. Bockisch, and J. Nagarajah, "Pioglitazone therapy in progressive differentiated thyroid carcinoma," *NuklearMedizin*, vol. 51, no. 4, pp. 111–115, 2012.

[109] M. Elola, A. Yoldi, J. I. Emparanza, T. Matteucci, I. Bilbao, and M. Goena, "Redifferentiation therapy with rosiglitazone in a case of differentiated thyroid cancer with pulmonary metastases and absence of radioiodine uptake," *Revista Espanola de Medicina Nuclear*, vol. 30, no. 4, pp. 241–243, 2011.

[110] A. N. Elias and P. Lizotte, "Enhanced radioiodine uptake in a patient with poorly differentiated papillary thyroid cancer after treatment with rosiglitazone," *Clinical Nuclear Medicine*, vol. 31, no. 9, pp. 517–519, 2006.

[111] J.-C. Philips, C. Petite, J.-P. Willi, F. Buchegger, and C. A. Meier, "Effect of peroxisome proliferator-activated receptor γ agonist, rosiglitazone, on dedifferentiated thyroid cancers," *Nuclear Medicine Communications*, vol. 25, no. 12, pp. 1183–1186, 2004.

[112] E. Kebebew, M. Peng, E. Reiff et al., "A phase II trial of rosiglitazone in patients with thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer," *Surgery*, vol. 140, no. 6, pp. 960–967, 2006.

[113] S. Tepmongkol, S. Keelawat, S. Honsawek, and P. Ruangvejvorachai, "Rosiglitazone effect on radioiodine uptake in thyroid carcinoma patients with high thyroglobulin but negative total body scan: a correlation with the expression of peroxisome proliferator-activated receptor-gamma," *Thyroid*, vol. 18, no. 7, pp. 697–704, 2008.

[114] E. Kebebew, S. Lindsay, O. H. Clark, K. A. Woeber, R. Hawkins, and F. S. Greenspan, "Results of rosiglitazone therapy in patients with thyroglobulin-positive and radioiodine-negative advanced differentiated thyroid cancer," *Thyroid*, vol. 19, no. 9, pp. 953–956, 2009.

[115] E. A. Eisenhauer, P. Therasse, J. Bogaerts et al., "New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1)," *European Journal of Cancer*, vol. 45, no. 2, pp. 228–247, 2009.

[116] J. D. Burton, D. M. Goldenberg, and R. D. Blumenthal, "Potential of peroxisome proliferator-activated receptor gamma antagonist compounds as therapeutic agents for a wide range of cancer types," *PPAR Research*, vol. 2008, Article ID 494161, 7 pages, 2008.

[117] S. Kakiuchi-Kiyota, J. A. Vetro, S. Suzuki et al., "Effects of the PPARγ agonist troglitazone on endothelial cells in vivo and in vitro: Differences between human and mouse," *Toxicology and Applied Pharmacology*, vol. 237, no. 1, pp. 83–90, 2009.

[118] S. Takano, T. Kubota, H. Nishibori et al., "Pioglitazone, a ligand for peroxisome proliferator-activated receptor-γ acts as an inhibitor of colon cancer liver metastasis," *Anticancer Research*, vol. 28, no. 6, pp. 3593–3599, 2008.

[119] S. J. Diede, Z. Yao, C. Chip Keyes et al., "Fundamental differences in promoter CpG island DNA hypermethylation between human cancer and genetically engineered mouse models of cancer," *Epigenetics*, vol. 8, no. 12, pp. 1254–1260, 2013.

[120] M. De Jong and T. Maina, "Of mice and humans: are they the same?—Implications in cancer translational research," *Journal of Nuclear Medicine*, vol. 51, no. 4, pp. 501–504, 2010.

[121] V. Vaish, L. Tanwar, and S. N. Sanyal, "The role of NF-κB and PPARγ in experimentally induced colorectal cancer and chemoprevention by cyclooxygenase-2 inhibitors," *Tumour Biology*, vol. 31, no. 5, pp. 427–436, 2010.

[122] M. V. Pino, M. F. Kelley, and Z. Jayyosi, "Promotion of colon tumors in C57BL/6J-APCmin/+ mice by thiazolidinedione PPARγ agonists and a structurally unrelated PPARγ agonist," *Toxicologic Pathology*, vol. 32, no. 1, pp. 58–63, 2004.

[123] H. Kohno, S. Yoshitani, S. Takashima et al., "Troglitazone, a ligand for peroxisome proliferator-activated receptor γ, inhibits chemically-induced aberrant crypt foci in rats," *Japanese Journal of Cancer Research*, vol. 92, no. 4, pp. 396–403, 2001.

[124] E. J. Han, C. N. Im, S. H. Park, E. Y. Moon, and S. H. Hong, "Combined treatment with peroxisome proliferator-activated receptor (PPAR) gamma ligands and gamma radiation induces apoptosis by PPARγ-independent up-regulation of reactive oxygen species-induced deoxyribonucleic acid damage signals in non-small cell lung cancer cells," *International Journal of Radiation Oncology Biology Physics*, vol. 85, no. 5, pp. e239–e248, 2013.

[125] S. Y. James, F. Lin, S. K. Kolluri, M. I. Dawson, and X.-K. Zhang, "Regulation of retinoic acid receptor β expression by peroxisome proliferator-activated receptor γ ligands in cancer cells," *Cancer Research*, vol. 63, no. 13, pp. 3531–3538, 2003.

[126] A. Papi, P. Rochhi, A. M. Ferreri, F. Guerra, and M. Orlandi, "Enhanced effects of PPARγ ligands and RXR selective retinoids in combination to inhibit migration and invasiveness in cancer cells," *Oncology Reports*, vol. 21, no. 4, pp. 1083–1089, 2009.

[127] A. Papi, L. Tatenhorst, D. Terwel et al., "PPARγ and RXRγ ligands act synergistically as potent antineoplastic agents in vitro and in vivo glioma models," *Journal of Neurochemistry*, vol. 109, no. 6, pp. 1779–1790, 2009.

[128] K. Bräutigam, J. Biernath-Wüpping, D. O. Bauerschlag et al., "Combined treatment with TRAIL and PPARγ ligands overcomes chemoresistance of ovarian cancer cell lines," *Journal of Cancer Research and Clinical Oncology*, vol. 137, no. 5, pp. 875–886, 2011.

[129] P. Mrówka, E. Glodkowska, D. Nowis et al., "Ciglitazone, an agonist of peroxisome proliferator-activated receptor γ, exerts potentiated cytostatic/cytotoxic effects against tumor cells when combined with lovastatin," *International Journal of Oncology*, vol. 32, no. 1, pp. 249–255, 2008.

[130] T. H. Chang and E. Szabo, "Enhanced growth inhibition by combination differentiation therapy with ligands of peroxisome proliferator-activated receptor-γ and inhibitors of histone deacetylase in adenocarcinoma of the lung," *Clinical Cancer Research*, vol. 8, no. 4, pp. 1206–1212, 2002.

[131] Y. Liu, Z.-A. Zhu, S.-N. Zhang et al., "Combinational effect of PPARγ agonist and RXR agonist on the growth of SGC7901 gastric carcinoma cells in vitro," *Tumor Biology*, vol. 34, no. 4, pp. 2409–2418, 2013.

[132] R. C. Reddy, A. Srirangam, K. Reddy et al., "Chemotherapeutic drugs induce PPAR-γ expression and show sequence-specific synergy with PPAR-γ ligands in inhibition of non-small cell lung cancer," *Neoplasia*, vol. 10, no. 6, pp. 597–603, 2008.

[133] E. Elstner, E. A. Williamson, C. Zang et al., "Novel therapeutic approach: ligands for PPARγ and retinoid receptors induce apoptosis in bcl-2-positive human breast cancer cells," *Breast Cancer Research and Treatment*, vol. 74, no. 2, pp. 155–165, 2002.

[134] B.-H. Park, S.-B. Lee, D. B. Stolz, Y. J. Lee, and B.-C. Lee, "Synergistic interactions between heregulin and peroxisome proliferator-activated receptor-γ (PPARγ) agonist in breast cancer cells," *Journal of Biological Chemistry*, vol. 286, no. 22, pp. 20087–20099, 2011.

[135] C.-J. Yao, G.-M. Lai, C.-F. Chan, A.-L. Cheng, Y.-Y. Yang, and S.-E. Chuang, "Dramatic synergistic anticancer effect of clinically achievable doses of lovastatin and troglitazone," *International Journal of Cancer*, vol. 118, no. 3, pp. 773–779, 2006.

[136] K.-H. Yan, C.-J. Yao, H.-Y. Chang, G.-M. Lai, A.-L. Cheng, and S.-E. Chuang, "The synergistic anticancer effect of troglitazone combined with aspirin causes cell cycle arrest and apoptosis in human lung cancer cells," *Molecular Carcinogenesis*, vol. 49, no. 3, pp. 235–246, 2010.

[137] H.-N. Yu, E.-M. Noh, Y.-R. Lee et al., "Troglitazone enhances tamoxifen-induced growth inhibitory activity of MCF-7 cells," *Biochemical and Biophysical Research Communications*, vol. 377, no. 1, pp. 242–247, 2008.

[138] Z. An, X. Liu, H. Song et al., "Effect of troglitazone on radiation sensitivity in cervix cancer cells," *Radiation Oncology Journal*, vol. 30, no. 2, pp. 78–87, 2012.

[139] Y.-Q. Zhang, X.-Q. Tang, L. Sun et al., "Rosiglitazone enhances fluorouracil-induced apoptosis of HT-29 cells by activating peroxisome proliferator-activated receptor γ," *World Journal of Gastroenterology*, vol. 13, no. 10, pp. 1534–1540, 2007.

[140] R. M. Cesario, J. Stone, W.-C. Yen, R. P. Bissonnette, and W. W. Lamph, "Differentiation and growth inhibition mediated via the RXR:PPARγ heterodimer in colon cancer," *Cancer Letters*, vol. 240, no. 2, pp. 225–233, 2006.

[141] M. Mody, N. Dharker, M. Bloomston et al., "Rosiglitazone sensitizes MDA-MB-231 breast cancer cells to anti-tumour effects of tumour necrosis factor-α, CH11 and CYC202," *Endocrine-Related Cancer*, vol. 14, no. 2, pp. 305–315, 2007.

[142] H. Koga, K. Selvendiran, R. Sivakumar et al., "PPARγ potentiates anticancer effects of gemcitabine on human pancreatic cancer cells," *International Journal of Oncology*, vol. 40, no. 3, pp. 679–685, 2012.

[143] C. Freudlsperger, A. Thies, U. Pfuller, and U. Schumacher, "The proteasome inhibitor bortezomib augments anti-proliferative effects of mistletoe lectin-I and the PPAR-γ agonist rosiglitazone in human melanoma cells," *Anticancer Research*, vol. 27, no. 1, pp. 207–213, 2007.

[144] S. Gehring, J. H. Tapia-Pérez, E. Kirches et al., "Cytotoxic effects of statins and thiazolidinediones on meningioma cells," *Journal of Neuro-Oncology*, vol. 102, no. 3, pp. 383–393, 2011.

[145] J. H. Tapia-Pérez, E. Kirches, C. Mawrin, R. Firsching, and T. Schneider, "Cytotoxic effect of different statins and thiazolidinediones on malignant glioma cells," *Cancer Chemotherapy and Pharmacology*, vol. 67, no. 5, pp. 1193–1201, 2011.

[146] E. Gottfried, S. Rogenhofer, H. Waibel et al., "Pioglitazone modulates tumor cell metabolism and proliferation in multicellular tumor spheroids," *Cancer Chemotherapy and Pharmacology*, vol. 67, no. 1, pp. 117–126, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 920618, 12 pages
http://dx.doi.org/10.1155/2015/920618

Review Article

The Association between Type 2 Diabetes Mellitus and Women Cancer: The Epidemiological Evidences and Putative Mechanisms

Kyong Hye Joung,[1] Jae-Wook Jeong,[2] and Bon Jeong Ku[1]

[1]*Department of Internal Medicine, Chungnam National University School of Medicine, 282 Munhwa-ro, Jung-gu, Daejeon 301-721, Republic of Korea*
[2]*Department of Obstetrics, Gynecology and Reproductive Biology, Michigan State University, College of Human Medicine, 333 Bostwick Avenue NE, Grand Rapids, MI 49503, USA*

Correspondence should be addressed to Jae-Wook Jeong; jaewook.jeong@hc.msu.edu and Bon Jeong Ku; bonjeong@cnu.ac.kr

Received 24 June 2014; Revised 12 September 2014; Accepted 8 October 2014

Academic Editor: Viroj Boonyaratanakornkit

Type 2 diabetes mellitus (T2DM), a chronic disease increasing rapidly worldwide, is well established as an important risk factor for various types of cancer. Although many factors impact the development of T2DM and cancer including sex, age, ethnicity, obesity, diet, physical activity levels, and environmental exposure, many epidemiological and experimental studies are gradually contributing to knowledge regarding the interrelationship between DM and cancer. The insulin resistance, hyperinsulinemia, and chronic inflammation associated with diabetes mellitus are all associated strongly with cancer. The changes in bioavailable ovarian steroid hormone that occur in diabetes mellitus (the increasing levels of estrogen and androgen and the decreasing level of progesterone) are also considered potentially carcinogenic conditions for the breast, endometrium, and ovaries in women. In addition, the interaction among insulin, insulin-like growth factors (IGFs), and ovarian steroid hormones, such as estrogen and progesterone, could act synergistically during cancer development. Here, we review the cancer-related mechanisms in T2DM, the epidemiological evidence linking T2DM and cancers in women, and the role of antidiabetic medication in these cancers.

1. Introduction

Diabetes mellitus (DM), mostly type 2 diabetes mellitus (T2DM), is one of the most common chronic diseases characterized by hyperglycemia. The World Health Organization (WHO) announced that the worldwide prevalence of DM in 2000 was 171 million and would reach approximately 366 million by 2030 [1]. However, the worldwide prevalence of DM has already reached 346 million as of 2010 [2]. The exponential growth and future burden of the high DM prevalence are responsible for most of the mortality and morbidity rates worldwide [3, 4]. Therefore, many studies have investigated the association and effects between DM and DM-related disease extensively, particularly the relationship between T2DM and cancer [5–8]. The recent consensus report sponsored by the American Association of Clinical Endocrinologists and the American College of Endocrinology (AACE/ACE Consensus Statement) highlighted that large and systemic studies are needed to investigate the relationship between T2DM and cancer [9].

The association between T2DM and cancer was reported more than 100 years ago [10]. Most epidemiological studies have suggested that cancers, particularly hepatic, pancreatic, colorectal, bladder, endometrial, and breast cancers, appear to be associated with T2DM, increasing the risk and mortality rates [1, 3, 6, 11–20]. Each value of the estimated risk may differ because of the impact of variable and intermingling factors, such as ethnic differences—including genetic susceptibility, life-style behavior, and environmental exposure—across populations [21]. However, recent studies have suggested that variable biological effects of diabetes may act synergistically with other definite cancer risk factors, particularly with

ovarian steroid hormones [21–25]. Although the different impacts of ovarian steroid hormones and biological differences between males and females may lead to variations in cancer incidence, prognosis, and clinical outcomes, dedicated research on the effects of T2DM and cancer in females is limited.

In the present review, we will discuss the epidemiological evidence and possible mechanisms behind the relationship between T2DM and cancer in women, as well as the effect of diabetes treatments on cancer incidence and comorbidity.

2. Mechanisms of Carcinogenesis in Diabetes Mellitus

Carcinogenesis is a multistep process that undergoes various genetic "hits," and diabetes may influence these processes by several mechanisms, particularly in females. DM and cancer share several common mechanisms, including increased insulin and insulin-like growth factor (IGF) signaling, dysregulation of ovarian steroid hormones, and chronic inflammation.

2.1. Insulin/IGF Signaling. Insulin and IGFs are well known for their involvement in cell survival and proliferation, as well as carbohydrate metabolism [26]. Unlike epidermal growth factor (EGF) and platelet-derived growth factor, which play roles at the cellular or tissue level as autocrine or paracrine factors, insulin and IGFs play important systemic regulatory roles at the whole organism level as a hormone [27–29]. Insulin and IGFs show hormonal effects through the insulin receptor (IR) and IGF receptors (IGFRs), which are widely expressed in normal tissues [30, 31]. Both types of receptors are membrane receptors with the tyrosine kinase domain located inside the cell membrane [32]. In terms of biological activity, these receptors form a holoreceptor characterized by two "half receptors," which comprise an extracellular α-chain and an intracellular β-chain [32]. Half receptors of insulin exist as two splice variant isoforms, "A" and "B." While the "B" [33] isoform recognizes only insulin, the "A" isoform recognizes both insulin and IGF-2 and is expressed most commonly by cancer cells [34]. The half IR and half IGF-1 receptor (IGF1R) can interact and form tetrameric structures known as "hybrid receptors," which exert similar, but not identical, downstream signaling to that of IR or IGF1R [34–36]. Autoregulated or ligand-recognized IRs activate insulin receptor substrates (IRS) through tyrosine phosphorylation, thereby activating the phosphatidylinositol 3-kinase (PI3K) pathway and Ras/mitogen-activated protein kinase (MAPK) pathway known as mitogenic signaling by insulin [34].

Many epidemiological studies have suggested that insulin and IGF-1 play important roles in the regulation of cancer. An increased insulin or IGF-1 level, which presents in T2DM, obesity, and acromegaly, is strongly associated with increased cancer risk and mortality [37–41]. Some studies have shown evidence that the rates of insulin secretion among individuals may influence the risk and progression of cancer [42, 43]. Additionally, the insulin and IGF-1 levels in cancer patients are proportional to cancer-related mortality [26, 44]. Many in vitro and in vivo studies have shown evidence supporting epidemiological studies. For example, insulin or IGF-1 increased cell proliferation and reduced apoptosis in cancer cells, even at physiologically relevant concentrations [45, 46]. On the other hand, insulin signaling deficiency caused by the downregulation of IRs was shown to inhibit the proliferation and metastasis of cancer cells in vitro and in vivo [47, 48]. Insulin has direct access to its receptors, but most circulating IGFs are bound to IGF binding proteins (IGFBPs) and thereby demonstrate limited and attenuated IGFR-mediated bioactivity [49]. Therefore, the biological activity of IGFs may be determined by the level of IGFBPs, which are influenced by various conditions associated with insulin resistance such as T2DM and obesity [50–52]. Insulin resistance and increased insulin levels are associated with increased risk and mortality in women with cancer, particularly breast, endometrial, and ovarian cancers [53–55].

Although mainstream studies have suggested that insulin and IGFs are associated strongly with cancer development, each study showed a different degree of cancer risk associated with insulin and IGFs [33]. The latter view may be due to the following reasons. First, the functional differences in receptors may be due to the various types of tyrosine kinases, leading to phosphorylation of different IRS members. Therefore, insulin and IGFs may lead to various effects depending on the combination of half receptors in cancer cells. Second, the insulin and IGF cancer-related signaling pathways have adapted from those of normal cells, particularly from insulin-sensitive tissues such as the liver, muscle, and adipose tissue. However, the internal signals of a cancer cell are very different from those of normal cells because of the changes in genetic and/or epigenetic factors, thereby stimulating another signaling pathway in response to insulin and IGF. Therefore, insulin and IGFs may induce a cancer cell-specific signaling pathway aberrant from that of normal cells [26, 56].

2.2. Ovarian Steroid Hormone/Sex Hormone-Binding Globulin Regulation. The ovarian steroid hormones are some of the most common types of hormones related to cancer generation and/or progression. The predominating theories suggest that the ovarian steroid hormones estrogen and progesterone have a strong association with endometrial, breast, and ovarian cancers [57–59]. Enhanced signaling by estrogen in particular has been considered a risk factor for females with cancer. This is based on the observation that the estrogen increase and endometrial proliferation rate during the follicular phase of the menstrual cycle drive ductal elongation in mammary gland development during puberty [60–62].

Epidemiological studies have demonstrated that postmenopausal women are at an increased risk of cancers from exogenous estrogen replacement without progesterone [63–69]. Other studies have shown that polycystic ovarian syndrome (PCOS) in premenopausal women is very closely related to increased cancer risk and results from increased androgen and decreased progesterone levels [70–74]. The sex hormone-binding globulin (SHBG) level is one of the most important factors in cancer generation and/or development

in postmenopausal women because its reduction leads to an increase in free ovarian steroid hormones [75–82].

Estrogen in cancer cells may trigger proliferation and cellular growth through the activation of estrogen receptor alpha (ERα) following the activation of PI3K and MAPK pathways [83, 84]. The role of estrogen is important because ovarian steroid hormone-sensitive tissues responding to ovarian steroid hormones exhibit increased levels of bioactive IGF-1 and gene expression of IGF1R, IRS-1, and IRS-2 [85–87]. In addition, activation of IR and IGF1R induces the phosphorylation of ERα, thereby potentiating ERα signaling [88, 89]. Therefore, cancer cells expressing higher levels of IR and/or IGF1R would result in resistance to antiestrogen therapy such as tamoxifen [89–91]. Although the role of androgen in promoting carcinogenesis and cell proliferation is well known in prostate cancer, in vitro studies have suggested that androgen could affect cell viability and proliferation through the regulation of inflammatory and Notch signaling pathways [92, 93].

Interestingly, hyperinsulinemia and/or insulin resistance, particularly in postmenopausal females with T2DM, result in increased bioavailable ovarian steroid hormone levels through suppressed hepatic SHBG production and induced ovarian steroid hormone production [23, 85, 94–97]. Additionally, the increased insulin and IGF-1 levels in females with T2DM potentiate ERα signaling by IR and/or IGF1R. Such observation and prediction suggest that diabetes, particularly T2DM, may involve cancer generation and/or development mechanisms through abnormal sex hormone signaling.

2.3. Chronic Inflammation. Most of our current knowledge indicates that the net effect of inflammation is the triggering of cancer development and progression [98–102]. Mediators of inflammatory pathways such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), and cyclooxygenase-2 (COX-2) are involved in cancer-related mechanisms that diminish tumor suppressor function, stimulate oncogene expression, and increase cell cycling [103]. Conversely, inhibition of inflammatory signaling such as that by nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) reduces cancer incidence [104, 105]. Additionally, many studies concerning the association of inflammation and cancer in females suggest that the inflammatory pathways activated through NF-κB signaling play an important role in the development and progression of cancers such as breast, endometrial, and ovarian cancers [106–109].

Insulin resistance and hyperinsulinemia in T2DM promote subclinical or low-grade chronic inflammation that aggravates insulin resistance [110, 111]. Ovarian steroid hormones, particularly estrogen, can activate NF-κB signaling, which induces the gene expression of inflammatory mediators such as IL-1, TNFα, and metalloproteinases (MMPs), thereby facilitating inflammatory processes [108, 112, 113]. As noted above, females with T2DM-increased bioavailable ovarian steroid hormones show more enhanced inflammatory effects. Therefore, a chronic inflammatory state in these patients may be the main mechanism associated with cancer development and progression.

3. The Link between Diabetes Mellitus and Cancer in Women

The risk of cancers in the female reproductive organs is increased in T2DM. Both breast and endometrial cancer risks are increased in diabetic females. Several biological mechanisms may be involved, mostly regarding ovarian steroid hormone abnormalities.

3.1. Breast Cancer. T2DM and breast cancer are both serious life-threatening diseases globally. Breast cancer, the most common cancer and second leading cause of cancer-related death in women, shares some risk factors with diabetes, such as age and obesity [114, 115]. Female diabetic patients were more likely to have increased risk of and mortality from breast cancer [3, 116–118]. A meta-analysis of 20 studies (five case-control and 15 cohort studies between 1966 and February 2007) indicated that females diabetics had an increased risk of breast cancer with a relative risk (RR) of 1.20 [95% confidence interval (CI): 1.12–1.28] [11]. A recently conducted meta-analysis of 12 studies (five case-control and seven cohort studies between 2000 and March 2010) showed a similar result in which women with diabetes have a significantly increased risk of breast cancer with a summary RR of 1.72 (95% CI: 1.47–2.00). Additionally, among postmenopausal or postmenopausal-age females, a strong relationship was demonstrated between diabetes and breast cancer with a summary RR of 1.25 (95% CI: 1.20–1.29) [119]. The Cancer Prevention Study II demonstrated that the incidence of diabetes in females was associated significantly with a 16% increased mortality from breast cancer with an age-adjusted RR of 1.24 (95% CI: 1.11–1.39) and multivariable-adjusted RR of 1.16 (95% CI: 1.03–1.29) [3]. These findings are similar to those of two retrospective cohort studies in the United Kingdom (UK) and Taiwan. Data in the UK study showed a reduced survival time for women with both diabetes and breast cancer (nondiabetes versus diabetes, 14.3 versus 10.4 years, resp.) [117]. In the Taiwan study, breast cancer patients with diabetes had a significantly increased mortality with a hazard ratio (HR) of 1.57 (95% CI: 1.15–2.15) [118].

In breast cancer, insulin and IGFs play important roles as mitogens. Breast cancer tissues showed increased levels of the "A" isoform of the IR (IR-A) activated by insulin and IGF [120]. PI3K and/or Ras/MAPK pathways induced through IR-A activation resulted in mitosis of breast cancer cells in vitro [53]. In vivo studies using nonobese, insulin-resistant, and hyperinsulinemic transgenic MKR mouse models showed that hyperinsulinemia results in mammary ductal hyperplasia and IR-expressing carcinoma [121]. Women with diabetes displayed hormonal changes resulting from increased production of estrogen and androgen with decreased liver production of SHBG [122]. These hormonal changes were also strongly associated with breast cancer risk in postmenopausal females [69]. Increased bioavailable estrogen stimulated the proliferation of ER-positive and/or estrogen-dependent breast cancer [123]. Hyperinsulinemia in T2DM induced the expression and increased the binding capacity of ER [124, 125]. The activation of ER can also enhance insulin mitogenicity by promoting IRS-1 function and activating PI3K

and Ras/MAPK signaling [126]. The inflammatory mediators, TNFα and IL-6, which are associated with insulin resistance in T2DM, enhanced estrogen production in both normal and breast cancer cells and could be expected to result in the development and proliferation of breast cancer cells [127].

3.2. Endometrial Cancer. Endometrial cancer is the most common gynecological cancer and is closely associated with endometrial hyperplasia, unopposed estrogen exposure, and genetic alterations [128, 129]. This cancer has been associated strongly with T2DM in most epidemiological studies [13, 15, 117, 118, 130–132]. Many studies have suggested that T2DM and endometrial cancer share characteristics regarding the major modifiable determinates, such as low physical activity and obesity [133–135]. A meta-analysis of 16 studies (13 case-control and three cohort studies between 1956 and June 2005) indicated that T2DM had a significantly increased risk and comorbidity with endometrial cancer with a summary RR of 2.10 (95% CI: 1.75–2.53). The risk was particularly strong among studies with age-adjusted estimates (RR 2.74; 95% CI: 1.87–4.00) [13]. Also, a recent population-based and retrospective cohort study demonstrated that endometrial cancer and diabetes were strongly associated, with an HR of 1.81 (95% CI: 1.37–2.41), and had an increased relationship, with an age-adjusted HR of 1.85 (95% CI: 1.36–2.50) [15]. Although some studies have shown that endometrial cancer and diabetes had no significant statistical association with mortality [117], most studies have indicated that diabetes is associated with an increased risk of death from endometrial cancer; for example, a prospective study reported a multivariable-adjusted RR of 1.33 (95% CI: 1.08–1.65) and an age-adjusted RR of 1.72 (95% CI: 1.40–2.12) [3].

Similar to breast cancer cells, in vitro studies have shown that endometrial cancer cell lines increased proliferation by activation of insulin, IGF-1, and ovarian steroid hormone signaling pathways, such as estrogen and androgen signaling pathways [54]. Although endometrial cancer has no direct correlation with insulin or IGF levels, additional factors such as ovarian steroid hormones and/or inflammatory cytokines may make it difficult to confirm a single effect of insulin or IGF activation through insulin or IGF serum levels. Estrogen can activate IGF1R on endometrial cancer cells, thereby enhancing cellular proliferation through PI3K signaling, a link to IGF1R activation [136]. The androgen receptor (AR) activated by the binding of androgen could enhance the proliferation of endometrial cancer cells by the Notch signaling pathway [93]. C-reactive protein (CRP), which is an inflammatory biomarker induced by IL-6, was increased by insulin resistance and was associated with an increased risk of endometrial cancer in postmenopausal women [137]. Therefore, endometrial cancer may be associated with chronic inflammation in T2DM.

3.3. Ovarian Cancer. Although ovarian cancer is the ninth most common cancer and represents the fifth leading cause of death in women worldwide [138], studies concerning the relationship between ovarian cancer and T2DM are limited. One reason could be due to well-known and very important factors such as familial history, genetic mutations, menstrual cycles, and usage of oral contraceptives [139, 140]. However, some incidences in which the ovary displays insulin sensitivity and steroidogenesis induced by insulin and IGFs suggest that T2DM may be an important risk factor for ovarian cancer [141, 142]. Although small-scale epidemiological studies have demonstrated inconsistent results regarding the relationship between ovarian cancer and T2DM, a recent meta-analysis of 19 studies (six case-case control, one nested case-control, and 12 cohort studies between 1976 and 2007) indicated that women with diabetes had an increased risk of ovarian cancer with a summary RR of 1.17 (95% CI: 1.02–1.33) [3, 5, 117, 118, 143–146]. Many epidemiological studies have shown that ovarian cancer is associated with increased serum androgen levels and decreased serum progesterone levels rather than altered serum estrogen levels [79, 147]. These hormonal changes appear in diabetes and may be one reason for the increased risk of ovarian cancer in T2DM.

Although there is no experimental evidence for the positive association between insulin and ovarian cancer, some studies have shown that the increased serum levels of IGF-1, IGF-1R, and IGFBP-2 were associated positively in patients with ovarian cancer [55]. One study demonstrated that IGF-1 in human ovarian OVCAR-3 cells enhanced the expression of KCl cotransport (KCC) and was associated with proliferation and invasiveness of ovarian cancer cells [148]. Other studies have also shown that IGF-1 and IGFBP-2 in human ovarian cancer cell lines resulted in the induction of proliferation and invasion through phosphorylation of AKT and ERK1/2 [149, 150]. The significance of inflammation in ovarian carcinogenesis stems from the relationship between increased ovulation and ovarian cancer risk [151]. The role of androgen in stimulating the proliferation of ovarian cancer cells may also be associated with increased IL-6 and decreased transforming growth factor beta (TGFβ), which were included in the proinflammatory network [92, 152].

4. The Role of Diabetes Medications in Cancer Development in Women

The potential effects of antidiabetic medications on cancer have sparked recent discussion and concern among the epidemiological and experimental studies related to the potential underlying mechanisms. In this section, we discuss the relationship between cancer risk and antidiabetic medication, including insulin and insulin analogs, metformin, and thiazolidinediones.

4.1. Insulin/Insulin Analogues. As noted above, because excessive insulin and IGF-1 signaling by hyperinsulinemia may be one of the most important causes of the development and proliferation of cancer, exogenous insulin is a suspected powerful carcinogenetic factor in diabetes patients. Increased circulating insulin levels over endogenous insulin secretion occur frequently with subcutaneous insulin injection, thereby making possible the association between insulin therapy and cancer [21, 153, 154]. Nevertheless, all patients with type 1 DM (T1DM) and approximately 40~80% of patients with

T2DM are considered for insulin therapy to maintain proper glycemic control [155].

A significant number of epidemiological studies have suggested that insulin use and daily doses, particularly of the long-acting insulin analog glargine, may be responsible for the association with and strong increase in the risk of cancer [156–160]. A recent meta-analysis of 15 studies (five case-control and 10 cohort studies) demonstrated that insulin treatment was associated significantly with an increased risk of overall cancer with a summary RR of 1.39 (95% CI: 1.14–1.70), particularly in case-control studies that evaluated T1DM, with a higher summary RR of 1.83 (95% CI; 0.99–3.38) [161]. Some studies showed a strong relationship between insulin glargine and breast cancer, particularly in T2DM patients treated with insulin for more than 5 years [162, 163]. Only a few studies with large-scale patient databases exist, such as the ORIGIN trial which enrolled 12,537 patients and followed them for 6.2 years (interquartile range, 5.8–6.7 years) and showed no statistically significant association between cancer risk and insulin glargine use; however, these results may be due to very well-controlled glucose levels, as well as the inclusion of prediabetic patients [9, 164]. Therefore, optional selection between insulin treatment and proper glucose control may be needed for patients with diabetes, particularly T2DM.

4.2. Metformin. Metformin is an oral antidiabetic drug classified as an insulin sensitizer and is the most widely used drug, prescribed as the initial or in combination therapy, for T2DM [165]. Metformin reduces serum glucose and insulin levels in diabetic patients via improved insulin sensitivity, which reduces glucose production in the liver and increases glucose uptake in the muscles [166, 167]. Another feature of metformin that attracts special attention is its anticancer effects supported by evidence from epidemiologic, in vitro, and in vivo model studies [3, 168–175].

In in vitro studies, metformin inhibits complex I of the mitochondrial respiratory chain, resulting in ATP/AMP imbalance, thereby activating AMP-activated protein kinase (AMPK) [176]. Activated AMPK inhibits mTOR signaling and interferes with the roles of cyclin D1 and p53. The latter two proteins not only alter glucose metabolism but also reduce cell proliferation through cell cycle interference [177–181].

The anticancer effects of metformin have been shown to reduce spontaneous mammary tumor development in rodent animal models [182, 183]. However, some studies using mouse models found that metformin induced insulin resistance and hyperinsulinemia, which suggested that the anticancer effect of metformin may be mediated by reduced serum insulin levels [184]. In other words, the insulin-lowering effect of metformin was associated with its anticancer effect, thereby having less of an impact on cancer in patients with normal or lower insulin levels.

Most epidemiological studies have suggested that metformin used for T2DM reduces the risk, progression, and mortality of overall cancer [169, 171, 185–189]. Although those studies were limited in their assessment of a detailed relationship between metformin and specific cancer types, a recent meta-analysis of 28 studies showed that metformin has a significant inverse association with cancer mortality, including endometrial and ovarian cancers [187].

4.3. Thiazolidinediones. Thiazolidinediones (TZDs) are insulin-sensitizing antidiabetic drugs belonging to the peroxisome proliferator-activated receptor (PPAR) agonist class that induce the transcription of genes associated with glucose and lipid metabolism through activation of PPARγ, a nuclear receptor, to reduce insulin resistance [190]. The effects of TZDs in reducing insulin resistance may be expected to result in anticancer activities similar to those of metformin. Some studies have indicated antiangiogenic and anti-inflammatory effects of TZDs, as well as anticancer effects, such as the inhibition of proliferation and induction of apoptosis and differentiation [191]. However, unlike metformin, the effects of TZDs are inconsistent between in vitro and in vivo studies and depend on parameters such as the animal model (rodent versus nonrodent and nonhuman primate versus human) and cancer type [192–197]. Some studies in rodents have suggested that TZDs can even potentiate tumorigenesis as multispecies and multisex carcinogens [192, 198–200].

Epidemiological studies evaluating the relationship between TZDs and cancer risk are limited and have shown inconsistent results [201–205]. One meta-analysis of randomized clinical trials concerning rosiglitazone and cancer risk indicated that rosiglitazone did not alter the risk of cancer, including breast and female genital tract cancers [206]. A recent meta-analysis indicated that pioglitazone, but not rosiglitazone, was associated significantly with a decreased risk of breast cancer (Mantel-Haenszel odds ratio (MH-OR): 0.28 [0.09–0.93]; $P = 0.038$), but neither pioglitazone nor rosiglitazone altered the risk of uterine cancer (MH-OR: 0.77 [0.34–1.73]; $P = 0.52$) [207].

5. Conclusion

The incidence and prevalence of gynecologic cancers are increased in patients with T2DM. Similar to other cancers, gynecologic cancers have several common mechanisms with T2DM, including increased insulin and IGF signaling and chronic inflammation. Unlike other cancers, dysregulation of ovarian steroid hormones is another commonly associated mechanism between T2DM and gynecologic cancer. Insulin resistance could induce or aggravate ovarian steroid hormone dysregulation and chronic inflammation in diabetic women. Most epidemiological studies have suggested that cancer in diabetic women can be modulated by insulin sensitizers such as metformin and TZDs. The management of insulin resistance is a main factor in controlling blood glucose and preventing cancer in female diabetic patients.

Thus, clinicians should recommend life-style changes such as weight-loss diets and exercise to overcome insulin resistance in women with T2DM. Additionally, clinicians should attend to and perform screening tests for gynecologic cancers according to currently established routines until screening protocols are developed for each specific gynecologic cancer in women with T2DM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank Amanda Sterling for paper preparation. This work was supported by the National Research Foundation of Korea Grant (NRF-2013R1A1A2004719) funded by the Korean Government (to Bon Jeong Ku), Grant NIH R01 HD057873, and American Cancer Society Research Grant RSG-12-084-01-TBG (to Jae-Wook Jeong).

References

[1] W. Rathmann, G. Giani, S. H. Wild et al., "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030," *Diabetes Care*, vol. 27, no. 10, pp. 2568–2569, 2004.

[2] P. Zhang, X. Zhang, J. Brown et al., "Global healthcare expenditure on diabetes for 2010 and 2030," *Diabetes Research and Clinical Practice*, vol. 87, no. 3, pp. 293–301, 2030.

[3] P. T. Campbell, E. J. Jacobs, C. C. Newton, S. M. Gapstur, and A. V. Patel, "Diabetes and cause-specific mortality in a prospective cohort of one million U.S. adults," *Diabetes Care*, vol. 35, no. 9, pp. 1835–1844, 2012.

[4] S. R. Seshasai, S. Kaptoge, A. Thompson et al., "Diabetes mellitus, fasting glucose, and risk of cause-specific death," *The New England Journal of Medicine*, vol. 364, no. 9, pp. 829–841, 2011.

[5] S. S. Coughlin, E. E. Calle, L. R. Teras, J. Petrelli, and M. J. Thun, "Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults," *American Journal of Epidemiology*, vol. 159, no. 12, pp. 1160–1167, 2004.

[6] S. D. Nath, S. L. Habib, H. E. Abboud et al., "Fasting serum glucose level and cancer risk in Korean men and women," *Journal of the American Medical Association*, vol. 293, no. 18, pp. 2210–2211, 2005.

[7] G. Chodick, A. D. Heymann, L. Rosenmann et al., "Diabetes and risk of incident cancer: a large population-based cohort study in Israel," *Cancer Causes and Control*, vol. 21, no. 6, pp. 879–887, 2010.

[8] E. K. K. Lam, G. D. Batty, R. R. Huxley et al., "Associations of diabetes mellitus with site-specific cancer mortality in the Asia-Pacific region," *Annals of Oncology*, vol. 22, no. 3, pp. 730–738, 2011.

[9] Y. Handelsman, D. Leroith, Z. T. Bloomgarden et al., "Diabetes and cancer—an AACE/ACE consensus statement," *Endocrine Practice*, vol. 19, no. 4, pp. 675–693, 2013.

[10] A. Czyzyk and Z. Szczepanik, "Diabetes mellitus and cancer," *European Journal of Internal Medicine*, vol. 11, no. 5, pp. 245–252, 2000.

[11] S. C. Larsson, C. S. Mantzoros, and A. Wolk, "Diabetes mellitus and risk of breast cancer: a meta-analysis," *International Journal of Cancer*, vol. 121, no. 4, pp. 856–862, 2007.

[12] S. C. Larsson, N. Orsini, and A. Wolk, "Diabetes mellitus and risk of colorectal cancer: a meta-analysis," *Journal of the National Cancer Institute*, vol. 97, no. 22, pp. 1679–1687, 2005.

[13] E. Friberg, N. Orsini, C. S. Mantzoros, and A. Wolk, "Diabetes mellitus and risk of endometrial cancer: a meta-analysis," *Diabetologia*, vol. 50, no. 7, pp. 1365–1374, 2007.

[14] E. M. Ko, P. Walter, L. Clark et al., "The complex triad of obesity, diabetes and race in Type I and II endometrial cancers: prevalence and prognostic significance," *Gynecologic Oncology*, vol. 133, no. 1, pp. 28–32, 2014.

[15] H.-F. Chen, M.-D. Liu, P. Chen et al., "Risks of breast and endometrial cancer in women with diabetes: a population-based cohort study," *PLoS ONE*, vol. 8, no. 6, Article ID e67420, 2013.

[16] R. Huxley, A. Ansary-Moghaddam, A. Berrington de González, F. Barzi, and M. Woodward, "Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies," *British Journal of Cancer*, vol. 92, no. 11, pp. 2076–2083, 2005.

[17] H. B. El-Serag, H. Hampel, and F. Javadi, "The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence," *Clinical Gastroenterology and Hepatology*, vol. 4, no. 3, pp. 369–380, 2006.

[18] H. Fang, B. Yao, Y. Yan et al., "Diabetes mellitus increases the risk of bladder cancer: an updated meta-analysis of observational studies," *Diabetes Technology and Therapeutics*, vol. 15, no. 11, pp. 914–922, 2013.

[19] B. B. Barone, H.-C. Yeh, C. F. Snyder et al., "Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis," *The Journal of the American Medical Association*, vol. 300, no. 23, pp. 2754–2764, 2008.

[20] R. Clarke, M. Shipley, S. Lewington et al., "Underestimation of risk associations due to regression dilution in long- term follow-up of prospective studies," *The American Journal of Epidemiology*, vol. 150, no. 4, pp. 341–353, 1999.

[21] S. L. Habib and M. Rojna, "Diabetes and risk of cancer," *ISRN Oncology*, vol. 2013, Article ID 583786, 16 pages, 2013.

[22] F. Ikeda, Y. Doi, K. Yonemoto et al., "Hyperglycemia increases risk of gastric cancer posed by Helicobacter pylori infection: a population-based cohort study," *Gastroenterology*, vol. 136, no. 4, pp. 1234–1241, 2009.

[23] T. N. Le, J. E. Nestler, J. F. Strauss, and E. P. Wickham, "Sex hormone-binding globulin and type 2 diabetes mellitus," *Trends in Endocrinology & Metabolism*, vol. 23, no. 1, pp. 32–40, 2012.

[24] S. K. Pal and A. Hurria, "Impact of age, sex, and comorbidity on cancer therapy and disease progression," *Journal of Clinical Oncology*, vol. 28, no. 26, pp. 4086–4093, 2010.

[25] J. H. Koo and R. W. L. Leong, "Sex differences in epidemiological, clinical and pathological characteristics of colorectal cancer," *Journal of Gastroenterology and Hepatology*, vol. 25, no. 1, pp. 33–42, 2010.

[26] M. Pollak, "Insulin and insulin-like growth factor signalling in neoplasia," *Nature Reviews Cancer*, vol. 8, no. 12, pp. 915–928, 2008.

[27] J. A. Cooper, D. F. Bowen-Pope, E. Raines, R. Ross, and T. Hunter, "Similar effects of platelet-derived growth factor and epidermal growth factor on the phosphorylation of tyrosine in cellular proteins," *Cell*, vol. 31, no. 1, pp. 263–273, 1982.

[28] N. Chegini, M. J. Rossi, and B. J. Masterson, "Platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and EGF and PDGF β-receptors in human endometrial tissue: localization and in vitro action," *Endocrinology*, vol. 130, no. 4, pp. 2373–2385, 1992.

[29] Z. Laron, "IGF-1 and insulin as growth hormones," *Novartis Foundation Symposium*, vol. 262, pp. 56–83, 265–268, 2004.

[30] V. Pezzino, A. Costantino, P. Russo, D. Gullo, and V. Papa, "Insulin receptor content in tissues of normal and diabetic

rats measured by radioimunoassay," *Journal of Endocrinological Investigation*, vol. 19, no. 9, pp. 593–597, 1996.

[31] C. Hernández-Sánchez, A. Mansilla, F. De Pablo, and R. Zardoya, "Evolution of the insulin receptor family and receptor isoform expression in vertebrates," *Molecular Biology and Evolution*, vol. 25, no. 6, pp. 1043–1053, 2008.

[32] P. de Meyts, "Insulin and its receptor: structure, function and evolution," *BioEssays*, vol. 26, no. 12, pp. 1351–1362, 2004.

[33] E. S. Schernhammer, J. M. Holly, D. J. Hunter, M. N. Pollak, and S. E. Hankinson, "Insulin-like growth factor-I, its binding proteins (IGFBP-1 and IGFBP-3), and growth hormone and breast cancer risk in The Nurses Health Study II," *Endocrine-Related Cancer*, vol. 13, no. 2, pp. 583–592, 2006.

[34] A. Belfiore, F. Frasca, G. Pandini, L. Sciacca, and R. Vigneri, "Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease," *Endocrine Reviews*, vol. 30, no. 6, pp. 586–623, 2009.

[35] S. Benyoucef, K. H. Surinya, D. Hadaschik, and K. Siddle, "Characterization of insulin/IGF hybrid receptors: contributions of the insulin receptor L2 and Fn1 domains and the alternatively spliced exon 11 sequence to ligand binding and receptor activation," *Biochemical Journal*, vol. 403, no. 3, pp. 603–613, 2007.

[36] D. LeRoith, "Insulin-like growth factor I receptor signaling—overlapping or redundant pathways?" *Endocrinology*, vol. 141, no. 4, pp. 1287–1288, 2000.

[37] P. J. Jenkins, "Cancers associated with acromegaly," *Neuroendocrinology*, vol. 83, no. 3-4, pp. 218–223, 2006.

[38] E. E. Calle, C. Rodriguez, K. Walker-Thurmond, and M. J. Thun, "Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. Adults," *The New England Journal of Medicine*, vol. 348, no. 17, pp. 1625–1638, 2003.

[39] D. L. Roberts, C. Dive, and A. G. Renehan, "Biological mechanisms linking obesity and cancer risk: new perspectives," *Annual Review of Medicine*, vol. 61, pp. 301–316, 2010.

[40] D. H. Cohen and D. LeRoith, "Obesity, type 2 diabetes, and cancer: the insulin and IGF connection," *Endocrine-Related Cancer*, vol. 19, no. 5, pp. F27–F45, 2012.

[41] Y. Gu, C. Wang, Y. Zheng et al., "Cancer incidence and mortality in patients with type 2 diabetes treated with human insulin: a cohort study in Shanghai," *PLoS ONE*, vol. 8, no. 1, Article ID e53411, 2013.

[42] J. Ma, H. Li, E. Giovannucci et al., "Prediagnostic body-mass index, plasma C-peptide concentration, and prostate cancer-specific mortality in men with prostate cancer: a long-term survival analysis," *The Lancet Oncology*, vol. 9, no. 11, pp. 1039–1047, 2008.

[43] J. Ma, E. Giovannucci, M. Pollak et al., "A prospective study of plasma C-peptide and colorectal cancer risk in men," *Journal of the National Cancer Institute*, vol. 96, no. 7, pp. 546–553, 2004.

[44] R. Dankner, M. H. Shanik, L. Keinan-Boker, C. Cohen, and A. Chetrit, "Effect of elevated basal insulin on cancer incidence and mortality in cancer incident patients: the Israel GOH 29-year follow-up study," *Diabetes Care*, vol. 35, no. 7, pp. 1538–1543, 2012.

[45] M. Lippman, G. Bolan, and K. Huff, "The effects of estrogens and antiestrogens on hormone responsive human breast cancer in long term tissue culture," *Cancer Research*, vol. 36, no. 12, pp. 4595–4601, 1976.

[46] C. K. Osborne, G. Bolan, M. E. Monaco, and M. E. Lippman, "Hormone responsive human breast cancer in long term tissue culture: effect of insulin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 73, no. 12, pp. 4536–4540, 1976.

[47] H. Zhang, D. H. Fagan, X. Zeng, K. T. Freeman, D. Sachdev, and D. Yee, "Inhibition of cancer cell proliferation and metastasis by insulin receptor downregulation," *Oncogene*, vol. 29, no. 17, pp. 2517–2527, 2010.

[48] J. C. Heuson and N. Legros, "Influence of insulin deprivation on growth of the 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in rats subjected to alloxan diabetes and food restriction.," *Cancer Research*, vol. 32, no. 2, pp. 226–232, 1972.

[49] S. M. Firth and R. C. Baxter, "Cellular actions of the insulin-like growth factor binding proteins," *Endocrine Reviews*, vol. 23, no. 6, pp. 824–854, 2002.

[50] V. A. Ezzat, E. R. Duncan, S. B. Wheatcroft, and M. T. Kearney, "The role of IGF-I and its binding proteins in the development of type 2 diabetes and cardiovascular disease," *Diabetes, Obesity and Metabolism*, vol. 10, no. 3, pp. 198–211, 2008.

[51] S. Söderberg, B. Ahrén, M. Eliasson, B. Dinesen, K. Brismar, and T. Olsson, "Circulating IGF binding protein-1 is inversely associated with leptin in non-obese men and obese postmenopausal women," *European Journal of Endocrinology*, vol. 144, no. 3, pp. 283–290, 2001.

[52] A. López-Bermejo, J. Khosravi, J. M. Fernández-Real et al., "Insulin resistance is associated with increased serum concentration of IGF-binding protein-related protein 1 (IGFBP-rP1/MAC25)," *Diabetes*, vol. 55, no. 8, pp. 2333–2339, 2006.

[53] D. P. Rose and L. Vona-Davis, "The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression," *Endocrine-Related Cancer*, vol. 19, no. 6, pp. R225–R241, 2012.

[54] N. Mu, Y. Zhu, Y. Wang, H. Zhang, and F. Xue, "Insulin resistance: a significant risk factor of endometrial cancer," *Gynecologic Oncology*, vol. 125, no. 3, pp. 751–757, 2012.

[55] M. C. Beauchamp, A. Yasmeen, A. Knafo, and W. H. Gotlieb, "Targeting insulin and insulin-like growth factor pathways in epithelial ovarian cancer," *Journal of Oncology*, vol. 2010, Article ID 257058, 11 pages, 2010.

[56] M. Pollak, "The insulin and insulin-like growth factor receptor family in neoplasia: an update," *Nature Reviews Cancer*, vol. 12, no. 3, pp. 159–169, 2012.

[57] P. K. Siiteri, "Steroid hormones and endometrial cancer," *Cancer Research*, vol. 38, no. 11, part 2, pp. 4360–4366, 1978.

[58] T. J. A. Key and M. C. Pike, "The dose-effect relationship between "unopposed" oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk," *British Journal of Cancer*, vol. 57, no. 2, pp. 205–212, 1988.

[59] A. Lukanova and R. Kaaks, "Endogenous hormones and ovarian cancer: epidemiology and current hypotheses," *Cancer Epidemiology Biomarkers and Prevention*, vol. 14, no. 1, pp. 98–107, 2005.

[60] A. Ferenczy, G. Bertrand, and M. M. Gelfand, "Proliferation kinetics of human endometrium during the normal menstrual cycle," *American Journal of Obstetrics & Gynecology*, vol. 133, no. 8, pp. 859–867, 1979.

[61] C. L. Wilson, A. H. Sims, A. Howell, C. J. Miller, and R. B. Clarke, "Effects of oestrogen on gene expression in epithelium and stroma of normal human breast tissue," *Endocrine-Related Cancer*, vol. 13, no. 2, pp. 617–628, 2006.

[62] J. Stingl, "Estrogen and progesterone in normal mammary gland development and in cancer," *Hormones and Cancer*, vol. 2, no. 2, pp. 85–90, 2011.

[63] R. F. Spengler, E. A. Clarke, C. A. Woolever, A. M. Newman, and R. W. Osborn, "Exogenous estrogens and endometrial cancer: a case-control study and assessment of potential biases," *American Journal of Epidemiology*, vol. 114, no. 4, pp. 497–506, 1981.

[64] E. J. Crosbie, M. Zwahlen, H. C. Kitchener, M. Egger, and A. G. Renehan, "Body mass index, hormone replacement therapy, and endometrial cancer risk: a meta-analysis," *Cancer Epidemiology Biomarkers and Prevention*, vol. 19, no. 12, pp. 3119–3130, 2010.

[65] K. K. Steinberg, S. B. Thacker, S. J. Smith et al., "A meta-analysis of the effect of estrogen replacement therapy on the risk of breast cancer," *The Journal of the American Medical Association*, vol. 265, no. 15, pp. 1985–1990, 1991.

[66] C. Magnusson, I. Persson, and H.-O. Adami, "More about: effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin," *Journal of the National Cancer Institute*, vol. 92, no. 14, pp. 1183–1184, 2000.

[67] P. G. Toniolo, M. Levitz, A. Zeleniuch-Jacquotte et al., "A prospective study of endogenous estrogens and breast cancer in postmenopausal women," *Journal of the National Cancer Institute*, vol. 87, no. 3, pp. 190–197, 1995.

[68] A. Akhmedkhanov, A. Zeleniuch-Jacquotte, and P. Toniolo, "Role of exogenous and endogenous hormones in endometrial cancer: review of the evidence and research perspectives," *Annals of the New York Academy of Sciences*, vol. 943, pp. 296–315, 2001.

[69] C. M. Friedenreich, "Review of anthropometric factors and breast cancer risk," *European Journal of Cancer Prevention*, vol. 10, no. 1, pp. 15–32, 2001.

[70] S. C. Sommers, A. T. Hertig, and H. Bengloff, "Genesis of endometrial carcinoma; cases 19 to 35 years old," *Cancer*, vol. 2, no. 6, pp. 957–963, 1949.

[71] E. Ron, B. Lunenfeld, J. Menczer et al., "Cancer incidence in a cohort of infertile women," *American Journal of Epidemiology*, vol. 125, no. 5, pp. 780–790, 1987.

[72] S. Wild, T. Pierpoint, H. Jacobs, and P. McKeigue, "Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study," *Human Fertility*, vol. 3, no. 2, pp. 101–105, 2000.

[73] C. B. Coulam, J. F. Annegers, and J. S. Kranz, "Chronic anovulation syndrome and associated neoplasia," *Obstetrics and Gynecology*, vol. 61, no. 4, pp. 403–407, 1983.

[74] A. Daniilidis and K. Dinas, "Long term health consequences of polycystic ovarian syndrome: a review analysis," *Hippokratia*, vol. 13, no. 2, pp. 90–92, 2009.

[75] N. Potischman, R. N. Hoover, L. A. Brinton et al., "Case-control study of endogenous steroid hormones and endometrial cancer," *Journal of the National Cancer Institute*, vol. 88, no. 16, pp. 1127–1135, 1996.

[76] B. Pettersson, R. Bergstrom, and E. D. B. Johansson, "Serum estrogens and androgens in women with endometrial carcinoma," *Gynecologic Oncology*, vol. 25, no. 2, pp. 223–233, 1986.

[77] H. C. J. Nyholm, A. L. Nielsen, J. Lyndrup, A. Dreisler, C. Hagen, and E. Haug, "Plasma oestrogens in postmenopausal women with endometrial cancer," *British Journal of Obstetrics and Gynaecology*, vol. 100, no. 12, pp. 1115–1119, 1993.

[78] J. A. Nisker, G. L. Hammond, B. J. Davidson et al., "Serum sex hormone-binding globulin capacity and the percentage of free estradiol in postmenopausal women with and without endometrial carcinoma. A new biochemical basis for the association between obesity and endometrial carcinoma," *American Journal of Obstetrics and Gynecology*, vol. 138, no. 6, pp. 637–642, 1980.

[79] J. M. Schildkraut, P. J. Schwingl, E. Bastos, A. Evanoff, C. Hughes, and J. P. Curtin, "Epithelial ovarian cancer risk among women with polycystic ovary syndrome," *Obstetrics & Gynecology*, vol. 88, no. 4, part 1, pp. 554–559, 1996.

[80] E. G. Silva, C. Tornos, H. A. Fritsche Jr. et al., "The induction of benign epithelial neoplasms of the ovaries of guinea pigs by testosterone stimulation: a potential animal model," *Modern Pathology*, vol. 10, no. 9, pp. 879–883, 1997.

[81] I. Wiegratz, C. Jung-Hoffmann, and H. Kuhl, "Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins," *Contraception*, vol. 51, no. 6, pp. 341–346, 1995.

[82] N. Fortunati, M. G. Catalano, G. Boccuzzi, and R. Frairia, "Sex Hormone-Binding Globulin (SHBG), estradiol and breast cancer," *Molecular and Cellular Endocrinology*, vol. 316, no. 1, pp. 86–92, 2010.

[83] G. N. Armaiz-Pena, L. S. Mangala, W. A. Spannuth et al., "Estrous cycle modulates ovarian carcinoma growth," *Clinical Cancer Research*, vol. 15, no. 9, pp. 2971–2978, 2009.

[84] C.-H. Chien, F.-F. Wang, and T. C. Hamilton, "Transcriptional activation of *c-myc* proto-oncogene by estrogen in human ovarian cancer cells," *Molecular and Cellular Endocrinology*, vol. 99, no. 1, pp. 11–19, 1994.

[85] A. V. Lee, J. G. Jackson, J. L. Gooch et al., "Enhancement of insulin-like growth factor signaling in human breast cancer: estrogen regulation of insulin receptor substrate-1 expression in vitro and in vivo," *Molecular Endocrinology*, vol. 13, no. 5, pp. 787–796, 1999.

[86] L. C. Giudice, G. Lamson, R. G. Rosenfeld, and J. C. Irwin, "Insulin-like growth factor-II (IGF-II) and IGF binding proteins in human endometrium," *Annals of the New York Academy of Sciences*, vol. 626, pp. 295–307, 1991.

[87] L. J. Murphy and A. Ghahary, "Uterine insulin-like growth factor-1: regulation of expression and its role in estrogen-induced uterine proliferation," *Endocrine Reviews*, vol. 11, no. 3, pp. 443–453, 1990.

[88] V. Bartella, P. de Marco, R. Malaguarnera, A. Belfiore, and M. Maggiolini, "New advances on the functional cross-talk between insulin-like growth factor-I and estrogen signaling in cancer," *Cellular Signalling*, vol. 24, no. 8, pp. 1515–1521, 2012.

[89] D. H. Fagan, R. R. Uselman, D. Sachdev, and D. Yee, "Acquired resistance to tamoxifen is associated with loss of the type I insulin-like growth factor receptor: implications for breast cancer treatment," *Cancer Research*, vol. 72, no. 13, pp. 3372–3380, 2012.

[90] T. Winder, G. Giamas, P. M. Wilson et al., "Insulin-like growth factor receptor polymorphism defines clinical outcome in estrogen receptor-positive breast cancer patients treated with tamoxifen," *Pharmacogenomics Journal*, vol. 14, no. 1, pp. 28–34, 2014.

[91] S. Periyasamy-Thandavan, S. Takhar, A. Singer et al., "Insulin-like growth factor 1 attenuates antiestrogen- and antiprogestin-induced apoptosis in ER^+ breast cancer cells by MEK1 regulation of the BH3-only pro-apoptotic protein Bim," *Breast Cancer Research*, vol. 14, no. 2, p. R52, 2012.

[92] V. Syed, G. Ulinski, S. C. Mok, and S.-M. Ho, "Reproductive hormone-induced, STAT3-mediated interleukin 6 action in normal and malignant human ovarian surface epithelial cells," *Journal of the National Cancer Institute*, vol. 94, no. 8, pp. 617–629, 2002.

[93] M. Qiu, W. Bao, J. Wang et al., "FOXA1 promotes tumor cell proliferation through AR involving the Notch pathway in endometrial cancer," *BMC Cancer*, vol. 14, no. 1, article 78, 2014.

[94] L. E. Underwood, J.-P. Thissen, S. Lemozy, J.-M. Ketelslegers, and D. R. Clemmons, "Hormonal and nutritional regulation of IGF-I and its binding proteins," *Hormone Research*, vol. 42, no. 4-5, pp. 145–151, 1994.

[95] J.-P. Thissen, J.-M. Ketelslegers, and L. E. Underwood, "Nutritional regulation of the insulin-like growth factors," *Endocrine Reviews*, vol. 15, no. 1, pp. 80–101, 1994.

[96] R. Pasquali, V. Vicennati, D. Bertazzo et al., "Determinants of sex hormone-binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status," *Metabolism: Clinical and Experimental*, vol. 46, no. 1, pp. 5–9, 1997.

[97] D. P. Rose, D. Komninou, and G. D. Stephenson, "Obesity, adipocytokines, and insulin resistance in breast cancer," *Obesity Reviews*, vol. 5, no. 3, pp. 153–165, 2004.

[98] D. Pardoll, "Does the immune system see tumors as foreign or self?" *Annual Review of Immunology*, vol. 21, pp. 807–839, 2003.

[99] M. Philip, D. A. Rowley, and H. Schreiber, "Inflammation as a tumor promoter in cancer induction," *Seminars in Cancer Biology*, vol. 14, no. 6, pp. 433–439, 2004.

[100] J.-L. Luo, S. Maeda, L.-C. Hsu, H. Yagita, and M. Karin, "Inhibition of NF-κB in cancer cells converts inflammation-induced tumor growth mediated by TNFα to TRAIL-mediated tumor regression," *Cancer Cell*, vol. 6, no. 3, pp. 297–305, 2004.

[101] M. Karin and F. R. Greten, "NF-κB: linking inflammation and immunity to cancer development and progression," *Nature Reviews Immunology*, vol. 5, no. 10, pp. 749–759, 2005.

[102] K. Esposito, P. Chiodini, A. Colao, A. Lenzi, and D. Giugliano, "Metabolic syndrome and risk of cancer: a systematic review and meta-analysis," *Diabetes Care*, vol. 35, no. 11, pp. 2402–2411, 2012.

[103] P. Pothiwala, S. K. Jain, and S. Yaturu, "Metabolic syndrome and cancer," *Metabolic Syndrome and Related Disorders*, vol. 7, no. 4, pp. 279–287, 2009.

[104] K. Garber, "Aspirin for cancer chemoprevention: still a headache?" *Journal of the National Cancer Institute*, vol. 96, no. 4, pp. 252–253, 2004.

[105] R. B. Ness and C. Cottreau, "Possible role of ovarian epithelial inflammation in ovarian cancer," *Journal of the National Cancer Institute*, vol. 91, no. 17, pp. 1459–1467, 1999.

[106] D. K. Biswas, Q. Shi, S. Baily et al., "NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 27, pp. 10137–10142, 2004.

[107] L. A. deGraffenried, B. Chandrasekar, W. E. Friedrichs et al., "NF-κB inhibition markedly enhances sensitivity of resistant breast cancer tumor cells to tamoxifen," *Annals of Oncology*, vol. 15, no. 6, pp. 885–890, 2004.

[108] A. E. Wallace, D. A. Gibson, P. T. K. Saunders, and H. N. Jabbour, "Inflammatory events in endometrial adenocarcinoma," *Journal of Endocrinology*, vol. 206, no. 2, pp. 141–157, 2010.

[109] A. B. Alvero, "Recent insights into the role of NF-κB in ovarian carcinogenesis," *Genome Medicine*, vol. 2, no. 8, article 56, 2010.

[110] C. de Luca and J. M. Olefsky, "Inflammation and insulin resistance," *FEBS Letters*, vol. 582, no. 1, pp. 97–105, 2008.

[111] M. Y. Donath and S. E. Shoelson, "Type 2 diabetes as an inflammatory disease," *Nature Reviews Immunology*, vol. 11, no. 2, pp. 98–107, 2011.

[112] K. H. Seo, H.-S. Lee, B. Jung et al., "Estrogen enhances angiogenesis through a pathway involving platelet-activating factor-mediated nuclear factor-κB activation," *Cancer Research*, vol. 64, no. 18, pp. 6482–6488, 2004.

[113] F. Modugno, R. B. Ness, C. Chen, and N. S. Weiss, "Inflammation and endometrial cancer: a hypothesis," *Cancer Epidemiology Biomarkers and Prevention*, vol. 14, no. 12, pp. 2840–2847, 2005.

[114] C. la Vecchia, S. H. Giordano, G. N. Hortobagyi, and B. Chabner, "Overweight, obesity, diabetes, and risk of breast cancer: interlocking pieces of the puzzle," *Oncologist*, vol. 16, no. 6, pp. 726–729, 2011.

[115] K. B. Michels, C. G. Solomon, F. B. Hu et al., "Type 2 diabetes and subsequent incidence of breast cancer in the nurses' health study," *Diabetes Care*, vol. 26, no. 6, pp. 1752–1758, 2003.

[116] L. L. Lipscombe, P. J. Goodwin, B. Zinman, J. R. McLaughlin, and J. E. Hux, "The impact of diabetes on survival following breast cancer," *Breast Cancer Research and Treatment*, vol. 109, no. 2, pp. 389–395, 2008.

[117] C. J. Currie, C. D. Poole, S. Jenkins-Jones, E. A. M. Gale, J. A. Johnson, and C. L. Morgan, "Mortality after incident cancer in people with and without type 2 diabetes: impact of metformin on survival," *Diabetes Care*, vol. 35, no. 2, pp. 299–304, 2012.

[118] J.-Y. Chen, W.-K. Chiou, W.-Y. Chou, and J.-D. Lin, "The impact of type 2 diabetes mellitus on mortality in hospitalized female cancer patients in Taiwan," *Asia-Pacific Journal of Clinical Oncology*, 2013.

[119] S. Liao, J. Li, W. Wei et al., "Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature," *Asian Pacific Journal of Cancer Prevention*, vol. 12, no. 4, pp. 1061–1065, 2011.

[120] L. Sciacca, A. Costantino, G. Pandini et al., "Insulin receptor activation by IGF-II in breast cancers: evidence for a new autocrine/paracrine mechanism," *Oncogene*, vol. 18, no. 15, pp. 2471–2479, 1999.

[121] R. Novosyadlyy, D. E. Lann, A. Vijayakumar et al., "Insulin-mediated acceleration of breast cancer development and progression in a nonobese model of type 2 diabetes," *Cancer Research*, vol. 70, no. 2, pp. 741–751, 2010.

[122] H. Nyholm, H. Djursing, C. Hagen, T. Agner, P. Bennett, and B. Svenstrup, "Androgens and estrogens in postmenopausal insulin-treated diabetic women," *The Journal of Clinical Endocrinology and Metabolism*, vol. 69, no. 5, pp. 946–949, 1989.

[123] L. Vona-Davis and D. P. Rose, "Type 2 diabetes and obesity metabolic interactions: common factors for breast cancer risk and novel approaches to prevention and therapy," *Current Diabetes Reviews*, vol. 8, no. 2, pp. 116–130, 2012.

[124] B. S. Katzenellenbogen and M. J. Norman, "Multihormonal regulation of the progesterone receptor in MCF-7 human breast cancer cells: Interrelationships among insulin/insulin-like growth factor-I, serum, and estrogen," *Endocrinology*, vol. 126, no. 2, pp. 891–898, 1990.

[125] M. L. Panno, M. Salerno, V. Pezzi et al., "Effect of oestradiol and insulin on the proliferative pattern and on oestrogen and progesterone receptor contents in MCF-7 cells," *Journal of Cancer Research and Clinical Oncology*, vol. 122, no. 12, pp. 745–749, 1996.

[126] L. Mauro, M. Salerno, M. L. Panno et al., "Estradiol increases IRS-1 gene expression and insulin signaling in breast cancer cells," *Biochemical and Biophysical Research Communications*, vol. 288, no. 3, pp. 685–689, 2001.

[127] A. Purohit, S. P. Newman, and M. J. Reed, "The role of cytokines in regulating estrogen synthesis: implications for the etiology of breast cancer," *Breast Cancer Research*, vol. 4, no. 2, pp. 65–69, 2002.

[128] A. Di Cristofano and L. H. Ellenson, "Endometrial carcinoma," *Annual Review of Pathology*, vol. 2, pp. 57–85, 2007.

[129] S. S. Jick, A. M. Walker, and H. Jick, "Estrogens, progesterone, and endometrial cancer," *Epidemiology*, vol. 4, no. 1, pp. 20–24, 1993.

[130] K. E. Anderson, E. Anderson, P. J. Mink et al., "Diabetes and endometrial cancer in the Iowa Women's Health Study," *Cancer Epidemiology Biomarkers and Prevention*, vol. 10, no. 6, pp. 611–616, 2001.

[131] Y. Zhang, Z. Liu, X. Yu et al., "The association between metabolic abnormality and endometrial cancer: a large case-control study in China," *Gynecologic Oncology*, vol. 117, no. 1, pp. 41–46, 2010.

[132] E. Friberg, C. S. Mantzoros, and A. Wolk, "Diabetes and risk of endometrial cancer: a population-based prospective cohort study," *Cancer Epidemiology Biomarkers and Prevention*, vol. 16, no. 2, pp. 276–280, 2007.

[133] H. Vainio, R. Kaaks, and F. Bianchini, "Weight control and physical activity in cancer prevention: international evaluation of the evidence," *European Journal of Cancer Prevention*, vol. 11, supplement 2, pp. S94–S100, 2002.

[134] L. J. Schouten, R. A. Goldbohm, and P. A. van den Brandt, "Anthropometry, physical activity, and endometrial cancer risk: results from the Netherlands Cohort Study," *Journal of the National Cancer Institute*, vol. 96, no. 21, pp. 1635–1638, 2004.

[135] S. C. Modesitt and J. R. Van Nagell Jr., "The impact of obesity on the incidence and treatment of gynecologic cancers: a review," *Obstetrical and Gynecological Survey*, vol. 60, no. 10, pp. 683–692, 2005.

[136] I. Bruchim, R. Sarfstein, and H. Werner, "The IGF hormonal network in endometrial cancer: functions, regulation, and targeting approaches," *Frontiers in Endocrinology*, vol. 5, p. 76, 2014.

[137] T. Wang, T. E. Rohan, M. J. Gunter et al., "A prospective study of inflammation markers and endometrial cancer risk in postmenopausal hormone nonusers," *Cancer Epidemiology Biomarkers and Prevention*, vol. 20, no. 5, pp. 971–977, 2011.

[138] R. Siegel, D. Naishadham, and A. Jemal, "Cancer statistics, 2012," *CA: A Cancer Journal for Clinicians*, vol. 62, no. 1, pp. 10–29, 2012.

[139] B. T. Hennessy, R. L. Coleman, and M. Markman, "Ovarian cancer," *The Lancet*, vol. 374, no. 9698, pp. 1371–1382, 2009.

[140] V. Beral, R. Doll, C. Hermon et al., "Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23 257 women with ovarian cancer and 87 303 controls," *The Lancet*, vol. 371, no. 9609, pp. 303–314, 2008.

[141] L. Poretsky and M. F. Kalin, "The gonadotropic function of insulin.," *Endocrine Reviews*, vol. 8, no. 2, pp. 132–141, 1987.

[142] R. J. Chang, R. M. Nakamura, H. L. Judd, and S. A. Kaplan, "Insulin resistance in nonobese patients with polycystic ovarian disease," *Journal of Clinical Endocrinology and Metabolism*, vol. 57, no. 2, pp. 356–359, 1983.

[143] A. I. Adler, N. S. Weiss, M. L. Kamb, and J. L. Lyon, "Is diabetes mellitus a risk factor for ovarian cancer? A case-control study in Utah and Washington (United States)," *Cancer Causes and Control*, vol. 7, no. 4, pp. 475–478, 1996.

[144] J.-Y. Lee, I. Jeon, J. W. Kim, Y.-S. Song, J.-M. Yoon, and S. M. Park, "Diabetes mellitus and ovarian cancer risk: a systematic review and meta-analysis of observational studies," *International Journal of Gynecological Cancer*, vol. 23, no. 3, pp. 402–412, 2013.

[145] L. Wideroff, G. Gridley, L. Mellemkjaer et al., "Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in denmark," *Journal of the National Cancer Institute*, vol. 89, no. 18, pp. 1360–1365, 1997.

[146] S.-F. Lo, S.-N. Chang, C.-H. Muo et al., "Modest increase in risk of specific types of cancer types in type 2 diabetes mellitus patients," *International Journal of Cancer*, vol. 132, no. 1, pp. 182–188, 2013.

[147] H. A. Risch, "Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone," *Journal of the National Cancer Institute*, vol. 90, no. 23, pp. 1774–1786, 1998.

[148] M.-R. Shen, A.-C. Lin, Y.-M. Hsu et al., "Insulin-like growth factor 1 stimulates KCl cotransport, which is necessary for invasion and proliferation of cervical cancer and ovarian cancer cells," *The Journal of Biological Chemistry*, vol. 279, no. 38, pp. 40017–40025, 2004.

[149] Y. Tanaka, H. Kobayashi, M. Suzuki, Y. Hirashima, N. Kanayama, and T. Terao, "Genetic downregulation of pregnancy-associated plasma protein-A (PAPP-A) by bikunin reduces IGF-I-dependent Akt and ERK1/2 activation and subsequently reduces ovarian cancer cell growth, invasion and metastasis," *International Journal of Cancer*, vol. 109, no. 3, pp. 336–347, 2004.

[150] E. J. Lee, C. Mircean, I. Shmulevich et al., "Insulin-like growth factor binding protein 2 promotes ovarian cancer cell invasion," *Molecular Cancer*, vol. 4, article 7, 2005.

[151] T. V. Clendenen, E. Lundin, A. Zeleniuch-Jacquotte et al., "Circulating inflammation markers and risk of epithelial ovarian cancer," *Cancer Epidemiology Biomarkers and Prevention*, vol. 20, no. 5, pp. 799–810, 2011.

[152] A. Evangelou, S. K. Jindal, T. J. Brown, and M. Letarte, "Down-regulation of transforming growth factor β receptors by androgen in ovarian cancer cells," *Cancer Research*, vol. 60, no. 4, pp. 929–935, 2000.

[153] E. Giovannucci, D. M. Harlan, M. C. Archer et al., "Diabetes and cancer: a consensus report," *CA: A Cancer Journal for Clinicians*, vol. 60, no. 4, pp. 207–221, 2010.

[154] L. G. Hemkens, R. Bender, U. Grouven, and P. T. Sawicki, "Insulin glargine and cancer," *The Lancet*, vol. 374, no. 9703, pp. 1743–1744, 2009.

[155] S. Jabbour, "Primary care physicians and insulin initiation: multiple barriers, lack of knowledge or both?" *International Journal of Clinical Practice*, vol. 62, no. 6, pp. 845–847, 2008.

[156] C. J. Currie, C. D. Poole, and E. A. M. Gale, "The influence of glucose-lowering therapies on cancer risk in type 2 diabetes," *Diabetologia*, vol. 52, no. 9, pp. 1766–1777, 2009.

[157] J. M. Jonasson, R. Ljung, M. Talbäck, B. Haglund, S. Gudbjörnsdòttir, and G. Steineck, "Insulin glargine use and short-term incidence of malignancies-a population-based follow-up study in Sweden," *Diabetologia*, vol. 52, no. 9, pp. 1745–1754, 2009.

[158] H. M. Colhoun, "Use of insulin glargine and cancer incidence in Scotland: a study from the Scottish Diabetes Research Network Epidemiology Group," *Diabetologia*, vol. 52, no. 9, pp. 1755–1765, 2009.

[159] L. G. Hemkens, U. Grouven, R. Bender et al., "Risk of malignancies in patients with diabetes treated with human insulin or insulin analogues: a cohort study," *Diabetologia*, vol. 52, no. 9, pp. 1732–1744, 2009.

[160] S. J. Pocock and L. Smeeth, "Insulin glargine and malignancy: an unwarranted alarm," *The Lancet*, vol. 374, no. 9689, pp. 511–513, 2009.

[161] M. Janghorbani, M. Dehghani, and M. Salehi-Marzijarani, "Systematic review and meta-analysis of insulin therapy and risk of cancer," *Hormones and Cancer*, vol. 3, no. 4, pp. 137–146, 2012.

[162] R. Ljung, M. Talbäck, B. Haglund, J. M. Jonasson, S. Gudbjörnsdòttir, and G. Steineck, "Insulin glargine use and short-term incidence of breast cancer a four-year population-based observation," *Acta Oncologica*, vol. 51, no. 3, pp. 400–402, 2012.

[163] S. Suissa, L. Azoulay, S. Dell'Aniello, M. Evans, J. Vora, and M. Pollak, "Long-term effects of insulin glargine on the risk of breast cancer," *Diabetologia*, vol. 54, no. 9, pp. 2254–2262, 2011.

[164] M. K. Leow, "Basal insulin and cardiovascular and other outcomes," *The New England Journal of Medicine*, vol. 367, no. 18, pp. 1763–1764, 2012.

[165] D. M. Nathan, J. B. Buse, and M. B. Davidson, "Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American diabetes association and the European association for the study of diabetes," *Diabetes Care*, vol. 32, no. 1, pp. 193–203, 2009.

[166] R. Giannarelli, M. Aragona, A. Coppelli, and S. Del Prato, "Reducing insulin resistance with metformin: the evidence today," *Diabetes & Metabolism*, vol. 29, no. 4, part 2, pp. 6–S28, 2003.

[167] R. J. Shaw, K. A. Lamia, D. Vasquez et al., "The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin," *Science*, vol. 310, no. 5754, pp. 1642–1646, 2005.

[168] A. DeCensi, M. Puntoni, P. Goodwin et al., "Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis," *Cancer Prevention Research*, vol. 3, no. 11, pp. 1451–1461, 2010.

[169] G. W. D. Landman, N. Kleefstra, K. J. J. van Hateren, K. H. Groenier, R. O. B. Gans, and H. J. G. Bilo, "Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16," *Diabetes Care*, vol. 33, no. 2, pp. 322–326, 2010.

[170] Z. B. Mei, Z. J. Zhang, C. Y. Liu et al., "Survival benefits of metformin for colorectal cancer patients with diabetes: a systematic review and meta-analysis," *PLoS ONE*, vol. 9, no. 3, Article ID e91818, 2014.

[171] H. Noto, A. Goto, T. Tsujimoto, and M. Noda, "Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis," *PLoS ONE*, vol. 7, no. 3, Article ID e33411, 2012.

[172] M. Martin and R. Marais, "Metformin: a diabetes drug for cancer, ora cancer drug for diabetics?" *Journal of Clinical Oncology*, vol. 30, no. 21, pp. 2698–2700, 2012.

[173] R. Rattan, R. Ali Fehmi, and A. Munkarah, "Metformin: an emerging new therapeutic option for targeting cancer stem cells and metastasis," *Journal of Oncology*, vol. 2012, Article ID 928127, 12 pages, 2012.

[174] E. J. Gallagher and D. Leroith, "Diabetes, cancer, and metformin: connections of metabolism and cell proliferation," *Annals of the New York Academy of Sciences*, vol. 1243, no. 1, pp. 54–68, 2011.

[175] H. Lee, H. J. Park, C.-S. Park et al., "Response of breast cancer cells and cancer stem cells to metformin and hyperthermia alone or combined," *PLoS ONE*, vol. 9, no. 2, Article ID e87979, 2014.

[176] D. G. Hardie, "Neither LKB1 nor AMPK are the direct targets of metformin," *Gastroenterology*, vol. 131, no. 3, pp. 973–975, 2006.

[177] R. J. O. Dowling, M. Zakikhani, I. G. Fantus, M. Pollak, and N. Sonenberg, "Metformin inhibits mammalian target of rapamycin—dependent translation initiation in breast cancer cells," *Cancer Research*, vol. 67, no. 22, pp. 10804–10812, 2007.

[178] M. Zakikhani, R. Dowling, I. G. Fantus, N. Sonenberg, and M. Pollak, "Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells," *Cancer Research*, vol. 66, no. 21, pp. 10269–10273, 2006.

[179] I. N. Alimova, B. Liu, Z. Fan et al., "Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro," *Cell Cycle*, vol. 8, no. 6, pp. 909–915, 2009.

[180] B. Liu, Z. Fan, S. M. Edgerton et al., "Metformin induces unique biological and molecular responses in triple negative breast cancer cells," *Cell Cycle*, vol. 8, no. 13, pp. 2031–2040, 2009.

[181] M. Zakikhani, R. J. O. Dowling, N. Sonenberg, and M. N. Pollak, "The effects of adiponectin and metformin on prostate and colon neoplasia involve activation of AMP-activated protein kinase," *Cancer Prevention Research*, vol. 1, no. 5, pp. 369–375, 2008.

[182] V. N. Anisimov, L. M. Berstein, P. A. Egormin et al., "Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice," *Experimental Gerontology*, vol. 40, no. 8-9, pp. 685–693, 2005.

[183] M. B. Schneider, H. Matsuzaki, J. Haorah et al., "Prevention of pancreatic cancer induction in hamsters by metformin," *Gastroenterology*, vol. 120, no. 5, pp. 1263–1270, 2001.

[184] C. Algire, M. Zakikhani, M.-J. Blouin, J. H. Shuai, and M. Pollak, "Metformin attenuates the stimulatory effect of a high-energy diet on *in vivo* LLC1 carcinoma growth," *Endocrine-Related Cancer*, vol. 15, no. 3, pp. 833–839, 2008.

[185] J. M. M. Evans, L. A. Donnelly, A. M. Emslie-Smith, D. R. Alessi, and A. D. Morris, "Metformin and reduced risk of cancer in diabetic patients," *British Medical Journal*, vol. 330, no. 7503, pp. 1304–1305, 2005.

[186] S. Jiralerspong, S. L. Palla, S. H. Giordano et al., "Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer," *Journal of Clinical Oncology*, vol. 27, no. 20, pp. 3297–3302, 2009.

[187] Z.-J. Zhang and S. Li, "The prognostic value of metformin for cancer patients with concurrent diabetes: a systematic review and meta-analysis," *Diabetes, Obesity and Metabolism*, vol. 16, no. 8, pp. 707–710, 2014.

[188] R. J. Stevens, R. Ali, C. R. Bankhead et al., "Cancer outcomes and all-cause mortality in adults allocated to metformin: systematic review and collaborative meta-analysis of randomised clinical trials," *Diabetologia*, vol. 55, no. 10, pp. 2593–2603, 2012.

[189] N. F. Col, L. Ochs, V. Springmann, A. K. Aragaki, and R. T. Chlebowski, "Metformin and breast cancer risk: a meta-analysis and critical literature review," *Breast Cancer Research and Treatment*, vol. 135, no. 3, pp. 639–646, 2012.

[190] H. Hauner, "The mode of action of thiazolidinediones," *Diabetes/Metabolism Research and Reviews*, vol. 18, supplement 2, pp. S10–S15, 2002.

[191] D. Panigrahy, S. Huang, M. W. Kieran, and A. Kaipainen, "PPARγ as a therapeutic target for tumor angiogenesis and

metastasis," *Cancer Biology and Therapy*, vol. 4, no. 7, pp. 687–693, 2005.

[192] A. Rubenstrunk, R. Hanf, D. W. Hum, J.-C. Fruchart, and B. Staels, "Safety issues and prospects for future generations of PPAR modulators," *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids*, vol. 1771, no. 8, pp. 1065–1081, 2007.

[193] K. Ohta, T. Endo, K. Haraguchi, J. M. Hershman, and T. Onaya, "Ligands for peroxisome proliferator-activated receptor γ inhibit growth and induce apoptosis of human papillary thyroid carcinoma cells," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 5, pp. 2170–2177, 2001.

[194] C. Blanquicett, J. Roman, and C. M. Hart, "Thiazolidinediones as anti-cancer agents," *Cancer Therapy*, vol. 6, pp. 25–34, 2008.

[195] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[196] D. Panigrahy, L. Q. Shen, M. W. Kieran, and A. Kaipainen, "Therapeutic potential of thiazolidinediones as anticancer agents," *Expert Opinion on Investigational Drugs*, vol. 12, no. 12, pp. 1925–1937, 2003.

[197] D. Panigrahy, S. Singer, L. Q. Shen et al., "PPARγ ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis," *The Journal of Clinical Investigation*, vol. 110, no. 7, pp. 923–932, 2002.

[198] E. Saez, J. Rosenfeld, A. Livolsi et al., "PPARγ signaling exacerbates mammary gland tumor development," *Genes & Development*, vol. 18, no. 5, pp. 528–540, 2004.

[199] K. Yang, K.-H. Fan, S. A. Lamprecht et al., "Peroxisome proliferator-activated receptor γ agonist troglitazone induces colon tumors in normal C57BL/6J mice and enhances colonic carcinogenesis in $Apc^{1638N/+}$ $Mlh1^{+/-}$ double mutant mice," *International Journal of Cancer*, vol. 116, no. 4, pp. 495–499, 2005.

[200] E. Saez, P. Tontonoz, M. C. Nelson et al., "Activators of the nuclear receptor PPARγ enhance colon polyp formation," *Nature Medicine*, vol. 4, no. 9, pp. 1058–1061, 1998.

[201] C. Koro, S. Barrett, and N. Qizilbash, "Cancer risks in thiazolidinedione users compared to other anti-diabetic agents," *Pharmacoepidemiology and Drug Safety*, vol. 16, no. 5, pp. 485–492, 2007.

[202] R. Govindarajan, L. Ratnasinghe, D. L. Simmons et al., "Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes," *Journal of Clinical Oncology*, vol. 25, no. 12, pp. 1476–1481, 2007.

[203] M. E. Ramos-Nino, C. D. MacLean, and B. Littenberg, "Association between cancer prevalence and use of thiazolidinediones: results from the vermont diabetes information system," *BMC Medicine*, vol. 5, article 17, 2007.

[204] X. He, F. J. Esteva, J. Ensor, G. N. Hortobagyi, M.-H. Lee, and S.-C. J. Yeung, "Metformin and thiazolidinediones are associated with improved breast cancer-specific survival of diabetic women with HER2+ breast cancer," *Annals of Oncology*, vol. 23, no. 7, Article ID mdr534, pp. 1771–1780, 2012.

[205] C.-H. Tseng, "Pioglitazone does not affect the risk of ovarian cancer: analysis of a nationwide reimbursement database in Taiwan," *Gynecologic Oncology*, vol. 131, no. 1, pp. 135–139, 2013.

[206] M. Monami, C. Lamanna, N. Marchionni, and E. Mannucci, "Rosiglitazone and risk of cancer: a meta-analysis of randomized clinical trials," *Diabetes Care*, vol. 31, no. 7, pp. 1455–1460, 2008.

[207] M. Monami, I. Dicembrini, and E. Mannucci, "Thiazolidinediones and cancer: results of a meta-analysis of randomized clinical trials," *Acta Diabetologica*, vol. 51, no. 1, pp. 91–101, 2014.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 548436, 13 pages
http://dx.doi.org/10.1155/2015/548436

Review Article

Recent Advances in the Use of Metformin: Can Treating Diabetes Prevent Breast Cancer?

Diana Hatoum[1] and Eileen M. McGowan[1,2]

[1] *School of Medical and Molecular Biosciences, Faculty of Science, University of Technology Sydney, Sydney, NSW 2007, Australia*
[2] *School of Medicine, University of Sydney, Camperdown, Sydney, NSW 2006, Australia*

Correspondence should be addressed to Eileen M. McGowan; eileen.mcgowan@uts.edu.au

Received 31 July 2014; Accepted 21 October 2014

Academic Editor: Viroj Boonyaratanakornkit

There is substantial epidemiological evidence pointing to an increased incidence of breast cancer and morbidity in obese, prediabetic, and diabetic patients. *In vitro* studies strongly support metformin, a diabetic medication, in breast cancer therapy. Although metformin has been heralded as an exciting new breast cancer treatment, the principal consideration is whether metformin can be used as a generic treatment for all breast cancer types. Importantly, will metformin be useful as an inexpensive therapy for patients with comorbidity of diabetes and breast cancer? In general, meta-analyses of clinical trial data from retrospective studies in which metformin treatment has been used for patients with diabetes and breast cancer have a positive trend; nevertheless, the supporting clinical data outcomes remain inconclusive. The heterogeneity of breast cancer, confounded by comorbidity of disease in the elderly population, makes it difficult to determine the actual benefits of metformin therapy. Despite the questionable evidence available from observational clinical studies and meta-analyses, randomized phases I–III clinical trials are ongoing to test the efficacy of metformin for breast cancer. This special issue review will focus on recent research, highlighting *in vitro* research and retrospective observational clinical studies and current clinical trials on metformin action in breast cancer.

1. Introduction

Cancer and diabetes are two of the most common chronic diseases worldwide [1] with a strong association between the two diseases [2, 3]. Substantial evidence exists indicating that the risk of developing and dying from breast cancer is higher in diabetic patients compared to nondiabetic patients, excluding all other diseases [2]. Metformin, a biguanide oral antidiabetic drug, commonly used to treat type 2 diabetes mellitus has aroused much interest in comorbidity (diabetes/cancer) treatment, and emerging evidence from *in vitro* and epidemiological studies suggests that metformin improves the overall survival for cancer/diabetic comorbidity patients [2, 3]. *In vitro* experimentation supports metformin as a strong candidate for treatment of breast cancer, where it has been shown to increase breast cancer cell death. However, the use of metformin as a comorbidity treatment, or breast cancer preventative therapy, in retrospective clinical meta-analyses studies is controversial. Metformin, on the one hand, has been shown to decrease cancer incidence and increase survival [3–6], while on the other hand no such association has been observed in other studies [7].

This special issue review brings together recent *in vitro* research supporting metformin as a wide-ranging treatment for most breast cancer subtypes, including the hard to treat triple negative subtype. Importantly, this paper will provide an overview of the recent contradicting meta-analyses and retrospective observational clinical studies focusing on metformin as a therapeutic agent for breast cancer.

1.1. Changing Metabolism Linking Diabetes and Cancer. For over a century, disturbances in cellular metabolism intrinsically linking diabetes and cancer have been recognized [8, 9]. One of the hallmarks of cancer is the reprogramming of energy metabolism to fuel cancer cell growth and division [10]. First proposed by Otto Warburg in 1924, cancer cells hijack cellular metabolism to favour aerobic glycolysis (high

glucose demand) for energy needs in preference to mitochondrial oxidative phosphorylation [11]. Although aerobic glycosylation is an inefficient energy process, the bioenergetic demands of a cancer cell favour fast nutrients in the form of excess glucose for fast bursts of energy to fuel all the molecular components for DNA replication and cell division. Simplistically, prediabetes is inefficient processing of intracellular glucose, which leads to insulin resistant cells, hyperinsulinemia (increased insulin), and hyperglycemia (increased blood glucose levels). Elevated insulin levels have been shown to have mitogenic effects and constitute an increased risk factor for breast cancer [12]. An excessive supply of glucose in the bloodstream, as evidenced in diabetic patients, may provide the necessary nutrients to feed cancer cells; hence, the proposal that diabetic treatments reduce glucose in the bloodstream may prove beneficial for cancer prevention and patient therapy [13]. Metformin is commonly used in the treatment of type 2 diabetes mellitus to combat insulin resistance by reducing the amount of available glucose in the blood, as aptly described by Jalving and colleagues [13] "taking away the candy." The antidiabetic drug metformin is emerging as a potential, efficient, preventative, and adjuvant therapy for many cancer types [14–17].

1.2. Safety of Metformin in Diabetic Treatment. For over than 50 years, metformin has been one of the most effective, well tolerated, antidiabetic treatments prescribed worldwide [18]. Metformin taken alone is a relatively safe drug for clinical use with only mild side effects documented including gastrointestinal disturbances (diarrhea, nausea, and irritation of the abdomen) [19]. The major toxicity reported is lactic acidosis, though this is very rare (9 per 100,000) [20]. A recent report suggests metformin is associated with impairment of cognitive function and these studies are ongoing [21]. The overall safety of metformin with minimal and rare side effects adds to its attractiveness as a potential breast cancer or comorbidity treatment for cancer patients with diabetes.

1.3. Diabetes and Breast Cancer. Evidence from epidemiological studies strongly supports that prediabetes, preexisting diabetes mellitus, and obesity are risk factors for cancer with a poorer outcome reported for breast cancers occurring in diabetic patients compared to nondiabetic patients [2, 22–26]. A meta-analysis of twenty clinical trials involving more than 1.9 million cancer patients with or without diabetes supported a significant increase in combined incidence and death from breast cancer [2]. This mega-study agreed with previous findings by Peairs and colleagues who reported comorbidity of breast cancer and diabetes was associated with a 49% increased risk of death from any cause and increased adverse effects in response to chemotherapy [24]. Prediabetes and hyperinsulinemia in breast cancer patients have also been associated with higher mortality rates [27–29]. Interestingly, a meta-analysis by Boyle and colleagues showed that the association between diabetes and breast cancer was restricted to diabetes mellitus type 2 (not type 1) in postmenopausal women and no such association was evident between diabetes and prediabetic conditions and breast cancer in premenopausal women [30]. The link between the onset of prediabetes, type 2 diabetes mellitus, and a higher risk of breast cancer diagnosis comes with new insights into how diabetic treatments influence breast cancer outcomes [2, 17, 24, 30]. Metformin, a well-tolerated insulin-sensitizer, has shown promise in reducing cancer risk or has no negative effect [29, 31–33]. Recently, mining of over 100,000 electronic medical records from Vanderbilt University Medical Center and Mayo Clinic by Xu and colleagues showed that the use of metformin significantly reduced cancer risk, including breast cancer, compared to patients who are not using metformin and are independent of diabetes status [6], thus providing additional support for metformin use in future cancer treatment regimens. Consequently, there has been much interest in understanding the mechanism of metformin action and exploring its efficacy in breast cancer therapy. Equally, there are a number of studies that do not support the observation of a reduction in breast cancer risk in diabetic and nondiabetic patients being treated with metformin and these findings are discussed.

In contrast, diabetic treatments, such as sulfonylureas, have been shown to increase mortality in patients with cancer and type 2 diabetes and insulin replacement has been shown to increase mortality due to its mitogenic effects [34–40]. However, it is noted that, in one meta-analysis retrospective study, data extracted from the Hong Kong Diabetes Registry reported that insulin replacement therapy reduced cancer risk [41]. An increase in body mass index (BMI) or obesity is associated with cancer risk and this study did not account for BMI [42]. Given the low BMI in the Asian population, this may contribute to the differences in the results [43].

2. Mechanism of Metformin Action to Inhibit Cancer

The exact molecular mechanism of metformin action is not clearly understood and has been hotly debated [44, 45]. Nevertheless, metformin action undisputedly has been shown to increase insulin sensitivity *in vivo*, resulting in reduced plasma glucose concentrations, increased glucose uptake, and decreased gluconeogenesis [46, 47]. High insulin levels are associated with increased breast cancer risk and poor patient survival outcome [17, 48]; therefore, metformin directly and indirectly reduces cancer cell proliferation through reduction of insulin levels and blood glucose levels. In the context of breast cancer risk, metformin has been shown to decrease circulating hormones such as androgen and estrogen where elevated levels are linked with postmenopausal breast cancer development [49, 50]. Thus metformin treatment may serve as a contributory factor in decreasing breast cancer risk.

The concept that cancer cells undergo metabolic reprogramming in favour of glycolysis is generally accepted. Metformin acts by interfering with cellular processes that facilitate insulin signalling and glucose synthesis. Some of these proposed signalling pathways are described in this section and illustrated in Figure 1.

There is general consensus that the organic cation transporter (OCT1) plays a major role in mediating the first

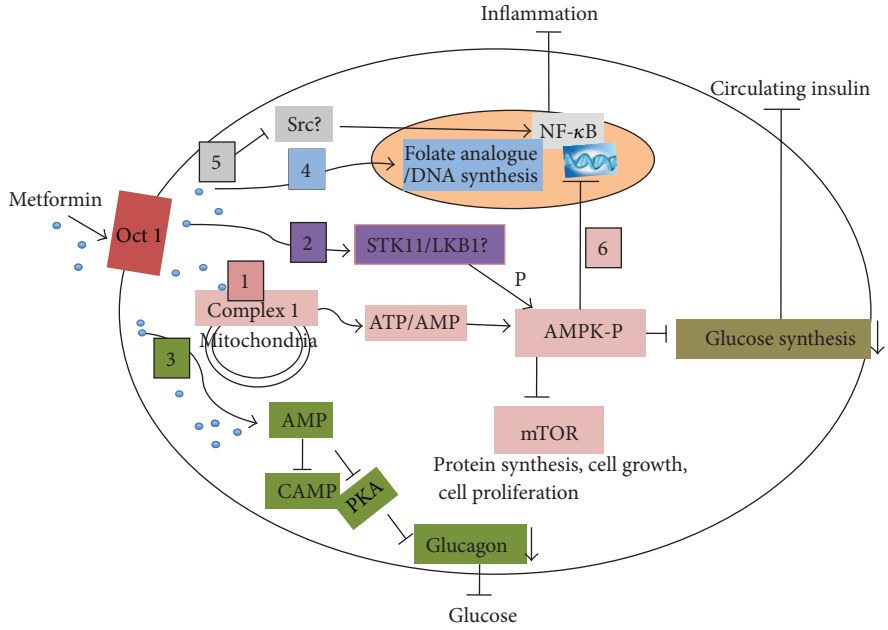


Figure 1: Mechanisms of metformin action to inhibit cancer. Metformin disrupts circulating glucose and insulin levels and reduces inflammation. The organic cation transporter (OCT1) mediates the first step in metformin cellular response [51–53]. (1) Metformin activates the AMPK-P pathway through inhibition of Complex 1 of the mitochondrial respiratory chain [54, 55]. This leads to the inhibition of mTOR and thus loss of cell proliferation and inhibition of glucose synthesis [56–59]. (2) LKB1 may act as an intermediatory of AMPK activation [60, 61]. (3) Metformin blocks cAMP and PKA, which in turn antagonizes glucagon action [62]. (4) Metformin acts as an antifolate hindering DNA replication [63]. (5) Metformin induces an anti-inflammatory response via the Src-mediated NF-κB pathway [64]. (6) Metformin action is implicated in both AMPK dependent and independent inhibition of the angiogenesis process [65].

step in metformin cellular response [51–53]. Shu and colleagues demonstrated that genetic variation in the OCT1 gene reduced hepatic uptake of metformin and altered the efficacy of metformin suggesting that patients with reduced response to metformin may be screened for OCT1 mutations [51]. The most widely accepted mechanism of metformin action is, by indirect activation of the central energy sensor, adenosine monophosphate-activated protein kinase (AMPK), which also plays a key role in insulin signalling [54, 55]. Activation of AMPK has been shown to inhibit the mammalian target of rapamycin (mTOR) and therefore inhibit pathological cell proliferation in different cancer cell lines [56–59] (Figure 1(1)). Phosphorylation of AMPK by serine-threonine kinase 11/liver kinase B1 (STK11/LKB1) has also been reported to be an upstream event in metformin action [60, 61] despite more recent evidence questions whether LKB1 is required for metformin action [58, 66] (Figure 1(2)). Whereas the focus of metformin action has been directed towards reduction of glucose synthesis through the AMPK pathway (Figure 1(1)), Miller and colleagues showed that metformin antagonism of glucagon action was responsible for reducing fasting glucose levels [62] (Figure 1(3)).

2.1. Metformin, Cancer, and the Mitochondria Conundrum. Upstream of AMPK-activation both mitochondria-dependent and -independent mechanisms have been described as precursors of AMPK activation. Metformin has been described as a "mitochondrial poison" through inhibition of Complex 1 of the mitochondrial respiratory chain leading to AMPK activation and reduction of glucose synthesis [54, 55] (Figure 1(1)). Based on the premise that metformin is a weak "poison," Salem and colleagues proposed that metformin could be useful as an anticancer therapy targeting mitochondrial metabolism [67]. Metformin also affects the mitochondrial redox state through inhibition of mitochondrial glycerophosphate dehydrogenase, which leads to suppression of gluconeogenesis [68]. These studies were confirmed in mouse and rat models using metformin treatment doses that achieved similar plasma concentrations to those observed in type 2 diabetes patients treated with metformin [68].

Alternatively, mitochondrial-independent AMPK activation has been described whereby metformin acts in a similar manner to an antifolate, a member of the antimetabolite class of chemotherapy drugs, and inhibits DNA replication and cell proliferation [63] (Figure 1(4)).

Hirsch and colleagues implicated metformin in blocking the inflammatory response through inhibition of a step(s) in the Src-mediated-nuclear factor kappa B (NF-κB) signaling pathway [64] (Figure 1(5)). These findings are especially relevant as a preventative measure in obesity-associated inflammation and cancer progression. Others have shown that metformin may be associated with inhibition of the angiogenesis process, as shown in endothelial cells, via AMPK-dependent and -independent pathways [65] (Figure 1(6)). As new vascular formation is essential for tumour growth, this effect would assist in the prevention of cancer development.

In summary, metformin has been reported to have both direct and indirect effects on a number of metabolic pathways. Whilst the majority of laboratory research has focused on the mitochondrial-AMPK signalling pathway, new research has elucidated new mechanisms of metformin action, some of which are highlighted in Figure 1. Nonetheless, the mode of metformin action is still unclear and under investigation. The consensus is that the most important therapeutic endpoints of metformin are reduction in blood glucose level, and action as an insulin sensitizer, which is beneficial to patients with diabetes and/or potentially reduces the risk of most cancers including breast cancer.

3. Metformin and Breast Cancer *In Vitro* Studies

Since the benefits of metformin treatment for breast cancer patients were reported in 2005 [32], an increasing number of articles assessing its anticancer properties have been published. Highlighted here are some of the important findings from the *in vitro* studies linking metformin treatment and breast cancer outcome.

3.1. Breast Cancer Classification. Breast cancer is heterogeneous and, as such, different breast cancer subtypes are known to have distinct molecular profiles [69–74] and variable responses to different treatments. Based on the differential expression of various genes, breast cancer has been categorised into five major distinct molecular subtypes with prognostic significance: luminal A; luminal B; overexpression of HER2; also known as ErbB2; breast-like; and basal-like/triple negative [69]. Triple negative breast cancers have been further classified into six distinct subtypes: immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor, basal-like 1, and basal-like 2 [75]. In addition, there are at least seventeen rare subtypes defined [76]. Response to therapy is dependent on the pathology and classification of the breast tumour. The most predominant subtype, luminal A, is known to have the best prognosis with HER2 and the basal-like triple negative subtype has the worst outcome [77]. Nevertheless, many breast cancers recur and acquire resistance to conventional treatments. Metformin is being investigated *in vitro* in different breast cancer cell types, reviewed below, and an understanding of the mode of action in diverse breast cancer cell types is providing some insights into drug resistance. One of the leading questions is can metformin be used as a generic therapy for all breast cancer subtypes?

3.2. Metformin as Mono- or Combinational Therapy for Breast Cancer. There are enormous differences in clinical response to metformin monotherapy in diabetic and cancer patients; hence, the drug is generally used in combination with other treatments. The current challenge is to understand why this drug has reduced efficacy in some patients and to modify drug therapy for better outcome for individual patients. There have been a number of recent reports showing synergistic or enhanced effects on endpoints such as increased apoptosis and cell death in breast cancer cell lines when metformin is used in combination with chemotherapeutic drugs and with targeted therapies, providing a strong rationale for the use of metformin in clinical treatment regimens [78–80]. Metformin monotherapy has been shown to promote cell cycle arrest in both ER+ and ER− breast cancer cell lines [78, 80]. Metformin was reported to markedly suppress, but not completely abrogate, proliferation of breast cancer and cancer stem cells whilst being less toxic to normal stem cells [81]. These findings are important as a small proportion of breast cancer stem cells are believed to be the source of cancer recurrence [64]. Interestingly, cell cycle inhibition in a study by Lee and colleagues was significantly enhanced when the temperature was increased to 42°C suggesting that metformin may be more toxic to breast cancer patients with elevated body temperature [81]. In these experiments, metformin cytotoxicity appeared to be mediated through AMPK/mTOR activation [81].

3.3. Metformin Effects on Basal-Like/Triple Negative Breast Cancers. Triple negative breast cancers occur in a minority of breast cancer patients and such patients have a very poor prognosis [82]. These types of tumours are very aggressive and are associated with high morbidity and mortality due to their fast proliferation and propensity for metastasis. Their failure to express ER/PR and HER2 makes them resistant to antihormonal therapies and herceptin. Many triple negative breast tumours demonstrate epithelial mesenchymal transition (EMT) and stem cell-like properties and may lie dormant making them extremely difficult to treat with current chemotherapy treatments. Metformin has been shown to be a promising adjuvant treatment for triple negative breast cancers [58, 67, 78, 82–87] where Stat3 has been shown to be a critical regulator of metformin action [87], and it has also been shown to directly inhibit the enzymatic function of hexokinase I and hexokinase II [86]. However, not all studies have shown that metformin induces apoptosis and cell cycle arrest in the triple negative cell model, MDA-MB-231, and it has been suggested that this is a function of glucose homeostasis [58, 85].

3.4. Metformin Efficacy Is Dependent on Glucose Homeostasis. Circulating glucose levels may prove to be an important factor in response to metformin treatment in cancer patients. Menendez and colleagues reported that metformin lethality was enhanced in breast cancer cells that had undergone glucose deprivation [88]. Their studies showed metformin was protective in normal cells in the presence of physiological amounts of glucose, whereas it caused cell cycle arrest in breast cancer cells. Conversely, withdrawal of glucose induced breast cancer cell death independent of the following subtypes: ER+, HER2+, and triple negative [88]. Further studies have also confirmed that the failure to maintain glucose homeostasis results in a more aggressive triple negative breast cancer phenotype [85]. Moreover, in hyperglycemic conditions, Zordoky and colleagues showed that a surplus of glucose supply rescued the triple negative MDA-MD-231 cells from metformin induced cell death and suggested

that the bypass was due to the generation of enough energy for proliferation through aerobic glycolysis using the excess glucose [58]. Based on the laboratory evidence, it has been advocated that glucose monitoring of breast cancer patients may provide some insight into patient response to metformin and that pharmacological deprivation of glucose combined with metformin treatment may benefit patients with high glucose levels [88].

3.5. Use of Metformin to Overcome Multidrug and Chemotherapy Resistance in Breast Cancer Cells. The emergence of multidrug/chemotherapy resistant cells within a tumour population is a major obstacle for many cancer patients. There is now compelling evidence to suggest that metformin resensitizes cells and cooperates with some anticancer drugs to improve efficacy through reprogramming of the metabolic cellular pathways [89, 90]. A recent study showed the reversal of multidrug resistance in breast cancer cells through activation of AMPK/mTOR by metformin [90]. In addition, metformin promoted 5-FU-induced apoptosis, consistent with its proposed role as a pseudo metabolite, and reversed epithelial mesenchymal transition (EMT), a critical phenotypic switch associated with enhanced capacity of cells for invasion, metastasis, and chemoresistance [90]. Metformin sensitisation to chemotherapy has also been demonstrated in breast cancer cells overexpressing aldehyde dehydrogenase (ALDH), an enzyme linked to chemoresistance in breast cancer cells that also feature an EMT phenotype [91]. Potentially, small doses of metformin could be used as an adjuvant therapy to prevent some chemotherapy resistant phenotypes and prevent EMT transition.

ErbB2-positive (HER2/neu) breast cancer cells are usually treated with lapatinib (a dual inhibitor of the EGRF and ERBB2/HER2 tyrosine kinase inhibitor) as a first line monotherapy [92–94]. Short-lived clinical responses in ErbB+ breast cancers are due to acquired resistance to lapatinib. Komurov and colleagues showed that forcing ErbB2 drug-sensitive cells into glucose-deprivation made them more resistant to lapatinib [95]. In line with the glucose-deprivation concept described above, metformin counteracted lapatinib-induced toxicity [95]. Combinational therapy of metformin and conventional chemotherapy treatment, such as carboplatin, doxorubicin, and paclitaxel, were shown to contribute to synergistic inhibition of cell proliferation in most breast cancer cell types [78]. The use of metformin to counteract or prevent tamoxifen resistance has also been explored in breast cancer cell lines with positive results. The combination of tamoxifen and metformin has been shown to augment the apoptotic effect of tamoxifen alone [79, 96]. As demonstrated, metformin-induced alteration in cancer cell metabolism appears to be an effective adjuvant therapy for many different types of chemoresistant breast tumours.

3.6. Metformin Failure in Prevention and Treatment of Breast Cancer. Resistance to treatment is inherent in breast cancer and metformin is proving to be no exception. The Menendez group used chronic metformin exposure to establish metformin resistant cells [97]. Acquired metformin resistance triggered a transcriptome reprogramming event in breast cancer cells and the cells developed a highly metastatic stem-like expression profile making these cancer cells more difficult to treat [97]. Metformin efficacy was also reduced in breast cancers overexpressing BCA2, a gene associated with an AMPK-suppressive function [98]. The BCA2 gene is overexpressed in >50% of breast cancer patients making it a potential target/adjuvant therapy for metformin resistant breast cancer cells [98]. These studies advocate an individualized genetic approach targeting specific genetic mutations, such as BCA2, with combinational treatment to reduce acquired resistance to metformin.

In summary, the heterogeneous nature of breast cancer makes the disease difficult to treat. However, *in vitro* studies strongly support a role for metformin, which is one of the most commonly used diabetic medications, as a generic therapy for most, if not all, breast cancer subtypes. Furthermore, the potential to use metformin as a dual treatment for cancer and diabetes is an important consideration with the increasing incidence of comorbidity worldwide. As highlighted in these *in vitro* studies, the mechanism of metformin action is still unclear and affects more than one cellular signaling pathway. Breast cancer is inherent and acquired resistance to metformin is still to be explored.

4. Breast Cancer Retrospective Observational Clinical Studies

In vitro studies examining the use of metformin as a breast cancer therapy for most breast cancer subtypes have been very promising; however, translating these positive findings into reduced breast cancer incidence and improved clinical outcomes with metformin use has come with very mixed and contradictory reviews. Table 1 summarises the important points arising from the recent meta-analyses as highlighted in this section.

The subtype of breast cancer, the presence or absence of hormones and hormone receptors; the age of the patient (pre- or postmenopausal); comorbidities, such as prediabetes, diabetes, and other diseases; and comorbidity treatments all impact on the efficacy of relapse-free survival (RDFS), metastasis-free survival (MDFS), and patient overall disease-free survival (OS). The majority of breast cancers are present in postmenopausal women where there is a higher risk of comorbidity with diabetes, obesity, and other age-related diseases. A number of meta-analyses of clinical study data support the use of metformin as a breast cancer adjuvant treatment with improved patient outcome in postmenopausal women [22, 23, 99, 114]. A study by Currie and colleagues showed that mortality increased in elderly breast cancer patients with diabetes, and metformin treatment improved survival rates in comparison with other diabetic treatments (sulfonylureas and insulin) and compared to a nondiabetic patient cohort [29]. In agreement with these findings, a study by Kiderlen and colleagues showed that metformin increased the RDFS in elderly breast cancer patients with diabetes compared to nondiabetic patients, with no difference between patients with other comorbidity diseases [100]. In

Table 1: BCa outcome with metformin treatment with/without diabetes mellitus.

References	Patient cohort	Age group	Ethnicity	BCa type	Key findings
Currie et al. 2012 [29]	112408 (8392 DM) (i) 24393 BCa (no DM) (ii) 1182 BCa (with DM)	>35 years	N/S	N/S	No mortality differences with/without metformin at diagnosis
Chlebwoski et al. 2012 [99]	68,019 patients (3401 DM) (i) 3273 invasive BCa	Postmenopausal	Mixed race	ER+ PR+ HER2+ HER2− Noninvasive Invasive	Women with diabetes were older and were more likely to be black and obese. Women with diabetes on metformin had a reduced BCa risk: Associated with PR+ ER+ Associated with HER2+? No association with HER2− Lower incidence of invasive BCa
Kiderlen et al. 2013 [100]	3124 (505 DM) (i) nonmetastatic BCa	Postmenopausal	N/S (Netherlands)	ER/PR+ ER/PR− Not defined	Patients with diabetes had overall better relapse-free survival (possibly through the effect of metformin, speculated not proven)
Xiao et al. 2014 [101]	5785 Luminal-type BCa (680 DM) (i) 1384 luminal A (201 diabetes) (ii) 3393 luminal B, high Ki67+ (341 DM) (iii) 1008 luminal B (her-2/neu+) (138 DM)	Pre-and postmenopausal	Asian	Luminal A Luminal B (Ki67) Luminal B (HER2+)	BMI was not a prognostic factor in these studies Metformin versus nonmetformin: Better prognosis for all subtypes Compared to metformin group, risk of death was higher in nonmetformin group. No significant difference between metformin and control groups. Diabetic patients: Metformin better prognosis for Luminal A and Luminal B (HER2+) Metformin poorer prognosis for Luminal B (high Ki67)
Bonanni et al. 2012 [102]	200 (non-DM) (i) 100 metformin (ii) 100 placebo	>18 years both pre and postmenopausal	N/S (Milan)	Luminal A Luminal B (Ki67) Luminal B (HER2+) TNBC	Metformin treatment: Overall, no change in Ki67 Overall, positive effect on insulin resistance Luminal B tumours-trend, decreased proliferation. Overweight or obese-trend, decreased proliferation
Besic et al. 2014 [103]	573 (invasive BCa) (i) 253 patients (+DM) (ii) 128 + metformin (iii) 125 no metformin. (iv) 320 BCa (no DM)	38–93 years (median age, 67)	N/S (Slovenia)	Luminal A Luminal B HER2 TNBC	DM + metformin—lower grade BCa compared to no metformin No change in ER/PR status with metformin Noted: Long-term metformin treatment was correlated with different BCa subtype distribution
Berstein et al. 2011 [104]	90 (BCa and DM)	48–82 years postmenopausal	N/S	ER+ PR+	Metformin increased PR in diabetic patients. Potentially increasing endocrine therapy success
Lega et al. 2014 [105]	Meta-analyses—Cancer patients with diabetes (all cancer types)	All ages	N/S	All types	No correlation between BCa and metformin and increased survival

Table 1: Continued.

References	Patient cohort	Age group	Ethnicity	BCa type	Key findings
Lega et al. 2013 [106]	2361 (BCa and DM)	>66 years	Ontario Ethnicity N/S	All types	No significant reduction in mortality or DFS in patients using metformin
Oppong et al. 2014 [107]	2889 (BCa + chemotherapy) (i) 141 (BCa + DM) (ii) 104 (DM at BCa diagnosis) (iii) 37 (DM + BCa diagnosed 6 mth)	38–80 years Majority Postmenopausal	Caucasian (72) African/American (52), Asian (10), Hispanic (4)	ER+, ER− PR+, PR− HER2+, HER2−	No difference between metformin and nonmetformin users in RFS, OS, and contralateral BCa
Bayraktar et al. 2012 [108]	1448 (triple negative BCa—TNBC) (i) 63 diabetic + metformin (ii) 67 diabetic no metformin (iii) 1318 non diabetic	More diabetic patients were postmenopausal	black, and obese	Triple negative	Metformin does not significantly impact on survival in TNBC. Trend toward decreased risk of distant metastasis in DM patients receiving metformin compared to non-DM
Ferro et al. 2013 [109]	110 (BCa) (i) 51 + metformin (DM) (ii) 28 no metformin (DM) (iii) 51 non-DM	>50	Mixed (white, black, other)	All types	Radiation therapy and metformin treatment Metformin associated with increased local radiation toxicity compared to nonmetformin users
Kim et al. 2014 [110]	208 (BCa—no DM) (i) 104 + Letrozole (ii) 104 + letrozole + metformin	Postmenopausal	Asian (Korean)	ER+	Study in progress
Kalinsky et al. 2014 [111]	33 non DM patients (Obese) (i) 9 DCIS (ii) 24 invasive BCa	>21 years	80% Hispanic	85% HR+ 20% triple negative	Metformin treatment pre-surgery—No reduction in proliferation of BCa tumour. Reduction in diabetic markers (insulin resistance)
Cazzaniga et al. 2013 [112]	100 BCa patients-Analysed (i) 45 metformin (ii) 42 placebo.	45–62 years	N/S (Milan)	Luminal A Luminal B Her2+ Triple negative	Metformin treatment pre-surgery—No reduction in proliferation of BCa tumour. Reduction in diabetic markers (insulin resistance)
Hadad et al. 2011 [113]	55 (BCa—no DM) (i) 25 completed met. (ii) 22 no metformin	41–82 years Pre- and postmenopausal	N/S	N/S	This trial supports antiproliferative effects of metformin in BC patients

BCa: breast cancer; DM: diabetes mellitus; N/S: not stated; TNBC: triple negative BCa.

a retrospective clinical meta-analysis of 28 separate studies by Zhang and Li, they found that, in breast cancer patients with existing diabetes, metformin reduced the mortality of breast cancer and reduced the risk of breast cancer by 6% [3]. In addition, elderly nondiabetic breast cancer patients had similar survival rates to diabetic breast cancer patients using metformin with elderly diabetic patients treated with metformin having a higher RFS period [100]. Metformin was also associated with reduced incidence of invasive breast cancer in postmenopausal women [99].

Can Metformin Be Used as a Generic Therapy for All Breast Cancer Sub-Types? Xiao and colleagues looked at specific breast cancer subtypes and found that the nondiabetic metformin group of patients with Luminal A (ER+/PR+), Luminal B (high ki67), and luminal B (HER-2/NUE+) had better prognosis compared to the nondiabetic group not treated with metformin. However, in diabetic groups, only luminal A and luminal B (HER-2/NUE+) metformin treated patients had better prognosis than nonmetformin group [101]. Concurring with these findings, in independent studies metformin showed decreased cell proliferation in insulin resistant, luminal B subtype breast cancer patients although overall metformin did not significantly alter cell proliferation in this patient cohort [102] and diabetic patients with HER2+ subtype had a better prognosis with metformin [115]. In contrast, looking at patients' data from 2005 to 2011, Besic and colleagues indicated that the long-term use of metformin in diabetic breast cancer patients does not associate with breast cancer subtype distribution [103]. Berstein and colleagues showed that postmenopausal diabetic breast cancer patients treated with metformin as a monotherapy or metformin and sulfonylurea were found to have higher progesterone receptor (PR) tumours than patients treated with other antidiabetic therapies leading to better response of these breast cancer patients to hormone therapy [104]. In contrast, Besic's group found that there was no change in the rate of PR between metformin and nonmetformin groups [103]. In this study, 253 patients (both pre- and postmenopausal patients) were reviewed.

Although the studies described showed metformin to have tantalizing promise as a comorbidity treatment for cancer patients with diabetes and treatment for breast cancer subtypes, most of these studies were inconclusive.

Not all meta-analyses reports showed a positive correlation with improved patient mortality and metformin treatment. Five recent reports, one comprised of a meta-analysis of twenty-one observational studies subgrouped by cancer type, did not show any significant reduction in mortality in breast cancer patients [105–107, 116, 117]. Although there have been very promising *in vitro* studies for the use of metformin in triple negative breast cancer therapy, these reports have not been confirmed in clinical observational studies where no significant impact on survival outcome has been observed, even though there was a trend towards reduced distant metastasis in these cohorts [108]. There are a number of examples where *in vitro* data did not correlate with clinical observations. Samarajeewa and colleagues found metformin specifically inhibited aromatase expression *in vitro* [118], whereas Bershtein's group found that this did not translate to clinical samples where they observed that metformin did not inhibit aromatase expression in tumour samples from diabetic breast cancer patients [119]. As metformin is a well-tolerated drug for diabetes with very few side effects, the important question is that can we continue to use this drug in combination with traditional cancer therapies for comorbidity patients? One study by Ferro and colleagues showed that metformin caused radiotoxicity in breast cancer patients with diabetes compared to nondiabetic patients and diabetic patients receiving alternative medications [109]. With the increasing comorbidity of breast cancer with diabetes and other diseases in postmenopausal women, combination comorbidity medication studies are imperative to determine metformin interactive efficacy.

4.1. Metformin as a Breast Cancer Treatment Independent of Diabetes. Despite the benefits of metformin to reduce breast cancer risk in diabetic patients metformin is still debatable; metformin is coming into prominence in its own right as a breast cancer adjuvant treatment independent of diabetes. As mentioned earlier, in addition to metformin's properties to reduce glucose and insulin in the bloodstream, it has also been shown to reduce circulating androgen and estrogen levels, which have well established mitogenic effects in breast cancer [49, 50]. Endocrine resistant breast cancer in obese postmenopausal women is partly mediated by insulin resistance and changes in estrogen metabolism metformin may also play a crucial role in preventing endocrine resistant tumours. However, early Phase I clinical trials with a combination of metformin with exemestane, an aromatase inhibitor, in a cohort of obese nondiabetic postmenopausal women, though well tolerated, showed no improved outcome [120]. A prospective phase II clinical trial to test neoadjuvant metformin with the aromatase inhibitor letrozole in ER+ postmenopausal nondiabetic women has been initiated to evaluate the direct antitumour effects of metformin [110] and it will be some time before the long-term benefits of metformin use is realised.

4.2. Metformin Presurgical Trials in Breast Cancer Patients without Diabetes. Four presurgical metformin clinical trials to determine if metformin was able to modulate breast tumour proliferation were conducted with mixed results. Three clinical trials showed no significant difference in apoptosis when metformin was given before the surgery [102, 111]; conversely, one trial indicated a potential benefit according to insulin-resistant status [112] and one trial provided support for antiproliferative effects with metformin [113]. The major limitation to of all these studies was the small sample size.

Despite the controversial retrospective meta-analyses studies reported, currently there are at least 20 recruiting and completed clinical trials, registered by the National Institute of Health (NIH) USA, addressing the use of metformin with combinational cancer therapies (https://clinicaltrials.gov/ct2/results?term=breast+cancer+and+metformin&Search=Search). To date, the results of one study have been posted on the NIH clinical trials site; however, due to the low numbers

in the patient cohorts, no meaningful results have been recorded.

5. Conclusion

Overwhelming evidence supporting that type 2 diabetes increases breast cancer risk makes the idea of using the diabetic drug metformin as a preventative drug for cancer a very exciting prospect. Still there are a number of unresolved issues in metformin use for breast cancer treatment outlined as follows.

Summary. Metformin Use for Breast Cancer Treatment. There is strong epidemiological evidence to support an increase in breast cancer incidence and death in prediabetic and diabetic patients.

There is mounting evidence to suggest that diabetic patients treated with metformin have reduced breast cancer risk supporting metformin use as a preventative medication for breast cancer.

In vitro studies strongly support the role for metformin in treatment for most of, if not all of, the subtypes of breast cancer, especially the hard to treat triple negative breast cancers.

To date, meta-analyses of retrospective clinical trial data on the use of metformin as a mono- or combined therapy for comorbidity (patients with diabetes and cancer) are equivocal supporting positive or no difference in survival outcomes. Most studies are inconclusive and recommend further confirmation.

Phase I clinical trials with a combination of metformin with exemestane in a cohort of obese, nondiabetic postmenopausal women, although well tolerated, showed no improved outcome.

The majority of breast cancers patients are postmenopausal women where there is increasing incidence of comorbidity, diabetes, and cancer. The heterogeneity of breast cancer, confounded by comorbidity of disease in the elderly population, makes it difficult to determine the actual benefits of metformin as a mono- or adjuvant therapy for breast cancer.

Prospective controlled clinical trial outcomes will be important to provide more definitive answers regarding the efficacy of metformin use in prevention and treatment for a breast cancer. Ongoing clinical trials are open for metformin as an adjuvant therapy for breast cancer.

The biology and mechanism of metformin action underpinning its use as an antidiabetic and antibreast cancer comorbidity treatment are likewise very compelling. Although the mechanism of metformin action is not fully understood, the *in vitro* evidence shows that metformin is an effective inhibitor of cell proliferation and an activator of apoptosis in breast cancer cells and supports the use of metformin as a mono- and/or adjuvant therapy for breast cancer with some limitations as discussed. Data from the retrospective meta-analyses investigating the use of metformin in breast cancer have suffered from a number of limitations and flawed assumptions. The meta-analyses are retrospective observational studies only and were not designed to specifically analyse the effects of metformin as a preventative or adjuvant treatment in defined breast cancer patient cohorts. Patient numbers and confounding comorbidities limited many of the studies. The majority of the studies report a significant increase in breast cancer incidence in postmenopausal type 2 diabetic, prediabetic, and obese patients with higher prevalence of other comorbidities such as cardiovascular disease. Retrospective studies to investigate if the use of metformin as a preferred diabetic medication actually reduced the incidence of breast cancer in these population studies have been contentious and divided into some meta-analyses showing a decreased risk of breast cancer incidence and others showing no effect. Other aspects that can alter patient outcome after metformin treatment include other medications taken, the different administration times of taking the drugs, and the drug dosage. These need to be taken into account in future studies. To date, metformin is not approved for clinical use in breast cancer treatment by the Food and Drugs Administration (FDA) and is still considered investigational. Even so, metformin is well established as an inexpensive, relatively safe, and effective drug for diabetes, prediabetes, and obesity and to extend this into breast cancer treatment regimens may have both economic and clinical benefits. Two important issues that are still to be resolved are the safety of metformin in comorbidity treatments for breast cancer and diabetes and the suitability of metformin as a breast cancer therapy independent of diabetes. The persuasive *in vitro* evidence and the optimistic retrospective observational clinical meta-analyses studies on metformin treatment for breast cancer have led to ongoing phases I–III clinical trials. These studies are important for clarification of the use of metformin in breast cancer prevention and breast cancer treatment, particularly as it is a commonly used FDA approved drug for diabetes. Prospective controlled clinical trial outcomes will be important to provide more definitive answers regarding the efficacy of metformin use in prevention and treatment for a breast cancer patients as well as its efficacy in comorbidity treatments for diabetes, breast cancer, and other diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Thanks are due to Najah Nassif who kindly helped in editing this paper. Diana Hatoum was the recipient of an Australian Postgraduate Award and top-up support Grant from UTS and Translational Cancer Research Network.

References

[1] WHO, *Global Status Report on Noncommunicable Diseases 2010, Description of the Global Burden of NCDs, Their Risk Factors and Determinants*, World Health Organisation, Geneva, Switzerland, 2011.

[2] K. M. J. de Bruijn, L. R. Arends, B. E. Hansen, S. Leeflang, R. Ruiter, and C. H. J. van Eijck, "Systematic review and meta-analysis of the association between diabetes mellitus and incidence and mortality in breast and colorectal cancer," *British Journal of Surgery*, vol. 100, no. 11, pp. 1421–1429, 2013.

[3] Z.-J. Zhang and S. Li, "The prognostic value of metformin for cancer patients with concurrent diabetes: a systematic review and meta-analysis," *Diabetes, Obesity and Metabolism*, vol. 16, pp. 707–710, 2014.

[4] P. Zhang, H. Li, X. Tan, L. Chen, and S. Wang, "Association of metformin use with cancer incidence and mortality: a meta-analysis," *Cancer Epidemiology*, vol. 37, no. 3, pp. 207–218, 2013.

[5] G. Hou, S. Zhang, X. Zhang, P. Wang, X. Hao, and J. Zhang, "Clinical pathological characteristics and prognostic analysis of 1,013 breast cancer patients with diabetes," *Breast Cancer Research and Treatment*, vol. 137, no. 3, pp. 807–816, 2013.

[6] H. Xu, M. C. Aldrich, Q. Chen et al., "Validating drug repurposing signals using electronic health records: a case study of metformin associated with reduced cancer mortality," *Journal of the American Medical Informatics Association*, 2014.

[7] K. K. Tsilidis, D. Capothanassi, N. E. Allen et al., "Metformin does not affect cancer risk: a cohort study in the U.K. Clinical Practice Research Datalink analyzed like an intention-to-treat trial," *Diabetes Care*, vol. 37, pp. 2522–2532, 2014.

[8] A. S. Glicksman and R. W. Rawson, "Diabetes and altered carbohydrate metabolism in patients with cancer," *Cancer*, vol. 9, pp. 1127–1134, 1956.

[9] E. Freund, "Diagnosis des Carcinomas," *Wiener Medizinische*, vol. B1, p. 268, 1885 (German).

[10] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *Cell*, vol. 144, no. 5, pp. 646–674, 2011.

[11] O. Warburg, "On the origin of cancer cells," *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[12] C. Catsburg, M. J. Gunter, C. Chen et al., "Insulin, estrogen, inflammatory markers, and risk of benign proliferative breast disease," *Cancer Research*, vol. 74, pp. 3248–3258, 2014.

[13] M. Jalving, J. A. Gietema, J. D. Lefrandt et al., "Metformin: taking away the candy for cancer?" *European Journal of Cancer*, vol. 46, no. 13, pp. 2369–2380, 2010.

[14] M. J. Khandekar, P. Cohen, and B. M. Spiegelman, "Molecular mechanisms of cancer development in obesity," *Nature Reviews Cancer*, vol. 11, no. 12, pp. 886–895, 2011.

[15] C. Li and D. Kong, "Cancer risks from diabetes therapies: evaluating the evidence," *Pharmacology & Therapeutics*, vol. 144, pp. 71–81, 2014.

[16] S. Sen, Y. He, D. Koya, and K. Kanasaki, "Cancer biology in diabetes," *Journal of Diabetes Investigation*, vol. 5, no. 3, pp. 251–264, 2014.

[17] H. Noto, A. Goto, T. Tsujimoto, K. Osame, and M. Noda, "Latest insights into the risk of cancer in diabetes," *Journal of Diabetes Investigation*, vol. 4, no. 3, pp. 225–232, 2013.

[18] C. J. Bailey and C. Day, "Metformin: its botanical background," *Practical Diabetes International*, vol. 21, no. 3, pp. 115–117, 2004.

[19] C. J. Bailey and R. C. Turner, "Metformin," *The New England Journal of Medicine*, vol. 334, no. 9, pp. 574–579, 1996.

[20] M. Stang, D. K. Wysowski, and D. Butler-Jones, "Incidence of lactic acidosis in metformin users," *Diabetes Care*, vol. 22, no. 6, pp. 925–927, 1999.

[21] E. M. Moore, A. G. Mander, D. Ames et al., "Increased risk of cognitive impairment in patients with diabetes is associated with metformin," *Diabetes Care*, vol. 36, no. 10, pp. 2981–2987, 2013.

[22] F. Xue and K. B. Michels, "Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence," *The American Journal of Clinical Nutrition*, vol. 86, no. 3, pp. s823–s835, 2007.

[23] S. Liao, J. Li, W. Wei et al., "Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature," *Asian Pacific Journal of Cancer Prevention*, vol. 12, no. 4, pp. 1061–1065, 2011.

[24] K. S. Peairs, B. B. Barone, C. F. Snyder et al., "Diabetes mellitus and breast cancer outcomes: a systematic review and meta-analysis," *Journal of Clinical Oncology*, vol. 29, no. 1, pp. 40–46, 2011.

[25] C. la Vecchia, S. H. Giordano, G. N. Hortobagyi, and B. Chabner, "Overweight, obesity, diabetes, and risk of breast cancer: interlocking pieces of the puzzle," *Oncologist*, vol. 16, no. 6, pp. 726–729, 2011.

[26] P. J. Hardefeldt, S. Edirimanne, and G. D. Eslick, "Diabetes increases the risk of breast cancer: a meta-analysis," *Endocrine-Related Cancer*, vol. 19, no. 6, pp. 793–803, 2012.

[27] C. Duggan, M. L. Irwin, L. Xiao et al., "Associations of insulin resistance and adiponectin with mortality in women with breast cancer," *Journal of Clinical Oncology*, vol. 29, no. 1, pp. 32–39, 2011.

[28] M. L. Irwin, C. Duggan, C.-Y. Wang et al., "Fasting C-peptide levels and death resulting from all causes and breast cancer: the health, eating, activity, and lifestyle study," *Journal of Clinical Oncology*, vol. 29, no. 1, pp. 47–53, 2011.

[29] C. J. Currie, C. D. Poole, S. Jenkins-Jones, E. A. M. Gale, J. A. Johnson, and C. L. Morgan, "Mortality after incident cancer in people with and without type 2 diabetes: impact of metformin on survival," *Diabetes Care*, vol. 35, no. 2, pp. 299–304, 2012.

[30] P. Boyle, M. Boniol, A. Koechlin et al., "Diabetes and breast cancer risk: a meta-analysis," *British Journal of Cancer*, vol. 107, no. 9, pp. 1608–1617, 2012.

[31] G. Libby, L. A. Donnelly, P. T. Donnan, D. R. Alessi, A. D. Morris, and J. M. M. Evans, "New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes," *Diabetes Care*, vol. 32, no. 9, pp. 1620–1625, 2009.

[32] J. M. M. Evans, L. A. Donnelly, A. M. Emslie-Smith, D. R. Alessi, and A. D. Morris, "Metformin and reduced risk of cancer in diabetic patients," *The British Medical Journal*, vol. 330, no. 7503, pp. 1304–1305, 2005.

[33] M. Bodmer, C. Meier, S. Krähenbühl, S. S. Jick, and C. R. Meier, "Long-term metformin use is associated with decreased risk of breast cancer," *Diabetes Care*, vol. 33, no. 6, pp. 1304–1308, 2010.

[34] S. L. Bowker, S. R. Majumdar, P. Veugelers, and J. A. Johnson, "Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin: response to Farooki and Schneider," *Diabetes Care*, vol. 29, no. 8, pp. 1990–1991, 2006.

[35] L. Sciacca, R. Vigneri, A. Tumminia et al., "Clinical and molecular mechanisms favoring cancer initiation and progression in diabetic patients," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 23, no. 9, pp. 808–815, 2013.

[36] P. Vigneri, F. Frasca, L. Sciacca, G. Pandini, and R. Vigneri, "Diabetes and cancer," *Endocrine-Related Cancer*, vol. 16, no. 4, pp. 1103–1123, 2009.

[37] R. Vigneri, "Diabetes: diabetes therapy and cancer risk," *Nature Reviews Endocrinology*, vol. 5, no. 12, pp. 651–652, 2009.

[38] B. Thakkar, K. N. Aronis, M. T. Vamvini, K. Shields, and C. S. Mantzoros, "Metformin and sulfonylureas in relation to cancer risk in type II diabetes patients: a meta-analysis using primary data of published studies," *Metabolism: Clinical and Experimental*, vol. 62, no. 7, pp. 922–934, 2013.

[39] R. Ruiter, L. E. Visser, M. P. van Herk-Sukel et al., "Lower risk of cancer in patients on metformin in comparison with those on sulfonylurea derivatives: Results from a large population-based follow-up study," *Diabetes Care*, vol. 35, no. 1, pp. 119–124, 2012.

[40] C. A. Bannister, S. E. Holden, S. Jenkins-Jones et al., "Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls," *Diabetes, Obesity and Metabolism*, vol. 16, no. 11, pp. 1165–1173, 2014.

[41] X. Yang, G. T. Ko, W. Y. So et al., "Associations of hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong diabetes registry," *Diabetes*, vol. 59, no. 5, pp. 1254–1260, 2010.

[42] J. C. Chan, V. Malik, W. Jia et al., "Diabetes in Asia: epidemiology, risk factors, and pathophysiology," *The Journal of the American Medical Association*, vol. 301, no. 20, pp. 2129–2140, 2009.

[43] D. Simon and B. Balkau, "Comment on: Yang et al. (2010) Associations of hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong Diabetes Registry. Diabetes; 59:1254–1260," *Diabetes*, vol. 59, no. 11, p. e25, 2010.

[44] S. W. Yee, L. Chen, and K. M. Giacomini, "The role of ATM in response to metformin treatment and activation of AMPK," *Nature Genetics*, vol. 44, no. 4, pp. 359–360, 2012.

[45] K. Zhou, C. Bellenguez, C. Sutherland et al., "The role of ATM in response to metformin treatment and activation of AMPK," *Nature Genetics*, vol. 44, pp. 361–362, 2012.

[46] R. J. Dowling, S. Niraula, V. Stambolic, and P. J. Goodwin, "Metformin in cancer: translational challenges," *Journal of Molecular Endocrinology*, vol. 48, no. 3, pp. R31–E43, 2012.

[47] A. M. Gonzalez-Angulo and F. Meric-Bernstam, "Metformin: a therapeutic opportunity in breast cancer," *Clinical Cancer Research*, vol. 16, no. 6, pp. 1695–1700, 2010.

[48] D. H. Cohen and D. LeRoith, "Obesity, type 2 diabetes, and cancer: the insulin and IGF connection," *Endocrine-Related Cancer*, vol. 19, no. 5, pp. F27–F45, 2012.

[49] C. Campagnoli, F. Berrino, E. Venturelli et al., "Metformin decreases circulating androgen and estrogen levels in nondiabetic women with breast cancer," *Clinical Breast Cancer*, vol. 13, no. 6, pp. 433–438, 2013.

[50] C. Campagnoli, P. Pasanisi, C. Abbà et al., "Effect of different doses of metformin on serum testosterone and insulin in non-diabetic women with breast cancer: a randomized study," *Clinical Breast Cancer*, vol. 12, no. 3, pp. 175–182, 2012.

[51] Y. Shu, S. A. Sheardown, C. Brown et al., "Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action," *The Journal of Clinical Investigation*, vol. 117, no. 5, pp. 1422–1431, 2007.

[52] E. Shikata, R. Yamamoto, H. Takane et al., "Human organic cation transporter (*OCT1* and *OCT2*) gene polymorphisms and therapeutic effects of metformin," *Journal of Human Genetics*, vol. 52, no. 2, pp. 117–122, 2007.

[53] A. T. Nies, H. Koepsell, K. Damme, and M. Schwab, "Organic cation transporters (OCTs, MATEs), *in vitro* and *in vivo* evidence for the importance in drug therapy," *Handbook of Experimental Pharmacology*, vol. 201, pp. 105–167, 2011.

[54] M.-Y. El-Mir, V. Nogueira, E. Fontaine, N. Avéret, M. Rigoulet, and X. Leverve, "Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I," *Journal of Biological Chemistry*, vol. 275, no. 1, pp. 223–228, 2000.

[55] M. R. Owen, E. Doran, and A. P. Halestrap, "Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain," *Biochemical Journal*, vol. 348, no. 3, pp. 607–614, 2000.

[56] Y. Storozhuk, S. N. Hopmans, T. Sanli et al., "Metformin inhibits growth and enhances radiation response of non-small cell lung cancer (NSCLC) through ATM and AMPK," *British Journal of Cancer*, vol. 108, no. 10, pp. 2021–2032, 2013.

[57] R. J. Dowling, M. Zakikhani, I. G. Fantus, M. Pollak, and N. Sonenberg, "Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells," *Cancer Research*, vol. 67, no. 22, pp. 10804–10812, 2007.

[58] B. N. M. Zordoky, D. Bark, C. L. Soltys, M. M. Sung, and J. R. B. Dyck, "The anti-proliferative effect of metformin in triple-negative MDA-MB-231 breast cancer cells is highly dependent on glucose concentration: implications for cancer therapy and prevention," *Biochimica et Biophysica Acta—General Subjects*, vol. 1840, no. 6, pp. 1943–1957, 2014.

[59] N. Li, Y. Deng, F. Qin, J. Yu, and F. Li, "Simultaneous quantification of metformin and glipizide in human plasma by high-performance liquid chromatography-tandem mass spectrometry and its application to a pharmacokinetic study," *Biomedical Chromatography*, vol. 27, no. 2, pp. 191–196, 2013.

[60] A. Woods, S. R. Johnstone, K. Dickerson et al., "LKB1 is the upstream kinase in the AMP-activated protein kinase cascade," *Current Biology*, vol. 13, no. 22, pp. 2004–2008, 2003.

[61] Z. Shen, X.-F. Wen, F. Lan, Z.-Z. Shen, and Z.-M. Shao, "The tumor suppressor gene LKB1 is associated with prognosis in human breast carcinoma," *Clinical Cancer Research*, vol. 8, no. 7, pp. 2085–2090, 2002.

[62] R. A. Miller, Q. Chu, J. Xie, M. Foretz, B. Viollet, and M. J. Birnbaum, "Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP," *Nature*, vol. 494, no. 7436, pp. 256–260, 2013.

[63] B. Corominas-Faja, R. Quirantes-Piné, C. Oliveras-Ferraros et al., "Metabolomic fingerprint reveals that metformin impairs one-carbon metabolism in a manner similar to the antifolate class of chemotherapy drugs," *Aging*, vol. 4, no. 7, pp. 480–498, 2012.

[64] H. A. Hirsch, D. Iliopoulos, and K. Struhl, "Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 3, pp. 972–977, 2013.

[65] K. Dallaglio, A. Bruno, A. R. Cantelmo et al., "Paradoxic effects of metformin on endothelial cells and angiogenesis," *Carcinogenesis*, vol. 35, no. 5, pp. 1055–1066, 2014.

[66] M. Foretz, S. Hébrard, J. Leclerc et al., "Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state," *The Journal of Clinical Investigation*, vol. 120, no. 7, pp. 2355–2369, 2010.

[67] A. F. Salem, D. Whitaker-Menezes, A. Howell, F. Sotgia, and M. P. Lisanti, "Mitochondrial biogenesis in epithelial cancer cells promotes breast cancer tumor growth and confers autophagy resistance," *Cell Cycle*, vol. 11, no. 22, pp. 4174–4180, 2012.

[68] A. K. Madiraju, D. M. Erion, Y. Rahimi et al., "Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase," *Nature*, vol. 510, no. 7506, pp. 542–546, 2014.

[69] K. M. Cornejo, D. Kandil, A. Khan, and E. F. Cosar, "Theranostic and molecular classification of breast cancer," *Archives of Pathology and Laboratory Medicine*, vol. 138, no. 1, pp. 44–56, 2014.

[70] C. M. Perou, T. Sørile, M. B. Eisen et al., "Molecular portraits of human breast tumours," *Nature*, vol. 406, no. 6797, pp. 747–752, 2000.

[71] B. Haibe-Kains, "Classification models for breast cancer molecular subtyping: what is the best candidate for a translation into clinic?" *Women's Health*, vol. 6, no. 5, pp. 623–625, 2010.

[72] A. Ahr, U. Holtrich, C. Solbach et al., "Molecular classification of breast cancer patients by gene expression profiling," *Journal of Pathology*, vol. 195, no. 3, pp. 312–320, 2001.

[73] M. C. Cummings, R. Chambers, P. T. Simpson, and S. R. Lakhani, "Molecular classification of breast cancer: is it time to pack up our microscopes?" *Pathology*, vol. 43, no. 1, pp. 1–8, 2011.

[74] A. M. Gruver, B. P. Portier, and R. R. Tubbs, "Molecular pathology of breast cancer the journey from traditional practice toward embracing the complexity of a molecular classification," *Archives of Pathology and Laboratory Medicine*, vol. 135, no. 5, pp. 544–557, 2011.

[75] B. D. Lehmann, J. A. Bauer, X. Chen et al., "Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies," *Journal of Clinical Investigation*, vol. 121, no. 7, pp. 2750–2767, 2011.

[76] J. S. Reis-Filho and S. R. Lakhani, "Breast cancer special types: why bother?" *Journal of Pathology*, vol. 216, no. 4, pp. 394–398, 2008.

[77] A. A. Onitilo, J. M. Engel, R. T. Greenlee, and B. N. Mukesh, "Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival," *Clinical Medicine and Research*, vol. 7, no. 1-2, pp. 4–13, 2009.

[78] H. Liu, C. Scholz, C. Zang et al., "Metformin and the mTOR inhibitor everolimus (RAD001) sensitize breast cancer cells to the cytotoxic effect of chemotherapeutic drugs in vitro," *Anticancer Research*, vol. 32, no. 5, pp. 1627–1637, 2012.

[79] J. Ma, Y. Guo, S. Chen et al., "Metformin enhances tamoxifen-mediated tumor growth inhibition in ER-positive breast carcinoma," *BMC Cancer*, vol. 14, no. 1, article 172, 2014.

[80] S. M. Hadad, D. G. Hardie, V. Appleyard, and A. M. Thompson, "Effects of metformin on breast cancer cell proliferation, the AMPK pathway and the cell cycle," *Clinical & Translational Oncology*, vol. 16, no. 8, pp. 746–752, 2013.

[81] H. Lee, H. J. Park, C. S. Park et al., "Response of breast cancer cells and cancer stem cells to metformin and hyperthermia alone or combined," *PLoS ONE*, vol. 9, p. e87979, 2014.

[82] W. D. Foulkes, I. E. Smith, and J. S. Reis-Filho, "Triple-negative breast cancer," *The New England Journal of Medicine*, vol. 363, no. 20, pp. 1938–1948, 2010.

[83] R. Schmadeka, B. E. Harmon, and M. Singh, "Triple-negative breast carcinoma: current and emerging concepts," *The American Journal of Clinical Pathology*, vol. 141, no. 4, pp. 462–477, 2014.

[84] B. C. Litzenburger and P. H. Brown, "Advances in preventive therapy for estrogen-receptor-negative breast cancer," *Current Breast Cancer Reports*, vol. 6, no. 2, pp. 96–109, 2014.

[85] R. Wahdan-Alaswad, Z. Fan, S. M. Edgerton et al., "Glucose promotes breast cancer aggression and reduces metformin efficacy," *Cell Cycle*, vol. 12, no. 24, pp. 3759–3769, 2013.

[86] C. Marini, B. Salani, M. Massollo et al., "Direct inhibition of hexokinase activity by metformin at least partially impairs glucose metabolism and tumor growth in experimental breast cancer," *Cell Cycle*, vol. 12, no. 22, pp. 3490–3499, 2013.

[87] X.-S. Deng, S. Wang, A. Deng et al., "Metformin targets Stat3 to inhibit cell growth and induce apoptosis in triple-negative breast cancers," *Cell Cycle*, vol. 11, no. 2, pp. 367–376, 2012.

[88] J. A. Menendez, C. Oliveras-Ferraros, S. Cufí et al., "Metformin is synthetically lethal with glucose withdrawal in cancer cells," *Cell Cycle*, vol. 11, no. 15, pp. 2782–2792, 2012.

[89] B. Liu, Z. Fan, S. M. Edgerton, X. Yang, S. E. Lind, and A. D. Thor, "Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of ErbB2/IGF-1 receptor interactions," *Cell Cycle*, vol. 10, no. 17, pp. 2959–2966, 2011.

[90] C. Qu, W. Zhang, G. Zheng, Z. Zhang, J. Yin, and Z. He, "Metformin reverses multidrug resistance and epithelial-mesenchymal transition (EMT) via activating AMP-activated protein kinase (AMPK) in human breast cancer cells," *Molecular and Cellular Biochemistry*, vol. 386, no. 1-2, pp. 63–71, 2014.

[91] M. Cioce, M. C. Valerio, L. Casadei et al., "Metformin-induced metabolic reprogramming of chemoresistant ALDHbright breast cancer cells," *Oncotarget*, vol. 5, no. 12, pp. 4129–4143, 2014.

[92] F. L. Opdam, H.-J. Guchelaar, J. H. Beijnen, and J. H. M. Schellens, "Lapatinib for advanced or metastatic breast cancer," *Oncologist*, vol. 17, no. 4, pp. 536–542, 2012.

[93] M. Toi, H. Iwata, Y. Fujiwara et al., "Lapatinib monotherapy in patients with relapsed, advanced, or metastatic breast cancer: efficacy, safety, and biomarker results from Japanese patients phase II studies," *British Journal of Cancer*, vol. 101, no. 10, pp. 1676–1682, 2009.

[94] J. E. Frampton, "Lapatinib: a review of its use in the treatment of HER2-overexpressing, trastuzumab-refractory, advanced or metastatic breast cancer," *Drugs*, vol. 69, no. 15, pp. 2125–2148, 2009.

[95] K. Komurov, J.-T. Tseng, M. Muller et al., "The glucose-deprivation network counteracts lapatinib-induced toxicity in resistant ErbB2-positive breast cancer cells," *Molecular Systems Biology*, vol. 8, article 596, 2012.

[96] R. Sanchez-Alvarez, U. E. Martinez-Outschoorn, R. Lamb et al., "Mitochondrial dysfunction in breast cancer cells prevents tumor growth: understanding chemoprevention with metformin," *Cell Cycle*, vol. 12, no. 1, pp. 172–182, 2013.

[97] C. Oliveras-Ferraros, A. Vazquez-Martin, E. Cuyas et al., "Acquired resistance to metformin in breast cancer cells triggers transcriptome reprogramming toward a degradome-related metastatic stem-like profile," *Cell Cycle*, vol. 13, no. 7, pp. 1132–1144, 2014.

[98] D. Buac, F. R. Kona, A. K. Seth, and Q. Ping Dou, "Regulation of metformin response by breast cancer associated gene 2," *Neoplasia*, vol. 15, no. 12, pp. 1379–1390, 2013.

[99] R. T. Chlebowski, A. McTiernan, J. Wactawski-Wende et al., "Diabetes, metformin, and breast cancer in postmenopausal women," *Journal of Clinical Oncology*, vol. 30, no. 23, pp. 2844–2852, 2012.

[100] M. Kiderlen, N. A. de Glas, E. Bastiaannet et al., "Diabetes in relation to breast cancer relapse and all-cause mortality in elderly breast cancer patients: a FOCUS study analysis," *Annals of Oncology*, vol. 24, no. 12, pp. 3011–3016, 2013.

[101] Y. Xiao, S. Zhang, G. Hou, X. Zhang, X. Hao, and J. Zhang, "Clinical pathological characteristics and prognostic analysis

of diabetic women with luminal subtype breast cancer," *Tumor Biology*, vol. 35, no. 3, pp. 2035–2045, 2014.

[102] B. Bonanni, M. Puntoni, M. Cazzaniga et al., "Dual effect of metformin on breast cancer proliferation in a randomized presurgical trial," *Journal of Clinical Oncology*, vol. 30, no. 21, pp. 2593–2600, 2012.

[103] N. Besic, N. Satej, I. Ratosa et al., "Long-term use of metformin and the molecular subtype in invasive breast carcinoma patients—a retrospective study of clinical and tumor characteristics," *BMC Cancer*, vol. 14, no. 1, article 298, 2014.

[104] L. M. Berstein, M. P. Boyarkina, E. V. Tsyrlina, E. A. Turkevich, and V. F. Semiglazov, "More favorable progesterone receptor phenotype of breast cancer in diabetics treated with metformin," *Medical Oncology*, vol. 28, no. 4, pp. 1260–1263, 2011.

[105] I. C. Lega, P. S. Shah, D. Margel, J. Beyene, P. A. Rochon, and L. L. Lipscombe, "The effect of metformin on mortality following cancer among patients with diabetes," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 23, pp. 1974–1984, 2014.

[106] I. C. Lega, P. C. Austin, A. Gruneir, P. J. Goodwin, P. A. Rochon, and L. L. Lipscombe, "Association between metformin therapy and mortality after breast cancer: A population-based study," *Diabetes Care*, vol. 36, no. 10, pp. 3018–3026, 2013.

[107] B. A. Oppong, L. A. Pharmer, S. Oskar et al., "The effect of metformin on breast cancer outcomes in patients with type 2 diabetes," *Cancer Medicine*, vol. 3, no. 4, pp. 1025–1034, 2014.

[108] S. Bayraktar, L. F. Hernadez-Aya, X. Lei et al., "Effect of metformin on survival outcomes in diabetic patients with triple receptor-negative breast cancer," *Cancer*, vol. 118, no. 5, pp. 1202–1211, 2012.

[109] A. Ferro, S. Goyal, S. Kim et al., "Evaluation of diabetic patients with breast cancer treated with metformin during adjuvant radiotherapy," *International Journal of Breast Cancer*, Article ID 659723, 2013.

[110] J. Kim, W. Lim, E.-K. Kim et al., "Phase II randomized trial of neoadjuvant metformin plus letrozole versus placebo plus letrozole for estrogen receptor positive postmenopausal breast cancer (METEOR)," *BMC Cancer*, vol. 14, no. 1, article 170, 2014.

[111] K. Kalinsky, K. D. Crew, S. Refice et al., "Presurgical trial of metformin in overweight and obese patients with newly diagnosed breast cancer," *Cancer Investigation*, vol. 32, no. 4, pp. 150–157, 2014.

[112] M. Cazzaniga, A. DeCensi, G. Pruneri et al., "The effect of metformin on apoptosis in a breast cancer presurgical trial," *British Journal of Cancer*, vol. 109, no. 11, pp. 2792–2797, 2013.

[113] S. Hadad, T. Iwamoto, L. Jordan et al., "Evidence for biological effects of metformin in operable breast cancer: a preoperative, window-of-opportunity, randomized trial," *Breast Cancer Research and Treatment*, vol. 128, no. 3, pp. 783–794, 2011.

[114] L. Vona-Davis and D. P. Rose, "Type 2 diabetes and obesity metabolic interactions: common factors for breast cancer risk and novel approaches to prevention and therapy," *Current Diabetes Reviews*, vol. 8, no. 2, pp. 116–130, 2012.

[115] X. He, F. J. Esteva, J. Ensor, G. N. Hortobagyi, M.-H. Lee, and S.-C. J. Yeung, "Metformin and thiazolidinediones are associated with improved breast cancer-specific survival of diabetic women with HER2+ breast cancer," *Annals of Oncology*, vol. 23, no. 7, pp. 1771–1780, 2012.

[116] R. J. Stevens, R. Ali, C. R. Bankhead et al., "Cancer outcomes and all-cause mortality in adults allocated to metformin: systematic review and collaborative meta-analysis of randomised clinical trials," *Diabetologia*, vol. 55, no. 10, pp. 2593–2603, 2012.

[117] H. Noto, A. Goto, T. Tsujimoto, and M. Noda, "Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis," *PLoS ONE*, vol. 7, no. 3, Article ID e33411, 2012.

[118] N. U. Samarajeewa, S. Ham, F. Yang, E. R. Simpson, and K. A. Brown, "Promoter-specific effects of metformin on aromatase transcript expression," *Steroids*, vol. 76, no. 8, pp. 768–771, 2011.

[119] L. M. Berstein, E. A. Turkevich, and M. P. Boyarkina, "Metformin doesn't suppress the aromatase expression breast cancer tissue of patients with concurrent type 2 diabetes," *Voprosy Onkologii*, vol. 58, no. 2, pp. 194–198, 2012.

[120] F. J. Esteva, S. L. Moulder, A. M. Gonzalez-Angulo et al., "Phase i trial of exemestane in combination with metformin and rosiglitazone in nondiabetic obese postmenopausal women with hormone receptor-positive metastatic breast cancer," *Cancer Chemotherapy and Pharmacology*, vol. 71, no. 1, pp. 63–72, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 165105, 9 pages
http://dx.doi.org/10.1155/2015/165105

Review Article

Switching the Sphingolipid Rheostat in the Treatment of Diabetes and Cancer Comorbidity from a Problem to an Advantage

Nikolas K. Haass,[1,2,3] Najah Nassif,[4] and Eileen M. McGowan[4,5]

[1] *The University of Queensland, The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, QLD, Australia*
[2] *The Centenary Institute, Newtown, NSW, Australia*
[3] *Discipline of Dermatology, University of Sydney, Camperdown, NSW, Australia*
[4] *School of Medical and Molecular Biosciences, University of Technology Sydney, Ultimo, NSW 2007, Australia*
[5] *Sydney Medical School, University of Sydney, Camperdown, NSW 2050, Australia*

Correspondence should be addressed to Eileen M. McGowan; eileen.mcgowan@uts.edu.au

Received 17 July 2014; Accepted 16 October 2014

Academic Editor: Anandwardhan Hardikar

Cancer and diabetes are among the most common diseases in western societies. Epidemiological studies have shown that diabetic patients have a significantly higher risk of developing a number of different types of cancers and that individuals with comorbidity (cancer and diabetes/prediabetes) have a poorer prognosis relative to nondiabetic cancer patients. The increasing frequency of comorbidity of cancer and diabetes mellitus, mainly type 2 diabetes, has driven the development of therapeutic interventions that target both disease states. There is strong evidence to suggest that balancing the sphingolipid rheostat, ceramide—sphingosine—sphingosine-1-phosphate (S1P) is crucial in the prevention of diabetes and cancer and sphingosine kinase/S1P modulators are currently under development for the treatment of cancer and diabetes. This paper will highlight some of the complexities inherent in the use of the emerging sphingosine kinase/S1P modulators in the treatment of comorbidity of diabetes and cancer.

1. Introduction

Cancer and diabetes mellitus are two of the most prevalent diseases worldwide. An estimated 347 million people worldwide suffer from diabetes [1]. The World Health Organization (WHO) projects this disease to become the 7th leading cause of death by 2030 [2]. Cancer is the 2nd most prevalent disease worldwide [3, 4]. Whilst there is an increasing awareness of a strong association between the two diseases, both for cancer incidence and prognosis, the biologic links between diabetes and cancer risk are not well defined [5–7]. Type 2 diabetic patients have a greater propensity to develop cancer, and cancer and diabetes share many risk factors [8]. Some epidemiological studies suggest increased mortality in cancer patients with preexisting diabetes [9]. With the increasing likelihood of comorbidity of cancer and diabetes and the potential of increased mortality in these patients [9–11], understanding the aetiology underlying both diseases will aid in the development of more efficacious treatments.

Sphingosine kinase (SphK) is an important signalling enzyme that catalyses the phosphorylation of the lipid sphingosine to form sphingosine-1-phosphate (S1P) and has been implicated in the pathology of both diabetes and cancer [7, 12–17]. SphK plays a critical role in balancing the relative levels of the two signalling molecules controlling cellular metabolic processes such as cell proliferation, survival, apoptosis, adhesion, and migration [18–20]. Hence there is a strong motivation for the development of SphK/S1P modulators for therapeutic interventions to target patients with comorbidity of diabetes and cancer. This paper, as part of the special issue on "Hijacking the metabolic regulation in cancer and diabetes," aims to highlight the complications arising from targeting the SphK1/S1P rheostat, by the S1P modulators, for cancer therapy in patients with prediabetes/diabetes.

2. Type 1 and Type 2 Diabetes

Type 1 and type 2 diabetes are complex diseases characterised by progressive failure of the insulin producing pancreatic β-cells [21]. The mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes have very few similarities [22]. Type 1 diabetes is caused by an autoimmune attack resulting in the loss of the insulin producing β-cells and loss of insulin secretion whereas type 2 diabetes is characterised by insulin resistance, which can lead to a relative state of hyperinsulinaemia (overproduction of insulin) to maintain normal glycaemia and eventually results in β-cell failure. Approximately 10% of diabetic patients have type 1 diabetes (usually starting in childhood or younger age), and these patients have an absolute requirement for insulin therapy requiring daily dosage of insulin. Type 2 diabetes is the most common, making up approximately 90% of all cases. In most instances these patients are noninsulin dependent; however, over time, they may require insulin to maintain glycaemic control. The onset of type 2 diabetes is usually later in life and is associated with obesity and a sedentary lifestyle. Saturated fatty acids associated with obesity, such as palmitate, are lipotoxic towards the pancreatic β-cells, exerting a double hit: insulin resistance and reduced pancreatic β-cell survival [23, 24]. Skeletal muscle also plays a major role in the pathology of insulin resistance as this tissue is important for whole body insulin-stimulated glucose removal [25]. Thus perturbation of insulin signalling in skeletal muscle is a key factor in type 2 diabetes development. Complications of both type 1 and type 2 diabetes include cardiovascular disease, neuropathy, retinopathy, and kidney failure [21].

3. Obesity, Diabetes, and Cancer

Obesity is a common risk factor linking type 2 diabetes and cancer and is covered extensively in a recent review [6]. Type 2 diabetes and obesity have been associated independently, and in common, with increased cancer risk [8]. This risk may be attributed to underlying metabolic conditions such as insulin resistance, hyperinsulinaemia, hyperglycaemia, and inflammation, which all influence the development and progression of neoplasia [26]. Treatment of diabetes with glucose-lowering therapies, such as metformin, has been reviewed extensively and, in general, the treatment of diabetic patients with metformin has been shown to lead to a reduced cancer risk and results in a better overall survival [5, 10, 11, 27]. The effects of cancer drugs on coexisting diabetes have been less well studied and in some cases cancer therapies may cause increased risk of diabetes development [27, 28]. A signalling pathway crucial to the onset/progression of cancer and diabetes is the phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway [29]. Hyperactivation of this pathway is known to result in increased cell proliferation, decreased apoptosis, and cancer [28]. Inhibitors of this pathway are used for cancer therapy but such drugs may result in impaired insulin responses and insulin resistance leading to the development of type 2 diabetes [28]. Cancer chemotherapy with drugs such as 5-fluorouracil, androgen-deprivation therapy, and carboplatin has been reported to be associated with drug-induced diabetes or the worsening of preexisting diabetes and is reviewed in [6]. More recently, manipulation of the sphingosine kinase/sphingosine-1-phosphate (SphK/S1P) signalling pathway using generic and specific inhibitors has been investigated as a potential cancer therapy [13–15, 30–34]. However, there is a fine balance between swinging the ceramide-SphK/S1P pendulum in favour of cancer prevention/treatment and the onset of diabetes (Figure 1). This conundrum is discussed in more detail below.

4. Sphingosine Kinase

There are two major isoforms of SphK (SphK1 and SphK2) with diverse and compensatory actions [35]. SphK mediates the balance between the proapoptotic effects of ceramide and sphingosine substrates and the antiapoptotic effects of sphingosine-1-phosphate (S1P), a phosphorylation balance system more aptly named "the sphingolipid rheostat" [18, 36, 37]. SphK phosphorylates sphingosine to produce sphingosine-1-phosphate (S1P) and modulates autocrine (intracellular) and paracrine (extracellular) functions. S1P binds mainly to five specific G-protein-coupled receptors (GPCRs), $S1P_{1-5}$ [38]. One or more of the five S1P receptor subtypes are found on the surface of most cells [38]. S1P activation and function is cell type and S1P receptor type specific. In skeletal muscle cells, S1P has been shown to increase glucose uptake through the transactivation of the insulin receptor [39] whereas in epithelial cells S1P inhibits AKT activity and interrupts insulin signalling and cell proliferation through the $S1P_2$ receptor subtype [40]. The SphK1 isoform has two major subtypes, SphK1a and SphK1b, and emerging evidence indicates that SphK1a and SphK1b have common and differing interacting partners [41] and, through such interactions, each subtype is able to influence diverse downstream signalling pathways [42]. Tipping the balance in favour of ceramide accumulation has been shown to cause insulin resistance whereas SphK1 prevents ceramide accumulation by promoting its metabolism to S1P and augmenting insulin action [16, 43, 44]. In contrast, overexpression of SphK1 is associated with increased cancer risk [7, 12]. As mentioned previously, inhibitors of SphK1 are currently being explored for cancer treatment; however, with the high probability of comorbidity of cancer and diabetes [5, 6], the possibility of cancer treatments such as SphK1 inhibitors promoting insulin resistance may have dire consequences for cancer survivors.

5. SphK and S1P Inhibitors and Diabetes/Obesity Complications

The drive towards the use of SphK/S1P pharmaceutical inhibitors for cancer treatment has key significance for diabetic patients. The "sphingolipid rheostat" is implicated in controlling the balance between cell proliferation and apoptosis. As such, activation of S1P has been shown to be critical in protecting pancreatic β-cells (the cells that produce, store, and release insulin) from apoptosis and preventing

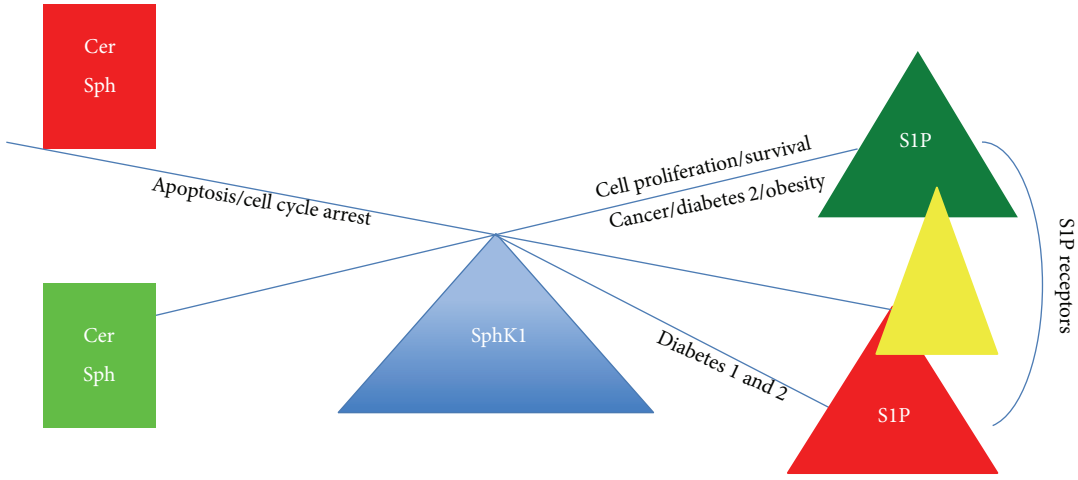


Figure 1: The swinging pendulum. Overexpression of SphK1 activates S1P and favours cell proliferation and survival. S1P overexpression is associated with cancer progression, type 2 diabetes complications such as inflammation, metabolic dysfunction, cardiovascular problems, nephropathy, retinopathy, and neuropathy. Loss of S1P can affect pancreatic β-cell proliferation and is associated with the progression of both type 1 and type 2 diabetes. Therapeutic intervention involving binding to specific S1P receptors may swing the pendulum in favour of more promising comorbidity treatments. Cer: ceramide, Sph: sphingosine, SphK1: sphingosine kinase 1, S1P: sphingosine-1-phosphate.

the development of diabetes in obese mice [43]. Abnormal islet function is central to the development of type 1 and type 2 diabetes [45]; therefore the danger of SphK/S1P inhibitors for cancer therapy is that they may increase the risk of diabetes development. In support of S1P activation in diabetic control, S1P has been shown to be important for insulin synthesis and secretion in a rat insulinoma cell line [46], muscle insulin resistance [16], and adiponectin action (increased sensitivity, decreased inflammation, and prosurvival) [47, 48]. The diabetic mouse model, KK/Ay, demonstrates a morbidly obese phenotype with metabolic abnormalities that are common in diabetic patients [49]. Overexpression of SphK1 in KK/Ay diabetic mice has been shown to significantly reduce blood glucose levels and improve the overall health of the animals whilst having no effect on normal animals [17].

There is a strong risk of cardiovascular diseases and heart failure in diabetic patients [50–55]. Several studies and reviews have emphasised the importance of SphK1/S1P in cardioprotection [17, 56–58]. A typical feature of the phenotype of animal models of diabetes is an increased accumulation of glycogen in the myocardium which leads to cardiomyopathy [59]. Such glycogen accumulation, which is typical of KK/Ay diabetic mice, was absent after adenoviral mediated (Ad-SphK1) overexpression of SphK1, potentially improving the function of the heart [17]. Moreover, impairment of liver and kidney function associated with the diabetic phenotype was also reversed in the Ad-SphK1 diabetic mice [17].

Atherosclerosis, the hardening of the arteries eventually leading to heart attacks and peripheral vascular disease, is accelerated in type 1 and type 2 diabetic patients [60–62]. Interactions between monocytes and endothelial cells are critical early events in the development of atherosclerosis [63]. In the nonobese diabetic mouse model (NOD/LtJ), a mouse model of spontaneous type 1 diabetes development (autoimmune destruction of the pancreatic islet cells), S1P minimises the monocyte/endothelial interaction that occurs in elevated glucose environments [64, 65].

Silent myocardial ischaemia is frequently presented in diabetic patients and this is reviewed in [66]. Activation of SphK1 has been shown to protect isolated mouse hearts against ischaemia/reperfusion injury [67], to have a cardioprotective effect of ischaemic preconditioning in mice and ischaemia/reperfusion injury [67–69] and to play a role in recovery of haemodynamic function after ischaemic injury [69]. In addition, SphK1 is important in the maintenance of blood vessel integrity and mice depleted of SphK1 have increased vascular leakiness [70]. Wound healing is also problematic in diabetic patients; however, SphK1/S1P activation has recently shown promise in the improvement of the wound healing process in diabetic rats [71].

Prevention of diabetes and improved pancreatic islet transplantation outcomes through pharmacological manipulation of the sphingolipid rheostat in favour of SphK1 has been shown to (i) promote insulin release, (ii) promote establishment and maintenance of intraislet vasculature, (iii) improve glucose sensing, and (iv) play a role in the prevention/treatment of the immune-mediated attack [45]. SphK1/S1P also plays a prosurvival role in primary hepatocytes and protects against liver injury [72].

On the other hand, S1P activation is not all positive for diabetic patients. S1P has been shown to be significantly increased in the blood of obese humans and mice and elevated S1P levels in humans have been correlated with metabolic dysfunction, cardiovascular problems, high body mass index (BMI), and large waist circumference, all factors associated with obesity [73]. Complications associated with obesity are also linked to cancer risk [10]. Wang and colleagues demonstrated that SphK1 overexpression was associated with adipose proinflammatory responses and insulin resistance in diet-induced obese mice and obese

diabetic humans [74]. In agreement with these findings Tous and colleagues demonstrated that activation of SphK1 in adipocytes (fat cells) triggered a cytokine inflammatory response whereas suppression of SphK1 activation lowered the expression of proinflammatory cytokines in adipose tissue of Zucker diabetic fatty rats [75]. In these experimental scenarios, inhibition of SphK1 was suggested as a therapeutic tool for the prevention and treatment of inflammation associated with obesity and type 2 diabetes [75]. Although there are several studies and reviews emphasising the importance of SphK1/S1P in cardioprotection (as mentioned above), elevated SphK1/S1P levels have also been associated with the negative effects of cardiovascular diseases linked to diabetes. For example, in one study SphK1 inhibition ameliorated angiotensin II-induced acute hypertension [76] and in another study deregulation of specific S1Ps played a role in cardiac microvascular dysfunction [77]. A growing list of adverse diabetic complications is believed to be involved with high levels of SphK1/S1P expression including neuropathy [36, 78, 79], retinopathy [80–84], nephropathy [85], and cancer [5, 6, 8]. The complexities of insulin resistance, with reference to the onset of diabetes and the modulation of S1P signalling, are discussed comprehensively in recent articles by Fayyaz and colleagues [86, 87]. In summary, the major apparent hurdle is that therapies targeting the SphK/S1P rheostat in cancer patients (for cancer therapy) may prove to be a double-edged sword where predisposing conditions such as obesity and diabetes are also presented. In addition, complications associated with the use of SphK1/S1P inhibitors may be that cancer patients are more susceptible to diabetes development. The multifaceted nature of SphK complicates the generation of SphK/S1P inhibitors as therapies for cancer.

6. SphK and S1P Inhibitors: Obesity/Diabetes/Cancer Conundrum

The development of treatment regimes to avoid complications arising from the presence of combined disease states, such as cancer and diabetes, is a major challenge: in this case, to balance cancer cell apoptosis and reduce disease complications whilst protecting pancreatic β-cell proliferation, it is becoming increasingly apparent that balancing the sphingosine rheostat is crucial in the development of many types of cancer and also diabetes; however, the opposing effects of SphK/S1P inhibitors on diabetes and cancer are a conundrum. It is unknown whether S1P activation influences both type 1 and type 2 diabetes outcomes such as mechanism of β-cell death or insulin resistance in skeletal muscle. Furthermore, obese cancer patients could be at heightened risk of diabetes if treated with SphK/S1P inhibitors and this concept needs to be considered in future research in SphK/S1P inhibitor design and treatment. S1P agonists and functional antagonists (S1P receptor modulators) are in development to target specific S1P receptor subtypes to maximise therapeutic efficacy [88, 89]. FTY720 (fingolimod) is a first generation S1P modulator under consideration for the treatment of cancer and diabetes, however not necessarily for comorbidity therapy. FTY720 is a S1P analogue that mimics S1P as an agonist of all the S1P receptors except $S1P_2$ [90–92]. Despite this, it also acts as a functional $S1P_1$ receptor antagonist, reviewed in [93]. The fact that FTY720 does not bind to $S1P_2$ has created much interest for diabetes/cancer therapy advocates. There are mixed results reported to date with the use of FTY720 for cancer treatment. Recent advances have shown the use of FTY720 and its derivatives to be promising potential therapies for cancers such as intestinal and colorectal cancer [94–97], leukaemia [95, 98, 99], ovarian cancer [100], triple-negative breast cancers [101], and increased sensitivity to radiation of breast cancer cells [102]. Moreover, FTY720 inhibits melanoma growth and invasion in 3D culture *in vitro* (NKH, unpublished results). On the other hand, FTY720 decreased sensitivity of breast cancer cells overexpressing the oncogene pp32r1 [103] and HER2 targeted therapy with lapatinib [104] potentially compromising the efficacy of FTY720 in some breast cancer clinical cotreatment regimes.

SphK1/S1P inhibitors as therapies for diabetes are also problematic. The effect of FTY720 in various animal models of type 1 diabetes is summarised by Jessup and colleagues [45]. The efficacy of FTY720 ranges from complete prevention of diabetes, short-term prevention, and—depending on the disease stage and time point of drug administration—diminished efficacy from 20–100% [45]. In recent studies, FTY720 has been shown to inhibit the development of obesity in high fat fed mice, by modulation of adipogenesis and lipolysis [105], and to attenuate the accumulation of ceramide in muscles, associated with a high fat diet, resulting in improved whole body glucose homeostasis [106] and amelioration of prediabetic type 2 disposition. Previous reports also provided promising results with complete reversal of diabetes (6/11 mice) in obese mice with continuous administration of FTY720 [107]. In addition, the recent study by Moon and colleagues demonstrated that FTY720 increased β-cell survival and restored β-cell function with improved glucose tolerance in a diabetic *(db/db)* mouse model [108]. Not all groups have found FTY720 beneficial in the prevention or cure of diabetes [86, 109]. Fayyaz and colleagues demonstrated FTY720 was unable to modulate S1P mediated insulin signalling in human and rat hepatocytes [86]. As mentioned, FTY720 does not bind the $S1P_2$ receptor. The importance of the $S1P_2$ receptor in insulin resistance was demonstrated by blocking the receptor using a specific antagonist (JTE-013), thereby increasing hepatic insulin signalling [86, 109]. Hence specific $S1P_2$ receptor antagonists such as JTE-013 have been suggested as targets for diabetes treatments (Figure 2).

The controversial function of current S1P agonists and functional antagonists has been associated with binding of differing S1P receptor transmembrane expression, such as demonstrated for FTY720. As discussed above, SphK1/S1P inhibitors can have positive and negative impact for diabetic patients depending on the patient's specific condition. Current second generation S1P receptor agonists hold much promise for comorbidity cancer/diabetes treatments and are reviewed in [88, 89]. A comparison of fingolimod (FTY720) and the most advanced next generation S1P modulators (siponimod, ponesimod, KRP-203,

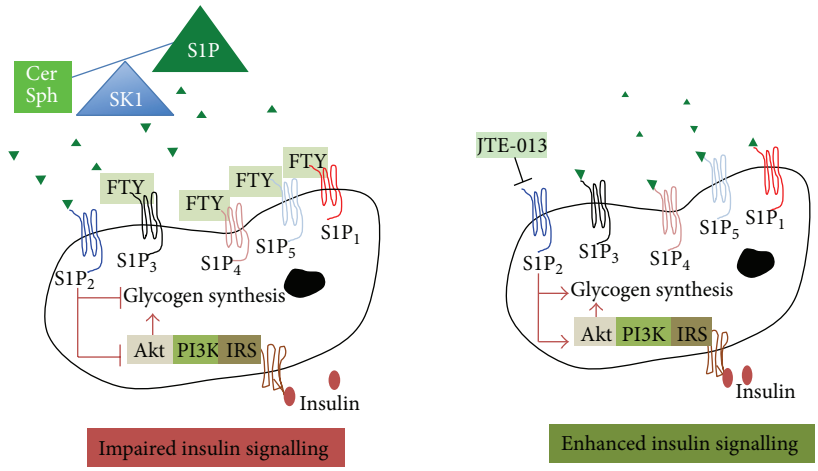


Figure 2: The $S1P_2$ receptor modulates hepatic insulin signalling. FTY720 binds to $S1P_{1,3-5}$ receptors and does not impact the normal signalling functions of $S1P_2$. $S1P_2$ has been associated with impaired insulin signalling [86, 109]. FTY720 is a $S1P_{1,3-5}$ agonist but also acts as a functional antagonist of $S1P_1$ [109]. FTY720 does not bind to $S1P_2$ and therefore does not affect $S1P_2$ function. In contrast, JTE-013 inhibits $S1P_2$.

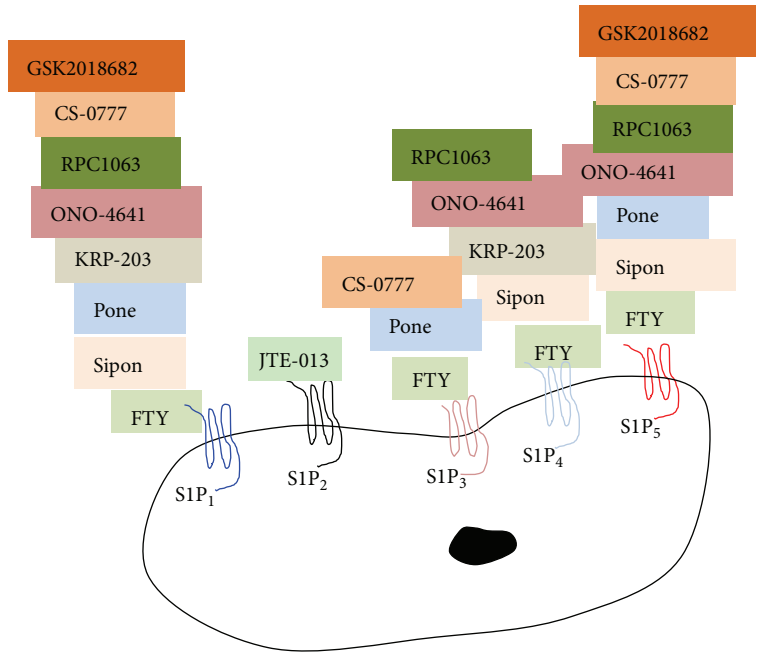


Figure 3: Balancing the SphK1/S1P rheostat for diabetes and cancer comorbidity treatments. Second generation S1P receptor modulators are currently being developed to target individual and multiple S1P receptors. Each of the receptor modulators binds to individual or multiple receptors to block or activate the S1P receptor. Siponimod is a $S1P_{1,4,5}$ modulator; ponesimod is an agonist for $S1P_{1,3,4}$; KRP-203 is an agonist for $S1P_{1,4}$; ONO-4641 is an agonist for $S1P_{1,4,5}$; RPC1063 is an agonist for $S1P_{1,4,5}$; Cs-0777 is an agonist for $S1P_{1,3,5}$; GSK2018682 is an agonist for $S1P_{1,5}$. FTY720 and JTE-013 are described in Figure 2. These novel S1P receptors and downstream signalling pathways and functions are reviewed in [88, 93]. Sipon: siponimod; pone: ponesimod; FTY: FTY720.

ONO-4641, RPC1063, CS-0777, and GSK2018682), each modulator targeting common and different S1P receptors, are illustrated in Figure 3 [88, 93]. Comparative selectivity of S1P modulator activation of specific S1P receptors is shown in Table 1. Knowledge of specific S1P receptor function provides some insight into how S1P receptor modulators may be targeted for comorbidity treatments.

7. The SphK1/S1P Rheostat Therapeutic Challenge

Targeting the sphingolipid rheostat for diabetes and cancer therapy holds great promise; however, the treatment for comorbidity will be the greatest hurdle to overcome. As portrayed in Figure 1, the challenge will be to balance cancer

Table 1: Comparative selectivity of the S1P modulators (adapted from [88]).

	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
FTY720	++++	−	+	++	+++
CS-0777	+++	−	+	−	++
Ponesimod	+++	−	+	−	++
RPC0163	++++	+/−	+/−	+/−	++
ONO-4641	++	−	−	+	++
Siponimod	++	−	−	+	++
GSK2018682	++	−	−	−	+
KRP-203	+++	−	−	++	−
JTE-013	−	+++	−	−	−

+ indicates comparative selectivity of S1P modulators binding to individual receptors.

cell apoptosis on the one hand and promote β-cell survival for insulin production on the other hand; it is a swinging pendulum (Figure 1). A greater understanding of the actions of SphK1/S1P in the context of diabetes, especially the onset of type 2 diabetes and cancer, is required if we are to switch the sphingolipid rheostat in the treatment of diabetes and cancer comorbidity from a problem to an advantage.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] G. Danaei, M. M. Finucane, Y. Lu et al., "National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants," *The Lancet*, vol. 378, no. 9785, pp. 31–40, 2011.

[2] WHO, *Global Status Report on Noncommunicable Diseases 2010*, WHO, Geneva, Switzerland, 2011.

[3] J. Ferlay, I. Soerjomataram, M. Ervik et al., *GLOBOCAN 2012 v1.0*, IARC Cancer Base No. 11, Cancer Incidence and Mortality Worldwide, 2012.

[4] F. Bray, J.-S. Ren, E. Masuyer, and J. Ferlay, "Global estimates of cancer prevalence for 27 sites in the adult population in 2008," *International Journal of Cancer*, vol. 132, no. 5, pp. 1133–1145, 2013.

[5] S. Sen, Y. He, D. Koya, and K. Kanasaki, "Cancer biology in diabetes," *Journal of Diabetes Investigation*, vol. 5, no. 3, pp. 251–264, 2014.

[6] S. K. Garg, H. Maurer, K. Reed, and R. Selagamsetty, "Diabetes and cancer: two diseases with obesity as a common risk factor," *Diabetes, Obesity and Metabolism*, vol. 16, no. 2, pp. 97–110, 2014.

[7] M. J. Khandekar, P. Cohen, and B. M. Spiegelman, "Molecular mechanisms of cancer development in obesity," *Nature Reviews Cancer*, vol. 11, no. 12, pp. 886–895, 2011.

[8] E. Giovannucci, D. M. Harlan, M. C. Archer et al., "Diabetes and cancer: a consensus report," *Diabetes Care*, vol. 33, no. 7, pp. 1674–1685, 2010.

[9] B. B. Barone, H.-C. Yeh, C. F. Snyder et al., "Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis," *The Journal of the American Medical Association*, vol. 300, no. 23, pp. 2754–2764, 2008.

[10] H. Noto, A. Goto, T. Tsujimoto, K. Osame, and M. Noda, "Latest insights into the risk of cancer in diabetes," *Journal of Diabetes Investigation*, vol. 4, no. 3, pp. 225–232, 2013.

[11] M. Jalving, J. A. Gietema, J. D. Lefrandt et al., "Metformin: taking away the candy for cancer?" *European Journal of Cancer*, vol. 46, no. 13, pp. 2369–2380, 2010.

[12] M. Maceyka, K. B. Harikumar, S. Milstien, and S. Spiegel, "Sphingosine-1-phosphate signaling and its role in disease," *Trends in Cell Biology*, vol. 22, no. 1, pp. 50–60, 2012.

[13] J.-P. Truman, M. García-Barros, L. M. Obeid, and Y. A. Hannun, "Evolving concepts in cancer therapy through targeting sphingolipid metabolism," *Biochimica et Biophysica Acta*, vol. 1841, no. 8, pp. 1174–1188, 2014.

[14] L. A. Heffernan-Stroud and L. M. Obeid, "Sphingosine kinase 1 in cancer," *Advances in Cancer Research*, vol. 117, pp. 201–235, 2013.

[15] D. Plano, S. Amin, and A. K. Sharma, "Importance of sphingosine kinase (SphK) as a target in developing cancer therapeutics and recent developments in the synthesis of novel SphK inhibitors," *Journal of Medicinal Chemistry*, vol. 7, no. 13, pp. 5509–5524, 2014.

[16] C. R. Bruce, S. Risis, J. R. Babb et al., "Overexpression of sphingosine kinase 1 prevents ceramide accumulation and ameliorates muscle insulin resistance in high-fat diet-fed mice," *Diabetes*, vol. 61, no. 12, pp. 3148–3155, 2012.

[17] M. M. Ma, J. L. Chen, G. G. Wang et al., "Sphingosine kinase 1 participates in insulin signalling and regulates glucose metabolism and homeostasis in KK/Ay diabetic mice," *Diabetologia*, vol. 50, no. 4, pp. 891–900, 2007.

[18] Y. A. Hannun and L. M. Obeid, "Principles of bioactive lipid signalling: lessons from sphingolipids," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 2, pp. 139–150, 2008.

[19] A. Olivera and S. Spiegel, "Sphingosine-1-phosphate as second messenger in cell proliferation induced by PDGF and FCS mitogens," *Nature*, vol. 365, no. 6446, pp. 557–560, 1993.

[20] J. R. van Brocklyn, M.-J. Lee, R. Menzeleev et al., "Dual actions of sphingosine-1-phosphate: extracellular through the G_i- coupled receptor Edg-1 and intracellular to regulate proliferation and survival," *The Journal of Cell Biology*, vol. 142, no. 1, pp. 229–240, 1998.

[21] "Report of the expert committee on the diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 26, supplement 1, pp. S5–S20, 2003.

[22] M. Cnop, N. Welsh, J.-C. Jonas, A. Jörns, S. Lenzen, and D. L. Eizirik, "Mechanisms of pancreatic β-cell death in type 1 and type 2 diabetes: many differences, few similarities," *Diabetes*, vol. 54, no. 2, pp. S97–S107, 2005.

[23] S. Shao, Y. Yang, G. Yuan, M. Zhang, and X. Yu, "Signaling molecules involved in lipid-induced pancreatic beta-cell dysfunction," *DNA and Cell Biology*, vol. 32, no. 2, pp. 41–49, 2013.

[24] P. Newsholme, D. Keane, H. J. Welters, and N. G. Morgan, "Life and death decisions of the pancreatic β-cell: the role of fatty acids," *Clinical Science*, vol. 112, no. 1-2, pp. 27–42, 2007.

[25] E. Phielix and M. Mensink, "Type 2 diabetes mellitus and skeletal muscle metabolic function," *Physiology and Behavior*, vol. 94, no. 2, pp. 252–258, 2008.

[26] J. A. Johnson and M. Pollak, "Insulin, glucose and the increased risk of cancer in patients with type 2 diabetes," *Diabetologia*, vol. 53, no. 10, pp. 2086–2088, 2010.

[27] C. Li and D. Kong, "Cancer risks from diabetes therapies: evaluating the evidence," *Pharmacology & Therapeutics*, vol. 144, no. 1, pp. 71–81, 2014.

[28] L. Braccini, E. Ciraolo, M. Martini et al., "PI3K keeps the balance between metabolism and cancer," *Advances in Biological Regulation*, vol. 52, no. 3, pp. 389–405, 2012.

[29] R. Zoncu, A. Efeyan, and D. M. Sabatini, "mTOR: From growth signal integration to cancer, diabetes and ageing," *Nature Reviews Molecular Cell Biology*, vol. 12, no. 1, pp. 21–35, 2011.

[30] K. A. Orr Gandy and L. M. Obeid, "Targeting the sphingosine kinase/sphingosine 1-phosphate pathway in disease: review of sphingosine kinase inhibitors," *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids*, vol. 1831, no. 1, pp. 157–166, 2013.

[31] N. J. Pyne and S. Pyne, "Sphingosine 1-phosphate and cancer," *Nature Reviews Cancer*, vol. 10, no. 7, pp. 489–503, 2010.

[32] N. J. Pyne, F. Tonelli, K. G. Lim et al., "Targeting sphingosine kinase 1 in cancer," *Biochemical Society Transactions*, vol. 40, no. 1, pp. 94–100, 2012.

[33] Y. A. Hannun, C. R. Loomis, A. H. Merrill Jr., and R. M. Bell, "Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets," *The Journal of Biological Chemistry*, vol. 261, no. 27, pp. 12604–12609, 1986.

[34] S. Milstien and S. Spiegel, "Targeting sphingosine-1-phosphate: a novel avenue for cancer therapeutics," *Cancer Cell*, vol. 9, no. 3, pp. 148–150, 2006.

[35] R. Alemany, C. J. van Koppen, K. Danneberg, M. Ter Braak, and D. M. Zu Heringdorf, "Regulation and functional roles of sphingosine kinases," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 374, no. 5-6, pp. 413–428, 2007.

[36] H. J. Abuhusain, A. Matin, Q. Qiao et al., "A metabolic shift favoring sphingosine 1-phosphate at the expense of ceramide controls glioblastoma angiogenesis," *The Journal of Biological Chemistry*, vol. 288, no. 52, pp. 37355–37364, 2013.

[37] N. C. Hait, C. A. Oskeritzian, S. W. Paugh, S. Milstien, and S. Spiegel, "Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases," *Biochimica et Biophysica Acta*, vol. 1758, no. 12, pp. 2016–2026, 2006.

[38] K. Mendelson, T. Evans, and T. Hla, "Sphingosine 1-phosphate signalling," *Development*, vol. 141, no. 1, pp. 5–9, 2014.

[39] E. Rapizzi, M. L. Taddei, T. Fiaschi, C. Donati, P. Bruni, and P. Chiarugi, "Sphingosine 1-phosphate increases glucose uptake through trans-activation of insulin receptor," *Cellular and Molecular Life Sciences*, vol. 66, no. 19, pp. 3207–3218, 2009.

[40] M. Schüppel, U. Kürschner, U. Kleuser, M. Schäfer-Korting, and B. Kleuser, "Sphingosine 1-phosphate restrains insulin-mediated keratinocyte proliferation via inhibition of Akt through the S1P2 receptor subtype," *The Journal of Investigative Dermatology*, vol. 128, no. 7, pp. 1747–1756, 2008.

[41] D. Yagoub, M. R. Wilkins, A. J. Lay et al., "Sphingosine kinase 1 isoform-specific interactions in breast cancer," *Molecular Endocrinology*, vol. 28, no. 11, pp. 1899–1915, 2014.

[42] K. G. Lim, F. Tonelli, E. Berdyshev et al., "Inhibition kinetics and regulation of sphingosine kinase 1 expression in prostate cancer cells: functional differences between sphingosine kinase 1a and 1b," *The International Journal of Biochemistry & Cell Biology*, vol. 44, no. 9, pp. 1457–1464, 2012.

[43] Y. Qi, J. Chen, A. Lay, A. Don, M. Vadas, and P. Xia, "Loss of sphingosine kinase 1 predisposes to the onset of diabetes via promoting pancreatic β-cell death in diet-induced obese mice," *FASEB Journal*, vol. 27, no. 10, pp. 4294–4304, 2013.

[44] W. L. Holland, J. T. Brozinick, L.-P. Wang et al., "Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance," *Cell Metabolism*, vol. 5, no. 3, pp. 167–179, 2007.

[45] C. F. Jessup, C. S. Bonder, S. M. Pitson, and P. T. H. Coates, "The sphingolipid rheostat: a potential target for improving pancreatic islet survival and function," *Endocrine, Metabolic and Immune Disorders: Drug Targets*, vol. 11, no. 4, pp. 262–272, 2011.

[46] N. M. Hasan, M. J. Longacre, S. W. Stoker et al., "Sphingosine kinase 1 knockdown reduces insulin synthesis and secretion in a rat insulinoma cell line," *Archives of Biochemistry and Biophysics*, vol. 518, no. 1, pp. 23–30, 2012.

[47] W. L. Holland, R. A. Miller, Z. V. Wang et al., "Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin," *Nature Medicine*, vol. 17, no. 1, pp. 55–63, 2011.

[48] C. Tao, A. Sifuentes, and W. L. Holland, "Regulation of glucose and lipid homeostasis by adiponectin: effects on hepatocytes, pancreatic β cells and adipocytes," *Best Practice & Research: Clinical Endocrinology and Metabolism*, vol. 28, no. 1, pp. 43–58, 2014.

[49] H. Iwatsuka, A. Shino, and Z. Suzuoki, "General survey of diabetic features of yellow KK mice," *Endocrinologia Japonica*, vol. 17, no. 1, pp. 23–35, 1970.

[50] O. Schnell, F. Cappuccio, S. Genovese, E. Standl, P. Valensi, and A. Ceriello, "Type 1 diabetes and cardiovascular disease," *Cardiovascular Diabetology*, vol. 12, no. 1, article 156, 2013.

[51] F. Paneni, J. A. Beckman, M. A. Creager, and F. Cosentino, "Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I," *European Heart Journal*, vol. 34, no. 31, pp. 2436–2443, 2013.

[52] S. M. Grundy, I. J. Benjamin, G. L. Burke et al., "Diabetes and cardiovascular disease: a statement for healthcare professionals from the american heart association," *Circulation*, vol. 100, no. 10, pp. 1134–1146, 1999.

[53] P. Fioretto, P. M. Dodson, D. Ziegler, and R. S. Rosenson, "Residual microvascular risk in diabetes: unmet needs and future directions," *Nature Reviews Endocrinology*, vol. 6, no. 1, pp. 19–25, 2010.

[54] F. Paneni, S. Costantino, and F. Cosentino, "Insulin resistance, diabetes, and cardiovascular risk," *Current Atherosclerosis Reports*, vol. 16, no. 7, article 419, 2014.

[55] H. Li, S. Horke, and U. Forstermann, "Vascular oxidative stress, nitric oxide and atherosclerosis," *Atherosclerosis*, vol. 237, pp. 208–219, 2014.

[56] P. Keul, K. Sattler, and B. Levkau, "HDL and its sphingosine-1-phosphate content in cardioprotection," *Heart Failure Reviews*, vol. 12, no. 3-4, pp. 301–306, 2007.

[57] J. S. Karliner, "Sphingosine kinase regulation and cardioprotection," *Cardiovascular Research*, vol. 82, no. 2, pp. 184–192, 2009.

[58] J. S. Karliner, "Sphingosine kinase and sphingosine 1-phosphate in the heart: a decade of progress," *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids*, vol. 1831, no. 1, pp. 203–212, 2013.

[59] S. Penpargkul, T. Schaible, T. Yipintsoi, and J. Scheuer, "The effect of diabetes on performance and metabolism of rat hearts," *Circulation Research*, vol. 47, no. 6, pp. 911–921, 1980.

[60] K. Dahl-Jørgensen, J. R. Larsen, and K. F. Hanssen, "Atherosclerosis in childhood and adolescent type 1 diabetes: early disease, early treatment?" *Diabetologia*, vol. 48, no. 8, pp. 1445–1453, 2005.

[61] M. Fisher, "Diabetes and atherogenesis," *Heart*, vol. 90, no. 3, pp. 336–340, 2004.

[62] R. Donnelly and K. R. Davis, "Type 2 diabetes and atherosclerosis," *Diabetes, Obesity and Metabolism*, vol. 2, no. 1, pp. S21–S30, 2000.

[63] J. Mestas and K. Ley, "Monocyte-endothelial cell interactions in the development of atherosclerosis," *Trends in Cardiovascular Medicine*, vol. 18, no. 6, pp. 228–232, 2008.

[64] A. M. Whetzel, D. T. Bolick, and C. C. Hedrick, "Sphingosine-1-phosphate inhibits high glucose-mediated ERK1/2 action in endothelium through induction of MAP kinase phosphatase-3," *American Journal of Physiology: Cell Physiology*, vol. 296, no. 2, pp. C339–C345, 2009.

[65] A. M. Whetzel, D. T. Bolick, S. Srinivasan et al., "Sphingosine-1 phosphate prevents monocyte/endothelial interactions in type 1 diabetic NOD mice through activation of the S1P1 receptor," *Circulation Research*, vol. 99, no. 7, pp. 731–739, 2006.

[66] H. A. Khafaji and J. M. Suwaidi, "A typical presentation of acute and chronic coronary artery disease in diabetics," *World Journal of Cardiology*, vol. 6, no. 8, pp. 802–813, 2014.

[67] Z.-Q. Jin, J. S. Karliner, and D. A. Vessey, "Ischaemic postconditioning protects isolated mouse hearts against ischaemia/reperfusion injury via sphingosine kinase isoform-1 activation," *Cardiovascular Research*, vol. 79, no. 1, pp. 134–140, 2008.

[68] D. A. Vessey, M. Kelley, L. Li, and Y. Huang, "Sphingosine protects aging hearts from ischemia/reperfusion injury: superiority to sphingosine 1-phosphate and ischemic pre- and post-conditioning," *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 3, pp. 146–151, 2009.

[69] D. A. Vessey, M. Kelley, L. Li et al., "Role of sphingosine kinase activity in protection of heart against ischemia reperfusion injury," *Medical Science Monitor*, vol. 12, no. 10, pp. BR318–BR324, 2006.

[70] C. S. Bonder, W. Y. Sun, T. Matthews et al., "Sphingosine kinase regulates the rate of endothelial progenitor cell differentiation," *Blood*, vol. 113, no. 9, pp. 2108–2117, 2009.

[71] H. Yu, L. Yuan, M. Xu, Z. Zhang, and H. Duan, "Sphingosine kinase 1 improves cutaneous wound healing in diabetic rats," *Injury*, vol. 45, no. 7, pp. 1054–1058, 2014.

[72] Y. Osawa, H. Uchinami, J. Bielawski, R. F. Schwabe, Y. A. Hannun, and D. A. Brenner, "Roles for C16-ceramide and sphingosine 1-phosphate in regulating hepatocyte apoptosis in response to tumor necrosis factor-α," *The Journal of Biological Chemistry*, vol. 280, no. 30, pp. 27879–27887, 2005.

[73] G. M. Kowalski, A. L. Carey, A. Selathurai, B. A. Kingwell, and C. R. Bruce, "Plasma sphingosine-1-phosphate is elevated in obesity," *PLoS ONE*, vol. 8, no. 9, Article ID e72449, 2013.

[74] J. Wang, L. Badeanlou, J. Bielawski, T. P. Ciaraldi, and F. Samad, "Sphingosine kinase 1 regulates adipose proinflammatory responses and insulin resistance," *American Journal of Physiology: Endocrinology and Metabolism*, vol. 306, no. 7, pp. E756–E768, 2014.

[75] M. Tous, R. Ferrer-Lorente, and L. Badimon, "Selective inhibition of sphingosine kinase-1 protects adipose tissue against LPS-induced inflammatory response in Zucker diabetic fatty rats," *The American Journal of Physiology, Endocrinology and Metabolism*, vol. 307, no. 5, pp. E437–E446, 2014.

[76] H. Furuya, M. Wada, Y. Shimizu et al., "Effect of sphingosine kinase 1 inhibition on blood pressure," *The FASEB Journal*, vol. 27, no. 2, pp. 656–664, 2013.

[77] Z. Yin, L. Fan, L. Wei et al., "FTY720 protects cardiac microvessels of diabetes: a critical role of S1P1/3 in diabetic heart disease," *PLoS ONE*, vol. 7, no. 8, Article ID e42900, 2012.

[78] K. Janes, J. W. Little, C. Li et al., "The development and maintenance of paclitaxel-induced neuropathic pain require activation of the sphingosine 1-phosphate receptor subtype 1," *The Journal of Biological Chemistry*, vol. 289, no. 30, pp. 21082–21097, 2014.

[79] H. Guan, L. Song, J. Cai et al., "Sphingosine kinase 1 regulates the Akt/FOXO3a/Bim pathway and contributes to apoptosis resistance in glioma cells," *PLoS ONE*, vol. 6, no. 5, Article ID e19946, 2011.

[80] B. Xie, J. Shen, A. Dong, A. Rashid, G. Stoller, and P. A. Campochiaro, "Blockade of sphingosine-1-phosphate reduces macrophage influx and retinal and choroidal neovascularization," *Journal of Cellular Physiology*, vol. 218, no. 1, pp. 192–198, 2009.

[81] L. W. Maines, K. J. French, E. B. Wolpert, D. A. Antonetti, and C. D. Smith, "Pharmacologic manipulation of sphingosine kinase in retinal endothelial cells: implications for angiogenic ocular diseases," *Investigative Ophthalmology and Visual Science*, vol. 47, no. 11, pp. 5022–5031, 2006.

[82] R. A. Sabbadini, "Sphingosine-1-phosphate antibodies as potential agents in the treatment of cancer and age-related macular degeneration," *British Journal of Pharmacology*, vol. 162, no. 6, pp. 1225–1238, 2011.

[83] B. Visentin, J. A. Vekich, B. J. Sibbald et al., "Validation of an anti-sphingosine-1-phosphate antibody as a potential therapeutic in reducing growth, invasion, and angiogenesis in multiple tumor lineages," *Cancer Cell*, vol. 9, no. 3, pp. 225–238, 2006.

[84] K. Mizugishi, T. Yamashita, A. Olivera, G. F. Miller, S. Spiegel, and R. L. Proia, "Essential role for sphingosine kinases in neural and vascular development," *Molecular and Cellular Biology*, vol. 25, no. 24, pp. 11113–11121, 2005.

[85] T. Lan, W. Liu, X. Xie et al., "Sphingosine kinase-1 pathway mediates high glucose-induced fibronectin expression in glomerular mesangial cells," *Molecular Endocrinology*, vol. 25, no. 12, pp. 2094–2105, 2011.

[86] S. Fayyaz, J. Henkel, L. Japtok et al., "Involvement of sphingosine 1-phosphate in palmitate-induced insulin resistance of hepatocytes via the S1P2 receptor subtype," *Diabetologia*, vol. 57, no. 2, pp. 373–382, 2014.

[87] S. Fayyaz, L. Japtok, and B. Kleuser, "Divergent role of sphingosine 1-phosphate on insulin resistance," *Cellular Physiology and Biochemistry*, vol. 34, pp. 134–147, 2014.

[88] M. Bigaud, D. Guerini, A. Billich, F. Bassilana, and V. Brinkmann, "Second generation S1P pathway modulators: research strategies and clinical developments," *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids*, vol. 1841, no. 5, pp. 745–758, 2014.

[89] A. Huwiler and J. Pfeilschifter, "New players on the center stage: sphingosine 1-phosphate and its receptors as drug targets," *Biochemical Pharmacology*, vol. 75, no. 10, pp. 1893–1900, 2008.

[90] P. Bandhuvula, Y. Y. Tam, B. Oskouian, and J. D. Saba, "The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity," *The Journal of Biological Chemistry*, vol. 280, no. 40, pp. 33697–33700, 2005.

[91] N. C. Kaneider, J. Lindner, C. Feistritzer et al., "The immune modulator FTY720 targets sphingosine-kinase-dependent

migration of human monocytes in response to amyloid beta-protein and its precursor," *The FASEB Journal*, vol. 18, no. 11, pp. 1309–1311, 2004.

[92] V. Brinkmann, M. D. Davis, C. E. Heise et al., "The immune modulator FTY720 targets sphingosine 1-phosphate receptors," *Journal of Biological Chemistry*, vol. 277, no. 24, pp. 21453–21457, 2002.

[93] V. A. Blaho and T. Hla, "An update on the biology of sphingosine 1-phosphate receptors," *The Journal of Lipid Research*, vol. 55, no. 8, pp. 1596–1608, 2014.

[94] I. Cristobal, R. Manso, and R. Rincon, "PP2A inhibition is a common event in colorectal cancer and its restoration using FTY720 shows promising therapeutic potential," *Molecular Cancer Therapeutics*, vol. 13, no. 4, pp. 938–947, 2014.

[95] P. Neviani, R. Santhanam, J. J. Oaks et al., "FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia," *The Journal of Clinical Investigation*, vol. 117, no. 9, pp. 2408–2421, 2007.

[96] M. Nagahashi, N. C. Hait, M. Maceyka et al., "Sphingosine-1-phosphate in chronic intestinal inflammation and cancer," *Advances in Biological Regulation*, vol. 54, no. 1, pp. 112–120, 2014.

[97] J. Liang, M. Nagahashi, E. Y. Kim et al., "Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer," *Cancer Cell*, vol. 23, no. 1, pp. 107–120, 2013.

[98] Q. Liu, X. Zhao, F. Frissora et al., "FTY720 demonstrates promising preclinical activity for chronic lymphocytic leukemia and lymphoblastic leukemia/lymphoma," *Blood*, vol. 111, no. 1, pp. 275–284, 2008.

[99] R. Mani, Y. Mao, F. W. Frissora et al., "Tumor antigen ROR1 targeted drug delivery mediated selective leukemic but not normal B cell cytotoxicity in chronic lymphocytic leukemia," *Leukemia*, 2014.

[100] N. Zhang, Y. Qi, C. Wadham et al., "FTY720 induces necrotic cell death and autophagy in ovarian cancer cells: a protective role of autophagy," *Autophagy*, vol. 6, no. 8, pp. 1157–1167, 2010.

[101] S. Baldacchino, C. Saliba, V. Petroni et al., "Deregulation of the phosphatase, PP2A is a common event in breast cancer, predicting sensitivity to FTY720," *EPMA Journal*, vol. 5, no. 1, article 3, 2014.

[102] G. Marvaso, A. Barone, N. Amodio et al., "Sphingosine analog fingolimod (FTY720) increases radiation sensitivity of human breast cancer cells in vitro," *Cancer Biology & Therapy*, vol. 15, no. 6, pp. 797–805, 2014.

[103] S. Buddaseth, W. Göttmann, R. Blasczyk, and T. Huyton, "Overexpression of the pp32r1 (ANP32C) oncogene or its functional mutant pp32r1Y140H confers enhanced resistance to FTY720 (Finguimod)," *Cancer Biology and Therapy*, vol. 15, no. 3, pp. 289–296, 2014.

[104] M. S. McDermott, B. C. Browne, N. T. Conlon et al., "PP2A inhibition overcomes acquired resistance to HER2 targeted therapy," *Molecular Cancer*, vol. 13, p. 157, 2014.

[105] S.-Y. Park, M.-H. Moon, J.-K. Jeong et al., "Antiobesity activity of a sphingosine 1-phosphate analogue FTY720 observed in adipocytes and obese mouse model," *Experimental and Molecular Medicine*, vol. 44, no. 10, pp. 603–614, 2012.

[106] C. R. Bruce, S. Risis, J. R. Babb et al., "The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice," *Endocrinology*, vol. 154, no. 1, pp. 65–76, 2013.

[107] T. Maki, R. Gottschalk, N. Ogawa, and A. P. Monaco, "Prevention and cure of autoimmune diabetes in nonobese diabetic mice by continuous administration of FTY720," *Transplantation*, vol. 79, no. 9, pp. 1051–1055, 2005.

[108] H. Moon, J. Chon, J. Joo et al., "FTY720 preserved islet β-cell mass by inhibiting apoptosis and increasing survival of β-cells in db/db mice," *Diabetes/Metabolism Research and Reviews*, vol. 29, no. 1, pp. 19–24, 2013.

[109] T. Imasawa, K. Koike, I. Ishii, J. Chun, and Y. Yatomi, "Blockade of sphingosine 1-phosphate receptor 2 signaling attenuates streptozotocin-induced apoptosis of pancreatic β-cells," *Biochemical and Biophysical Research Communications*, vol. 392, no. 2, pp. 207–211, 2010.