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Review Article

Targeting Oncogene-Induced Autophagy: A New Approach in Cancer Therapy?

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Autophagy is a tightly controlled self-degradation process utilised by cells to sustain cellular homeostasis and to support cell survival in response to metabolic stress and starvation. Thus, autophagy plays a critical role in promoting cell integrity and maintaining proper function of cellular processes. Defects in autophagy, however, can have drastic implications in human health and diseases, including cancer. Described as a double-edged sword in the context of cancer, autophagy can act as both suppressor and facilitator of tumorigenesis. As such, defining the precise role of autophagy in a multistep event like cancer progression can be complex. Recent findings have implicated a role for components of the autophagy pathway in oncogene-mediated cell transformation, tumour growth, and survival. Notably, aggressive cancers driven by Ras oncoproteins rely on autophagy to sustain a reprogrammed mitochondrial metabolic signature and evade cell death. In this review, we summarize our current understanding of the role of oncogene-induced autophagy in cancer progression and discuss how modulators of autophagic responses can bring about therapeutic benefit and eradication of a subset of cancers that are addicted to this ancient recycling machinery.

1. Introduction

Almost two decades ago, the Ohsumi laboratory first discovered and characterized the autophagy-related (*ATG*) genes in yeast [1, 2]. Since then, researchers around the world work relentlessly to unravel the biology of autophagy and its roles in a variety of human diseases.

Autophagy can be broadly categorized into macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy (hereafter autophagy) is a tightly regulated catabolic mechanism in the cell. It involves the sequestration of dysfunctional cytoplasmic constituents, ranging from misfolded proteins, proteoglycans and damaged organelles into double membrane vesicles, known as the autophagosomes. These autophagic vesicles eventually fuse with lysosomes, within which the dysfunctional cytoplasmic cargoes are degraded. This self-cannibalisation process in the cell appears to play a crucial role in supporting the bioenergetics and biosynthetic programs in response to nutrient deprivation and metabolic duress [3].

Under optimal growth conditions of a normal cell, the autophagic activity is kept in a minimal or basal state. Such basal autophagy is important for maintaining intracellular protein homeostasis and preservation of cellular integrity, through effective clearance of protein aggregates and damaged organelles. Under physiologic stress like starvation, hypoxia, and oncogene activation, this autophagic activity can be upregulated to provide efficient nutrient recycling for damage mitigation, stress tolerance, and sustain short-term viability of the cell. Given that autophagy-mediated management of energy homeostasis and nutrient recycling are important to the well-being of a cell, it is not surprising that a defective autophagic recycling chain results in human diseases, such as cancer, neurological disorders, and hepatic malfunction [4, 5].

Of particular interest to us is the emerging role of basal autophagy as a result of oncogene activation during cancer progression. Due to an increasing number of evidence demonstrating that autophagy can be both pro-tumorigenic and tumor suppressive in cells undergoing the different phases of an oncogene-induced transformation [6, 7], oncogene-induced autophagy is perceived as a "double-edged sword" [8]. As such, it remains an open question whether one should promote or inhibit autophagy to improve the efficacy

of our existing anticancer regimens. We herein summarize recent experimental findings in the area of oncogene-induced autophagy and speculate how they can help us to devise new diagnostic tools for early cancer detection as well as to develop novel anticancer therapeutics.

2. Role of Autophagy in Ras-Driven Cancers

The Ras oncoproteins are members of a large superfamily of guanosine-5′-triphosphate- (GTP-) binding proteins important in controlling activities of signaling pathways regulating normal cellular proliferation [9]. Three closely related Ras oncoproteins have been known so far: H-ras, K-ras, and N-ras. In 20–25% of all human tumors and up to 90% in specific tumor types, activating mutations in different *RAS* oncogene members have been reported [10].

2.1. How Does Oncogenic Ras Modulate Basal Autophagy of the Cell? A number of reports have shown that the activation of oncogenic Ras alone can promote both tumorigenesis and cell death, which are associated with features of autophagy [11–15]. For instance, Ras-induced autophagy can induce cancer cell death via the upregulation of BH3-only protein Noxa and a key regulator of autophagy, Beclin-1 [15], and yet it is indispensable for tumorigenesis [11–14]. As such, how does one resolve this discrepancy in survival outcomes conferred by oncogenic Ras overexpression and/or activation? It has been argued that varying intensities of Ras activation can lead to different cellular survival outcomes. If acute overexpression/activation of Ras occurs in the absence of cooperating mutations, cells may undergo cellular senescence or autophagic death via Beclin-1/Vps34 lipid kinase activation complex. In the case of chronic overexpression/activation of Ras, cells may be capable of overcoming the initial phase of oncogene activation stress by undergoing growth selection pressure through a "bottle-neck" effect to create favorable mutations for development into a cancerous state [16]. Chronic overexpression/activation of Ras in the cell may, via a weaker activation of its PI3K/Akt/mTOR effector pathway, limit the scale of basal autophagy elevation to favor cell survival over death. This elevated level of basal autophagy was observed in Ras-driven cells from multiple organ/tissue origins [11–14], suggesting that the matter of life and death in the cell may be determined by the "strength/dose" of Ras overexpression/activation rather than differences in cell type/origin.

Intriguingly, it was recently reported that active cross talk between different cellular responses induced by oncogenic Ras exists [17]. Briefly, cellular senescence, not autophagy, was shown to be a dominant outcome of mouse embryonic fibroblasts (MEFs) stably overexpressing oncogenic Ras. This bias towards Ras-induced senescence is mediated by N-terminal apoptosis-stimulating of p53 protein 2 (ASPP2). ASPP2, by competing with ATG16 for binding to the ATG5-ATG12 complex, prevents the formation of ATG16-ATG5-ATG12 ternary complex for autophagy initiation. Remarkably, overexpression of ATG5 but not autophagy-deficient ATG5 mutant K130R bypasses oncogenic Ras-induced senescence in ASPP2 wild type MEFs. Conversely, ATG5 or ATG3 depletion sensitizes MEFs to Ras-induced senescence. Taken together, these data indicate that different levels of autophagic activity may dictate the cellular response to Ras-induced senescence. In other words, high levels of autophagy bypass senescence and a significant reduction or lack of autophagy sensitizes mouse cells to senescence.

2.2. Opposing Roles of Autophagy in Cancer. During cancer progression, autophagy also plays discretely opposite roles. At the early stages of cancer, the antitumoral effect of autophagy is characterized by active maintenance of cellular quality control for cytosolic prooncogenic proteins, such as p62, to prevent malignant transformation of normal cells. Furthermore, the supply of energy provided through activation of autophagy reduces the dependence on glycolysis, while assuring the energy required for maintenance of a stable genome, further preventing oncogenesis [18, 19]. The gradual reduction in autophagic activity at the end of the early stages of cancer development eventually favours malignant transformation of cells, as the accumulation of proteins like p62 activates signaling pathways that promote necrosis and inflammation. Poor quality control as a result of diminished autophagy can also lead to the accumulation of defective mitochondria, which release cytochrome c and reactive oxygen species (ROS) to further alter genome integrity. As cancer progresses into the later stages, reactivation of autophagy becomes necessary, in part to compensate for the poor nutritional supply associated with rapidly growing tumors and to defend cancer cells against damage induced by antioncogenic therapies. In cancer cells and tumors with activating mutations of H-ras and K-ras, the upregulation of autophagy appears to support the proper maintenance of mitochondrial metabolism for robust cell growth [11–13]. Enhanced removal of dysfunctional mitochondria may contribute to the upregulation of glycolysis to maintain the energetic balance (Warburg effect) characteristic of malignant cells *in vitro* and *in vivo* [11–14]. Importantly, inhibition of autophagy via genetic knockdown/knockout of autophagy-related genes or chloroquine treatment (to alter lysosomal pH) leads to tumor regression in pancreatic cancer xenografts in mice [14]. Thus, the precise regulation of autophagy may be critical for transformation in these oncogenic Ras-activated cancer cells.

An interesting aspect of a subset of Ras-driven cancers (such as those of colonic, lung, and pancreatic origin) that the White, Kimmelman, Debnath, and Lee labs have described is their reliance on an elevated level of basal autophagy for survival [11–14]. For instance, human cancer cells with oncogenic Ras activation displayed high sensitivity to genetic impairment of essential autophagy proteins, ATG5 or ATG7, leading either to apoptosis or growth arrest [11]. Furthermore, Ras-driven proliferation and transformation of autophagy-competent cells appear to be more sensitive to diminished glucose availability than autophagy-deficient cells [13]. It is perhaps not entirely surprising that Ras-driven cancer cells hijack the autophagic recycling process to deal with the reprogramming of energy metabolism induced by oncogenic Ras. Persistent oncogenic Ras signaling may lead to the rapid depletion of biosynthetic intermediates from tricarboxylic

acid (TCA) cycle and high energy demand for its anabolic programs. These quickly result in the impairment of mitochondrial respiration function and the accumulation of dysfunctional mitochondria. The failure to clear dysfunctional mitochondria from these cancer cells can be deleterious. To overcome this problem, it seems reasonable to propose that Ras-driven cancer cells rely on the catabolic nature of autophagy to rapidly derive biosynthetic intermediates from the cargoes of autolysosomes to fuel growth and survival, both *in vitro* and *in vivo* [20, 21].

However, much remains to be answered in the interplay between autophagy and cellular metabolism during cancer progression. Apart from the PI3K/Akt/mTOR effector pathway of Ras signaling, do cancer cell utilize other signaling pathways to prevent self-cannibalism? What is the maximum threshold of autophagic vesicles that a Ras-driven cancer cell can contain before triggering its death signal? Is a Ras-driven cancer cell more sensitive to no autophagic recycling or too much autophagic recycling? In spite of all these questions, the White and Kimmelman labs demonstrate that targeting the autophagy pathway may be an effective therapeutic approach to combat Ras-driven cancers in mice and humans [20, 22].

3. Role of Autophagy in Myc-Driven Cancers

Following the discovery of Ras-induced autophagy by the White lab, one immediate question to be addressed is *do other oncogenes also elevate the level of autophagic activity to favor carcinogenesis?* Of note, the overexpression of c-Myc robustly induces autophagy in rat 3Y1 fibroblasts [23]. Koumenis and coworkers recently showed that the *MYC* oncogene activation elicits an adaptation-remodelling program called the unfolded protein response (UPR), where the module of the UPR containing PKR-like ER stress kinase (PERK) can promote tumorigenesis via endoplasmic reticulum (ER) stress-induced autophagy activation [24]. This new finding supports the idea that autophagy may be controlled by other oncogenes during cancer progression. In other words, cancers with oncogene addiction may also likely be addicted to autophagy as a secondary consequence.

The Myc family of protooncogenes consists of *MYC*, *MYCL*, and *MYCN*. It regulates gene transcription, cell-cycle progression, stemness, metabolic reprogramming, and many other important cellular processes [25]. Like *RAS*, *MYC* is frequently mutated in many human cancers. Chromosomal translocation, involving *MYC*, has been found in multiple myeloma and Burkitt's lymphoma. In addition, *MYCN* is commonly amplified in neuroblastoma, small-cell lung cancer, medulloblastoma, and neuroendocrine prostate cancer [26]. Due to the oncogenic role that the Myc family plays in blood cancers and solid tumors, many researchers are in hot pursuit of therapeutically targeting the Myc family and/or the cellular processes regulated by these transcription factors. However, it remains a daunting task to design therapeutic strategies to directly target this family because of their unpredictable pleiotropic effects in the cell, and more importantly, these transcription factors do not have a druggable domain. Hence, targeting UPR or specific kinases like PERK may provide a feasible therapeutic avenue to combat Myc-driven malignancies.

4. Targeting Oncogene-Induced Autophagy: A Novel Therapeutic Approach in Cancer Clinics

Although careful and selective targeting of the components of autophagy in the right cancer cell types may yield beneficial outcomes in the treatment of aggressive cancers that are specifically driven by Ras, Myc, or other oncogenes, one needs to appreciate the complexity of pursuing such a novel therapeutic strategy in the oncology clinics. A number of reports have highlighted that many current anticancer regimens can either be cytotoxic or protective to cancer cells by promoting autophagy [22, 27, 28]. For example, autophagy can confer cytoprotection and chemoresistance to colon and esophageal cancer cells in response to 5-fluorouracil (5-FU) treatment [29, 30]. Furthermore, elevated levels of autophagy can render breast cancer cells insensitive to ionizing radiation killing [31, 32]. On the other hand, von Hippel-Lindau (VHL) tumor suppressor-null renal carcinoma cells treated with an inducer of autophagy, STF-62247, undergo cancer-selective cell death and a reduction of tumor burden in a mouse xenograft model [33].

Should clinicians promote or inhibit autophagy to improve prognostics of cancer patients, and what do they need to consider before including a growing list of autophagy modulators (Table 1) in their therapeutic strategies? There are several possible reasons that can explain such contradictory outcomes of targeting autophagy in different contexts. Firstly, reported small molecule modulators of autophagy and ATG genes-targeted ablation may hit targets other than those involved in the autophagic pathway. For instance, it has been discussed that a high dose of chloroquine (CQ), a common lysosomotropic agent, is capable of inhibiting other cellular pathways, such as those implicated in DNA damage and immune modulation [27]. Although existing preclinical reports have shown that cancer cells with increased autophagy undergo cell death in response to lysosomal inhibitors such as CQ [70], more studies need to be done to demonstrate unequivocally that these cytotoxic effects arise particularly from autophagy inhibition rather than from off-target lysosome-independent effects.

Secondly, inhibiting autophagy in different stages of cancer progression can lead to different therapeutic outcomes. For example, it was demonstrated that inhibition of autophagy at early stages attenuated imatinib-induced cytotoxicity in human malignant glioma. In contrast, imatinib-induced cytotoxicity was augmented only when the later stage of the autophagy pathway was inhibited [71]. In the case of liver cancer, preventing autophagy inhibition may play an important role in suppressing the development of hepatocarcinogenesis at the dysplastic stage by promoting cell stability. However, the inhibition of autophagy may be a useful strategy to combat hepatocarcinogenesis in the tumor-forming stage by enhancing ROS-mediated cellular damage and suppressing

Table 1: Chemical modulators of autophagy and their modes of action.

Compound	Mechanism	Cancer type	Role of autophagy	References
Inducers of autophagy				
AZD8055	Inhibits mTORC1 activity	Nonsmall cell lung cancer	Prodeath	[34]
Bortezomib	Blocks Bax degradation	Chronic lymphocytic leukemia	Prodeath	[35]
Bufalin	Synergizes with JNK pathway and increases expression of TNF, BECN-1, MAPK, and ATG8	Liver cancer Colorectal cancer	Prodeath Prodeath	[36] [37]
Chlorpromazine	Inhibits PI3K/Akt/mTOR signaling pathway	Brain cancer	Prodeath	[38]
Cisplatin	Activation of AMPK signaling pathway	Liver cancer	Prodeath	[39]
Compound C (Dorsomorphin)	Inhibits AMPK activity	Colorectal cancer	Prodeath	[40]
Everolimus	Inhibits mTORC1 activity	Acute lymphoblastic leukemia Prostate cancer	Prodeath Prodeath	[41] [42]
Fangchinoline	Transactivation of sestrin2 gene, leading to AMPK signaling activation	Liver cancer	Prodeath	[43]
Imatinib	Increases expression of Beclin-1 and ATG5	Chronic myeloid leukemia Gastrointestinal stromal tumor	Prodeath Prosurvival	[44] [45]
Nilotinib	Induces PP2A-regulated AMPK phosphorylation and activation	Liver cancer	Prodeath	[46]
Obatoclax (GX15-070)	Induces ATG5-dependent autophagic cell death	Acute lymphoblastic leukemia	Prodeath	[47]
Pemetrexed	Increases levels of Akt, p70 S6K, and/or phosphorylated-mTOR	Nonsmall cell lung cancer	Prodeath	[48]
Perifosine	Inhibits PI3K/Akt/mTOR signaling pathway	Chronic myeloid leukemia	Prosurvival	[49]
PI-103 hydrochloride	Inhibits PI3K/Akt/mTOR signaling pathway	Brain cancer	Prodeath	[50]
PP242	Inhibits mTORC1 activity	Multiple cancers	Prodeath	[51]
Resveratrol	Accumulation of intracellular calcium and activation of AMPK/mTOR signaling pathway	Nonsmall cell lung cancer	Prodeath	[52]
Safingol	Increases ROS and/or AMPK activation Inhibits PKCs and PI3K pathway directly	Breast cancer Colorectal cancer	Prodeath Prodeath	[53] [54]
Salinomycin	Increases ER stress via ATF4-DDIT3/CHOP-TRIB3-Akt-mTOR signaling pathway	Nonsmall cell lung cancer	Prodeath	[55]
Sodium selenite	Activates Nrf2 Dephosphorylates PP2A and lead to DAPK activation	Nonsmall cell lung cancer Promyelocytic leukemia	Prosurvival Prodeath	[56] [57]
Sorafenib SC-59 (Sorafenib derivative)	Inhibits phospho-STAT3 signaling pathway and reduces expression of myeloid cell leukemia (MCL-1), which disrupts Beclin-1-Mcl-1 complex	Brain, breast, liver, and lung cancers	Prodeath Prodeath	[48] [58]
STF-62247	PI3K signaling pathway and golgi trafficking	VHL-deficient renal cancer	Prodeath	[33]
Temsirolimus	Inhibits mTORC1 activity	Mantle cell lymphoma	Prodeath	[59]
Torin-1	ATP-competitive mTOR inhibitor that inhibits both mTORC1 and mTORC2 directly	Multiple cancers	Prodeath	[60]
Wogonin	Inhibits p70S6K/Akt pathway	Nasopharyngeal cancer	Prodeath	[61]
Inhibitors of autophagy				
3-Methyladenine (3-MA)	Inhibits PI3K signaling pathway	Colorectal cancer Esophageal cancer	Prosurvival Prosurvival	[62] [63]
Bafilomycin A1	Prevents fusion of autophagosomes with lysosomes by acting as a V-ATPase inhibitor	Breast cancer Colorectal cancer	Prosurvival Prosurvival	[64] [65]
Chloroquine	Inhibits lysosomal acidification and prevents fusion of autophagosomes with lysosomes	Brain cancer Colorectal cancer	Prosurvival Prosurvival	[66] [67]

Table 1: Continued.

Compound	Mechanism	Cancer type	Role of autophagy	References
Lucanthone	Induces vacuolization and interferes with lysosomal function	Breast cancer	Prodeath	[68]
Spautin-1	Promotes ubiquitylation of Beclin-1	Breast cancer	Prodeath	[69]

cell metabolism [72]. Hence, we envisage that the development of diagnostic tools to specifically monitor the dynamic changes in the autophagy pathway *in vivo* will provide more precise interpretation of experimental or treatment outcomes. The recent creation of GFP-LC3 transgenic mouse [73] to study the dynamic changes in autophagic response to genetic or pharmacological manipulations has been unanimously hailed as a significant breakthrough, but it remains technically demanding to extend this scientific advancement to human patients at this point in time.

Thirdly, the discordant outcomes of targeting autophagy could be due to the specificity of approaches used to modulate and measure autophagy. Immunohistochemical staining to detect changes in LC3 expression levels remains as the most direct functional readout for autophagic processing. However, given the dynamic and complex process of autophagy, potential caveats exist when LC3 immunostaining data are being interpreted. Indeed, it has been reported that LC3 expression levels can differ significantly between cell types and conditions. Hence, immunostaining assays reflect only a static "snapshot in time" and not a true representation of the overall autophagic flux in cancer cells [74]. These discrepancies in measuring the extent of autophagic response may eventually lead to gross data misinterpretation. We support the notion that the differential scoring of cells undergoing death upon therapeutic treatments could be performed by taking account of the cell type, cell growth kinetics, and stages of tumor progression. Alternatively, the difference in selecting experimental end points in a timed study may also lead to differential cell survival outcomes [75].

Clearly, more should be done to delineate the role of autophagy in cancer therapy. Importantly, we should define and identify which cancer subtypes would exhibit higher sensitivity to autophagic modulation. According to findings from the White lab, oncogenic Ras-driven cancers appear to be particularly sensitive to the inhibition of autophagy [14, 20]. This observation seems to be at odds with the wide-accepted dogma that the PI3K/Akt/mTOR signaling axis, which is one of the three major Ras effector pathways, suppresses autophagy. Perhaps one way to explain this discrepancy is that Ras-driven cancers thrive on their ability to tightly control the increase in basal autophagy and that this mode of regulation on basal autophagy status will be lost, following inhibition of the PI3K/Akt/mTOR signaling cascade by either genetic or pharmacologic means. For example, rapamycin is a naturally occurring allosteric mTOR inhibitor, and its analogs temsirolimus (CCI-779), everolimus (RAD-001), and deforolimus (AP-23573) selectively target mTORC1 to stimulate autophagy. However, with the exception of renal cell and neuroendocrine carcinomas and lymphoma, rapamycin and its analogs (rapalogs) showed little efficacy in the clinical setting [76]. This may be attributed to the fact that rapamycin and rapalogs fail to inhibit mTORC2 activity and are thus unable to abrogate the S6K/IRS1–mediated negative feedback that induces AKT reactivation [77]. These limitations are circumvented by the development of ATP-competitive inhibitors of both mTORC1 and mTORC2 (e.g., PP242, Torin1, AZD8055, and WYE132) and the dual PI3K-mTOR inhibitor, NVP-BEZ235. In preclinical studies, dual inhibitors of mTORC1 and mTORC2 showed superior anticancer activity [34, 78, 79] and were shown to be more potent autophagy inducers compared to mTORC1 inhibitors alone [60, 80]. Notably, the dual PI3K-mTOR inhibitor PI-103 only induces autophagy in glioma cells and cancer cell killing via apoptosis was achieved when it was combined with autophagy inhibitors. In addition, the PI3K-mTOR inhibitors, NVP-BEZ235 and XL765, synergize with CQ in the induction of apoptosis in glioma and malignant peripheral nerve sheath tumor xenografts, respectively [81, 82]. It remains unclear whether the use of these dual PI3K-mTOR inhibitors alone will induce autophagy that will further fuel cancer progression.

Apart from PI3K pathway inhibitors, many ongoing studies and clinical trials are aiming to repurpose the antimalarial lysosomotropic drugs, CQ and hydroxychloroquine (HCQ), as anticancer therapeutics via the inhibition of lysosomal degradation of autophagic cargo and preferential killing of mouse cells expressing *MYC* oncogene in a p53-dependent manner. In addition, inhibition of autophagy can induce apoptosis and delay tumor recurrence following p53 reactivation in a p53-null, Myc-induced lymphoma mouse model [21, 22, 27, 91, 92]. At the face value, CQ and HCQ appear to be good anticancer drug candidates because they are well tolerated and safe for human use [64, 93, 94]. However, in most Ras-driven human cancer cell lines that are dependent on an elevated level of basal autophagy a suprahigh dose of CQ is required for growth inhibition [11]. This may be attributed to poor pharmacokinetics of CQ and low potency of the drug to inhibit autophagy [94]. In other words, only a high dose of these lysosomotropic drugs can induce the accumulation of ineffective autophagic vesicles beyond a certain threshold that is tolerable to the cell. Hence, one feasible way to maximize cancer cell growth inhibition/killing may likely be the optimal combination of autophagy inducers, inhibitors, and/or other anticancer regimens (Table 2).

Indeed, drug combination therapy has recently emerged as a promising alternative anticancer strategy [27]. For

Table 2: Preclinical trials evaluating the potential of combinatorial drug therapies as anticancer treatments.

Combinatorial drug therapies				
Compounds	Mechanism	Cancer type	Role of autophagy	References
5-Flurouracil and si-Beclin-1	Inhibit nucleic acid synthesis	Esophageal cancer Liver cancer	Prodeath Prodeath	[29] [39]
CQ, Oxaliplatin and Bevacizumab	Inhibition of autophagy by CQ sensitizes cells to Oxaliplatin and Bevacizumab treatment	Colorectal cancer	Prodeath	[83]
Compound C and Bafilomycin	Inhibit AMPK activity	Brain cancer	Prodeath	[84]
CQ and ADZ5363	Inhibit Akt signaling pathway downstream and reduce phosphorylated-mTOR and p-RPS6KB/p70S6K	Prostate cancer	Prodeath	[85]
CQ and NVP-BEZ235 CQ and XL765	Inhibition of autophagy by CQ sensitizes cells to dual PI3K/mTOR treatment	Brain cancer	Prodeath Prodeath	[81] [82]
Gemcitabine and Cannabinoid	Increase ceramide	Pancreatic cancer	Prodeath	[86]
Lucanthone and Vorinostat	Enhance activity of histone deacetylase inhibitor, Vorinostat	Breast cancer	Prodeath	[68]
Obatoclax and Lapatinib	Increase expression of NOXA, leading to displacement of Mcl-1 from Beclin-1	Breast cancer	Prodeath	[87]
Pyrvinium pamoate and 2-DG	Pyrvinium pamoate inhibits 2-DG-triggered accumulation of LC3 puncta	Cervical cancer Colorectal cancer	Prodeath Prodeath	[88]
Sorafenib and Pemetrexed	Increase levels of Akt, p70 S6K, and/or phosphorylated-mTOR	Brain, breast, liver, and lung cancers	Prodeath	[48]
Tamoxifen and deacetylase	Prevent HMGB1: Beclin-1-mediated autophagy from promoting drug resistance	Osteosarcoma	Prodeath	[89]
Valproic acid and Tubacin	Inhibit HDAC6 specifically	Ovarian cancer	Prodeath	[90]

instance, human colon cancer lines treated with a combination of Oxaliplatin, Bevacizumab, and chloroquine result in a significant reduction in tumor growth in a synergistic manner [83]. Given that successful drug combination therapies have been reported in many recent studies [83, 95–97], we support the idea that an increasing number of ongoing and future clinical trials will explore the amalgamation use of autophagic modulators with other treatment modalities, such as radiation or chemotherapy, to potentiate its antitumorigenic effect [27]. However, the cellular effect of these combination therapies involving autophagy modulation is likely to be complex and requires thorough understanding of their mechanism of action prior to their deployment in the clinics. For example, the capacity for sensitization by CQ appears to be quite wide ranging, with dramatic effects for some drugs/tumor models and modest or minimal effects in others. We noted a recent study published by Bristol et al. demonstrating that neither chloroquine nor silencing of an autophagy regulatory gene was effective in conferring radiation sensitivity in the 4T1 syngeneic mouse breast tumor model [98]. Bristol et al. argued that a fully functional immune system may play a central role in determining the effectiveness of autophagy inhibition in chemosensitization or radiosensitization, since most successful combination therapies involving autophagy modulation have generally been performed in xenograft models. This study opens a new Pandora's box that suggests the existence of many other unknown determinants of autophagy-targeting combination cancer therapies. Hence, much remains to be done to investigate whether experimental findings from *in vitro* cultured human cancer cells and *in vivo* animal models of cancers can ultimately be translated to humans.

5. Concluding Remarks

Similar to its bilateral roles as both a tumour suppressor and prosurvival mediator in cancer progression, autophagy can have cytotoxic and protective roles in anticancer therapies [8]. Depending on different cellular conditions and distinct cancer subtypes, the role of autophagy can vary significantly. In addition to sustaining cancer cell survival in response to environmental and metabolic stress, autophagy is also utilized by normal cells to maintain cellular homeostasis. Even though autophagy inhibitors can be exploited as alternative anticancer therapeutics, we ought to be mindful that their nonselective usage can also inflict serious collateral damages on normal tissues at the same time. In this regard, we need a better understanding of how signaling pathways or other cellular processes govern oncogene-induced autophagic responses. This will allow us to target important proteins of these signaling pathways or cellular processes that regulate autophagy specifically. As autophagy inhibitors proceed into early clinical trial phase to treat oncogenic Ras-driven cancers in human, future work will shed light on whether targeting Ras/Myc-, or at large, oncogene-induced autophagy is a viable anticancer therapeutic strategy.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Fuquan Zhang wrote the paper. Jit Kong Cheong edited the paper.

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