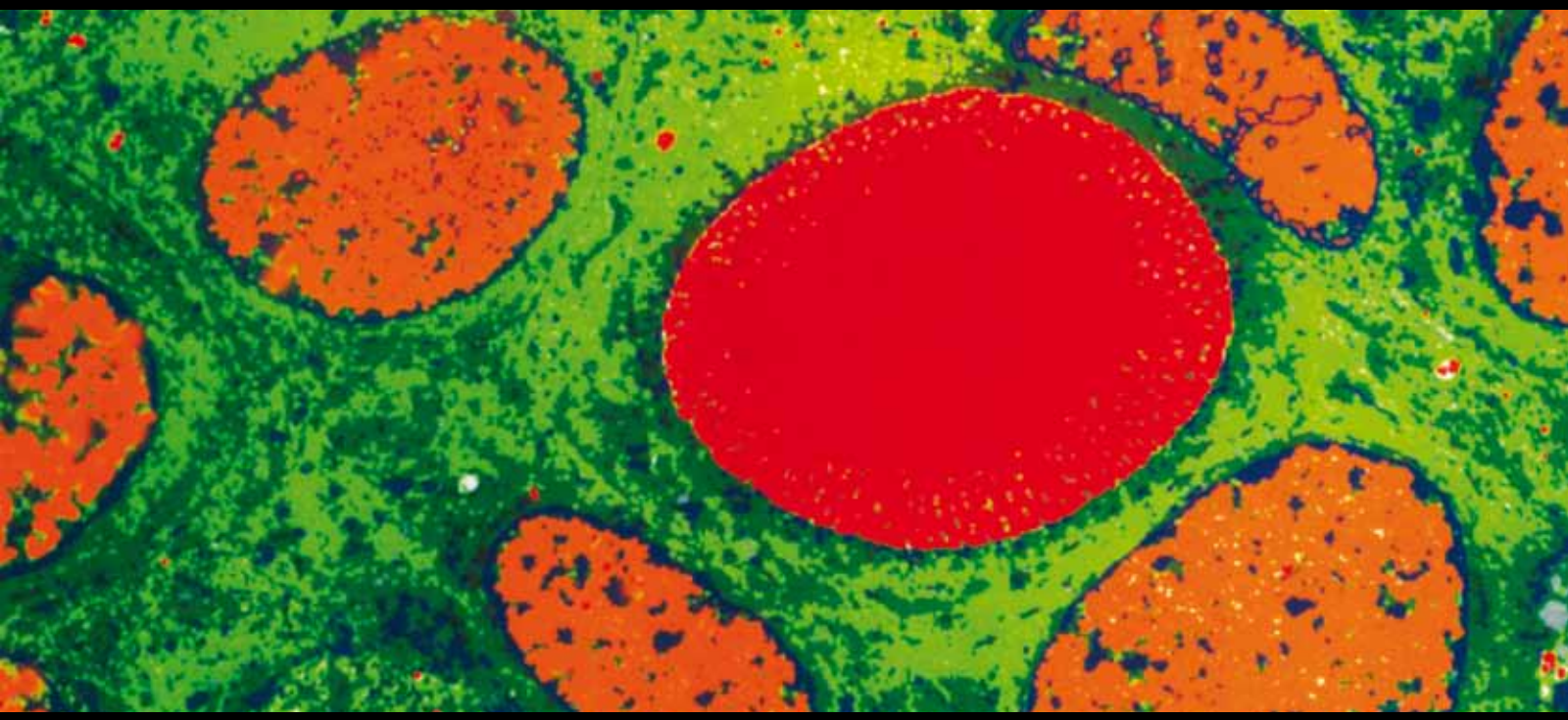
International Journal of Breast Cancer

Triple-Negative Breast Cancer

Guest Editors: Quyen D. Chu, Tari King, and Thelma Hurd



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Editorial Board

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International Journal of Breast Cancer
Volume 2012, Article ID 671684, 1 page
doi:10.1155/2012/671684

Editorial

Triple-Negative Breast Cancer

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Received 8 February 2012; Accepted 8 February 2012

Ever since the seminal work that was published by Perou et al., triple-negative breast cancer (TNBC) has become a common lexicon among clinicians who care for patients with breast cancer. Beside portending a poor outcome, TNBC is unique in that, unlike the hormone-positive and Her-2-positive cancer cells, it lacks target specific therapy. This is likely the result of our lack of having a clear understanding of its biology.

In this special issue, we were highly selective of only papers that we thought might further advance our understanding of the disease. Thus, the papers vary widely from the role of race/ethnicity to the metabolic/molecular influence that can potentially impact the biology of the disease.

We hope that this special issue will serve as a focal point to continue our ongoing discussion about this challenging entity.

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Hindawi Publishing Corporation
International Journal of Breast Cancer
Volume 2012, Article ID 764570, 6 pages
doi:10.1155/2012/764570

Clinical Study

Outcome for Patients with Triple-Negative Breast Cancer Is Not Dependent on Race/Ethnicity

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Received 11 July 2011; Accepted 18 January 2012

Academic Editor: Thelma Hurd

Introduction. Triple negative breast cancer (TNBC) is biologically aggressive and is associated with a worse prognosis. To understand the impact of race/ethnicity on outcome for patients with TNBC, confounding factors such as socioeconomic status (SES) need to be controlled. We examined the impact of race/ethnicity on a cohort of patients of low SES who have TNBC. *Methods*. 786 patients with Stage 0–III breast cancer were evaluated. Of these, 202 patients had TNBC (26%). Primary endpoints were cancer recurrence and death. ZIP code-based income tract and institutional financial data were used to assess SES. Data were analyzed using Kaplan-Meier survival analysis, log-rank tests, Cox Proportional hazard regression, chi square test, and t-tests. A P value ≤ 0.05 was considered statistically significant. *Results*. Of the 468 African-Americans (60%) in the database, 138 had TNBC; 64 of 318 Caucasians had TNBC. 80% of patients had an annual income of $\leq$ \$20,000. The 5-year overall survival was 77% for African-American women versus 72% for Caucasian women ($P = 0.95$). On multivariate analysis, race/ethnicity had an impact on disease-free survival ($P = 0.027$) but not on overall survival ($P = 0.98$). *Conclusion*. In a predominantly indigent population, race/ethnicity had no impact on overall survival for patients with triple negative breast cancer.

1. Introduction

Breast cancer is the most frequently diagnosed cancer in women and is associated with substantial morbidity and mortality. Breast cancer is a heterogeneous disease with different subtypes that are based upon the expression level of estrogen receptor (ER), progesterone receptor (PR), and HER-2/*neu* receptor (HER-2) [1]. Triple-negative breast cancers (TNBC), are breast tumors characterized by lack of expression of estrogen (ER), progesterone (PR), and HER2*neu* receptors and comprise 15% of all breast cancers. Most TNBC have a basal-like molecular phenotype by gene expression profiling [2, 3]. TNBC also shares clinical and pathologic features with hereditary BRCA1-related breast cancers including lack of ER/PR and HER2*neu*, presence of p53 mutation, basal gene expression patterns, and BRCA1 inactivation by either mutation or pathway dysfunction [4–6]. Most of these tumors are high grade or poorly differentiated tumors [7, 8]. TNBC has been shown to be associated with a poorer prognosis compared to receptor positive breast cancer subtype [9]. TNBC is not responsive to hormonal therapies such as Tamoxifen and aromatase inhibitors nor to inhibitors of HER-2 such as trastuzumab. Whether the poorer outcome with TNBC is the result of the loss of these therapeutic options or is the result of a more aggressive tumor biology or both is unknown.

African-American women have higher breast cancer death rates compared to Caucasian women, despite having a lower incidence of breast cancer [10]. Based on the 2001–2005 data from the Louisiana Tumor Registry, breast cancer mortality was 25.3% for Caucasian females and 40.9% for African-American females. The causes for this disparity in outcomes are not known. Some investigators believe that socioeconomic factors play a significant role in breast cancer disparities, while others speculate that it is the biologic differences that play a central role in outcome disparities. TNBC has been demonstrated to be more prevalent among young African-American females when compared to Caucasian

women and is the basis for the argument in favor of biology as the culprit for breast cancer disparities. However, many of these studies do not adequately control for socioeconomic status (SES). Possibly related to SES is the observation that African American women are less likely to be diagnosed at an early stage when treatment can improve survival. Additional other potentially SES-related factors that may contribute to the survival difference include unequal access to medical care, health insurance status, treatment noncompliance, and socioeconomic status [11].

Louisiana State University Health Sciences Center in Shreveport (LSUHSC-S) is a public hospital and is unique in that the majority of the patients treated are uninsured or receive Medicaid and are of low socioeconomic status. We have previously demonstrated that at LSUHSC-S overall survival of breast cancer patients is similar between African-American and Caucasian women when controlled for SES. However, this study did not specifically evaluate whether such parity of outcome occurred in the subset of patients with TNBC. Our current study is aimed to address the following question: among patients with TNBC who are of similar socioeconomic status and given equal access to medical care, does the survival disparity between the racial/ethnic groups still exist?

2. Methods

A prospectively maintained breast cancer database was created in 1998. Details of this database have previously been reported [12]. Briefly, patients with stage 0 to 3 breast cancer who were treated before October 2008 were accrued and analyzed. We obtained approval to conduct the study from our Institutional Review Board. Of the 803 breast cancer patients in the database, we excluded 17 patients because of the patients belonging to other ethnicities (Hispanics or Asians) or having incomplete data. Of the remaining 786 patients, 468 patients were African-Americans and 318 patients were Caucasians. Triple-negative breast cancers (TNBCs) are defined as tumors that lack estrogen, progesterone, and HER-2 expressions. We identified 202 patients (25.7%) with TNBC. The majority of patients (~90%) were treated at FWCC/LSUHSC-S and the remaining patients were treated at a safety-net hospital, the EA Conway Hospital, a sister public hospital managed by LSUHSC-S. The American Joint Committee on Cancer (AJCC) 6th Edition was used to stage patients [13].

Two Society-of-Surgical-Oncology-(SSO) fellowship trained surgical oncologists performed the surgeries at FWCC/LSUHSC-S. Three general surgeons, each of whom had more than 10 years of surgical experience, performed surgeries at E. A. Conway Hospital. A weekly multidisciplinary tumor board conference was held to discuss all breast cancer cases performed for the previous week. Discussion of care of patients treated at E. A. Conway was conducted via telemedicine conferencing. Attendants of the weekly tumor board included a myriad of specialists (surgical oncologists, medical oncologists, radiation oncologists, radiologists, geneticists, residents, fellows, nurses, researchers, coordinators, and educators).

All treatment and surveillance protocols were standardized in order to ensure study homogeneity. All patients were offered standard treatment protocols for adjuvant and neoadjuvant chemotherapy and radiation therapy. Antiestrogen therapy and herceptin were not used in this cohort. Definitive surgeries included either breast conservation therapy (BCT, lumpectomy with tumor-free margin, sentinel lymph node dissection and/or axillary lymph node dissection, and breast irradiation) or a mastectomy (±axillary lymph node dissection in select cases). After BCT, fractionated megavoltage external beam irradiation (encompassing the whole breast) to a total dose of 50 Gy/25 fractions was administered using tangential treatment portals; the supraclavicular area is irradiated (to the same total dose) when indicated (i.e., presence of disease to four or more axillary lymph nodes). Adjuvant systemic chemotherapy was offered and administered as indicated per current standard of care.

Patient follow-up consisted of a history and physical examination every 3 months for 3 years, every 6 months for years 4 and 5, and annually thereafter. A chest X-ray, mammogram, complete blood count, and liver function tests were obtained annually. Additional radiologic and/or histologic evaluation was performed based on clinical indications. Clinical data were accrued and recorded prospectively and included age at diagnosis, comorbid conditions, stage of disease, treatment protocol, surveillance protocol compliance, cancer recurrence, and death. Compliance with treatment and surveillance protocols was over 90%.

Socioeconomic statuses were assigned to each patient based on two sources: the Internal Revenue Service 2001 ZIP-code-based income tract and the LSU Hospital Computer Service database. These sources did not differ between the two racial/ethnic groups and the data were not combined across methods. The Internal Revenue Service 2001 ZIP-code based income tract reports income as median annual income (MAI) per ZIP code stratified into quintiles based on ten thousand dollar increments. If the percentage of patients falls within 1% of either stratification group, the average of both groups was used to estimate the MAI. Because the 2001 tax year approximates the middle of dates of surgery for our patient population, the data from 2001 was chosen. All patients were assigned an MAI and stratified accordingly.

Our hospital Computer Services database was used to link patients' financial code with their names, medical record numbers, initial dates of diagnosis, and ICD-9 diagnosis code 174.0–174.9. These financial codes were then used to stratify patients into the following subsets: commercial insurance, Medicare, Medicaid, or indigent/free care. Because this database only tracks patients for the past 7 years, only 57% of patients (115) were identified from this database.

The impact of race/ethnicity on the outcome of patients with TNBC breast cancers was assessed by comparing outcomes between Caucasian and African-American women. Asian and Hispanic women comprised less than 5 patients in our large database and therefore were excluded from analysis. Clinical outcomes were then compared to five reports on outcome for patients with TNBC (Table 4) [1, 8, 14–16].

All statistical analyses were performed using MedCalc software (Microsoft, Inc.). The chi-square test was used to

Table 1: Distribution of patient, clinicopathologic, and socioeconomic characteristics of 202 Patients with triple negative breast cancer.

	African-American ($N = 138$) 68%	Caucasian ($N = 64$) 32%	P value
Characteristics			
Mean age years (range)	54 (28–33)	60 (36–87)	0.38
Mean tumor size (cm)	3.39	3.16	0.35
Tumor size distribution			
T1 (28%)	33 (24%)	24 (38%)	
T2 (53%)	76 (55%)	30 (47%)	0.25
T3 (13%)	19 (14%)	7 (11%)	
T4 (6%)	10 (7%)	3 (4%)	
Nodal distribution			
N0 (55%)	71 (51%)	40 (62%)	
N1 (25%)	37 (27%)	14 (22%)	0.5
N2 (15%)	23 (17%)	7 (11%)	
N3 (5%)	7 (5%)	3 (5%)	
Stage distribution			
Stage 1 (21%)	25 (18%)	17 (26%)	
Stage 2 (52%)	73 (53%)	33 (52%)	0.31
Stage 3 (27%)	40 (29%)	14 (22%)	
Tumor grade			
I/II (38%)	40/125 (32%)	29/59 (49%)	0.04
III (62%)	85/125 (68%)	30/59 (51%)	
Definitive surgery			
Breast-conserving Rx (31%)	51 (37%)	12 (19%)	0.01
Mastectomy (69%)	87 (63%)	52 (81%)	
Systemic treatment			
Adriamycin alone (19%)	28 (20%)	11 (17%)	
Adriamycin + Taxane (41%)	60 (44%)	22 (34%)	
Taxane alone (3%)	3 (2%)	3 (5%)	0.33
Hormone therapy alone (3%)	2 (1%)	3 (5%)	
Hormone therapy + chemotherapy (16%)	19 (14%)	14 (22%)	
Others (18%)	26 (19%)	11 (17%)	
Median annual income	\$16,493	\$16,667	
Mean (range) annual income	\$17,873 (\$15,367–\$36,772)	\$21,081 (\$15,795–\$36,787)	<0.001
Financial class			
Commercial (11%)	8/80 (10%)	5/35 (14%)	
Medicare (10%)	7/80 (9%)	4/35 (11%)	0.69
Medicaid (6%)	6/80 (7%)	1/35 (3%)	
Free care (73%)	59/80 (74%)	25/35 (72%)	

analyze categorical data, and the independent samples t-test was used to compare means. Disease-free survival (DFS) was calculated from the date of surgery to the date of first recurrence (local or distant) or date of last follow-up. Overall survival (OS) was calculated from the date of surgery to the date of death from any cause or date of last follow-up.

The Kaplan-Meier survival method and the log-rank test were used to generate and compare survival curves. The Cox proportional hazard regression model was used to perform multivariate analyses. Risk ratios and 95% confidence intervals (CI) were calculated from the model. A P value ≤ 0.05 was considered statistically significant.

3. Results

Two-hundred and two patients with TNBC were identified. This represents approximately 26% (202/786) of all patients in our database. Table 1 demonstrates patient,

clinicopathologic, and socioeconomic characteristics of our cohort. There were 138 African-American women (68%) and 64 Caucasian women (32%) with TNBC representing 29% (138/468) of the African-American women and 20% (64/318) of the total number of Caucasian women in our database. The mean age at diagnosis was 54 years for African-American women and 60 years for Caucasian women ($P = 0.38$), and the mean follow-up time was 52.8 months.

The median annual income by ZIPcode for the entire group of patients with TNBC was \$16,577 (range, \$15,367 to \$36,788). The median annual income was \$16,493 (range: \$15,367 to \$36,772) for African-American women and was \$16,667 (range: \$15,795 to \$36,787) for Caucasian women. The differences between the median incomes were statistically significant ($P < 0.001$) although the magnitude of such differences does not appear to be clinically relevant. All patients resided within geographical areas with reported median annual incomes of \$40,000 or less, and approximately 90% (181/202) were in areas with a reported median annual income of less than or equal to \$30,000. The financial data at the time of diagnosis indicated no difference in the percent of patients with commercial insurance, Medicare, Medicaid, or free care (Table 1).

Of all the clinicopathologic parameters examined, only tumor grade ($P = 0.04$), type of definitive operation ($P = 0.01$), and median annual income ($P < 0.001$) were significantly different between the two racial/ethnic groups. Mean age at diagnosis ($P = 0.38$), mean tumor size ($P = 0.35$), tumor size distribution ($P = 0.25$), nodal distribution ($P = 0.50$), stage distribution ($P = 0.31$), receipt of adjuvant therapy ($P = 0.33$), and financial class distribution ($P = 0.69$) were not significantly different between the two racial/ethnic groups (Table 1).

Overall, locoregional recurrences occurred in 13.8% (28 of 202 patients) of patients. The locoregional recurrence rate for African-American women was 20% (13/64) for Caucasian women ($P = 0.08$). Additionally, 41/202 (20.3%) of the entire TNBC cohort died by the time of last follow-up (December 2009) with a mortality rate of 20% (28/138) for African-American women and 20% (13/64) for Caucasian women ($P = 0.85$).

To discern the impact of race/ethnicity on the outcome for patients with TNBC, we evaluated OS and DFS between African-American and Caucasian women (Figures 1 and 2). In our previous studies, we demonstrated that neither OS nor DFS was significantly different between the two racial/ethnic groups, specifically in a large cohort of 786 patients with stage 0–3 breast cancers and a cohort of 375 patients with ER-negative tumors. Within the ER-negative tumors, we were able to identify a significant proportion of patients to have TNBC (54%). Therefore, this cohort was evaluated separately.

Similar to our previous findings, in the subgroup of women with TNBC, we found no statistically significant difference in DFS or OS between the two racial/ethnic groups. The 5-year DFS was 66% for African-American women and 50% for Caucasian women; the median DFS was 99 months for African-American women and 60 months for Caucasian women ($P = 0.16$) (Figure 1). The 5-year OS was 77% for African-American women and 72% for Caucasian women; the median OS was 138 months for African-American women and 64 months for Caucasian women ($P = 0.95$) (Figure 2).

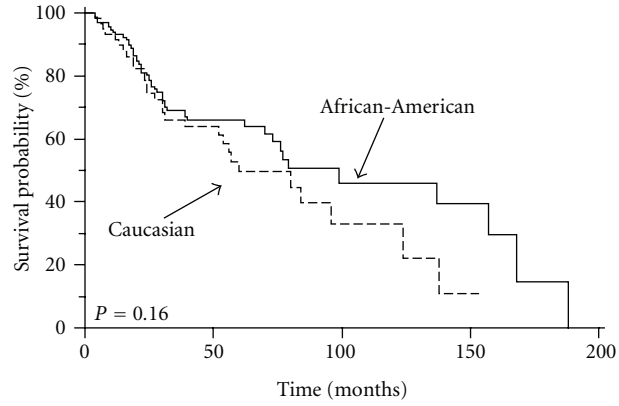

Figure 1: Effect of race/ethnicity on disease-free survival for 202 patients with triple-receptor negative breast cancer: shown is the DFS for 202 African-American and Caucasian patients with TNBC as described in section 2. The 5-year DFS was 66% for African-American women and 50% for Caucasian women ($P = 0.16$).

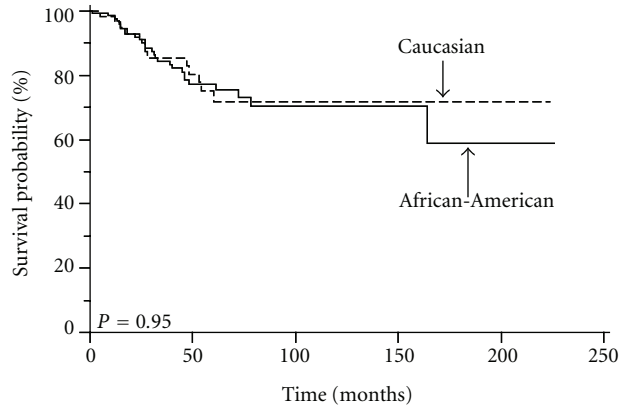

Figure 2: Effect of race/ethnicity on overall survival for 202 patients with triple-receptor negative breast cancer: shown is the OS for 202 African-American and Caucasian patients with TNBC as described in section 2. The 5-year OS was 77% for African-American women and 72% for Caucasian women ($P = 0.95$).

The Cox proportional hazard model was used to compare race/ethnicity, age at diagnosis, tumor grade, median income, T-stage, and N-stage for risk of cancer recurrence and overall survival (Tables 2 and 3). Note that although race/ethnicity was an independent predictor for DFS ($P = 0.027$), it was not an independent predictor for OS ($P = 0.98$). Clinical independent predictors for DFS were T-stage ($P = 0.001$) and N-stage ($P = 0.05$). Only N-stage ($P = 0.01$) was an independent predictor for OS.

Suboptimal results run the risk of masking any potential significant differences in outcomes between African-American women and Caucasian women. Therefore, we compared our outcomes for women with TNBC with outcomes reported in the literature [1, 8, 14–16]. In selected published

Table 2: Effect of race/ethnicity on cancer recurrence for patients with triple-receptor-negative breast cancer (Cox proportional hazard model).

	Relative Risk	95% CI	*P* value
Race/ethnicity	1.84	1.07 to 3.14	0.027
Age at diagnosis	1.00	0.98 to 1.03	0.80
Grade	1.20	0.71 to 2.03	0.49
Income level	0.87	0.54 to 1.40	0.58
T-stage	1.68	1.22 to 2.30	0.001
N-stage	1.29	1.0 to 1.68	0.05

Table 3: Effect of race/ethnicity on overall survival for patients with triple-receptor negative breast cancer (Cox proportional hazard model).

	Relative risk	95% CI	*P* value
Race/ethnicity	1.00	0.48 to 2.06	0.98
Age at diagnosis	1.01	0.98 to 1.05	0.41
Grade	1.89	0.89 to 3.97	0.09
Income level	1.15	0.63 to 2.09	0.65
T-stage	1.43	0.95 to 2.17	0.09
N-Stage	1.53	1.09 to 2.16	0.01

Table 4: Comparison of clinical outcomes for patients with triple-receptor negative breast cancer.

	Overall survival (%)	Disease free survival (%)
Chu (FWCC)	75	60; 66(AA), 50 (C)
Haffty	80	72
Bauer	77	—
Kyndi	50 (high-risk cohort)	—
Lund	59.6	30.8
Dawood	71 (3-yr OS)	68 (AA), 62 (C)

FWCC: Feist-Weiller Cancer Center, AA: African-american, and C: Caucasian.

series, the 5-year OS rate ranges from 59.6% to 80% and the 5-year DFS rate ranges from 30.8% to 72%. Our figures compare favorably with these historic figures (Table 4).

4. Discussion

African-American women have a lower incidence of breast cancer but a higher breast cancer mortality rate when compared to Caucasian women [17–19]. Such disparity has been the focus of recent debates. Confounding variables make it difficult to establish the exact nature of such disparity. While some investigators attribute it to differences in income and social status, which affect access to and receipt of treatment, others accredit it to racial/ethnic differences in tumor biology and responsiveness to treatment [10–12, 14, 17, 18, 20–23]. Race/ethnicity as an independent predictor of survival in breast cancer has been reported in several studies, although most do not adequately control for socioeconomic status (SES) and/or tumor subtype (i.e., TNBC) [16].

In our initial study of 786 patients with operable breast cancer (stage 0–III), we demonstrated that race/ethnicity had no impact on outcome when equal access was rendered, regardless of patients' financial statuses. In that study, outcomes at LSUHSC-S rivaled those reported by the National Cancer Data Base (NCDB). These results were achieved in a population that has historically been associated with poorer outcomes; over two-thirds of our patients were classified as having either Medicaid or free care and the median annual income for both groups was less than $17,000 [12].

A potential confounder of the above study was an imbalance of the different breast cancer subtypes between African-American and Caucasian women. We noted that African-American women had a significantly higher proportion of ER-negative tumors than Caucasian women. To address this, we separately evaluated outcomes for 375 patients with ER-negative breast cancers to determine whether there was disparity between the two racial/ethnic groups [11]. However, similar to the results of our initial study, we found that there were no significant differences in breast cancer mortality rates between African-American and Caucasian women who had ER-negative tumors [11]. Again, these results were achieved in a relatively homogenous cohort of patients with low SES.

One of the limitations of our ER-negative study was that it did not delineate the proportion of patients who had TNBC. TNBC is used by clinicians in reference to the basal-like subtype of breast cancer although only 85% of TNBCs are basal-like. Numerous studies have shown that TNBC is associated with a decreased overall survival when compared to receptor-positive tumors and that TNBC is more prevalent among African American women [24]. This fact has popularly been thought of as being one of the major contributors of disparity in outcomes between African-American women and Caucasian women [24]. However, our results demonstrated that even within the TNBC cohorts race/ethnicity had no impact on outcome. These results were obtained despite African-American women having had a significantly higher tumor grade than Caucasian women (grade 3 = 68% versus 51%; $P = 0.04$) and that TNBC was more predominant among African-American women than Caucasian women.

The principle that race/ethnicity should have no impact on outcome for patients with TNBC was further reinforced by a study by Dawood et al. [8]. In this study of nearly 500 patients who were treated with primary systemic chemotherapy followed by definitive surgery, neither pathologic complete response rates (pCR) nor survival outcomes differ between the two racial/ethnic groups [8].

The five-year overall survival rate for all breast cancer subtypes is approximately 89% and this rate drops precipitously for patients with TNBC (77% to 80%) [1, 16]. Our data seemed to support these results. What is unique about our cohort is that we were able to control for socioeconomic status and receipt of systemic therapy, thus eliminating any potential socioeconomic biases.

Based on our previous and current data and findings, we can conclude that disparity in survival between African-American females and Caucasian females can be mitigated

when all patients are provided with the same standard of care breast cancer treatment. This paradigm seems to be applicable for wide variety of breast cancer, including those with TNBC. In addition, our data do not support the idea of biological differences in tumor subtypes between compared and African-Americans. The higher proportion of younger African-Americans developing TNBCs compared with Caucasians may still contribute to the overall worse outcomes, even though the responses to treatment are similar.

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Hindawi Publishing Corporation
International Journal of Breast Cancer
Volume 2012, Article ID 740353, 9 pages
doi:10.1155/2012/740353

Research Article

PKCα and ERβ Are Associated with Triple-Negative Breast Cancers in African American and Caucasian Patients

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Received 22 July 2011; Accepted 18 December 2011

Academic Editor: Thelma Hurd

Although the incidence of breast cancer in the United States is higher in Caucasian women compared with African American women, African-American patients have more aggressive disease as characterized by a higher percentage of triple-negative breast cancers (TNBCs), high-grade tumors, and a higher mortality rate. PKCα is a biomarker associated with endocrine resistance and poor prognosis and ERβ is emerging as a protective biomarker. Immunohistochemical analysis of ERβ and PKCα expression was performed on 198 formalin-fixed paraffin-embedded primary infiltrating ductal carcinomas from 105 African-American and 93 Caucasian patients. PKCα is positively correlated with TNBC in patients of both races and with high tumor grade in African-American patients. Patients with TNBC express less nuclear ERβ compared with all other subtypes. We find no difference in frequency or intensity of PKCα or ERβ expression between African-American and Caucasian patients. PKCα and ERβ are discussed as potential therapeutic targets for the treatment of patients with TNBC.

1. Introduction

Although African American women have a lower incidence of breast cancer than Caucasians, repeated studies have shown that they suffer from more aggressive disease characterized by diagnosis at an earlier age, later stage, higher grade, and greater mortality [1–4]. While socioeconomic factors contribute in part to this disparity in survival, they do not account for all differences noted between these two racial groups [3, 5, 6]. In particular, premenopausal African American women present with a higher incidence of triple-negative breast cancer (TNBC), a molecular subtype that has limited targeted therapeutic options [3, 7]. Current investigations are focused upon the identification of new therapeutic targets specific to the aggressive TNBC form of breast cancers found more frequently in young African American women and the development of more effective treatment modalities.

One potential biomarker contributing to the aggressive nature of this disease in African American women is protein kinase Cα (PKCα). PKC is a serine/threonine protein kinase family of enzymes comprised of at least 12 isozymes that regulate numerous cellular functions [8]. PKCα in particular is involved in cell migration, apoptosis, differentiation, and proliferation and plays a critical role in several disease processes including cancer [9]. Overexpression of PKCα is a marker of poor prognosis of breast cancers and is associated with antiestrogen resistance, ERα-negative tumors, and tumor aggressiveness [10–13]. Therefore, differential expression of PKCα may underpin the observed racial disparity in breast cancer and may be a potential therapeutic target.

Although the clinical significance of estrogen receptor β (ERβ) in breast cancer is not yet firmly established, differential expression of ERβ in breast cancers between racial groups may provide further insight [14]. Recent reports suggest ERβ isoform expression and subcellular localization may correlate with endocrine response and breast cancer outcome [15–18]. When coexpressed with ERα, ERβ appears to dampen the proliferative program of ERα bound to estradiol and is generally considered to be antiproliferative [19, 20]. However, understanding the effects of ERβ is complicated by the fact that several ERβ isoforms exist, named ERβ 1–5 [21], and they have different implications in breast cancer development and progression. While most studies conclude that ERβ confers a good prognosis [16] and is predictive of response to tamoxifen [22], others report association with more aggressive disease and decreased overall survival [15, 23]. The accumulated evidence thus far indicates that although ERβ expression may predict good prognosis, the expression in relation to breast cancer subtypes and subcellular localization may influence the effect upon prognosis.

Since African American patients have more aggressive disease and lower overall survival than Caucasian patients, we tested the hypothesis that breast cancers from African American patients have higher PKCα expression and lower nuclear ERβ expression and/or higher cytoplasmic ERβ expression. We analyzed 198 primary invasive ductal carcinomas from African American and Caucasian patients for expression of PKCα and ERβ to determine whether differential expression of PKCα and/or localization of ERβ differed in breast cancers from African American and Caucasian women.

2. Materials and Methods

2.1. Patient Population. PKCα and ERβ expression was determined by immunohistochemical (IHC) staining of 198 formalin-fixed, paraffin-embedded primary infiltrating ductal carcinomas from 105 African American and 93 Caucasian patients from the Department of Pathology, Rush University Medical Center. Complete clinicopathological characteristics were obtained from the pathology reports and the number of evaluable patients for each characteristic is given in Table 1. This study was approved by the Institutional Review Board at Rush University Medical Center and the University of Illinois at Chicago. All specimens were obtained retrospectively and posed minimal risk; therefore informed consent was waived.

2.2. Immunohistochemical Staining for PKCα and ERβ. IHC was performed on 5 μM sections of formalin-fixed paraffin-embedded tissue with the Ventana Benchmark automated staining platform using the iView DAB detection kit according to company protocol using CC1 Standard antigen retrieval. The PKCα antibody (rabbit polyclonal, Santa Cruz Biotechnology, sc-208) was previously validated [11] and used at a dilution of 1 : 200 and incubated at 37°C for 30 minutes. The ERβ mouse monoclonal antibody 14C8 (Novus Biologicals Inc., Littleton, CO) was previously validated [24] and used at a 1 : 100 dilution and incubated for 30 min with HRP-rabbit Envision. This ERβ monoclonal antibody recognizes all isoforms of ERβ known to be expressed in breast cancer. Frequency and intensity of PKCα and ERβ staining of all tumor cells on each slide were scored on a scale of 0 to 4 without knowledge of clinical patient data. Frequency of positive staining in less than 1% of tumor cells was scored as 0, 1%–10% as 1, 11% to-35% as 2, 36%–70% as 3, and over 70% as 4. A composite score is also reported based on the Allred scoring system which is a sum of the frequency and intensity scores yielding numerical values from 0 to 8 [25].

2.3. Statistical Analysis. We analyzed the expression of ERβ and PKCα by comparing them with prognostic factors such as age, tumor grade, subtypes, and race. Chi-square tests were used for testing association between race and prognostics factors. For univariate analysis, nonparametric tests were conducted for nonnormal data. Wilcoxon Rank Sum test was performed for two groups' comparisons and Kruskal-Wallis test was performed for more than two groups' comparisons. Median, minimum, and maximum along with P values were reported. For multivariate analysis, to take into account prognostic factor effects, general linear regression was conducted. The interaction effects of race by prognostic factors were examined. P values were reported based on the type III sum of squares. P value < 0.05 was considered to be statistically significant. Freq, UNIVARIATE, NPAR1WAY, and GLM procedures in SAS version 9.2 (Cary, NC) were used in these analyses.

3. Results

3.1. PKCα and ERβ Expression in Tumors from African American and Caucasian Breast Cancer Patients. Since PKCα overexpression and ERβ expression and localization are reported to be associated with more aggressive breast cancers, we first asked whether these markers are differentially expressed based on race. Upon examination of breast cancers from 93 Caucasian and 105 African American patients, we evaluated both frequency and intensity of PKCα and ERβ immunostaining in addition to subcellular localization of ERβ. Cases exhibiting both high and low frequency and intensity of PKCα and ERβ were evident including both nuclear and cytoplasmic ERβ localization (Figures 1(a) and 1(b)). Examination of the total patient population (Table 1) revealed that 78% of all patients were positive for PKCα cytoplasmic staining. When patients were stratified by race, 76% of tumors from Caucasian patients and 79% of African American patients stained positively for PKCα. There was no statistical difference in the incidence of PKCα expression between races. Sixty-nine percent of patients stained positive for ERβ including nuclear and/or cytoplasmic staining. Of these ERβ positive cases, 57% exhibited only nuclear ERβ staining, 20% only cytoplasmic staining, and 23% both nuclear and cytoplasmic staining. When stratified by race, there is no statistical difference in the incidence of ERβ expression. As anticipated, there is a statistically significant

Table 1: Clinicopathological characteristics of 198 infiltrating ductal carcinomas.

	Caucasian *N* (%)	African American *N* (%)	Total *N* (%)	*P* value*
PKCα+	71 (76)	83 (79)	154 (78)	0.648
PKCα−	22 (24)	22 (21)	44 (22)	
ERα+	63 (68)	55 (52)	118 (60)	0.028**
ERα−	30 (32)	50 (48)	80 (40)	
ERβ+	65 (70)	72 (69)	137 (69)	0.847
ERβ−	28 (30)	33 (31)	61 (31)	
ERβ+ (nuc + cyto)	11 (17)	21 (29)	32 (23)	0.221
ERβ+ (nuc)	41 (63)	37 (51)	78 (57)	
ERβ+ (cyto)	13 (20)	14 (19)	27 (20)	
ERα+/ERβ+	49 (53)	40 (38)	89 (45)	0.108
ERα+/ERβ−	14 (15)	15 (14)	29 (15)	
ERα−/ERβ+	16 (17)	32 (30)	48 (24)	
ERα−/ERβ−	14 (15)	18 (17)	32 (16)	
Subtype#				0.205
Luminal A	32 (35)	26 (26)	58 (30)	
Luminal B	30 (33)	29 (28)	59 (31)	
Her2+	13 (14)	18 (18)	31 (16)	
TNBC	16 (18)	29 (28)	45 (23)	
Grade†				0.068
1	6 (13)	6 (8)	12 (10)	
2	21 (46)	21 (29)	42 (35)	
3	19 (41)	46 (63)	65 (55)	
Lymph node+	28 (35)	52 (58)	80 (47)	0.0024**
Lymph node−	53 (65)	38 (42)	91 (53)	
Tumor size Mean (SD)	2.17 (1.47)	2.97 (1.87)	2.60 (1.73)	0.0007**
Age				0.064
<50	28 (30)	45 (43)	73 (37)	
≥50	65 (70)	60 (57)	125 (63)	

*All *P* values were calculated using the Chi-square test. **$P < 0.05$; #Five patients categorized as ER−/PR+/Her2− were not assigned to a subtype category (3 African American, 2 Caucasian patients).
†Tumor grade was available on 46/93 Caucasian patients and 73/105 African American patients.

difference in ERα expression between races reflecting the higher proportion of ERα-negative tumors in the African American patient population. We also observed larger tumors and more lymph node positive cases in the African American population. When the intensity and frequency of PKCα and ERβ was compared by race, there was no difference in IHC staining between breast cancers from African American and Caucasian patients (Tables 2(a) and 2(b)).

3.2. PKCα Expression in ERα-Negative and Triple-Negative Breast Cancers. We and others previously reported the inverse relationship between PKCα and ERα expression [12, 26, 27]. Upon stratification by race (Table 3(a)), the intensity of PKCα expression achieves statistical significance in the African American patients, whereas the frequency of expression does not. Conversely in the Caucasian patients, PKCα frequency of expression achieves statistical significance, whereas intensity of staining does not. The composite score as determined by the sum of frequency and intensity achieves statistical significance only in the African American population. When Caucasian and African American patient populations are combined, there is a statistically significant inverse relationship between PKCα and ERα frequency and intensity of expression (Table 3(b)).

We next examined PKCα expression stratified by breast cancer subtype categorized as luminal A (ERα+/PR+/Her2−), luminal B (ERα+/PR+/Her2+), HER2 (ERα−/PR−/Her2+), and triple-negative breast cancer (TNBC, ERα−/PR−/Her2−) based solely on receptor expression as determined by IHC. There is a strong association of PKCα expression and breast cancer subtypes ($P < 0.001$) that is maintained when stratified by race (see supplemental

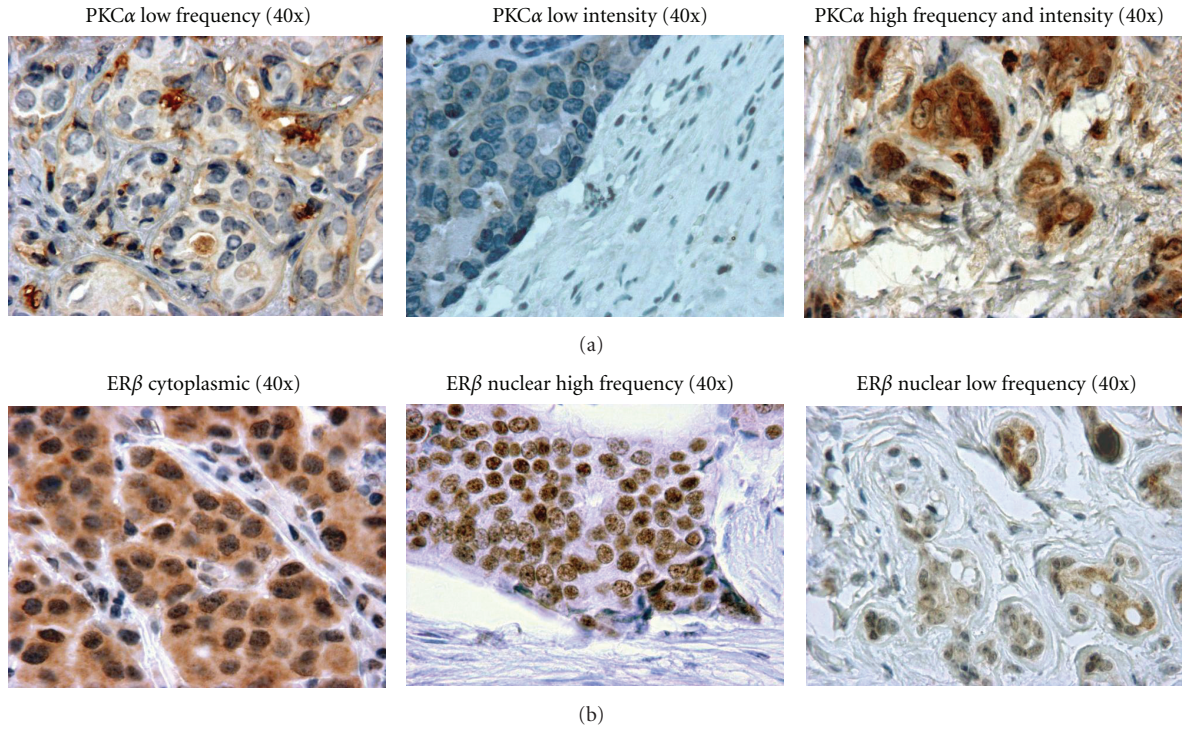

Figure 1: (a) Expression of PKCα (brown immunoperoxidase stain, blue hematoxylin counterstain). (b) Expression of ERβ (brown immunoperoxidase stain, blue hematoxylin counterstain).

Table 2: (a) PKCα expression is similar in African American and Caucasian patients. (b) ERβ expression and subcellular localization is similar in African American and Caucasian patients.

(a)

Outcome	Median (minimum, maximum)		P value*
	AA	Caucasian	
PKCα (freq)	2 (0,4)	2 (0,4)	0.46
PKCα (int)	2 (0,4)	1 (0,4)	0.52
PKCα (sum)	4 (0,8)	4 (0,8)	0.49

* P value based on the Wilcoxon rank-(sum) test.

(b)

Outcome	Median (minimum, maximum)		P value*
	AA	Caucasian	
ERβ freq(n)	2 (0, 4)	2 (0, 4)	1.00
ERβ int (n)	1 (0, 4)	1 (0, 4)	0.76
ERβ freq (c)	3 (1, 4)	3 (2, 4)	0.83
ERβ int (c)	1 (1, 3)	1 (1, 4)	0.84

* P value based on Wilcoxon rank-Sum test. n: nuclear; c: cytoplasmic.

Tables 1(a) and 1(b) in Supplementary Material available online at doi:10.155/2012/740353). Since the incidence of TNBC is higher in African American patients compared to Caucasian patients, we asked whether PKCα expression is associated with TNBCs. Combining all patients we find a strong association of PKCα expression with the TNBC subtype compared to all other subtypes (Table 4(a)). When stratified by race, frequency of PKCα expression but not intensity is similarly associated with the TNBC subtype in African American and Caucasian patients (Table 4(b)).

To determine whether TNBC is an independent predictor of PKCα, we performed general regression analysis with adjustment for tumor grade, patient age, lymph node status, and tumor size and found that the frequency and intensity of PKCα expression no longer correlates with TNBC (freq, $P = 0.262$; int, $P = 0.957$). This prompted us to ask whether PKCα expression correlates with the other known independent predictors of TNBC (tumor grade, patient age, lymph node status, and tumor size). Combining all patients, we find that grade 3 tumors have the highest frequency and intensity of PKCα expression (Table 5(a)); however there is no correlation of PKCα with patient age, tumor size, or lymph node status. Interestingly, when the patients are stratified by race, the positive relationship of PKCα and tumor grade is statistically significant only in the African American patients, but not in tumors from Caucasian patients (Table 5(b)).

In our population of TNBC cases, we found a statistically significant correlation with tumor grade ($P < 0.0001$), patient age ($P = 0.002$), and tumor size ($P = 0.004$); however there is no correlation between TNBC and lymph node status ($P = 0.1334$).

3.3. ERβ Expression and Localization in Triple-Negative Tumors. Since both expression and subcellular localization of ERβ are reported to influence clinical outcome and

Table 3: (a) Relationship of ERα status and PKCα expression based on race. (b) ERα status and PKCα expression is inversely related.

(a)

Race	Outcome	Median (minimum, maximum) ER(−) ($N = 80$)	Median (minimum, maximum) ER(+) ($N = 118$)	P value*
AA	PKC (freq)	2 (0, 4)	2 (0, 4)	0.09
	PKC (int)	2 (0, 4)	1 (0, 4)	0.01††
	PKC (sum)	5 (0, 8)	4 (0, 8)	0.02†
Caucasian	PKC (freq)	3 (0, 4)	2 (0, 4)	0.02†
	PKC (int)	1.5 (0, 4)	1 (0, 4)	0.38
	PKC (sum)	4.5 (0, 8)	3 (0, 7)	0.07

* P value based on the Wilcoxon Rank (sum) Test. † P value <0.05; †† P value <0.01.

(b)

Outcome	Median (minimum, maximum) ER(−)	Median (minimum, maximum) ER(+)	P value*
PKCα (freq)	3 (0, 4)	2 (0, 4)	0.004††
PKCα (int)	2 (0, 4)	1 (0, 4)	0.012†
PKCα (sum)	5 (0, 8)	3 (0, 8)	0.002††

* P value based on the Wilcoxon Rank (sum) Test. † P value <0.05; †† P value <0.01.

Table 4: (a) PKCα expression is higher in triple-negative breast cancers compared to other subtypes. (b) Correlation of PKCα expression and triple-negative breast cancer is similar in African American and Caucasian patients.

(a)

Outcome	Median (minimum, maximum) TNBC ($N = 45$)	Median (minimum, maximum) All other subtypes ($N = 153$)	P value*
PKCα (freq)	3 (0, 4)	2 (0, 4)	0.001†††
PKCα (int)	2 (0, 4)	1 (0, 4)	0.010†
PKCα (sum)	5 (0, 8)	4 (0, 8)	0.001†††

* P value based on the Wilcoxon Rank-(sum) test. † P value <0.05; †† P value <0.01; ††† P value <0.001.

(b)

Race	Outcome	Median (minimum, maximum) TNBC ($N = 45$)	Median (minimum, maximum) All other subtypes ($N = 153$)	P value*
AA ($N = 105$)	PKC (freq)	3 (0, 4)	2 (0, 4)	0.019†
	PKC (int)	2 (0, 4)	1 (0, 4)	0.061
	PKC (sum)	5 (0, 8)	4 (0, 8)	0.014†
Caucasian ($N = 93$)	PKC (freq)	3 (0, 4)	2 (0, 4)	0.010††
	PKC (int)	2 (0, 3)	1 (0, 4)	0.087
	PKC (sum)	5 (0, 7)	3 (0, 8)	0.018†

* P value based on the Wilcoxon Rank-(sum) test. † P value <0.05; †† P value <0.01.

response to therapy, we examined whether ERβ is differentially expressed in the various breast cancer subtypes. Upon stratification of all patients by subtype as previously categorized, we find there is no association of ERβ with any particular subtype (supplemental Table 2). However when we compared TNBC to all other subtypes, we find that nuclear ERβ expression is lower in TNBC compared to all other subtypes (Table 6). Interestingly when patients are stratified by age (<50 yrs versus ≥50 yrs), the inverse relationship of nuclear ERβ with TNBC is statistically significant only in the younger patients (freq, $P = 0.021$), whereas when stratified by race, statistical significance is

Table 5: (a) High tumor grade correlates with elevated PKCα expression. (b) PKCα and tumor grade is correlative in AA but not in Caucasian patients.

(a)

Outcome	Median (minimum, maximum)			P value*
	Grade = 1 ($N = 12$)	Grade = 2 ($N = 42$)	Grade = 3 ($N = 65$)	
PKCα (freq)	0.50 (0, 3)	2.00 (0, 4)	3.00 (0, 4)	0.010††
PKCα (int)	0.50 (0, 3)	1.00 (0, 3)	2.00 (0, 4)	0.012†
PKCα (sum)	1.00 (0, 6)	3.50 (0, 7)	5.00 (0, 8)	0.004††

* P value is based on the Kruskal-Wallis Test. † P value <0.05; †† P value <0.01.

(b)

Race	Outcome	Median (minimum, maximum)			P value*
		Grade=1 ($N = 6$)		Grade=3 ($N = 46$)	
AA ($N = 73$)	PKCα (freq)	0 (0, 1)	2 (0, 3)	2 (0, 4)	0.007††
	PKCα (int))	0 (0, 3)	1 (0, 3)	2 (0, 3)	0.017†
	PKCα (sum)	0 (0, 4)	3 (0, 5)	5 (0, 7)	0.003††
		Grade = 1 ($N = 6$)	Grade = 2 ($N = 21$)	Grade = 3 ($N = 19$)	
Caucasian ($N = 46$)	PKCα (freq)	2.5 (0, 3)	2 (0, 4)	3 (0, 4)	0.248
	PKCα (int)	1.5 (0, 3)	2 (0, 3)	2 (0, 4)	0.277
	PKCα (sum)	4.0 (0, 6)	4 (0, 7)	5 (0, 8)	0.169

* P value is based on the Kruskal-Wallis Test. † P value <0.05; †† P value < 0.01.

Table 6: Nuclear ERβ expression is lower in triple-negative patients.

Outcome	Median (minimum, maximum)		P value*
	TNBC ($N = 45$)	All other subtypes ($N = 153$)	
ERβ (freq) (n)	0 (0, 4)	2 (0, 4)	0.022†
ERβ (int) (n)	0 (0, 4)	1 (0, 4)	0.024†
ERβ (freq) (c)	3 (1, 3)	3 (2, 4)	0.079
ERβ (int) (c)	1 (1, 3)	1 (1, 4)	0.378

* P value is based on the Wilcoxon Rank-(sum) test. n: nuclear; c: cytoplasmic;
† P value < 0.05.

achieved only in Caucasian patients (freq, $P = 0.023$; int, $P = 0.015$) (results not shown). No association between ERβ and tumor grade was found.

4. Discussion

This is the first report to our knowledge to examine PKCα and ERβ protein expression using IHC comparing breast cancers from Caucasian and African American patients. We chose to examine the expression of these two biomarkers since both are known to be associated with endocrine response and African American patients have a higher incidence of endocrine-resistant breast cancer. PKCα expression is inversely related to ERα status [12, 13, 27], associated with more aggressive breast cancers [13] and endocrine resistance [11, 12]. Although there is less clarity regarding the clinical relevance of ERβ, with the availability of more reliable ERβ antibodies, the current consensus is that ERβ expression is associated with better prognosis [28], whereas cytoplasmic localization of the ERβ2 isoform may indicate worse prognosis [17]. Earlier studies that utilized ERβ mRNA expression in breast cancers yielded conflicting findings correlating ERβ expression with good prognosis while others report association with poor prognosis [29, 30]. Although we find no difference in the expression level of PKCα and ERβ comparing the two races, we find a highly significant association of PKCα with TNBCs (Table 4(a)). Multivariate analysis revealed that the association of PKCα expression with higher tumor grade is likely to account for the significant association of PKCα with TNBC since PKCα

does not correlate with patient age, tumor size, or lymph node status.

The African American patients in this study exhibit a higher incidence of TNBC (28% versus 18%) and more grade 3 tumors (63% versus 41%) (Table 1). This is an intriguing finding that presents a potential therapeutic opportunity since there are few treatment options available for this aggressive breast cancer subtype. PKCα was targeted in breast cancer patients using the antisense compound Affinitak [31]; however since the patients were not preselected for high tumor PKCα expression, the response to treatment was modest. We speculate that preselection of patients with TNBC with high-grade tumors in addition to elevated PKCα expression may improve the response rate to a PKCα-directed therapy. Another potential therapeutic approach may be to revisit the administration of estradiol treatment [32, 33]. Prior to the introduction of tamoxifen, high dose estrogen and diethylstilbestrol (DES) was used to treat breast cancers with similar response rates as tamoxifen, but with greater side effects [32, 34]. A recent phase 2 randomized trial was conducted comparing 2 doses of estrogen (6 mg and 30 mg) in patients with metastatic disease resistant to aromatase inhibitor therapy [35]. The majority of these patients were ERα positive and the clinical benefit rate of 28-29% was similar between the two dosing regimens, whereas the number of adverse events was much lower with the 6 mg estrogen dose. With the completion of this phase 2 study, we propose that the 6 mg estrogen dose be tested in patients with PKCα-overexpressing TNBCs. In our T47D/PKCα xenograft preclinical model, we reported complete tumor regression following 17β-estradiol (E2) administration [36] and subsequently determined that ERα is likely to be required for E2-triggered tumor regression. Interestingly, our preliminary studies suggest that it is extranuclear and not nuclear ERα that may be most important for mediating the inhibitory signal [37]. TNBCs by definition do not express nuclear ERα; however pathologists do not routinely score extranuclear ERα since optimal clinical IHC methods for detection of extranuclear or membrane ERα have not yet been developed. It is possible that a subset of TNBCs may in fact express extranuclear ERα. With the recent focus on the clinical significance of membrane and extranuclear ERα, detection methods for clinical use are likely to soon become available [38]. We propose further investigation is warranted to determine whether the PKCα/extranuclear ERα pathway is a feasible therapeutic target in TNBCs.

The first study to address the role of ERβ expression and racial disparity reported a greater decrease in the protective ERβ in breast cancers in African American patients compared with their matched adjacent normal tissue than levels found in Caucasian patients [39]. In a follow-up study using isoform-specific ERβ primer-probe pairs, these investigators reported higher ERβ isoform expression in ERα-negative breast cancers in African American patients than in Caucasian patients [40]. This finding is in agreement with our results that African American patients have a higher percentage of ERα-negative/ERβ-positive breast cancers (Table 1, Caucasian, 17% ER$\alpha-$/ER$\beta+$, African American, 30% ER$\alpha-$/ER$\beta+$). Interestingly patients with ERα-negative/ERβ-positive breast cancers are associated with increased survival compared to patients with ERα-negative/ERβ-negative breast cancers [41], suggesting that these ERα-negative patients would benefit from tamoxifen treatment. Although we hypothesized that African American patients would have higher cytoplasmic ERβ expression, in fact we find no difference in the level of cytoplasmic ERβ comparing the two races (Table 2(b)). However 29% of African American patients exhibit both nuclear and cytoplasmic ERβ expression whereas only 11% of Caucasian patients express ERβ in both subcellular locations (Table 1). The finding that nuclear ERβ is not associated with TNBC supports the observation that nuclear localization of ERβ is associated with better prognosis (Table 6(b)). However, since the 14C8 antibody recognizes all isoforms of ERβ, it is not possible to determine the specific presence and localization of ERβ2, the isoform reported to be associated with worse prognosis when localized to the cytoplasm [17]. Therefore, the significance of the subcellular distribution of ERβ with respect to prognosis cannot be determined in our study.

For the first time this study examined the association of two potential prognostic biomarkers, PKCα and ERβ, comparing African American and Caucasian patient populations. A significant limitation of our study is that we did not have access to treatment or follow-up information on these patients; therefore it was not possible to determine whether these biomarkers are associated with response to therapy, time to progression, or overall survival. Further investigation is warranted to determine the utility of PKCα as a potential therapeutic target and ERβ as a potential biomarker for tamoxifen therapy in ERα-negative and TNBCs in patients of all races.

5. Conclusions

Our findings suggest that PKCα is a potential therapeutic target for the treatment of ERα-negative disease, TNBCs, and high-grade tumors. Whereas lack of nuclear ERβ in TNBCs may be a biomarker of poor prognosis, further investigation is warranted to determine the significance of ERβ subcellular localization. While TNBCs occur more frequently in African American patients, all patients that present with this breast cancer subtype may benefit from the clinical application of these biomarkers. Further investigation into these potential therapeutic and prognostic approaches is warranted.

Abbreviations

E2: 17β-estradiol
ER: Estrogen receptor
IHC: Immunohistochemistry
PKC: Protein kinase C
TNBC: Triple-negative breast cancer.

Conflict of Interests

The authors declare they have no competing interests.

Acknowledgments

This study was supported by pilot project funds provided by NIH/NCI P50 CA106743 and in part by NIH/NCI R01 CA122914 to D. A. Tonetti. This project was supported by the University of Illinois at Chicago (UIC) Center for Clinical and Translational Science (CCTS), Award no. UL1RR029879 from the National Center For Research Resources. The authors thank Dr. Jonna Frasor for critical reading of the paper and helpful suggestions. Special thanks to Dr. Richard Warnecke for many helpful discussions and support. They are grateful to Sybille Lupee for performing excel sheet data sorting.

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Hindawi Publishing Corporation
International Journal of Breast Cancer
Volume 2012, Article ID 385978, 7 pages
doi:10.1155/2012/385978

Review Article

Triple-Negative Breast Cancer: An Update on Neoadjuvant Clinical Trials

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Received 18 July 2011; Revised 13 October 2011; Accepted 13 October 2011

Academic Editor: Quyen D. Chu

Triple-negative breast cancer (TNBC) is an aggressive malignancy with a poor prognosis despite the high rates of response to chemotherapy. This scenario highlights the need to develop novel therapies and/or treatment strategies to reduce the mortality associated with TNBC. The neoadjuvant setting provides a model for rapid assessment of treatment efficacy with smaller patient accruals and over shorter periods of time compared to the traditional adjuvant setting. In addition, a clear surrogate endpoint of improved survival, known as pathologic complete response, already exists in this setting. Here, we review current data from completed and ongoing neoadjuvant clinical trials for TNBC.

1. Introduction

Triple-negative breast cancer (TNBC) is defined histologically as invasive carcinoma of the breast that lacks staining for estrogen receptor, progesterone receptor, and HER2/neu. Approximately 15–20% of breast cancers illustrate this phenotype [1]. TNBC is associated with high proliferative rates, early recurrence, and poor survival rates. This aggressive disease is insensitive to widely used targeted therapies such as trastuzumab and endocrine therapies, tamoxifen and aromatase inhibitors, which have been effective at reducing breast cancer mortality. Younger women and women of African descent have a high prevalence of TNBC [1]. There are limited and often ineffective therapeutic treatment options for patients with stage IV TNBC.

2. The Concept of Neoadjuvant Chemotherapy

The use of neoadjuvant chemotherapy for patients with locally advanced breast cancer has increased significantly over several decades. Neoadjuvant chemotherapy was first used in patients with unresectable or marginally resectable breast cancer [2, 3]. The results from initial studies showed high rates of tumor response and regression. Additional clinical trials were performed with the primary objective of determining whether breast conserving surgery could be offered after neoadjuvant chemotherapy to patients who would have traditionally required mastectomy.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 study randomized 1,523 women with operable breast cancer to receive 4 cycles of adriamycin and cyclophosphamide either in the preoperative or postoperative setting [4]. This study showed that neoadjuvant chemotherapy improved breast conservation rates (67.8% versus 59.8%). Although there was no difference in overall survival (OS) between neoadjuvant and adjuvant therapy groups, patients treated in the neoadjuvant setting whose tumors obtained a pathologic complete response (pCR) at surgery (defined as no histologic evidence of invasive tumor cells in the breast) showed improved disease-free survival (DFS) and OS rates

compared to those with residual disease. The association of pCR with survival outcomes has also been observed by other neoadjuvant studies [5, 6]. Thus, pCR is now considered to be an important endpoint in clinical trials assessing the efficacy of neoadjuvant chemotherapy.

Just as breast cancer has been classified into subtypes with distinct gene expression and associated clinical outcomes [7, 8], response to neoadjuvant chemotherapy by subtype is also unique. For example, the pCR rate for patients with hormone receptor (HR)-positive tumors was 8% after anthracycline-based or anthracycline/taxane-based chemotherapy [9]. In contrast, the pCR rate for TNBC patients undergoing similar therapies was found to be 25% [9], despite poorer overall outcome when compared to those with HR-positive disease. This phenomenon, termed the "triple negative paradox," is supported by data from several notable clinical studies; however, the reason for this phenomenon is largely unknown [5, 6, 10].

Recognizing the clinical heterogeneity of breast cancer, a group of investigators sought to determine if different molecular subclasses of breast cancer responded differently to anthracycline- and paclitaxel-containing preoperative chemotherapy [10]. To answer this question, fine needle aspirations of breast cancer were obtained from 82 patients prior to initiation of neoadjuvant paclitaxel followed by 5-fluorouracil, doxorubicin, and cyclophosphamide chemotherapy. Gene expression profiling was performed and each breast cancer was assigned a unique molecular class—luminal ($n = 30$), basal-like ($n = 22$), and HER2-positive (HER2+; $n = 20$) breast cancers. The rates of pCR, defined as no residual invasive cancer in the breast and axillary lymph nodes, differed significantly among these three molecular classes of breast cancer. Basal-like breast cancers, of which greater than 85% were either estrogen receptor and/or HER2 negative, were associated with high rates of pCR 45% (95% confidence interval (CI) 24–68). Similarly, the HER2+ subgroup was associated with high rates of pCR (45%, CI 23–68), whereas those with luminal tumors illustrated much lower pCR rates (6%, CI 1–21). Genes associated with pCR were examined between the basal-like and HER2+ subtypes, and there was no overlap in these gene sets. This data indicates that genes associated with chemotherapy sensitivity likely differ between these two molecular subgroups of breast cancer.

Not only has response to preoperative chemotherapy been shown to differ by breast cancer subtype, but also prognosis, particularly as it relates to residual disease following neoadjuvant therapies. Carey et al. sought to examine the relationship between neoadjuvant response and long-term end points, including distant DFS (DDFS) and OS [6]. In this landmark study, 107 patients with stage II-III breast cancer were treated with 4 cycles of neoadjuvant doxorubicin/cyclophosphamide chemotherapy (75% also received preoperative taxanes) between the years 1998 and 2003. Breast cancer subtypes were defined as follows using immunohistochemistry-surrogate markers: 34% for luminal A (HER2−/HR-positive), 24% for luminal B (HER2+/HR-positive), 10% for HER2+ (HER2+/HR negative), and 32% for basal-like (HER2−/HR negative). Similar to the Rouzier et al. study, pCR was higher among patients with basal-like and HER2+ breast cancer (27% and 36%, resp.) and only 7% in luminal breast cancers ($P < 0.05$ in both comparisons). Although pCR was higher among those with HER2+ and basal-like breast cancer, patients of either subtype experienced inferior DDFS and OS compared to luminal breast cancer patients. Overall, only 2 of 17 patients across subtypes with pCR relapsed. Thus, the overall worse outcome observed within basal-like and HER2+ subtypes was due to higher relapse rates among those with residual disease.

A subsequent analysis conducted by Liedtke et al. performed a similar analysis that evaluated 1,118 patients who received neoadjuvant anthracycline and/or taxane-based chemotherapy at MD Anderson Cancer Center between the years 1985–2004 [5]. In this cohort of patients, 255 patients (23%) were classified as having TNBC, while 863 patients (77%) had non-TNBC. Consistent with prior reports, increased pCR rates were observed for patients with TNBC compared with non-TNBC (22% versus 11%; odds ratio [OR] = 1.53, $P = 0.034$). Despite this difference in pCR, a significant decrease in 3-year RFS and OS was observed for patients with TNBC compared with non-TNBC (63% versus 76%, $P = 0.0001$ and 74% versus 89%, $P = 0.0001$ resp.). Moreover, if a pCR was achieved, patients with TNBC and non-TNBC had similar survival (HR = 1.7, $P = 0.24$). Conversely, patients with residual disease experienced worse OS if they had TNBC compared with non-TNBC (HR = 1.5; $P < 0.0001$). This data supports the continued efforts to identify novel neoadjuvant approaches that will enhance pCR rates among women with TNBC (and non-TNBC). In parallel, there is a need to develop therapeutic strategies for TNBC with residual disease following neoadjuvant therapy.

3. Ongoing and Completed Neoadjuvant Therapeutic Strategies for TNBC

As per the most recent National Cancer Comprehensive Network (NCCN) guidelines for the treatment of invasive breast cancer, women with stage IIA–IIIA breast cancer who, with the exception of tumor size, are otherwise candidates for breast-conserving therapy, may be considered for preoperative chemotherapy with a number of anthracycline and/or taxane-based regimens (http://www.nccn.com/). While these chemotherapy regimens remain the mainstay to treat operable TNBC [11], salient efforts are being made to improve outcomes for women diagnosed with this aggressive disease. Some of these strategies include the addition of chemotherapeutic agents to the anthracycline/taxane backbone, as well as the incorporation of biologic and targeted agents to standard regimens. Many of the completed and ongoing clinical trials testing novel neoadjuvant treatment strategies for TNBC will be reviewed here (see Table 1).

4. Chemotherapy

Building on experiences in the metastatic setting where select combination chemotherapies have led to improved breast cancer outcomes compared to single agent regimens [20, 21],

Table 1: Summary of completed neoadjuvant chemotherapy trials.*

Clinical trials	Design	Drugs	Population	pCR rate
Silver et al. [12]	Phase II single arm	Cisplatin × 4	TNBC	6/28 (21%)
Byrski et al. [13]	Retrosp.	All; CMF; AD; AC/FAC; cisplatin	*BRCA1* mut.	All: 24/102 (24%) CMF: 1/14 (7%) AD: 2/25 (8%) AC/FAC: 11/51 (22%) Cisplatin: 10/12 (83%)
Bear et al. [14]	Phase III random.	Arm 1A: D × 4 → AC × 4 Arm 1B: D + X × 4 → AC × 4 Arm 1C: D + G × 4 cycles → Ac × 4	HER2−	Arm 1A: 102/393 (26%) Arm 1B: 91/390 (23%) Arm 1C: 106/388 (27%)
Alba et al. [15]	Phase II random.	Arm A: EC × 4 cycles → D × 4 Arm B: EC × 4 cycles → D + Carbo × 4	Basal-like	Arm A: 14/46 (30%) Arm B: 14/47 (30%)
Zelnak et al. [16]	Phase II random.	Arm A: D × 4 cycles → X × 4; Arm B: D + X × 8 cycles.	HER2−	Arm A: 2/25 (8%) Arm B: 3/26 (12%) Arm A/B (TNBC): 4/21 (19%)
Von Minckwitz et al. [17] Huober et al. [18]	Phase III random.	Arm 1 (responder): TAC × 4 Arm 2 (responder): TAC × 6 Arm 3 (nonresponder): TAC × 4 Arm 4 (nonresponder): VX × 4	Any breast cancer	Arm 1–4 TNBC: 77/198 (39%) Non-TNBC: 22/147 (15%)
Baselga et al. [19]	Phase II single arm	Ixabepilone × 4	Any breast cancer	TNBC: 11/42 (26%) Non-TNBC: 18/119 (15%)

*TNBC: triple-negative breast cancer; pCR: pathological complete response; M: methotrexate; F: 5-fluorouracil; Retrosp.: retrospective study; T: paclitaxel; Carbo: carboplatin; D: docetaxel; C: cyclophosphamide; A: doxorubicin; E: epirubicin; X: capecitabine; G: gemcitabine; V: vinorelbine.

several neoadjuvant studies have sought to determine the additive benefit of incorporating novel chemotherapeutics with standard anthracycline and/or taxanes. These additional chemotherapeutics have included antimetabolites, platinum agents, and novel microtubule stabilizing agents.

4.1. Antimetabolites. The recently reported National Surgical Adjuvant Breast and Bowel Project (NSABP) B-40 protocol asked two fundamental questions: (1) was the addition of the antimetabolite either capecitabine (X) or gemcitabine (G) to docetaxel (T) followed by AC, and/or (2) does the addition of bevacizumab to docetaxel/anthracycline-based regimens increase pCR rates for women with HER2-negative breast cancer [14]. While this study was not restricted with women with TNBC, 41% of the 1,206 patients had HER2-negative/HR-negative breast tumors (thus, triple negative). Complete clinical response as assessed by physical exam was not significantly different by treatment arm ($P > 0.4$). Similarly, no statistically significant difference was observed for pCR in both breast and lymph nodes across all treatment arms: T → AC 26%; TX → AC 23.3%; TG → AC 27.3% ($P > 0.4$; Table 1). Toxicity was reported for 1,191 patients, including all grade 3 and 4 adverse events and was numerically higher for the TX → AC (55% Grade 3 and 14% Grade 4) and TG → AC (61% Grade 3 and 12% Grade 4) arms compared to the T → AC arm (48% Grade 3 and 7% Grade 4).

A second study sought to determine the additional benefit of preoperative capecitabine to docetaxel—either sequentially or in combination—to treat women with HER2-negative breast cancer [16]. In this study, 51 women were treated with either 4 cycles of docetaxel followed by 4 cycles of capecitabine (Arm A, $n = 25$) or 8 cycles of concurrent docetaxel/capecitabine. Median tumor size was 6.1 cm, 68% of patients were clinically lymph node positive, and 41.2% had TNBC. Overall, treatment was well-tolerated with expected grade 3 and 4 toxicities (15.7% neutropenia, 5.9% neuropathy, and 3.9% neuropathy). For the entire study cohort, pCR rates were 8% and 11.5% for Arm A and B, respectively. Among those with TNBC, pCR rate in both arms combined was 19%.

While the results of these two studies illustrate modest, at best, activity for the addition of antimetabolites to anthracycline/taxane and/or taxane-based therapy, results as they pertain to TNBC should be interpreted with caution as only 40% of study populations were classified as triple negative. In addition, and given the higher toxicity profile associated with doublet chemotherapy, biomarker strategies to both enrich for responders and minimize toxicities associated with antimetabolites should be considered and incorporated into future neoadjuvant studies examining combination strategies.

4.2. Platinum Therapy. Given the inherent genomic instability of TNBC/basal-like with and without *BRCA* germline mutations and respectable sensitivity to platinums in the metastatic setting [22–24], several neoadjuvant studies have evaluated these agents as monotherapy or in different combination strategies. In Silver et al., 28 women with Stage II or III TNBC (of which 2 harbored a germline *BRCA1*

Table 2: Summary of neoadjuvant bevacizumab-based chemotherapy trials.*

Clinical trials	Design	Drugs	Population	Status	pCR rate
Gerber et al. [31] (GeparQuinto)	Phase III	Arm 1: EC × 4 → D × 4 Arm 2: EC+ Bev × 4 → D + Bev × 4	TNBC	Completed	Arm 1: 96/342 (28%) Arm 2: 119/327 (36.4%)
Bear et al. [14] (NSABP B-40)	Phase III random	Arm 1A-C: Anthracycline-taxane-based chemotherapy Arm 2A-C: Anthracycline-taxane-based chemotherapy + Bev	HER2−	Completed	All Arms Bev: 203/588 (35%) All Arms/no Bev: 168/592 (28%) TNBC Bev: 121/236 (51%) TNBC/no Bev: 115/243 (47%) HR+ Bev: 82/352 (23%) HR+/no Bev: 53/349 (15%)
CALGB-40603	Phase II random	Arm 1: T → AC Arm 2: T + Bev → AC + Bev Arm 3: T + Carbo → AC Arm 4: T + Carbo + B → AC + Bev	TNBC	Ongoing	—

*TNBC: triple-negative breast cancer; pCR: pathological complete response; Bev: bevacizumab; T: paclitaxel; Carbo: carboplatin; D: docetaxel; C: cyclophosphamide; A: doxorubicin; E: epirubicin.

mutation) were treated with 4 cycles of cisplatin monotherapy 75 mg/m^2 every 21 days. The pCR rate was 21% (6/28), and the partial and complete clinical response was 64% (18/28). Several variables were associated with response: young age, low *BRCA1* mRNA expression, *BRCA1* promoter methylation, p53 nonsense or frameshift mutations, and a gene expression signature of E2F3 activation. In a subsequent study of two Polish series of women with *BRCA1*-mutated breast cancer largely triple-negative treated with cisplatin monotherapy (75 mg/m^2 every 21 days), the pCR rates were as high as 80–90% [13, 25]. Further studies are needed to determine if *BRCA1* mutations are predictive of cisplatin benefit in TNBC.

The recently reported GEICAM 2006-03-A study sought to determine the additional benefit of carboplatin to conventional neoadjuvant chemotherapy in women with TNBC/basal-like breast cancer patients (defined as ER−/PR−/HER2− and cytokeratin 5/6+ and/or epithelial growth factor receptor [EGFR]+) [15]. In this Phase II multicenter study, 94 patients with ≥2 cm tumors were randomized to receive epirubicin/cyclophosphamide for 4 cycles followed by either docetaxel with or without carboplatin for 4 cycles. pCR in both the breast and axilla was reported to be 30% in both arms; Grade 3/4 toxicities between arms were similar (54% and 53%).

Ongoing studies will continue to help us define the role, timing, and optimal patient population of platinums in the preoperative treatment of TNBC. As an example, the Cancer and Leukemia Group B (CALGB) 40603 clinical trial is actively enrolling patients to standard anthracycline/taxane-based neoadjuvant therapy without carboplatin (NCT00861705). Pretreatment breast core biopsies are required at study entry. Both the clinical outcomes and correlative endpoints of this study will help guide future use of platinum agents in this setting.

4.3. Microtubule Stabilizing Agents. Ixabepilone, a novel semisynthetic antineoplastic agent derived from natural epothilones and their analogs, promotes tumor cell death by stabilizing microtubules and inducing cell cycle arrest and subsequent apoptosis. A large, randomized, Phase III study illustrated improvement in PFS by the addition of ixabepilone to capecitabine to treat women with metastatic breast cancer, including those with TNBC [26]. This has led investigators to evaluate the benefit of ixabepilone in the neoadjuvant treatment of invasive breast cancer not amenable to breast conservation surgery [19]. In this study, 161 women with inoperable breast cancer (of which 42 [26%] were triple negative) were treated with 4 or fewer cycles of single agent ixabepilone. pCR rates in the breast were 18% for the entire study population; 22% in ER negative/HER2 negative; 46.1% in ER negative/HER2+; 10.6% in ER positive/HER2-negative; 20% in ER positive/HER2+. Gene expression studies from pretreatment core breast biopsies confirmed the inverse relationship between ER expression and ixabepilone sensitivity. An ongoing clinical trial evaluating differential responses to neoadjuvant paclitaxel versus ixabepilone following AC chemotherapy in the preoperative setting of early stage breast cancer is eagerly awaited (NCT00455533).

5. Antiangiogenic Agents

It is well established in both the laboratory and clinical settings that angiogenesis is a key mediator of breast cancer progression [27]. Multiple studies have evaluated the benefit of targeting vascular endothelial growth factor receptor (VEGF) with the humanized monoclonal antibody, bevacizumab (Avastin, Genentech/Roche). Although results were more impressive in the E2100 study as compared to others, the addition of bevacizumab has consistently led to improvements in response rates, while PFS benefit has been more modest [28–30]. However, as some benefit has been seen in the TNBC subset and given the relative paucity of "targets" in TNBC, several investigators have sought to determine the benefit of targeting VEGF with bevacizumab in the neoadjuvant setting (see Table 2).

The GeparQuinto study was designed to determine the benefit to adding bevacizumab to anthracycline/taxane-based preoperative chemotherapy among 1,948 women with HER2-negative breast cancer [32]. Patients were randomized

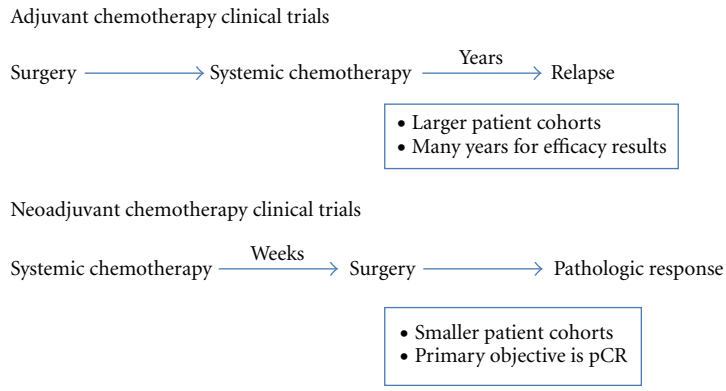

Figure 1: Clinical trial design schematic.

to receive 4 cycles of epirubicin/cyclophosphamide (EC) followed by 4 cycles of docetaxel (D) with or without bevacizumab. Approximately 35% of patients in both arms had TNBC. For the entire study cohort, there was no statistical significant difference in pCR (defined as no invasive/noninvasive residual in breast and nodes) between groups (15% EC → D and 17.5 EC → D plus bevacizumab). In a predefined stratification by subtype, patients with TNBC had a significantly higher likelihood of pCR by the addition of bevacizumab compared to the other subtypes (OR = 1.42). In a subsequent analysis in TNBC patients only ($n = 684$) reported at ASCO 2011 annual meeting, pCR rates in both breast and lymph nodes were higher for patients who received EC → T plus bevacizumab compared to EC → D alone (36.4% versus 28%, $P = 0.021$) [31]. A large biomarker program is ongoing to try to identify subgroups within TNBC who achieve greater benefit from bevacizumab.

In addition to evaluating the benefit of adding antimetabolites to standard anthracycline/taxane-based chemotherapy, the recently reported NSABP B-40 study also sought to determine if the addition of bevacizumab would enhance pCR rates for >1,200 women with HER2-negative breast cancer [14]. In this study, patients were treated with AC followed by docetaxel with or without bevacizumab. Complete clinical responses were higher among women who received bevacizumab (64.3 versus 55.8%, $P = 0.006$). This effect was more dramatic in those with HR-positive breast cancer (64.5% versus 53.7% with and without bevacizumab, resp., $P = 0.007$) compared to those with TNBC (63.9% versus 59.1% with and without bevacizumab, resp., $P = 0.371$). Similar to clinical response, pCR was higher for patients who received bevacizumab compared to those who did not (34.5 versus 28.4%; OR = 1.33, $P = 0.027$), and the positive effect was more prominent in patients with HR-positive tumors (OR = 1.7, $P = 0.008$) as compared to those with TNBC (OR = 1.17, $P = 0.44$). Given the apparent differences in response rates between the GeparQuinto and B40 studies within TNBC, the results of the ongoing CALGB study 40603 (NCT00861705) evaluating both the addition to platinum and bevacizumab to standard anthracycline/taxane chemotherapy are eagerly awaited.

6. Novel Targeted Strategies: Small Molecule Inhibitors

In addition to advances in combination chemotherapeutics and antiangiogenic agents, substantial effort is being made to optimize preoperative response rates through the use of novel agents targeting important oncogenic signaling pathways in breast cancer. These strategies include the inhibition of mammalian target of rapamycin (mTOR), histone deacetylase (HDAC), and poly-ADP-ribose polymerase (PARP).

Given that activation of the PI3K/mTOR pathway activation occurs frequently in TNBC, investigators sought to determine the benefit of adding RAD001 (Novartis), an mTOR inhibitor, to neoadjuvant anthracycline/taxane chemotherapy [33]. Fifty patients with TNBC were randomized to receive paclitaxel weekly for 12 weeks with or without weekly RAD001 for 12 weeks, both followed by 5FU/epirubicin/cyclophosphamide (FEC) every 3 weeks for 4 cycles. Although pCR rates did not differ by treatment arm (30.4% versus 25.9%, $P = 0.761$), investigators collected breast tumor biopsies to evaluate molecular changes in the PI3K pathway at baseline, 48 hours, 12 weeks after-therapy and at surgery. Ongoing correlative science studies are likely to help refine the selection of patients most likely to respond to these targeted agents.

Epigenetic mechanisms are another potential target for TNBC. For example, studies have shown that the loss of ER-α by gene methylation might be occurring in ER-negative breast tumors, and that demethylation could restore the expression of ER and sensitize the tumor cells to hormonal therapies [34]. In addition, preclinical and early phase clinical studies have illustrated efficacy for targeting endocrine-resistant breast cancers with HDAC inhibitors [34, 35]. Building on these results, an ongoing study (NCT00262834) is evaluating change in tumor morphology, tissue and blood (peripheral blood mononuclear cells) histone acetylation, and safety of short term exposure to the HDAC inhibitor, vorinostat (Merck), for newly diagnosed breast cancers. These results will undoubtedly inform future trials evaluating

HDAC inhibitors in the neoadjuvant treatment of breast cancer.

Finally, the I-SPY 2 trial (investigation of serial studies to predict your therapeutic response with imaging and molecular analysis 2) is a multicenter, neoadjuvant study projected to enroll over 800 women with breast cancer of all phenotypes (NCT01042379). This trial is integrating novel imaging and biomarker analysis to improve response prediction to a variety of novel targeted agents in combination with standard chemotherapeutics. Pertinent to TNBC, a subset known to share clinicopathologic features with *BRCA*-deficient breast cancers [36] will be treated with the PARP inhibitor, ABT-888.

7. Conclusions

Although TNBC has an overall poor prognosis, TNBC patients undergoing neoadjuvant chemotherapy have improved breast conservation rates and high response rates. In this setting, pCR is an appropriate endpoint for predicting improved longer-term outcome. However, this endpoint is only achieved by current treatment strategies in 20–40% of the cases. Thus, we recommend that patients presenting with operable TNBC be encouraged to participate in neoadjuvant clinical trials since there are a number of novel targeted agents that are currently being evaluated.

Treatment in the neoadjuvant setting provides an ideal model for evaluating the efficacy of new targeted therapies for TNBC. Such an approach allows for smaller patient accrual, shorter timeframes to obtain results and routine tissue collection for correlative studies compared to traditional adjuvant trials (see Figure 1). Neoadjuvant trials allow for more rapid evaluation of novel therapies for TNBC. In addition, primary tumor core biopsies can be obtained before initiation of systemic therapy and during therapy for correlative studies to assess the status of particular biomarkers and test if the presumed targets are being inhibited by these novel therapies. For example, proliferation-related biomarker Ki-67 has been shown to be a useful surrogate for response during or after neoadjuvant endocrine therapy [37].

In closing, there are numerous ongoing clinical neoadjuvant trials aimed at improving outcome for patients with TNBC. Moreover, the use of neoadjuvant chemotherapy as the primary model for clinical research for TNBC will advance our understanding of molecular response to novel agents and our ability to efficiently assess the efficacy of promising therapies with the ultimate goal of improving patient survival.

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Hindawi Publishing Corporation
International Journal of Breast Cancer
Volume 2012, Article ID 217185, 7 pages
doi:10.1155/2012/217185

Review Article

Molecular Basis of Triple Negative Breast Cancer and Implications for Therapy

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Received 15 July 2011; Accepted 15 September 2011

Academic Editor: Quyen D. Chu

Triple negative breast cancer is an aggressive form of breast cancer with limited treatment options and is without proven targeted therapy. Understanding the molecular basis of triple negative breast cancer is crucial for effective new drug development. Recent genomewide gene expression and DNA sequencing studies indicate that this cancer type is composed of a molecularly heterogeneous group of diseases that carry multiple somatic mutations and genomic structural changes. These findings have implications for therapeutic target identification and the design of future clinical trials for this aggressive group of breast cancer.

1. Introduction

Triple negative breast cancer (TNBC) is defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER-2 Overexpression. It accounts for 15–20% of all breast cancer cases [1, 2], but occurs at a higher frequency in young premenopausal women with African Ancestry (AA) [3]. High body mass index (BMI) and high parity, instead of low parity in other types of breast cancer, have been associated with increased risk for TNBC [4–6]. TNBC is associated with an overall poor prognosis as exemplified by a higher rate of early recurrence and distant metastasis to brain and lungs compared to other breast cancer subtypes [7, 8]. The unfavorable clinical outcome is partly explained by its aggressive pathologic features including a higher histology grade and mitotic index [9].

Chemotherapy is the only systemic therapy currently available for TNBC and is curative in a subset of patients with chemotherapy-sensitive disease. A higher rate of pathologic complete response (pCR) to standard chemotherapy has been observed in patients with TNBC compared to ER+ disease. A pCR rate of 22% in TNBC versus 11% in ER+ disease was reported in a study of over 1 000 patients treated with neoadjuvant anthracycline and taxane-based chemotherapy regimens [10]. The excellent outcome associated with the pCR, however, is in contrast to the high risk of recurrence and cancer-related deaths in those with residue disease. Although alternative agents such as platinum compounds have demonstrated promising activity, up to 70–80% of patients have residual cancer following neoadjuvant cisplatin [11]. In the metastatic setting, TNBC is typically associated with an initially higher response rate, but in a shorter time to progression following treatment with existing chemotherapy agents, resulting a shorter overall survival compared to ER+ breast cancer in multiple studies [12]. The underlying molecular mechanism for this paradox is yet to be elucidated, although one could hypothesize that the inherent genomic instability of TNBC renders the possibility of a faster adaptation to the cytotoxic effect of chemotherapy.

The treatment options for chemotherapy-resistant TNBC are limited. The established targeted therapies, including endocrine treatment and HER2-targeted agents, are ineffective. Although several small molecule inhibitors and monoclonal antibodies against important cellular pathways have been tested in clinical trials, none has entered clinical practice due to limited efficacy. A better understanding of the underlying biology of TNBC is therefore needed to identify new therapeutic targets and to pinpoint which TNBC patients may benefit from them. Recent advances in microarray and DNA sequencing

technologies have made it possible to analyze the tumor at the genomic level for therapeutic target discovery. These studies indicate that TNBC is a molecularly heterogeneous group of diseases with highly complex genomic aberrations. A further classification at the molecular level may be possible to facilitate drug development. In this paper, we will examine recent publications on the molecular basis of TNBC, with a particular focus on genomewide studies and their implication for future clinical trials.

2. Molecular Subclassification of TNBC Based on Gene Expression Profiling

In the seminal paper by Sørlie et al. breast cancer was subdivided into five intrinsic molecular subtypes, including luminal A, luminal B, HER-2 enriched, normallike, and basallike, based on hierarchical clustering analysis of approximately 500 genes (termed the intrinsic gene set because expression was not modulated by treatment) on a cDNA microarray study of 65 breast tumors obtained from 42 different individuals (Table 1) [8]. The term luminal A and luminal B subtypes was coined to reflect the presumed luminal epithelial cells origin of these cancers because of similarities in gene expression pattern and the expression of ER [13]. In contrast, HER-2-enriched subtype has high expression of HER-2 and genes that are close to HER-2 in the genome such as *GRB7*, but low expression of luminal and hormone receptor-related genes. Some of the clinical HER-2-positive cancers actually do not fall into HER-2-enriched subtype but belong to the luminal categories because of the coexpression of ER. These tumors are likely biologically different from those of the HER-2-enriched intrinsic subtype. Normal-like subtypes have, as their name implies, similar expression pattern to normal breast tissue. The significance of this subtype has yet to be determined, and some argue that it may represent a mere contamination of samples with normal breast tissue. The intrinsic subtypes carry prognostic significance with the basal-like subtype having the worse clinical outcome. The recent development of the 50-gene subtype predictor (PAM50), a RT-PCR assay that assigns intrinsic subtypes using RNA from formalin fixed and paraffin embedded tissue, has created a possible gold standard intrinsic subtype test for clinical application [14]. Although all intrinsic subtypes have been identified in TNBC, basal-like subtype is the most common, followed by the recently identified Claudin-low subtype [15].

Table 1: Intrinsic subtypes of breast cancer.

Intrinsic subtypes of breast cancer	Characteristics
Luminal A	High level expression of ER and ER-associated genes, associated with a favorable clinical outcome.
Luminal B	Low level expression of ER and ER-associated genes, associated with a higher tumor cell proliferation rate and a worse clinical outcome compared to the luminal A subtype.
HER-2 Enriched	High level expression of HER2 and GRB7, associated with a poor outcome before the era of HER2-targeted agents.
Basal-like	Positive for the expression of basal cytokeratin but negative for the expression of luminal- and HER2-related genes, associated with a high tumor cell proliferation rate and a poor clinical outcome.
Normal-like	Similar expression compared to normal breast, suspicious for normal cell contamination.
Claudin-low	Lack the expression of claudin proteins that are implicated in cell-cell adhesion, but high expression of EMT and putative stem cell markers, associated with ER and HER2 negativity but low in basal cytokeratin expression.

2.1. Basal-Like Subtype. TNBC is most commonly associated with basal-like intrinsic subtype. Basal-like subtype is termed after the basal epithelial layer cells due to their similarities in gene expression pattern. Basal-like breast cancers typically express basal cytokeratins such as CK5/6, CK17 as well as cadherin, and epidermal growth factor receptor (EGFR) [16]. They are also frequently triple negative (negative for ER, PR, and HER2). In one study, about 70% of TNBC belonged to basal-like subtype, and 76% of basal-like cancers were found to be triple negative [1]. Many studies have used the two interchangeably, however, and it is important to note that although there is significant overlap, basal-like subtype does not encompass all of TNBC and may itself be another too broad of a classification.

An association has been described between the basal-like subtype and *BRCA1*-gene-related breast cancers. The majority of *BRCA1*-related tumors are basallike by microarray analysis [12, 17], and sporadic basal-like breast cancers have been associated with "*BRCA*ness," which is characterized by high tumor grade, lymphocytic infiltrate, pushing margins, ER and HER2 negativity, association with *TP53* mutations, c-myc amplification, and multiple chromosome abnormalities including X-chromosome isodisomy [18]. Although somatic mutations in *BRCA1/2* rarely occur in sporadic breast cancer [19–21], a rather high incidence, approaching 20%, of germline mutations in *BRCA1* or *2* has been reported in patients with TNBC [21]. In a study of 77 cases of sporadic TNBC from MD Anderson, *BRCA1* mutation was identified in 12 (15.6%) (only one somatic) and *BRCA2* mutation was identified in 3 (3.9%) [21]. More commonly, loss of *BRCA* expression due to gene silencing by promoter methylation has been shown in TNBCs [22]. It has been demonstrated that *BRCA1* normally suppresses the expression of basal-like-related genes, which could provide an explanation for "*BRCA*ness" of basal-like sporadic cancers [23].

The molecular similarity between basal-like sporadic breast cancers with *BRCA*-related cancers has raised the possibility and excitement that PARP inhibitors could be effective in this patient population. *BRCA1*/2 is important for homologous DNA repair. In the background of *BRCA* deficiency, DNA damage repair relies on alternative pathways such as base excision repair pathway provided by PARP,

therefore inhibition of PARP would lead to accumulation of unrepaired DNA damage and cell death. This synthetic lethal approach has been shown to be effective in *BRCA*-related cancers in both preclinical and clinical settings [24–26]. The effectiveness of PARP inhibitors in TNBC, on the other hand, is not as clear. A recently published phase III trial of Iniparib in patients with TNBC did not yield positive results [27]. However Iniparib, which was originally thought to be a PARP inhibitor, turned out to have a more complicated mechanism of action unrelated to PARP so the results of this study may not be applicable to *bona fide* PARP inhibitors. The question also remains as to how to best identify patients who may benefit from these agents. In the absence of a robust biomarker predictor of treatment response, trials of PARP inhibitors in TNBC are being conducted in all comers rather than a defined molecular subtype.

2.2. Claudin-Low Subtype. Claudin-low is the latest subtype being identified by gene expression profiling studies [28]. It is characterized by the lack of expression of claudin proteins, which are important components of tight junctions that seal the potential space between adjacent epithelial cells, and epithelial cell adhesion molecules E-cadherin, EpCAM, and mucin-1 [15]. Claudin-low tumors are typically triple negative (61–71%), and conversely 25 to 39% of triple negative breast cancers are of the claudin-low subtype [15]. This subtype differs from the basal-like tumors, however, due to inconsistent expression of basal keratins and a significantly lower expression of proliferation genes [15]. They also have low expression of luminal markers, high expression of epithelial-to-mesenchymal transition (EMT) markers, and cancer stem-cell-like features. The expression of EMT markers is especially important in this group since it has been associated with resistance to therapy and higher metastatic potential [29, 30]. Claudin-low subtype accordingly was found to have a lower pathologic complete remission (pCR) rate with neoadjuvant chemotherapy than basal-like but higher than that of the luminal subtype putting their prognosis in between the two [15]. The identification of this subtype has provided further evidence of the broad underlying biology of TNBC and the need for a better understanding of the underlying biology of different subtypes of breast cancer and their therapeutic implications.

2.3. More Subtypes? To specifically subclassify TNBC, Lehmann et al. analyzed the gene expression profile of 587 TNBC cases from 21 breast cancer databases and performed clustering analysis. Six subtypes were identified which may have therapeutic implications (Table 2) [31]. Two basal-like subtypes, BL1 and BL2, were the most prevalent and were so named because of their similarity to the previously described basal-like intrinsic subtype. These tumors have high expression of genes involved in cell cycle and cell division such as Aurora kinase and *MYC* and are highly proliferative as marked by high Ki-67 nuclear staining (BL1+BL2: 70% versus other subtypes: 42%). These results suggest that chemotherapies that target cell division and mitosis, such as taxanes, would be most applicable in this class. Indeed, BL1 and BL2 subtypes were associated with a significantly higher rate of pCR (63%; $P = 0.042$) with taxane-based therapies as compared to mesenchymal-like (31%) or luminal androgen receptor (14%) subtypes [32]. In addition, elevated expression of DNA damage response pathway genes such as *CHEK1* and *RAD51* were present in the BL1 subtype, and representative cell lines were found to be preferentially responsive to cisplatin which induces DNA damage [31].

Table 2: Six subtypes of triple negative breast cancer based on gene expression profiling.

Subtype	Gene expression profile
Basal-like 1 (BL-1)	High in the expression of genes involved in cell cycle progression, cell division, and DNA damage response pathways.
Basal-like 2 (BL-2)	High in the expression of genes involved in cell cycle progression, cell division, and growth factor signaling.
Immunomodulatory (IM)	High in the expression of genes involved in immune processes and cell signaling.
Mesenchymal (M)	High in the expression of genes involved in motility and extracellular matrix.
Mesenchymal stem-like (MSL)	High in the expression of genes involved in motility, extracellular matrix, and growth factor signaling; consistent with claudin-low Intrinsic subtype.
Luminal androgen receptor (LAR)	High in the expression of genes involved in hormonally regulated pathways.

A third subtype, immunomodulatory (IM), was found to be enriched in genes involved in immune processes. These include immune transduction pathways (NFKB, TNF, JAK), cytokine signaling such as IL-2 pathway, and antigen processing, among others. This subtype may represent medullary breast cancer, a subtype of TNBC that has a good prognosis, based on a similar expression profile reported in another study [33].

Mesenchymal (M) and mesenchymal stemlike (MSL) subtypes were characterized by expression of cell motility genes and proteins of the extracellular matrix. The MSL subtype displayed low expression of claudins 3, 4, and 7, consistent with the claudin-low subtype of breast cancer as previously discussed. MSL subtype also expressed genes involved in growth factor signaling such as EGFR and PDGFR pointing to possible therapeutic options in this subtype. Cell line models of M and MSL responded to inhibitors of PI3K/mTOR or Src.

The sixth subtype, luminal androgen receptor (LAR), was found to be enriched in genes involved in steroid synthesis and androgen metabolism. It has been reported previously that a proportion of TNBC may use or be dependent on

the endocrine pathway despite being negative for ER and PR [34]. This was replicated in the study by Lehmann et al. in that a distinct subtype of TNBC, LAR subtype, was identified that has high expressions of hormonal related genes. Androgen receptor mRNA was expressed at an average of 9-fold higher level in this subtype than all the other subtypes [31]. Interestingly, LAR subtype belongs to either luminal A or luminal B intrinsic subtype despite being negative for ER expression. The finding of LAR subtype presents an exciting venue for endocrine treatment for at least a proportion of TNBC patients.

2.4. Subclassification of ER Breast Cancer Based on Kinase Gene Expression. In an attempt to identity kinase targets, Speers et al. investigated global kinase gene expression pattern and identified 52 kinases that are differentially expressed between ER-positive and ER-negative tumors [35]. The authors were able to further classify ER negative cancers into four types based on the expression of these kinases. One subtype was defined by the expression of cell cycle control kinases such as AK2, TTK, and CHK1. The second expressed kinases in the S6 pathway. Third subtype was defined by kinases involved in modulating the immune system such as LYN, IRAK1 and the fourth subtype defined by expression of MAPKs. Some of these tumors overexpressed HER-2 so this classification cannot be used specifically for TNBC, but these kinase-based subtypes may have therapeutic implications in targeting a particular pathway in TNBC.

3. Whole Genome Sequencing

The first comprehensive genomic analysis of a basal-like breast cancer was performed by using massively parallel sequencing technology and was published in 2010 [36]. The genome of the primary breast tumor obtained at initial diagnosis was compared with a brain metastasis developed at recurrence and a xenograft generated from the primary breast tumor in an immunodeficient mouse. Fifty novel somatic point mutations and small indels as well as 28 large deletions, 6 inversions, and 7 translocations were identified, including mutations in *TP53*, *JAK2*, and *MAP3K8,* among others. There was a wider range of mutation frequencies in the primary tumor compared to the brain metastasis and the xenograft, suggesting the existence of genetically heterogeneous tumor cell populations in the primary breast tumor that underwent clonal selection during the metastasis process and the generation of xenograft. Overall this basal-like breast cancer proved to possess an impressively complex genome. Compared to the genome of the two acute myeloid leukemia (AML) cases that were recently published, this basal-like cancer genome had 3- to 4-fold more single nucleotide variations (SNVs) [37, 38]. More genomic studies like this, however, are needed to create a genetic landscape of TNBC to guide therapeutics development. Importantly, as more genomic data is being generated, a significant challenge remains to differentiate "driver mutations" from "carrier mutations." Individualized treatment would not be possible before we fully understand the biology of these genetic abnormalities.

4. Potential Therapeutic Targets for TNBC

4.1. SRC Inhibition. Src is a nonreceptor tyrosine kinase involved in cell adhesion and motility [39]. In preclinical studies, TNBC cell lines showed the highest sensitivity to dasatinib, a small molecule kinase inhibitor of Src, Abl, and KIT [31, 40]. Clinical studies have been disappointing however. A phase II trial of single-agent dasatinib in patients with advanced TNBC (CA180059) was reported in an abstract form. Dasatinib was found to have modest single-agent activity in these unselected TNBC patients with partial response in 5% and disease control rate in around 10% of 44 treated patients [41]. A smaller study using single-agent saracatinib, also a Src inhibitor, on nine ER/PR negative patients, failed to provide positive results [42]. A specific subtype of TNBC with Src dependence likely needs to be targeted to provide a benefit from this class of medications. Preclinical work indicates that the mesenchymal-like subtypes are more sensitive to Src inhibitors [31]. Mesenchymal-like subtypes are enriched in cell motility pathways, and Src is known to play an important role in cell migration likely explaining their sensitivity to this class of drugs.

4.2. PARP Inhibition. As mentioned earlier, PARP inhibitors have been an area of enthusiastic research in recent years for the treatment of TNBCs given their similarity to *BRCA*-related breast cancers. Promising results were found in a phase II trial of Iniparib in TNBC [38]. But as mentioned, the benefits were not confirmed in the subsequent phase III trial [27]. Given that the mechanism of action of Iniparib is now questioned and it is likely not a PARP inhibitor as originally thought, it is unclear what the implications of this study are for true PARP inhibitors. The PARP inhibitor Olaparib (AZD2281), which has been shown to be safe and effective in *BRCA*-related cancers, as well as other PARP inhibitors is currently being tested in clinical trials of TNBCs [26]. The results of these studies are eagerly awaited. Given the similarity of *BRCA*-related tumors and basal-like subtype, targeting this subtype of TNBC may provide the most benefit from these medications.

4.3. Androgen Receptor Inhibition. An interesting target that is currently under investigation is the androgen receptor (AR). As mentioned earlier, despite being negative for ER and PR, some TNBCs are positive for downstream targets of the endocrine pathway such as the androgen receptor. These cancers are likely concentrated in the LAR subtype of TNBC described by Lehmann et al.[31] and could be still dependent on an endocrine therapy responsive pathway. Although there have been no studies targeting this particular subtype with an AR inhibitor, a phase II trial (NCT00468715) is currently ongoing evaluating bicalutamide, a commonly used androgen receptor antagonist, in patients with ER/PR-negative breast cancer. If effective, this pathway has the potential to provide a nontoxic and targeted treatment strategy in this subtype of TNBC.

4.4. Targeting Epigenetics. There is evidence of gene silencing in patients with TNBC by methylation and/or histone

acetylation [22]. In a recently published abstract on the first whole genome methylation analysis, TNBCs were indeed found to have a distinct methylation pattern from hormone receptor positive breast cancers [43]. Therefore it was hypothesized that epigenetic silencing may be involved in the lack of hormone receptors in TNBCs and demethylating or deacetylating agents could possibly reactivate genes involved in the endocrine pathway and subsequently restore sensitivity to endocrine therapy in these TNBCs. One study published in abstract form showed reexpression of ER and PR in TNBC after treatment with the combination of LBH589, a histone deacetylase inhibitor, and decitabine, a known hypomethylating agent [44]. There is another ongoing trial using single-agent decitabine in patients with TNBC followed by examination for ER expression and treatment with Tamoxifen (NCT01194908). It is not clear whether benefit from epigenetic manipulation would apply to all TNBCs or a specific subtype since gene methylation analysis was not tested in the published subtyping analysis.

4.5. EGFR Inhibition. Overexpression of EGFR is common in patients with TNBC and is seen in up to 60% of basal-like breast cancers [16]. It is associated with lower response to chemotherapy and poor overall survival [16]. As stated above, BL-2 and MSL subtypes of TNBC have been found to have higher expression of EGFR pathway genes. Trials have not targeted these specific subtypes with EGFR inhibition, and results have been disappointing in several published abstracts [45, 46]. In a study of 102 patients with TNBC, patients were randomized to weekly cetuximab plus or minus carboplatin at AUC of 2. Patients who received the combination therapy with carboplatin had a better response rate (18% versus 6%) and clinical benefit (PR or SD > 6 mo) of 27% versus 10% [45]. A second study tested carboplatin and irinotecan plus or minus cetuximab. Despite a higher response rate in the cetuximab-containing regimen (49% versus 30%), PFS was similar between the two groups (5.1 months versus 4.7 months) [46]. Activation of downstream EGFR targets, such as PI3K, may be responsible for limiting responses to EGFR directed therapy [47].

4.6. PI3K Pathway Inhibition. Phosphatidylinositol 3-kinase (PI3K), which is downstream of growth factor receptor signaling pathway, plays an important role in cell survival and proliferation and has been shown to be activated in a subset of TNBC, due to PTEN loss or less commonly *PIK3CA* mutation [48, 49]. Low PTEN expression was present in more than 60% of TNBC tumors in one study [48]. Since loss of PTEN is associated with increased activation of downstream Akt and predicts response to PI3K pathway inhibitors in preclinical models [50, 51], inhibitors of PI3K pathways could potentially have therapeutic efficacy in a subset of TNBC. For example, NVP-BEZ235, a PI3kinase inhibitor, has shown significant antitumor effect in the mesenchymal-like subtype of TNBCs, which are known to have higher expression of genes involved in EGFR pathway [31]. Clinical trials of PI3K pathway inhibitors are needed to confirm the preclinical findings.

Table 3: Potential Therapeutic Strategies and agent examples for TNBC.

Potential therapeutic strategies	Agent examples
Src inhibition	Dasatinib; Saracatanib
PARP inhibition	Olaparib; ABT-888
Androgen receptor inhibition	Bicalutamide
Targeting epigenetics	Decitabine LBH589
EGFR pathway inhibition	Cetuximab
PI3K pathway inhibition	NVP-BEZ235; Everolimus

5. Conclusions

Chemotherapy-resistant triple negative breast cancer remains a major cause of mortality and currently lacks any proven targeted therapy. The search for new therapeutic targets is complicated by the tremendous complexity of this disease, as demonstrated by the recent report of the first completed genome of a basal-like breast cancer. At the level of gene expression, the TNBC group also is actually comprised of distinct subtypes with very different biological signatures. All these subtypes would benefit from comprehensive analysis at the genomic, epigenomic, and proteomic levels and the results of the cancer genome atlas project are awaited with great interest.

There were more than 120 ongoing trials focusing on TNBC at the time of writing of this paper per clinicaltrials.gov. As stated above, potential targeted therapy can be applied to TNBC depending on the subtype (Table 3). However, most of the current trials are conducted in otherwise unselected patients and not directed by predictive biomarkers or mechanistic hypotheses. If this relatively large number of trials does not produce a breakthrough, we must rethink our investigational approach for this highly heterogeneous group of breast cancers. The development of "genome-first approaches" where patients are stratified upfront and prospectively placed into clinical trials designed to address the therapeutic hypotheses generated by analysis of individual tumor profiles is surely the most logical approach to consider.

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Hindawi Publishing Corporation
International Journal of Breast Cancer
Volume 2012, Article ID 829315, 6 pages
doi:10.1155/2012/829315

Review Article

Current Status of Poly(ADP-ribose) Polymerase Inhibitors as Novel Therapeutic Agents for Triple-Negative Breast Cancer

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Received 16 July 2011; Accepted 23 August 2011

Academic Editor: Tari King

Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer that is clinically defined as lacking estrogen and progesterone receptors, as well as being ERBB2 (HER-2) negative. Without specific therapeutic targets, TNBC carries a worse prognosis than other types of breast cancer in the absence of therapy. Research has now further differentiated breast cancer into subtypes based on genetic expression patterns. One of these subtypes, basal-like, frequently overlaps with the clinical picture of TNBC. Additionally, both TNBC and basal-like breast cancer link to BRCA mutations. Recent pharmaceutical advances have created a class of drugs, poly(ADP-ribose) polymerase (PARP) inhibitors, which are showing potential to effectively treat these patients. The aim of this paper is to summarize the basis behind PARP inhibitors and update the current status of their development in clinical trials for the treatment of TNBC.

1. Introduction

Breast cancer is a multifaceted, heterogeneous disease whose treatment is evolving as genetic profiles shed more light on potential targets. The understanding of breast cancer became more complex with Perou et al.'s 2000 publication detailing the classification of breast cancer based on gene-expression assays [1]. Among this classification was the basal-like subtype, described as frequently (but not always) being ER, PR, and HER-2 deficient while also expressing basal cytokeratins 5/6 and 17 and epidermal growth factor (EGFR) [1, 2]. These basal-like breast cancers make up 17 to 37% of all breast cancers [2–4]. Having genetic profiles outlining the inherent differences in breast cancer has allowed for new research paths attempting to develop novel therapeutics that are subtype dependent.

The definition of triple-negative breast cancer is based on clinical observations; the tumor must lack estrogen receptors (ERs), progesterone receptors (PRs), and hormone epidermal growth factor receptor type 2 (HER-2) expression. These tumors are particularly vexing for physicians because there are no known endocrine targets nor are there specific receptors to block. Women diagnosed with TNBC tend to be younger [5] and are more likely to present with poorly differentiated tumors [6]. Although TNBC is responsive to chemotherapy and features a higher pathologic complete response (pCR) rate compared to other breast cancer types (in the presence of neoadjuvant therapy) [7], the prognosis for TNBC patients is still poor [7, 8].

There are many similarities between TNBC and basal-like breast cancer, but the two terms are not synonymous (Figure 1). They share demographic characteristics such as age of first menarche and increased incidence in the African-American [9] and Hispanic [10] female population. It has been noted that roughly 80% of TNBC tumors are basal-like breast cancers [11]. However, immunohistochemical studies have shown that 17–40% of basal-like breast cancers do not have a triple-negative phenotype [12]. Up to 20% of basal-like breast cancers actually express ER or HER-2 to some extent [13].

One important similarity between TNBC and basal-like breast cancer is the incidence of mutations in the

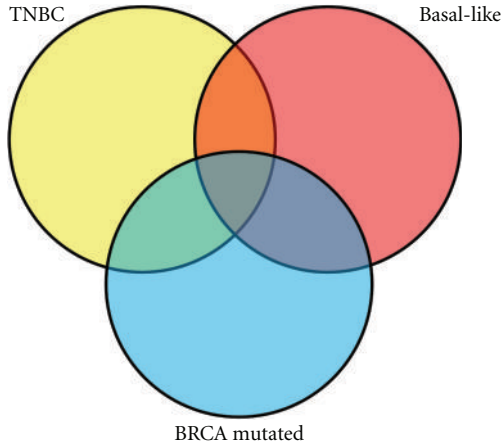


Figure 1: A Venn diagram representing the connection of TNBC, basal-like breast cancer, and BRCA-mutated breast cancer.

breast cancer susceptibility gene 1 and 2 (BRCA1 and 2). BRCA mutations are only 2-3% of all breast cancers but signify an increased lifetime risk of breast and ovarian cancer [14]. Somatic BRCA mutations or inactivation of the gene can also occur. It is estimated that methylation of the BRCA1 promoter can be found in 11–14% of sporadic breast cancers [15–17]. BRCA1 is a key player in mammary gland development [18], and both BRCA1 and BRCA2 are connected with DNA repair [14]. A majority of tumors in women with BRCA mutations feature similar expression patterns as basal-like tumors [18–20], clouding the picture of where BRCA-mutated cancers, basal-like breast cancers, and TNBC originate (Figure 1).

Researchers have found the links between TNBC, basal-like breast cancer, and BRCA mutations to be a potential source of directed therapy. One notable avenue is through synthetic lethality. This is a strategy to target and kill specific cell types, without collateral damage. It is achieved by locating a gene that, when inhibited, will kill cancerous cells that contain a specific genetic signature. The inhibitor would not damage normal cells that lack the cancer-specific gene. The design and exploration of poly(ADP-ribose) polymerase (PARP) inhibitors have emerged as a potential target to cause synthetic lethality in cancerous cells while sparing normal mammary tissue. The aim of this paper is to discuss the molecular basis behind PARP inhibitors and an update on their current status in several clinical trials.

2. PARP1 Inhibitors

Poly(ADP-ribose) polymerase (PARP) is a nuclear protein that is activated in the presence of DNA damage. While several PARP proteins have been detected, PARP1 and PARP2 have been associated with DNA stability [21]. When single strand DNA (ssDNA) damage occurs, it is identified and repaired by a cellular process that includes PARP and base excision repair [22]. If ssDNA breaks are not repaired (e.g., PARP inhibition), the breaks build up and are converted at the replication fork to double-strand DNA (dsDNA) breaks [23–25]. At this point, homologous recombination or nonhomologous end joining repairs the double-stranded breaks in DNA [23, 25].

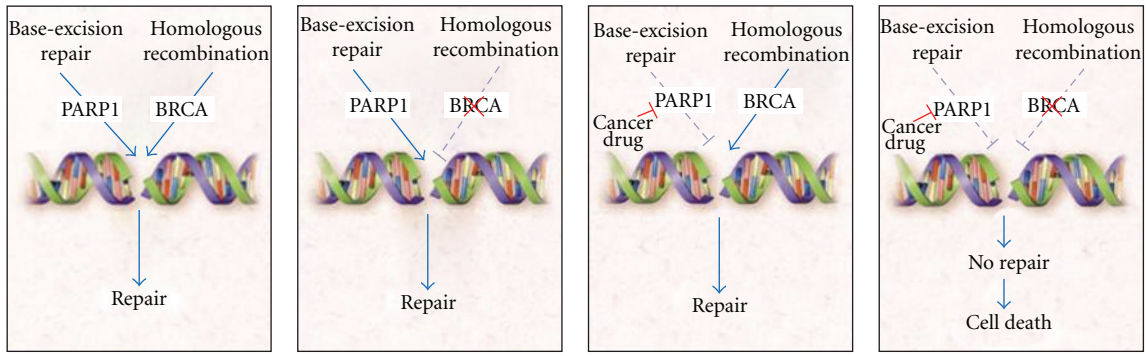
Homologous recombination is mediated by several factors, including BRCA1, BRCA2, and RAD51 [26–28]. Cells deficient in functioning homologous recombination, such as ones with defective BRCA1 and/or BRCA2 genes, are forced into less precise repair pathways that make them more susceptible to cell death when overwhelmed with defects to repair [29]. These alternate pathways include nonhomologous end joining. The incorrect pairing of ends of DNA then possibly leads to genomic instability, ultimately ending in apoptosis (Figure 2). Interestingly, PARP is also involved in dsDNA repair in combination with nonhomologous end joining, so PARP inhibition also hinders the cell's other repair routes [24]. PARP1 inhibitors are being investigated as pharmacologic interventions for metastatic TNBC due to a theory of selectivity: if only BRCA-defective genes are terminated, then other cells that maintain a normal, functioning BRCA allele will not be killed by a PARP inhibitor. This synthetic lethality is being developed to create a new class of drugs that aim to efficiently kill cancer cells.

3. Current Therapeutic Strategy

Several PARP1 inhibitors are being studied at the clinical trial level, and this paper will focus specifically on iniparib, olaparib, and veliparib (Table 1, http://www.clinicaltrials.gov/). Results of an open-label phase II trial for iniparib (BSI-201, Sanofi-Aventis) combined with chemotherapy on metastatic TNBC patients were recently published [30]. This trial compared the use of gemcitabine and carboplatin alone versus those two agents and iniparib. The median progression-free survival increased when iniparib was added, from 3.6 to 5.9 months. The median overall survival was also significantly increased in the iniparib group, up to 12.3 months from 7.7 months. A complete or partial response was seen in 56% of patients receiving iniparib, while only 34% exhibited such a response in the gemcitabine/carboplatin arm. Common side effects seen amongst the 116 patients were nausea, fatigue, anemia, and neutropenia. It is notable that these side effects did not increase when iniparib was added to the regimen, suggesting that the side effects originate from gemcitabine and/or carboplatin.

A notable component of this study is that BRCA1/2 status was not assessed on the patients. Domagala et al. have claimed that 18% of BRCA1-associated cancers have low or no nuclear expression of PARP1 [32] and low PARP1 expression in 21% of triple-negative BRCA1-associated breast cancers [33]. When looking at cytoplasmic and nuclear PARP, another group has observed its presence in all intrinsic types of breast cancer, albeit with different frequencies [34]. There was a significant correlation between cytoplasmic and nuclear PARP in that study. Clearly, the expression pattern and full mechanism of PARP1 needs to be investigated to better understand if it will be an effective target for TNBC.

At this year's meeting of the American Society of Clinical Oncology, O'Shaughnessy and colleagues presented their results of the phase III iniparib trial. This trial enrolled 519 women and again looked at gemcitabine and carboplatin



(a) (b) (c) (d)

Figure 2: Depiction of BRCA mutations and PARP1 inhibitors blocking DNA repair and causing cell death [31]. Copyright © 2009 Massachusetts Medical Society. All rights reserved.

Table 1: Partial list of ongoing clinical trials for PARP inhibitors on TNBC.

Drug/company	Trial ID	Trial	Phase
Olaparib (AZD2281)/AstraZeneca	NCT01116648	Cediranib and olaparib	II
	NCT00647062	AZD2281 and carboplatin	I
	NCT00516724	In combination with carboplatin and/or paclitaxel	I
	NCT00707707	In combination with paclitaxel	I
	NCT00679783	In known BRCA/TNBC	II
Iniparib (BSI-201)/Sanofi-Aventis	NCT01173497	Iniparib + irinotecan	II
	NCT00813956	Neoadjuvant with gemcitabine and carboplatin	II
	NCT01045304	Metastatic with gemcitabine and carboplatin	II
	NCT01204125	Neoadjuvant with paclitaxel	II
	NCT01130259	In combination with gemcitabine and carboplatin	III
Veliparib (ABT-888)/Abbott	NCT01009788	With temozolomide	II
	NCT01104259	With cisplatin and vinorelbine ditartrate	I
	NCT01306032	With cyclophosphamide	II
	NCT01042379	I-SPY2 trial	II
	NCT01251874	With carboplatin	I

Data obtained from http://www.clinicaltrials.gov, June 15, 2011.

versus the same regimen with added iniparib. The results did find an increase in progression-free survival amongst the iniparib/gemcitabine/carboplatin arm (5.1 versus 4.1, $P = 0.027$), but this did not achieve the prespecified criteria for significance ($P = 0.01$) [35]. A possible explanation behind the change in results from phase II to phase III is that the heterogenous nature of TNBC will continue to make finding a single agent problematic in treating all comers. By not stratifying the patients based on BRCA status or TNBC subtype, it leaves questions as to which patients will truly benefit from this drug and which have a genetic makeup that is not conducive to iniparib. Iniparib is continuing to be studied in other phase III clinical trials, including its effects on nonsmall cell lung cancer and ovarian cancer. Iniparib evidently is not being discontinued completely from breast cancer research; rather, the drug maker has continued with phase II trials analyzing different doses, schedules, and chemotherapy combinations.

Olaparib (AZD2281, AstraZeneca) is another PARP1 inhibitor that is being tested on various cancers, including breast. Preclinical models showed an increased selective potency for this compound [36]. The subsequent phase I trial revealed 400 mg twice daily to be the maximum dose. With a BRCA1- or BRCA2-defective cohort of 22 patients, antitumor efficacy was observed once the dosages reached 100 mg twice daily [37]. Results of a phase II trial detailed how olaparib is effective in breast cancer patients with a BRCA1 or BRCA2 mutation and advanced disease [38]. While admittedly not a flawless design, such as lacking randomization, the results showed promise. All patients in the study had locally advanced breast cancer (LABC) or metastatic breast cancer. For the TNBC and BRCA1/2 carrier patients in this cohort, twice daily 400 mg dosages of olaparib were more effective than twice daily 100 mg dosages when analyzing objective response (54% versus 25%) and progressive disease (15% versus 31%). These data were

observed, but it must be noted that this trial was not designed or powered for this comparison. When looking at all of the women in the trial, 41% of the BRCA1- or BRCA2-mutated breast cancer patients had an objective response when assigned 400 mg twice-daily olaparib.

Despite these encouraging results, London-based drug maker AstraZeneca has decided to suspend olaparib prior to a phase III trial. AstraZeneca has shifted its olaparib focus to ovarian cancer and currently has a phase II trial to study its effects on that cancer type [39].

Veliparib (ABT-888, Abbot Laboratories) has been investigated as a single agent [40] and also has been shown to improve laboratory outcomes when paired with platinum agents and radiotherapy. Donawho et al. were able to show that 5 and 25 mg/kg/d of veliparib combined with cisplatin were significant in tumor regression of murine models compared to cisplatin alone [41]. 10 mg/kg/d of veliparib was also shown to be effective in combination with carboplatin when compared to carboplatin alone. In addition to improving the effectiveness of platinum agents on murine models of breast cancer, veliparib has shown to assist in radiation therapy. In mice, 3 Gy with added veliparib was significantly more effective in inducing early cellular senescence than just the radiation alone [42]. A phase II trial recently studied the effects of veliparib combined with temozolomide on metastatic breast cancer and included TNBC patients [43]. Of the 51 patients in the study, only 8 had a BRCA mutation. Progression-free survival was 5.5 months in the BRCA-mutated group versus 1.8 months for patients without a BRCA mutation. This suggests that veliparib might only be effective in patients carrying BRCA mutations.

4. Conclusion

TNBC is a clinical term used to describe women whose tumors lack expression of ER, PR, and HER-2. This subset of breast cancer partially fits into a molecular subtype known as basal-like breast cancer. Regardless of whether one looks at data through a TNBC or basal-like spectrum, the prognosis is worse compared to other subtypes. While there is no specific treatment regimen for TNBC patients, neoadjuvant therapy has been effective in achieving complete pathologic response (pCR) that subsequently correlates to improved outcome [7, 44]. TNBC patients who achieve pCR had similar overall survival rates to non-TNBC patients who achieved pCR. However, TNBC patients that did not reach pCR had a worse outcome compared to non-TNBC patients that did not reach pCR.

Therapeutic options for TNBC have the potential to drastically increase in the near future. Combinations of platinum compounds for neoadjuvant therapy are being tested in various clinical trials. Epidermal growth factor receptors (EGFRs) are noted in 45–70% of TNBC [45, 46], resulting in EGFR antagonists such as cetuximab (Merck Serono) to be explored. Linderholm et al. noted VEGF to be increased in their TNBC patients compared to non-TNBC [47], and the antiangiogenic agent bevacizumab is being studied in combination with several chemotherapy agents in clinical trials. Still other emerging avenues for treatment include mammalian target of rapamycin (mTOR) inhibitors and SRC tyrosine kinase inhibitors.

Many potential therapeutic agents are in the pipeline in laboratories worldwide, but PARP inhibitors have the potential to alter the outcome of TNBC patients. In addition to iniparib, olaparib, and veliparib, there are more being constructed. These include CEP-9722 (Cephalon), INO-1001 (Genentech), PF-01367338 (Clovis/Pfizer), and MK-4827 (Merck).

Several challenges must still be met to continue advancing PARP inhibitors. Most notably is the fact that recent trial data have landed huge blows to the momentum of PARP inhibitors for breast cancer. At the 2011 ASOC, it was announced that iniparib did not perform at its expected effectiveness in a phase III trial with metastatic TNBC patients. AstraZeneca has maintained an interest in PARP inhibitors, but is doing so through further trials in other organs, such as ovarian. Yet another complication that has emerged is resistance to PARP inhibitors that is being observed in the laboratory [48]. Norquist et al. recently reported to observe cell lines with BRCA1/2 restoration mutations exhibiting resistance to platinum therapy in patients with hereditary ovarian cancer. They also observed these restoration mutations to predict resistance to PARP inhibitors, but did not have a large sample size [49]. More research must be done on these compounds to prepare for these and other, unknown, complications.

It will be imperative to continue exploring the pathway connecting TNBC, basal-like breast cancer, and BRCA. There appears to be more questions to explore and compounds to test in the TNBC population with these therapeutics. Also, further testing is necessary to identify the optimal doses of not only the PARP inhibitor but also any combined chemotherapy. These key components of PARP inhibitor development will hopefully improve the quality of this class of cancer-fighting drugs and provide hope for patients currently facing such bleak diagnoses.

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Hindawi Publishing Corporation
International Journal of Breast Cancer
Volume 2012, Article ID 809291, 10 pages
doi:10.1155/2012/809291

Review Article

Metabolic Syndrome and Triple-Negative Breast Cancer: A New Paradigm

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Received 25 July 2011; Accepted 15 August 2011

Academic Editor: Quyen D. Chu

Triple-negative breast cancers (TNBCs) are aggressive tumors with poor prognosis compared to other breast cancer subtypes. The evidence linking TNBC with the metabolic syndrome, which consists of central obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension, has emerged from clinical studies and experiments using cell lines and mouse models. Epidemiological studies have associated abdominal obesity with increased incidence of TNBC. Additionally, insulin resistance, dyslipidemia, and hypertension are associated with increased incidence of breast cancer across all subtypes. The insulin-leptin-adiponectin axis has been implicated mechanistically in breast cancer tumorigenesis. Specifically, increased leptin and decreased adiponectin levels disrupt homeostatic signaling pathways involved in cell proliferation, survival, cell-cycle regulation, and angiogenesis. Insulin, insulin-like growth factor I (IGF-I), and epidermal growth factor receptor (EGFR) may mediate interactions between these two hormones. Further research will facilitate the development of targeted therapeutics and programs to modify lifestyle factors to modulate the insulin-leptin-adiponectin axis for TNBC.

1. Introduction

Triple-negative breast cancers (TNBCs) lack expression of the steroid receptors estrogen (ER) and progesterone (PR) and the tyrosine kinase human epidermal growth factor receptor 2 (HER-2). Therefore, TNBCs are a diagnosis of exclusion, typically characterized by upregulation of cytokeratins 5, 14, and 17 and elevation of the epidermal growth factor receptor (EGFR) [1–3]. Studies estimate that approximately 15–20% of breast cancers meet these criteria [4–6]. Compared to other breast cancer subtypes, TNBCs are typically aggressive, invasive (ductal, medullary, or metaplastic), grade III tumors with high rates of mitotic division, of which approximately half contain a high rate of p53 mutations [7]. For these reasons, they account for a disproportionately high percentage of metastases, distant recurrence, and death among patients with breast cancer. Metastases in TNBCs are most common to visceral organs including liver, lungs, and central nervous system. As a diagnosis of exclusion, TNBC overlaps considerably with basal-like breast cancer (BLBC) although differences between the two subtypes exist, especially at a genetic level. Other molecular subtypes defined by gene expression patterns include luminal A, luminal B, HER-2-enriched group, and claudin-low, all of which may include TNBCs to some extent [8, 9]. TNCBs are most common among premenopausal women, especially those of African American descent [4–6, 10]. In addition, TNBCs are common among patients with BRCA1 mutations [11, 12].

Since the first molecular characterization of TNBCs in the literature in 2005, the topic has quickly emerged as an active area of research [13]. While initial studies focused on molecular and clinical characterizations of patients with the diagnosis, more recent studies have identified subgroups of patients with TNBC, proposed molecular mechanisms that may contribute to tumorigenesis, and explored potential therapeutic interventions for patients. In this paper, we examine the connection between TNBC and the metabolic

syndrome, which consists of central obesity, insulin resistance, impaired glucose tolerance, dyslipidemia, and hypertension. Our analysis of the literature will encompass *in vitro* and *in vivo* studies in cell lines and mouse models of TNBC, respectively, as well as clinical studies examining epidemiology and treatment of TNBC.

2. Risk Factors for TNBC

Obesity, which is associated with insulin resistance and type 2 diabetes mellitus (DM), is an established risk factor for cancer incidence. In a meta-analysis of 141 articles, body mass index (BMI) was positively associated with an increased incidence of postmenopausal breast cancer, along with colon, endometrial, esophageal, gallbladder, pancreas, renal, thyroid cancers, leukemia, multiple myeloma, and non-Hodgkin's lymphoma in women [14]. The results were less clear, however, for premenopausal breast cancer as a positive association between obesity and premenopausal breast cancer was found in Asia-Pacific women (risk ratio (RR) = 1.16; 95% CI, 1.01–1.32), while inverse relations were reported in North American women (RR = 0.91; 95% CI, 0.85–0.98) and European and Australian women (RR = 0.89; 95% CI, 0.84–0.94) These findings suggest that different subpopulations of women possess different risk factors for breast cancer. It may also suggest that BMI is not an ideal measure of adiposity. Instead, other measures such as waist-to-hip ratio (WHR) or waist circumference, which are specific measures of central or abdominal adiposity, may be preferential to assess cancer risk. Two meta-analyses that examined a correlation between elevated WHR and risk of breast cancer in premenopausal women reported positive associations [15, 16]. The study by Connolly et al. reported that elevated WHR was associated with a 79% (summary risk (SR) = 1.79; 95% CI, 1.22–2.62) increased risk of breast cancer for premenopausal women and a 50% (SR = 1.50; 95% CI, 1.10–2.04) increased risk for postmenopausal women [15]. Similarly, the study by Harvie et al. reported that small WHR was associated with a 37% decreased risk (RR = 0.63, 95% CI, 0.45–0.88) in premenopausal women only after adjusting for BMI [16]. The authors hypothesized that general obesity may not modulate risk, but central obesity increases risk in premenopausal women. In contrast, the authors reported that general obesity and not central obesity increased cancer risk in postmenopausal women. This interesting result led the authors to hypothesize that insulin resistance and insulin-like growth factors, which are associated with central obesity, may play a larger role in modifying breast cancer risk for premenopausal women, while estrogen may play a greater role in postmenopausal breast cancer [16].

While the link connecting obesity and incidence of all types of breast cancers is well established, the data examining obesity and TNBC are much less prevalent. In the Carolina Breast Cancer Study, WHR was compared between the highest (≥0.84) and lowest (<0.77) groups in relation to BLBC [17]. Across all women, there was an increased risk (odds ratio (OR) = 2.3; 95% CI, 1.4–3.6) for developing BLBC with higher WHR. Premenopausal women (OR = 1.8; 95% CI, 1.0–3.4) and postmenopausal women (OR = 2.7; 95% CI, 1.3–5.4) with high WHR both had elevated risk of developing breast cancer compared to the lowest WHR group. Weight gain in women as reported since fifth grade was highest in African American women in this sample. In contrast, no significant trend was reported for BMI and risk of breast cancer. A 2008 study examining 620 predominantly white women in rural Appalachia, 117 of whom had TNBC, reported a significant association between obesity and incidence of TNBC [18]. In this sample, approximately 50% of patients with TNBCs were obese as compared to 36% of non-TNBCs. Obesity in this study was defined as a BMI ≥30. The preponderance of evidence suggests an association between TNBC and obesity when obesity is defined as an elevated WHR, but more contradictory evidence exists when using BMI as a measure of obesity. Clearly, the conflicting results warrant additional research. Future epidemiological studies would benefit from measurement of all three receptor markers and studies that concurrently examine multiple definitions of obesity.

A common corollary of metabolic syndrome, type 2 DM, has been associated with increased risk of breast cancer. A 2007 meta-analysis of twenty studies estimated a 20% increased risk of breast cancer for women with type 2 DM (RR = 1.20; 95% CI, 1.12–1.28) [19]. For TNBC, one study reported a significant relation with 58% of patients with TNBC possessing a comorbid diagnosis of metabolic syndrome compared to 37% of patients without TNBC in a sample of 176 individuals using criteria of the National Cholesterol Education Program and 52% compared to 34% using criteria of the American Association of Clinical Endocrinologists [20]. In addition, a 2011 study reported a 75% increase in the risk of postmenopausal breast cancer (RR = 1.75; 95% CI, 1.37–2.22) for women who were found to have at least three of the four components of metabolic syndrome [21]. However, the Carolina Breast Cancer Study reported no elevated prevalence of type 2 DM in TNBC compared to other breast cancer subtypes [17].

Recently, epidemiological studies have associated dyslipidemia and hypertension with breast cancer risk. In a prospective study examining all-cancer incidence of 1,189,719 Korean men and women, Kitahara et al. reported a positive association between total cholesterol and breast cancer risk in women (hazard ratio (HR) = 1.17; 95% CI, 1.03–1.33) [22]. The researchers compared individuals with total cholesterol ≥240 mg/dL to individuals with cholesterol <160 mg/dL and adjusted for cigarette smoking, alcohol consumption, BMI, fasting serum glucose, hypertension, and physical activity. In addition, hypertension was independently predictive of breast cancer risk in a sample of 3,869 postmenopausal women with breast cancer as compared to 4,082 controls (OR = 1.19; 95% CI, 1.07–1.33) [21]. Another study reported a 23% increased risk of breast cancer for hypertensive women [23]. However, after adjustment of confounders including BMI, the elevated risk was no longer significant (HR = 1.14; 95% CI, 0.93–1.40).

Epidemiological studies suggest a positive association between the metabolic syndrome as a whole, along with

many of its individual components, and breast cancer risk. The many confounding variables that may mediate this effect need to be considered in order to determine whether this is a causative effect. Studies would benefit from multi-institution designs to assess geographically diverse populations. Further studies should also address how changes in components of metabolic syndrome, such as weight, affect incidence of disease, and treatment outcomes after initial diagnosis of TNBC. Larger sample sizes will determine whether subpopulations of patients with TNBC (e.g., pre- versus postmenopausal women) possess unique clinical and molecular characteristics.

3. Risk of Recurrence and Mortality in TNBC

In addition to exploring risk factors that influence incidence of TNBC (primary prevention), it is also essential to understand factors that influence recurrence of TNBC (secondary prevention). Compared to other subtypes of breast cancer, TNBCs are more often diagnosed as aggressive, invasive, grade III, and lymph-node positive tumors [7]. These outcomes are predictive of increased morbidity and mortality. In addition, TNBCs have a high rate of recurrence with visceral metastases compared to other subtypes of breast cancer, especially within the first five years after diagnosis [24]. After five years, the risk of recurrence drops dramatically.

Obese patients with breast cancer have more frequent recurrence and worse prognosis as compared to lean patients. In a sample of 495,477 U.S. women, increasing BMI was significantly associated with increased death rates for breast cancer [25]. As compared to the lowest BMI group (18.5–24.9), there was an elevated risk of 34% for BMI of 25.0–29.9 (RR = 1.34; 95% CI, 1.23–1.46), 63% for BMI of 30.0–34.9 (RR = 1.63; 95% CI, 1.44–1.85), 70% for BMI of 35.0–39.9 (RR = 1.70; 95% CI, 1.33–2.17), and 112% for BMI $\geq$ 40.0 (RR = 2.12; 95% CI, 1.41–3.19) of dying of breast cancer. Furthermore, in a sample of 18,967 patients in Denmark with early-stage breast cancer, BMI at diagnosis was correlated with disease prognosis. Patients with BMI $\geq$ 30 kg/m^2 had a 46% higher risk of distant metastases (HR = 1.46; 95% CI, 1.11–1.92) after 10 years and 38% increased risk of mortality from breast cancer (HR = 1.38; 95% CI, 1.11–1.71)) as compared to patients with BMI < 25 kg/m^2 [26]. The authors also suggested that adjuvant chemotherapy and endocrine therapy were less effective over time periods greater than 10 years for patients with BMI > 30 although it was unclear whether this effect was mediated by poor responsiveness to treatment or differences in biology. Even though obese patients were more likely to present with advanced tumors in terms of size and spread to lymph nodes, obesity was still an independent predictor after controlling for these confounders. A recent, single institution study examined BMI in 418 patients treated for TNBC [27]. The study measured BMI after diagnosis of TNBC and then counted the number of recurrences and deaths. After controlling for clinically significant factors, no significant relation was found between BMI and overall survival (HR = 0.94; 95% CI, 0.54–1.64) or recurrence-free survival (HR = 0.81; 95% CI, 0.49–1.34). In a sample of 1,169 patients diagnosed with invasive breast cancer, the relationship between general obesity and response to neoadjuvant chemotherapy was examined [28]. When comparing overweight (BMI 25 to <30 kg/m^2) and obese (BMI $\geq$ 30 kg/m^2) groups to the normal/underweight group (BMI < 25), a significant association was present for pathologic complete response to neoadjuvant chemotherapy (OR = 0.67; 95% CI, 0.45–0.99) in the normal/underweight group. While high BMI was associated with worse overall survival, no significant effects were seen for breast-cancer specific or progression-free survival. Finally, although data linking risk of recurrence and mortality in patients with hypertension and TNBC are limited, a 2011 study retrospectively examined the use of beta blockers on prognosis for patients with breast cancer [29]. After adjustment for a number of covariates, patients with TNBC who were taking beta blockers had significantly improved relapse-free survival (HR = 0.30; 95% CI, 0.10–0.87), and while overall survival was improved (HR = 0.35; 95% CI, 0.12–1.00), it only approached a significance level (P = 0.05). Similar findings were also reported for non-TNBC subtypes.

A number of epidemiological studies have suggested that physical activity and weight loss are inversely related breast cancer risk and recurrence. The Women's Healthy Eating and Living (WHEL) Study prospectively examined 1,490 women with breast cancer [30]. The authors reported that performing exercise equivalent to walking 30 min, six days per week, and consuming $\geq$5 daily servings of fruits and vegetables decreased mortality by 46% (HR = 0.56; 95% CI, 0.31–0.98). While ER+ tumors were associated with decreased mortality with these lifestyle interventions (P < 0.05), no significant effect was observed for ER−, PR− tumors (P = 0.40). To the best of our knowledge, the largest study to date examining the link between physical activity and invasive breast cancer was a meta-analysis of 12,108 patients, which included six studies [31]. While physical activity prior to diagnosis had no effect on breast cancer deaths across all patients, physical activity after diagnosis reduced breast cancer deaths by 34% (HR = 0.66, 95% CI, 0.57–0.77) and disease recurrence by 24% (HR = 0.76, 95% CI, 0.66–0.87). Postdiagnosis exercise only provided significant benefits for patients with BMI $\geq$ 25 kg/m^2. Interestingly, physical activity after diagnosis reduced breast cancer deaths by 50% (HR = 0.50, 95% CI, 0.34–0.74) for ER+ tumors with no significant effect for patients with ER− tumors. When looking at the individual studies that composed the meta-analysis, the studies that examined postdiagnosis physical activity were prospective, observational, and questionnaire-based studies, while those that examined prediagnosis physical activity had case-control designs [32–37]. While the definition of physical activity varied somewhat from study to study, the studies generally defined physical activity as moderate recreational activity, and for the purpose of their analyses, the authors combined these forms of exercise into metabolic equivalent task (MET) hours per week. Examples of moderate physical activity included walking, jogging, running, biking, swimming, tennis, calisthenics/aerobics, and squash/racquetball.

One study included in the meta-analysis specifically examined the relation between risk reduction of breast cancer and duration of exercise [32]. In a sample of 2,987 women diagnosed with breast cancer, the number of hours an individual exercised per week was categorized. Compared to women who performed the equivalent of walking at an average pace less than 3 MET-hours per week, there was a nonsignificant 20% risk reduction of death from breast cancer for 3 to 8.9 MET-hours per week (RR = 0.80; 95% CI, 0.60–1.06), a significant 50% risk reduction for 9 to 14.9 MET-hours per week (RR = 0.50; 95% CI, 0.31–0.82), 44% risk reduction for 15 to 23.9 MET-hours per week (RR = 0.56; 95% CI, 0.38–0.84), and 40% risk reduction for 24 or more MET-hours per week (RR = 0.60; 95% CI, 0.40–0.89). This study, however, did not find a significant effect for exercise, even for 9 or more MET-hours per week, for ER−, PR− tumors.

These studies provide an insight on the role of physical activity as a potentially beneficial breast cancer treatment that may be used in conjunction with existing radiation and chemotherapy treatments [32–37]. Although studies explicitly targeting patients with TNBC have not been performed, a potential mechanism behind this link may be decreased concentrations of estrogen via reduction in body fat or decreased androgens via increase in globulins that bind testosterone [38]. Improvements in insulin resistance or blood glucose may also mediate this effect.

In addition to exercise, two large randomized studies have examined whether diet interventions are effective in reducing breast cancer recurrence and mortality [39, 40]. The Women's Intervention Nutrition Study (WINS) examined 2,437 women with breast cancer [39]. The randomized study involved a dietary intervention group with a goal of reducing calories from fat to 15% without compromising nutrition compared to control with median followup of 60 months. The intervention group had statistically lower fat intake ($P < 0.001$). When comparing relapse events between the two groups, relapse was lower in the intervention group as compared to the control group (HR = 0.76; 95% CI, 0.60–0.98, $P = 0.077$ for stratified log rank and $P = 0.034$ for adjusted Cox model analysis). The authors reported a trend for a stronger effect for dietary fat reduction for hormone receptor-negative cancers (HR = 0.58; 95% CI, 0.37–0.91) compared to ER+ tumors (HR = 0.85; 95% CI, 0.63–1.14), although no significant effect was found (interaction test, $P = 0.15$). One of the criticisms of the WINS study was the fact that the intervention group lost about 6 pounds more than the control arm over the duration of the study ($P = 0.005$). As a result, it was unclear whether the outcomes were due to decreased weight or decreased fat intake. Furthermore, the dietary intervention was relatively strict, making it hard to implement in everyday practice. In addition, the WHEL study evaluated the potential benefit of physical activity and a diet rich in vegetables and fruit in breast cancer survivors [40]. The study included 3,088 women with early-stage breast cancer. The arm randomized to a diet rich in vegetables, fruit, and fiber, but low in fat did not have a significantly lower mortality (HR = 0.91; 95% CI, 0.72–1.15) or a lower incidence of second invasive breast cancer (HR = 0.96; 95% CI, 0.80–1.14) during a 7.3-year follow-up period. In this study, the intervention and comparison groups had an average weight difference of 1-kg or less based on measurements at baseline, 1 year, 2 or 3 years, 4 years, and 6 years. In an analysis of the comparison group only, consuming ≥5 daily servings of fruits and vegetables and performing exercise equivalent to walking 30 min, six days per week at baseline was associated with lower mortality [30]. No effect, however, was reported in the randomized trial based on physical activity at baseline for additional breast cancer events or all-cause mortality. These conflicting findings warrant further research, especially to assess diet interventions for patients with TNBC.

Alcohol consumption also appears to moderate recurrence and mortality for breast cancer survivors. In a recent study of 1,897 individuals, consumption of three to four alcoholic drinks or more per week was associated with a 35% (HR = 1.35; 95% CI, 1.00–1.83) increased risk of breast cancer recurrence and 51% (HR = 1.51; 95% CI, 1.00–2.29) increased risk of death due to breast cancer [41]. No difference was found between ER+ versus ER− subgroups although the authors noted that this lack of effect may have been due to a small sample size of patients with ER− tumors. Further studies will be important to assess whether different subtypes of breast cancer are affected differently by diet and alcohol in order to further probe the mechanism of these effects.

4. Insulin and TNBC

Insulin is implicated as a link between obesity and breast cancer risk. In particular, upregulation of insulin has been hypothesized to directly increase proliferation of breast tissue and breast cancer cells. A 2009 study, which measured insulin at baseline and at 1, 3, and 6 years of followup, reported a HR of 2.22 (95% CI, 1.39–3.53) for incidence of breast cancer in postmenopausal women when comparing the highest baseline insulin concentration group to the lowest group [42]. Another study demonstrated that a high homeostatic model assessment score, which is associated with serum levels of insulin and glucose, was correlated with increased breast cancer mortality in a sample of 527 women [43]. Samples were collected at a single time point, 30 months postdiagnosis. Similarly, a 2011 study of 604 women in the Health, Eating, Activity, and Lifestyle (HEAL) Study measured serum C-peptide, a marker of insulin secretion, three years after diagnosis [44]. An increased C-peptide concentration of 1 ng/mL was associated with a 35% increased risk of death from breast cancer (HR = 1.35; 95% CI, 1.02–1.87). Collectively, these data suggest that hyperglycemia and hyperinsulinemia are associated with poor prognosis for patients with breast cancer. In contrast, a 2007 case-control study examining blood samples in predominantly premenopausal women reported that increased levels of insulin and C-peptide were not risk factors for breast cancer [45]. This study, however, did not examine ER−, PR− tumors. A recent study by Erickson et al. examined type 2 DM and associated prognosis in patients with breast cancer

[46]. Baseline hemoglobin A1C (HbA1C) levels among 3,003 patients were examined for recurrence and all-cause mortality. The authors reported a significant increase in all-cause mortality after adjustment for confounders for women with HbA1C $\geq$7.0% as compared to <6.5% (HR = 2.35; 95% CI, 1.56–3.54).

The actions of insulin may also occur indirectly via decreased availability of globulin and insulin-like growth factor- (IGF-) binding proteins and increased blood concentration of testosterone, estrogens, or IGFs. Elevated concentrations of unbound estradiol and testosterone have been associated with increased breast cancer risk in pre- and postmenopausal women [47–50]. These compounds have been proposed as molecular links between obesity and breast cancer risk. Insulin also inhibits sex hormone-binding globulin (SHBG) production and increases the levels of IGF-I in blood, which results in increased mitogenic activity [51]. This link is consistent with approximately 50% of breast cancer tumors overexpressing IGF-I receptor [52]. A recent laboratory study found that seven cell lines that serve as models of TNBC expressed IGF receptors [53]. Surprisingly, expression was at similar levels to ER+ cell lines even though type I IGF receptor levels are increased by estrogen in ER+ cell lines. In all cases, IGF-I increased proliferation and survival of the cancer cell lines.

Although studies have reported a positive association between type 2 DM and breast cancer, a potential confounding variable in establishing this relation is treatment regimen [54]. Insulin has recently been implicated to have cancer promoting effects, while recent evidence suggests metformin to have cancer protecting effects in patients with type 2 DM [55]. Most patients with type 2 DM are prescribed either insulin or metformin. Insulin glargine use, especially when prolonged, may increase the incidence of breast cancer. In one study, this effect was especially prominent for individuals who had received insulin for an average of 5.6 years before starting insulin glargine (HR = 2.7; 95% CI, 1.1–6.5) [56]. In contrast, metformin has been shown to inhibit proliferation and colony formation of TNBC cells *in vitro* [57]. Further experiments extended these findings into *in vivo* mice. Metformin resulted in decreased tumor growth if injected in TNBC tumor xenograft mice and decreased tumor incidence if added before injecting TNBC cells. While the molecular mechanism of how metformin reduces breast cancer incidence and survival is unclear, potential mechanisms include (1) acting as a general growth inhibitor, (2) reducing serum insulin levels, and (3) reducing body weight [54, 57]. Interestingly, the drug only exhibited an antiapoptotic effect in TNBC cell lines, an effect which was not present for luminal A, B, and HER-2 subtypes [58]. Recently, observational studies were performed suggesting that metformin reduces the risk of breast cancer in humans. In one study, metformin use was associated with a 38% lower incidence of ER+, PR+ tumors in postmenopausal women with type 2 DM [59]. No significant effect was demonstrated for ER−, PR− tumors, however, although the sample size for TNBCs was limited. In addition, prospective studies are under way on the role of metformin in breast cancer recurrence. Further studies are necessary to determine whether elevated levels of insulin and C-peptide are risk factors for women with TNBC, as well as to elucidate the mechanism behind this association.

5. Leptin and TNBC

Leptin is the product of the obesity (ob) gene and is primarily synthesized and secreted by adipose tissue, with increasing adiposity associated with higher circulating leptin levels. [60]. Leptin helps regulate food intake and metabolism via its actions on the arcuate nucleus of the hypothalamus. It is hypothesized that leptin resistance in obese individuals may be analogous to insulin resistance in diabetics [61]. This resistance has been proposed to develop via impaired transport of leptin across the blood brain barrier and circumventricular organs and leptin receptor signal attenuation [62]. Clinical studies have reported a positive association between circulating blood leptin and breast cancer risk with particular elevation of mRNA expression in adipocytes in close proximity to the tumor [63].

On a molecular level, it has been hypothesized that elevated leptin expression in epithelial mammary cells may promote tumorigenesis via mechanisms including cell proliferation (aromatase, MAPK, STAT3, and cyclin D1), angiogenesis (VEGF), apoptosis (p53 and caspase 9), cell-cycle regulation (p21), and cell survival (Akt) in breast cancer cell lines [64]. In TNBC cell lines, a study by Saxena et al. reported that leptin directly increased activity of the IGF-I receptor [65]. Similarly, IGF-I reciprocally increased activity of the leptin receptor via phosphorylation. In addition, bidirectional crosstalk between leptin and IGF-I upregulated EGFR promoting proliferation and migration of TNBC cells. The study further reported that using the EGFR inhibitors, lapatinib and erlotinib, in an *in vitro* model system for metastasis after application of leptin and IGF-I reduced invasion and migration of breast cancer cells [65]. Collectively, these data suggest a possible therapeutic route for treatment of TNBC with EGFR inhibitors, because up to 70% of TNBCs overexpress EGFR [7]. In addition to leptin and IGF-I, a 2011 study by Burga et al. reported another potential mechanism for elevated levels of EGFR protein [66]. After RNA knockdown of BRCA1 in mammary epithelial cells, EGFR protein was upregulated due to transcriptional modification and posttranslational stabilization of EGFR. This is important to our understanding of TNBCs, because BRCA1 mutations are highly correlated with TNBCs. Interestingly, EGFR inhibition with erlotinib in female BRCA1 knockout mice, *in vivo*, prevented or delayed development of ER−, but not ER+ tumors. However, the treatment was not effective in shrinking the tumor after tumorigenesis [66].

A causal link between leptin and breast cancer is supported by animal studies in which obese mice that overexpressed transforming growth factor-alpha (TGF-α), but were deficient in leptin, did not develop mammary tumors, while heterozygous and homozygous wild type leptin mice developed tumors in 50% and 67% of cases, respectively [67]. However, these findings were difficult to interpret, because leptin deficient mice possessed limited mammary tissue.

Further studies in mouse models, *in vivo*, suggest a therapeutic potential for leptin receptor, antagonists. In a recent study of 69 TNBC tumors, 92% of breast tumors expressed leptin receptor and 86% expressed leptin [68]. In this study, the peptide Allo-aca, a leptin receptor antagonist, extended survival time by up to 80% in a TNBC mouse xenograft model, *in vivo*. Clinical studies are needed to determine whether leptin antagonists may hold promise as a therapy in humans, especially in obese patients who overexpress leptin.

Clinical trials in humans are currently underway to test the efficacy of EGFR inhibitors in TNBC. These studies have focused on using cetuximab, a humanized antiEGFR IgG1 antibody in conjunction with ixabepilone, cisplatin, carboplatin, or a taxane. (NCT00633464, NCT00463788, [69–71]). In one study, 12 patients with metastatic TNBC were treated with either paclitaxel or docetaxel with cetuximab weekly [69]. Of the eleven patients assessable to followup, nine (82%) exhibited decrease in size of metastasis, but three (27%) developed brain metastasis during treatment (133). Other studies by Carey et al. and O'Shaughnessy et al. have reported therapeutic value of using EGFR inhibitors in conjunction with other chemotherapy agents including (1) carboplatin plus cetuximab and (2) irinotecan and carboplatin, plus cetuximab [70, 71]. The study by Carey et al. compared cetuximab alone to carboplatin plus cetuximab in patients with TNBC metastases [70]. Of the 71 patients who received both drugs, 13 (18%) responded to treatment as compared to only 2 of 31 (6%) of patients who received cetuximab alone. In addition, the preliminary results of the randomized phase II study of metastatic patients with TNBC by O'Shaughnessy et al. reported no improvement in objective response rate (ORR), progression-free survival, and overall survival across all patients with metastatic disease when comparing cetuximab in conjunction with irinotecan and carboplatin as compared to irinotecan and carboplatin [71]. However, subset analysis of revealed that ORR was increased in metastatic patients with TNBC when using all three drugs (19 of 39; 49%) as compared to only irinotecan and carboplatin (10 of 33; 30%). These findings may suggest a therapeutic benefit of using EGFR inhibitors for a subset of patients with TNBC. Larger experimental and control groups and increased number of follow-up years will benefit our understanding of the potential for these treatments.

6. Adiponectin and TNBC

Adiponectin, a protein secreted exclusively by adipose tissue, is an endogenous insulin sensitizer. Levels of adiponectin are inversely correlated with obesity. In contrast to the procarcinogenic effects of leptin, adiponectin may possess anticarcinogenic effects. After controlling for BMI, studies have reported that women with increased adiponectin concentrations possessed a 65% reduced risk for breast cancer [72–74]. In another sample of 527 women diagnosed with stage I–IIIA breast cancer, adiponectin levels above 15.5 μg/mL were associated with improved breast cancer survival (HR = 0.39; 95% CI, 0.15–0.95) [43]. Interestingly, in a 2011 study by Oh et al. the authors reported prognostic value of adipokines in ER−, PR− tumors but not ER+, PR+ tumors (P for trend =0.027) [75]. Patients with low adiponectin levels as defined by the first quartile in the study had a significantly increased likelihood of cancer recurrence as compared to patients in the fourth quartile (HR = 2.82; 95% CI, 1.03–7.68). These results were significant even after adjustment for BMI and homeostasis model assessment scores for insulin resistance. Serum leptin levels were not correlated with diseased outcome in this study. Genetic data also links adiponectin to breast cancer risk. We recently evaluated the role of adiponectin pathway single nucleotide polymorphisms (SNPs) in breast cancer risk. We performed a case-control study on 733 breast cancer cases and 839 controls and genotyped 10 haplotype-tagging SNPs of adiponectin (*ADIPOQ*) and the type I adiponectin receptor (*ADIPOR1*) genes [76]. We showed that two functional polymorphisms of *ADIPOQ*, and one functional polymorphism which has been shown to alter mRNA levels of *ADIPOR1* was significantly associated with risk of breast cancer. When categorized by signaling status, low adiponectin signalers had a 6.56-fold increase in breast cancer risk (95% CI, 0.78–54.89), and intermediate adiponectin signalers had a 4.16-fold increase in risk (95% CI, 0.49–35.19) compared to high signalers (P for trend =0.001). Although these data are preliminary, they provide evidence for a significant role for adiponectin in predicting breast cancer risk.

The mechanisms underlying the association between adiponectin and breast cancer risk have been studied by several investigators. Components of the adiponectin signaling pathway have been implicated in breast tumorigenesis. More specifically, a number of compounds related to cell proliferation (aromatase, MAPK, and cyclin D1), apoptosis (Bcl2 and caspase 8), cell-cycle regulation (AMPK), and cell survival (Akt) have been implicated to mediate tumorigenesis in breast cancer cell lines [64]. While adiponectin has been shown to have an antiproliferative effect on cell growth in both ER+ and ER− cell lines, the dominant mechanisms responsible for these effects in ER+ and ER− cell lines are likely different [72]. For example, in MCF-7 cells, 24 hour treatment with adiponectin resulted in an antiproliferative effect lasting up to 96 hours [77]. Whether adiponectin induces cell apoptosis is controversial and depends on the particular breast cancer cell line and the duration of the adiponectin incubation period [64]. One study reported that increased cleavage of poly (ADP-ribose) polymerase (PARP), which serves as an early apoptotic biomarker, was only detected in ER+ cell lines [78]. Other studies have reported that adiponectin inhibits aromatase and estrogen receptor activity, mechanisms which would primarily act on ER+ tumors [64]. Collectively, these data suggest that adiponectin acts via multiple signaling pathways with different mechanisms predominating in ER+ and ER− cell lines.

Animal studies have demonstrated that overexpression of adiponectin, both locally and systemically, reduces mammary tumor size [79]. In contrast, reduced expression of adiponectin accelerates tumor onset and progression [80]. The proposed mechanisms linking low adiponectin levels and breast carcinogenesis are (1) interaction with insulin [60, 81], (2) interaction with leptin [64], (3) inhibition of TNF-α

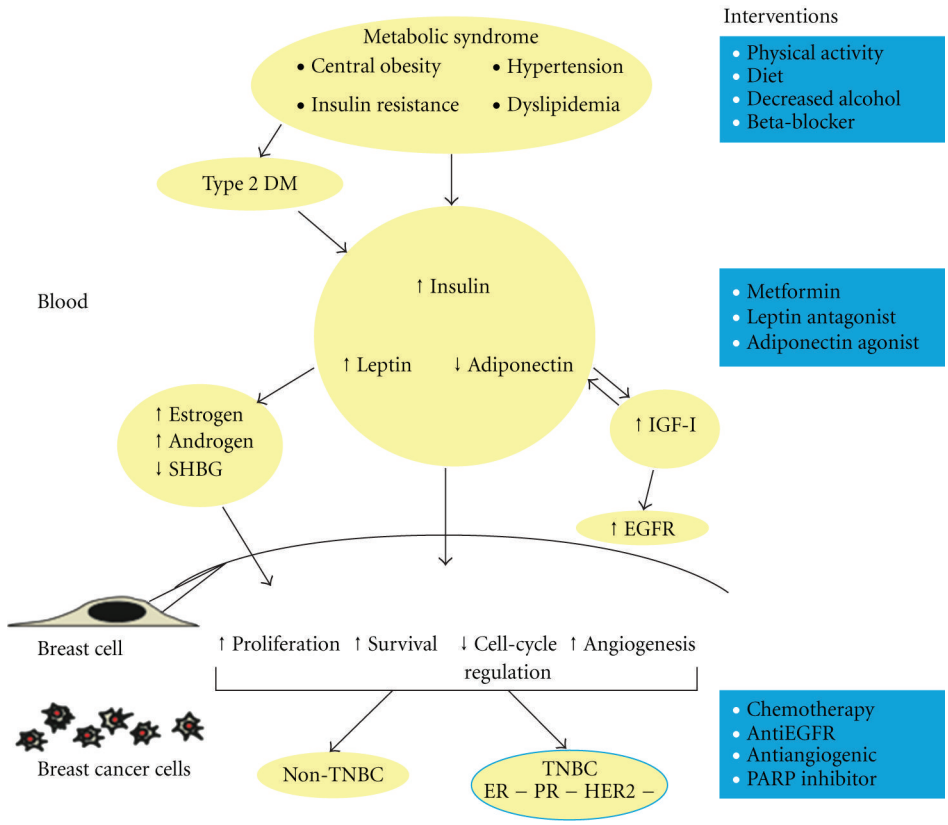

Figure 1: The insulin-leptin-adiponectin axis and risk of TNBC. Schematic representation demonstrates interactions of components in blood. After the compounds enter a normal breast cell, changes in proliferation, survival, cell-cycle regulation, and angiogenesis result in tumorigenesis of either TNBC or non-TNBC. Potential interventions for TNBC, at different levels, are included on the right.

in macrophages [82], (4) binding of fibroblast growth factor and platelet-derived growth factor-beta polypeptide [82], (5) inhibition of nuclear factor κB [83], and (6) promotion of angiogenesis [84]. Further research exploring the link between adiponectin levels over time and breast cancer risk is needed in order to elucidate dominant mechanisms in different breast cancer subtypes. Furthermore, monitoring changes in adiponectin levels in conjunction with different pharmacological and/or behavioral modifications such as diet or exercise in human patients may contribute to a better understanding of its role in TNBC. Finally, treatments aimed at increasing adiponectin levels should be explored for their potential therapeutic and preventive benefit in breast cancer.

7. Conclusions

Considerable evidence links the components of metabolic syndrome, including central obesity, insulin resistance, glucose intolerance, dyslipidemia, and hypertension, with the different breast cancer subtypes. Although data on the connection between TNBC and the metabolic syndrome are limited, several studies have provided evidence for this association. Studies have reported an association between elevated abdominal obesity, as defined by a high WHR, and increased incidence of TNBC, but the evidence for BMI is more contradictory [17, 18]. In addition, while type 2 DM and insulin resistance are associated with elevated breast cancer incidence, early evidence suggests that TNBCs do not have increased prevalence of type 2 DM compared to non-TNBCs [17]. In terms of disease progression, obesity is associated with worse prognosis and increased recurrence across all breast cancer subtypes [25, 26, 28]. Hyperglycemia and hyperinsulinemia have also recently been associated with increased incidence and poor prognosis [42–44]. Additionally, behavioral modifications including moderate physical activity, a diet rich in fruits, vegetables, and micronutrients, and reduced alcohol consumption show promise across all breast cancer subtypes [32–37, 39, 41]. It remains to be seen whether these alternative therapies may prove useful in conjunction with chemotherapy for patients with TNBC.

Molecular mechanisms of how these components of metabolic syndrome may mediate tumorigenesis and disease progression have been proposed. Insulin may mediate breast cancer risk via both direct and indirect effects, resulting in increased concentration of androgens and estrogens, along with increased concentration of IGF-I [47–53]. Leptin and adiponectin, which are both secreted by adipose tissue and often by breast tumors, act via a number of downstream signaling pathways involved in cell proliferation, apoptosis, cell-cycle regulation, angiogenesis, and cell survival [64]. It is likely that normal cells must maintain a fine balance between

leptin and adiponectin in order to maintain proper cell and tissue homeostasis, and the components of metabolic syndrome appear to disrupt this balance by increasing leptin and decreasing adiponectin levels [61, 62, 64]. In addition, insulin, IGF-I, and EGFR may play a pivotal role in mediating the potential interactions between these two hormones [65, 66].

We propose that components of the metabolic syndrome and the insulin-leptin-adiponectin axis play a pivotal role in the pathogenesis and progression of TNBC (Figure 1). At present, treatments for TNBC are limited compared to other subtypes of breast cancer, because these tumors are resistant to hormone therapy and drugs that target the HER-2 protein. Clinical trials have shown efficacy of treatments such as chemotherapy, anti-EGFR drugs, antiangiogenic drugs, and PARP inhibitors in the treatment of TNBC [7]. Lifestyle factors including diet, reduced alcohol consumption, and physical activity, which may modulate components of the metabolic syndrome, may also play a pivotal role in decreasing incidence and risk of recurrence of TNBC. Trials that incorporate agents such as metformin or leptin antagonists as well as other therapies that modify the insulin-leptin-adiponectin axis may prove very beneficial for prevention and treatment of TNBC.

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