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Targeting the Autophagy Process in Breast Cancer Development and Treatment

Joanna Magdalena Zarzynska

Additional information is available at the end of the chapter

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Abstract

Autophagy is a homeostatic process that degrades long-lived or damaged proteins and organelles. By recycling intracellular constituents, it is buffering metabolic stress under starvation conditions. The autophagy role in cancer remains unclear and complicated as it appears to be involved in tumorigenesis, cancer development and treatment outcome in different ways. Autophagy can act as both tumor-promoting and tumor-suppressing agent depending on the stage of cancer progression. During the initiation of cancer, autophagy prevents cells from further DNA damage and genomic instability. It could also be a cell death mechanism in cancer cells with apoptotic defect. Autophagy can also promote tumor growth by facilitating oncogene-induced senescence or protecting tumors against necrosis and inflammation. Once the cancer is formed, autophagy can contribute to tumor progression (by allowing cells to survive in stressful conditions) and metastasis. There is evidence that breast cancer could also be controlled by autophagy. Regulation of this process, correlated proteins and active factors are currently under scientific study in the aspect of breast cancer effective therapeutic strategies.

Keywords: Autophagy, breast cancer, tumorigenesis, Beclin-1, miRNA

1. Introduction

Breast cancer (BC) is a potentially life-threatening malignant tumor that still causes high mortality among women. One of the mechanisms through which cancer development could

be controlled is autophagy. This process exerts different effects during the stages of cancer initiation and progression due to the occurring superimposition of signaling pathways of autophagy and carcinogenesis. Chronic inhibition of autophagy or autophagy deficiency promotes cancer due to instability of the genome and defective cell growth as a result of cell stress. However, increased induction of autophagy can become a mechanism which allows tumor cells to survive the conditions of hypoxia, acidosis or chemotherapy. Therefore, in the development of cancer, autophagy is regarded as a double-edged sword. There are different ways of autophagy process control under scientific research for potential therapeutic treatment in anti-cancer strategies.

2. The process of autophagy

Autophagy is a process regulated genetically and/or controlled by a group of evolutionarily conserved genes (ATGs; autophagy-related genes). Initially, autophagy was identified as a cell survival mechanism protecting from nutrient deprivation. It ensures homeostasis by maintaining proteins and organelles turnover. Removing excess or damaged intracellular components in response to stress as well as microorganisms allows cells to restrain damage (including genome instability which limits initiation and progression of cancer) and subsequent inflammation. Cellular stress can be caused by a variety of chemical and physical agents like nutrient starvation, pro-inflammatory state, hypoxia, oxidants, infectious agents and xenobiotics [1, 2]. Under the influence of autophagic pathway, biological and morphological changes have been observed [3]. In certain developmental conditions like in cell's response to metabolic stress or under cytotoxic stimuli, autophagy results in a form of cell death described as programmed cell death type II [4].

Currently, over 35 proteins are believed to be essential for autophagy occurrence and progression [5]. The complete macroautophagy (referred to hereafter as autophagy) is generally divided into the following stages: induction, vesicle nucleation, vesicle elongation and completion, docking and fusion, degradation and then recycling [1, 6]. Vesicle nucleation is the initial step in which proteins and lipids are recruited for construction of the autophagosomal membrane. Nucleation consists of the formation of the phagophore or isolation membrane. In mammalian cells, this process is initiated by activation of the class III PI3K/Beclin-1 complex including the core members hVps34/PIK3C3, Beclin-1 (BECN1) and p150. Numerous additional binding partners of this complex function as either positive or negative regulators and include BAX-interacting factor-1 (BIF-1), Atg14L, UVRAG (UV irradiation resistance-associated gene), Ambra1 (activating molecule in Beclin-1-regulated autophagy protein 1) and Rubicon [1, 5, 7-9]. Rubicon (RUN domain Beclin 1-interacting cysteine-rich-containing protein) has also been shown to negatively regulate autophagy. Subsequently, the phagophore is elongated by several ATG proteins. During this elongation step, microtubule-associated protein 1 light chain 3 (LC3)-I is lipidated to LC3-II. Then, the phagophore is matured primarily upon the action of LC3-II and BECN1 proteins. Maturation leads to the formation of autophagosome (enclosed vesicle). The regulation of the maturation process of the autophagosome is multi-factorial and involves Rab GTPase, SNAREs (soluble N-ethylma-

leimide-sensitive fusion attachment protein receptors) and ESCRT (endosomal sorting complexes required for transport) proteins, molecules of the acidic lysosomal compartment (e.g. v-ATPase, LAMP proteins- lysosome-associated membrane glycoproteins; lysosomal carriers and hydrolases) and Beclin-1. Finally, the autophagosome fuses with the lysosome to form an autolysosome. The internal material of the autophagic vacuole is degraded by the lysosomal hydrolases.

Basal levels of macroautophagy are kept in check by mTORC1 (mammalian target of rapamycin complex 1) which phosphorylates Atg13 and ULK1 (uncoordinated 51-like kinase 1/Atg1) or ULK2. This activity in consequence is giving the inhibition of FIP200 (focal adhesion kinase interacting protein of 200 kD/Atg17) phosphorylation by ULK1 [10]. The mTORC1 complex is an important component of a network that accordingly maintains homeostasis by controlling the levels of anabolism and catabolism. For example, high levels of amino acids maintain mTORC1 in an active state by enhancing its binding to regulatory GTPases, Rag (Ras-related GTPase) and Rheb (Ras homolog enriched in brain) [11]. mTORC1 activity could be indirectly induced by insulin and IGF1 (insulin like growth factor 1) [6]. Low glucose levels or high AMP levels (adenosine 5'-monophosphate), indicators of low cellular energy status or stress, could activate AMPK (AMP-activated protein kinase) which in turn inhibits mTORC1 and stimulates autophagy [2,7].

The role of the PI3K/Akt pathway is to suppress autophagy. This pathway activation was shown to decrease autophagy through mTOR activation. It has been considered for cancer treatment. The MAPK pathway also plays a significant role in autophagy. Ras may play a dual role in autophagy [12]. When Ras activates PI3KCA, autophagy is inhibited; however, when it selectively activates the MAPK pathway, autophagy is stimulated.

3. Breast cancer

Breast cancer (BC) is the most common and fatal cancer in women worldwide. Decreasing mortality rates can be observed that result mostly from efficient screening strategies [13] but still BC is ranked on the second place in mortality among cancer types [14]. It has been estimated that approximately 1.3 million females develop BC each year with around 465.000 expected to succumb to the disease [15,16]. It is causing death of about 350.000 women in both developed and developing countries every year (with slightly more cases in less developed than in more developed regions) [17]. According to another data presented by DeSantis et al., there are still 500.000 breast cancer deaths per year worldwide [18]. More than 90% of lethality in patients is caused by metastasis and the occurrence of distant metastases (distinct metastatic pattern involving the regional lymph nodes, bone marrow, lung and liver) severely limits the prognosis [19,20]. The 5-year survival rate for patients with BC drops sharply from 98% for individuals with localized disease to 23% for those with metastatic disease (cancer statistics from 2012) [21]. A significant subpopulation of patients with metastasis risk has a median survival time of 18–30 months [22].

In the pathogenesis and progression of BC are involved many factors including genetic, biological and environmental factors as well as a lifestyle [17]. For example, Bcl-2 protooncogene is overexpressed in half of all human malignancies and more than 60% of BC and exerts its oncogenic role by preventing cells from undergoing apoptosis [23]. Still, the disease background is not fully clear because it has been estimated that 75% of women with sporadic invasive BC have no known epidemiological risk factors [24].

4. Autophagy regulation in breast tumors development

In tumor genesis and treatment responsiveness, autophagy role is complicated and context-dependent. It presumably differs in different stages of cancer development. At the initial stages, autophagy may represent a protective role thanks to its catabolic functions by degrading and/or recycling cell components (e.g. damaged organelles and misfolded proteins) [25-27]. It also protects against the deleterious effects of ROS (reactive oxygen species) in the cells. Proliferation of cells with cancer-linked mutations may be retarded by autophagy. It can also limit propagation of this type of mutations and consequently suppress tumorigenesis by facilitating the cellular senescence phenomenon (biological aging). However, once a tumor develops, the cancer cells can utilize autophagy for their own cytoprotection and use enhanced autophagy to survive under metabolic and therapeutic stress [27]. Autophagy might increase oxidative stress, hence promoting genome instability and malignant transformation [25-27]. As an example, autophagy has been shown to be required for the transformation of mouse embryonic fibroblasts by the Ras oncogene and this effect is linked to its role in nutrients recycling, such as glucose uptake and increased glycolytic flux [28]. What's more, it has been suggested that metastatic cancer cells can escape from anoikis (process of apoptosis induced by lack of correct cell-ECM attachment) through the autophagy induction [29, 30]. The AMP-activated protein kinase (AMPK) stress response pathway is involved in mediating anoikis resistance by inhibiting mTOR and suppression of protein synthesis. The Ras/MAPK and PI3K/Akt pathways are common mechanisms utilized by cancer cells to evade anoikis. Autophagy is necessary for cancer cells survival in hypoxic conditions during the later stages of *in vivo* tumor formation before the vascularization of tumor takes place [31, 32]. However, the induction of autophagy is associated with cell death in normal cells and in some cancer cells [23]. Autophagic cell death has been described e.g. in anti-estrogen-treated cultured human mammary carcinoma MCF-7 cells [33]. Some studies have shown that cancer cells express lower levels of the autophagy-related proteins LC3-II and Beclin-1 than normal epithelial cells. There is evidence that heterozygous disruption of BECN1 promotes tumorigenesis and the overexpression inhibits tumorigenesis, which support the assertion, that defective autophagy or inhibition of autophagy playing a role in malignant transformation [23].

The ability of cancer cells to invade and metastasize is closely correlated with the process of epithelial-mesenchymal transition (EMT). It was recently demonstrated that ectopic expression of the DEDD gene in the MDA-MB-231 metastatic BC cell line led to the degradation of the EMT inducers Snail and Twist through autophagy activation [34]. Reversely, knock-down

of DEDD in the MCF7 non-metastatic BC cell line led to autophagy reduction and EMT promotion [34].

Regulation of autophagy in tumors is governed by principles similar to normal cells only in a much more complicated manner. Abnormal PI3K activation in cancer cells is frequently observed. The multitude of interactions between the PI3K/Akt/mTOR pathway and other cell signaling cascades could often be deregulated [7]. For example, Ras/Raf/ERK pathway, indicated as one of the most commonly deregulated pathways identified in tumors, frequently are observed activating mutations in Ras or B-Raf oncogenes [4]. ERK activity has been associated with autophagy and autophagic cell death in many cellular models in response to different stresses [4]. It also happens in TNF α treatment in MCF-7 cells. A deregulated PI3K/Akt/mTOR axis not only suppresses autophagy process but also induces protein translation, cell growth and proliferation thereby can force tumorigenesis. Tumors with constitutively active PI3K mutations, PTEN loss or Akt activation would be expected to be dependent on autophagy for energy homeostasis and survival. Suppression of autophagy by the PI3K cascade is disadvantageous for rapidly proliferating tumor cells and there are theses that compensatory mechanisms (like deregulated apoptosis and/or metabolism) might be concurrently activated to prevent the negative implications of defective autophagy on tumor cell survival.

Many proteins and active factors correlated with autophagy are reported to be associated with human cancers [35]. Various tumor suppressors (e.g. PTEN, TSC1/2, p53, and DAPK) are autophagy inducers whereas some inhibitors of autophagy (e.g. Akt and Ras) possess oncogenic activity [36]. Some studies [37, 38] showed that the more advanced stages of breast cancer over-express several other oncogenic and signaling proteins such as IGF-1R, Cyclin D1, c myc, pERK, Stat3 and Pak4. Some are known activators of Akt/mTOR pathway. Several other autophagy regulators like mitogen-activated kinases (BNIP3) [39] and HSpin 1 (human homologue of the *Drosophila* spin gene product) [40] play a critical role in cancer cells. Deletion of the essential autophagy gene *FIP200* impairs oncogene-induced tumorigenesis in a mouse model of breast cancer [41].

PTEN, a critical regulator of the PI3K pathway, has a stimulatory effect on autophagy by downregulating PI3K/Akt signaling through inhibition of the Akt/PKB activation. Akt inhibition leads to mTOR signaling suppression and the induction of autophagy [42]. AMPK (AMP-activated protein kinase) pathway has a negative effect on mTOR signaling and promotes autophagy (e.g. upon starvation conditions by activation of Tuberin -TSC2 and/or mTOR signaling inhibitor).

EI24/PIG8 (etoposide induced gene) can be mentioned as another critical factor of autophagic degradation. It remains under control of p53 [43, 44] - well known critical tumor suppressor. p53 effects protectively by cell cycle arrest initiation, removal of cells with incurred DNA damage, senescence and apoptosis. The human EI24 genomic locus is on chromosome 11 in region frequently altered in cancers and was reported to be mutated in aggressive breast cancers. Furthermore, since EI24/PIG8 (induced by p53) is also known as important apoptotic effector [43, 45], its role may contribute to tumor suppression. EI24/PIG8 loss was associated with tumor invasiveness but not with the development of the primary tumor [45].

mTORC1, class I PI3K, Akt, class III PI3K, Beclin-1 and p53 are critical components of the autophagic pathway that have become major targets of autophagy-related drug design. As an example, rapamycin and its derivatives (e.g. rottlerin, PP242 and AZD8055) target the PI3K/Akt/mTOR signaling pathway to induce autophagy. Spautin-1 and tamoxifen regulate Beclin-1 activity to respectively inhibit and promote autophagy. Oridonin and metformin trigger p53-mediated autophagy and cell death [46].

4.1. Beclin-1 role

The most important evidence linking dysfunctional autophagy and cancer come from studies on mice demonstrating that the inhibition of autophagy by disruption of *BECN1* increases cellular proliferation, the frequency of spontaneous malignancies (i.e. lung cancer, liver cancer and lymphomas) as well as mammary hyperplasia. It also accelerates the development of carcinogen-induced premalignant lesions [23]. Additionally, low levels of Beclin1 in human MCF-7 BC cell line can inhibit tumor cell growth [47].

Human breast cancer cell lines FISH analysis with the Beclin-1-containing PAC 45208 as a probe revealed that 9 out of 22 cell lines had allelic *BECN1* deletions [7]. Monoallelic deletion of *BECN1* has been also detected in 40-75% cases of human breast, ovarian and prostate tumors [2, 26]. Thereby *BECN1* is considered as a tumor suppressor gene [48]. Deletions of Beclin-1 have recently been found mostly associated with *BRCA1* in breast and ovarian human tumors (suggesting that *BRCA1* loss is the mutation driver and that Beclin-1 is lost because of its proximity to it) [49]. Many breast carcinoma cell lines, although polyploid for chromosome 17 (*BECN1* gene is placed in 17q21 loci), exhibit deletions of one or more *BECN1* alleles. The aberrant expression of Beclin-1 in many kinds of tumors correlates with poor prognosis [26]. Also, heterozygous deletion of *BECN1* in mice (*BECN1*^{+/-}) resulted in increased incidence of spontaneous tumors [48]. Those mice do not have increased incidence of mammary tumors but rather are susceptible to lymphomas and lung carcinomas. *BECN1*^{+/-} mice tumors express wild-type *BECN1* mRNA and protein indicating that Beclin-1 is a haploinsufficient tumor suppressor [7, 8]. The Beclin-1 loss occurring in BC could have important effects independent of autophagy through its interaction with Bcl-2. Bcl-2 is overexpressed in 50%-70% of cancers including BC. An inverse correlation of Beclin-1 and Bcl-2 expression has been described in breast cancer tissue. Bcl-2 expression was correlated with histological grade, tubule formation, nuclear pleomorphism, mitotic count, ER and distant metastasis [49].

Beclin-1 also alters the expression of several autophagy proteins such as Atg5 and UVRAG [26].

4.2. Adipokines role

Adipokines, auto-/ endocrine and paracrine-acting bioactive molecules secreted by adipose tissue are one of the recently discovered factors correlating with autophagy and BC [50]. Adiponectin (AdipoQ) is the cytokine secreted in greatest abundance. The prevalence correlate low levels of AdipoQ in the blood circulation with higher BC risk and poorer prognosis. In breast tissue, AdipoQ has a direct anti-carcinogenic effect at the site of tumor growth. This cytokine is potentially capable of regulation of autophagy through AMP kinase (5'AMP-

activated protein kinase) and its activation has been observed in breast cancer cells [50]. Liu and colleagues observed that AdipoQ caused upregulation of autophagy in MDA-MB-231 cells *in vitro* and *in vivo* in cholesterol induced mammary tumorigenesis [51].

4.3. microRNA role

MicroRNAs (miRNAs) are endogenous ~22 nucleotide RNAs that suppress gene expression via messenger RNA (mRNA) cleavage and/or translational repression. Unregulated miRNAs of lymphoma, prostate, lung and breast cancers have been also detected in blood plasma and serum. Circulating miRNAs are currently assessed as proxy biomarkers for BC [52]. There is evidence that miRNAs can influence autophagy process in BC cells at many points. MiR-20a, miR-101, miR-106a/b and miR-885-3p have been reported to have direct possibility of targeting ULK1/2 [53]. Also, miR-155 might target multiple players in mTOR signaling including Rheb, RICTOR (RPTOR independent companion of mTOR) and RPS6KB2 (ribosomal protein S6 kinase). MiR-30a and miR-519a can directly target Beclin-1 causing negative regulation in the autophagic flow thereby resulting in decreased autophagic activity. Action of miR-30a was shown in the *in vitro* study on human BC cell lines MDA-MB-468 and MCF-7 [54] by Zhu *et al.* Tumor cells treatment with the mimic of miR-30a decreased the expression of Beclin-1 mRNA and protein whereas administration of the miR-30a antagomir had opposite effects. Furthermore, high expression of miR-30a blunted the rapamycin-induced autophagy activation [54]. Another miRNA, miR-376b also regulates Beclin-1 and it is also targeting directly Atg4C [9, 55] in MCF-7 cells. The antagomir-mediated inactivation of the endogenous miR-376b results in an increased level of Atg4C and Beclin-1 [56]. MiR-374a and miR-630 can modulate the direct regulation of UVRAG. The tumor suppressive miR-101 could act as a potent inhibitor of basal, etoposide-induced and rapamycin-induced autophagy in MCF-7 cells. Also, the miR-101-mediated inhibition of autophagy sensitized BC cells to 4-hydroxytamoxifen-induced apoptotic cell death and thus miR-101 was suggested to modulate the chemosensitivity of cancer cells [9]. Elevated levels of autophagy due to the progressive loss of miR-101, at least in breast cancer cells, have the potential to trigger cancer cell survival [9, 57]. Three components including STMN1 (stathmin1), RAB5A (ras related protein 5A) and Atg4D have been identified as targets of miR-101 among which the over-expression of STMN1 could partially rescue cells from miR-101-mediated inhibition of autophagy. Previously described RAB5A and STMN1 had uncertain roles in autophagy. RAB5A have been shown to regulate ATG5-ATG12 conjugation in the autophagosome completion while STMN1 plays an important role in cell-cycle regulation [58]. Another miRNA, miR-221/222, might inhibit the cell cycle inhibitor, p27Kip1, a downstream modulator of PI3K/Akt thereby leading to autophagic cell death in HER2/neu-positive primary human breast carcinoma MCF-7 cells. The ectopic expression of miR-221/222 renders the parental MCF-7 cells resistant to tamoxifen [59].

4.4. Cancer Stem Cells (CSC) and autophagy

Autophagy is thought to be a critical process for cancer stem cells (CSC) or tumor initiating cell maintenance but the mechanisms through which autophagy supports survival of CSCs

remain poorly understood [60]. The CSC theory proposes that heterogeneity within a tumor is driven by a small population of cells which have ability to differentiate and/or self-renewal, increased membrane transporter activity, anchorage independence and ability to migrate, tumorigenic capacities and pluripotency [61, 62]. Breast cancer follows this model since it has been shown that the CD44+/CD24 low/-phenotype of cell surface markers (which can be found also in normal stem cells in the breast), have an increased ability to form tumors in immunosuppressed mice than the bulk of the tumor cells. It has been predicted that a quality control mechanism like autophagy is important for maintaining normal and cancer stem cell homeostasis [63]. Maycotte *et al.* have previously reported that a subset of BC cell lines enriched in the triple negative (TN) type is particularly dependent on autophagy for survival even in nutrient rich conditions [49]. This process is regulated by autophagy through modulation of STAT3 activity (often activated in TNBC). STAT3 activity is known to be regulated by IL-6 (interleukin 6) paracrine signaling in breast cancer cell lines [64]. The IL-6/STAT3 pathway has been also shown to be important for TNBC xenograft growth and breast CSC maintenance [65]. Maycotte *et al.* have found that the pathways most affected by autophagy inhibition were related to stem cells, secretion and epithelial to mesenchymal transition [49]. We also show that autophagy regulates the CD44+/CD24 low/-phenotype and mammosphere formation in both the MCF7 and MDA-MB-468 breast cancer cell lines. Although autophagy regulates IL-6 secretion in both the autophagy dependent (MDA-MB-468) and independent (MCF7) cell lines, autophagy inhibition increased IL6 secretion in MCF7 cells while it decreased it in MDA-MB-468 cells. Decreased mammosphere formation in MDA-MB-468 cells induced by autophagy inhibition was reversed with conditioned media from autophagy proficient MDA-MB-468 cells or with IL-6 treatment. This identifies a mechanism by which autophagy selectively regulates CSC maintenance in autophagy-dependent breast cancer cells. Maycotte *et al.* had used a flow cytometry based assay to analyze CD24 and CD44 staining in cells with different levels of autophagic flux ("autophagic flux" represents the synthesis of autophagosomes, transportation of different substrates and degradation of autophagy inside the lysosome) [49]. In both MCF7 and MDA-MB-468 cell lines, the cells with low autophagic flux had decreased CD24 staining. Cells expressing a shRNA for ATG7 or BECN1 had lower levels of CD24 staining in both cell lines and no changes in CD44 were observed indicating that cells with lower levels of autophagy also have lower levels of CD24 expression.

CSCs are characteristically resistant to conventional anticancer therapy which may contribute to treatment failure and tumor relapse. CSCs exhibit the potential for regeneration which may promote tumor metastasis [66]. Recently, autophagy has been shown to be a critical factor for CSC survival and drug resistance [67].

5. Autophagy in anti-breast cancer therapies

There are different ways of autophagy process usage and/or influence recognized according to potential therapeutic treatment in anti-cancer strategies.

Inducing protective autophagy and prosurvival mechanism in human cancer cell lines have been shown in a number of currently used antineoplastic therapies including radiation

therapy, chemotherapy (e.g. doxorubicin, temozolomide and/or etoposide), histone deacetylase inhibitors, arsenic trioxide, TNF α , IFN γ , imatinib, rapamycin and anti-estrogen hormonal therapy (e.g. tamoxifen) [12, 23]. In fact, the therapeutic efficacy of these agents can be increased if autophagy is inhibited [23].

The scientific evidence suggests that autophagy leads to cell death in response to several compounds including etoposide, rottlerin, cytosine arabinoside and staurosporine as well as deprivation of growth-factors. A link has been demonstrated between autophagy and related autophagic cell death with usage of pharmacological inhibitors (e.g. 3-MA (3-methyl adenine), CQ (chloroquine), bafilomycin A1 or ammonium chloride) and genetic silencing or knock-down (silencing of *BECN1* and *ATG5*, *ATG7* and/or *ATG12*) approaches for autophagy suppression. This is connected with **cytoprotective form of autophagy** [68].

Autophagy has also been shown to protect against cellular stress induced by the anti-cancer chemotherapeutic drugs (**nonprotective autophagy**) [68]. The cell is apparently carrying out autophagy-mediated degradative functions but where autophagy inhibition does not lead to perceptible alterations in drug or radiation sensitivity. Furthermore, because autophagy is frequently upregulated in tumors in response to therapy, it may protect the tumors against therapy-induced apoptosis [69]. Gewirtz reported that ionizing radiation could promote autophagy in BC cells in cell culture but autophagy inhibition did not alter sensitivity to radiation [68]. Furthermore, the group showed that chloroquine did not sensitize murine breast tumor cells (4T1) to radiation in an immunocompetent animal model. Based on the results obtained, it was impossible to determine whether radiation had promoted autophagy process or the chloroquine actually effectively inhibited autophagy in the tumor-bearing animals. Supposedly, that the lack of sensitization could be related to findings [5] that autophagy inhibition interferes with the immune system's capability for recognition of the tumor undergoing a stress response.

Such disclosures have led to several clinical trials involving the use of the autophagy flux inhibitors as a combination therapy [70] to radiotherapy efficacy improvement in BC patients. For example, to such inhibitors could be included hydroxychloroquine (HCQ). HCQ is a less toxic version of CQ and the best autophagy inhibitor currently commercially available for clinical trials [71]. Irradiated cancer cells can induce damage in neighboring un-irradiated cells by intracellular gap-junction communication or signals released outside of the cells [72]. Huang *et al.* indicated that radiation-induced senescent MDA-MB- 231-2A cells are secreting multiple cytokines and chemokines including CSF2 (colony stimulating factor; expressed in the highest level), CXCL1(C-X-C motif ligand 1), IL-6 and IL-8 (interleukin 8) [73]. These factors are involved in multiple functions during cancer progression. Autophagy inhibition in MDAMB- 231-2A cells significantly decreased the release of CSF2 suggesting that autophagy plays an important role in promoting the secretion of SASPs (senescence-associated secretory phenotypes). In support of this notion, it has been reported that inhibition of autophagy delays the secretion of several senescence-associated cytokines such as IL-6 and IL-8.

Cytotoxic autophagy is the next form of autophagy which should be taken under consideration in the field of cancer treatment. Functionally, this form is associated with a reduction in the number of viable cells and/or reduced clonogenic survival upon treatment [74]. For example,

Bristol *et al.* reported that vitamin D (or the vitamin D analog, EB 1089) can be combined with radiation to promote a cytotoxic form of autophagy in breast tumor cell lines (MCF-7 and ZR-75) [75]. Other research groups also showed that the generation of cytotoxic autophagy may either independently lead to cells death or act as a precursor to apoptosis [76]. Gewirtz identified an additional form of autophagy, termed **cytostatic autophagy**, in non-small cell lung cancer cells (A549 and H460) which was induced in similar conditions to the ones previously described with regards to breast tumor cells [74]. What distinguishes cytostatic autophagy from the cytoprotective form is the failure to detect evidence of cell killing reported in the breast tumor cells [74]. Both Gewirtz and Kroemer's group demonstrated cytoprotective autophagy by radiation alone but the addition of vitamin D or EB 1089 converted cytoprotective autophagy to cytostatic autophagy [74,77]. Kroemer's group observed that the depletion of essential autophagy-relevant gene products such as ATG5 and Beclin-1 increased the sensitivity of human or mouse cancer cell lines (H460, A549 and CT26 cells) to irradiation both *in vitro* (where autophagy inhibition increased radiation-induced cell death and decreased clonogenic survival) and *in vivo* after transplantation of the cell lines into immunodeficient BALB/c nude mice (where autophagy inhibition potentiated the tumor growth-inhibitory effect of radiotherapy) [77].

As Ras/Raf/ERK pathway belongs to the most commonly deregulated pathways identified in tumors and is currently the target of new antitumor strategies based on the inhibition of upstream ERK regulators. Inhibiting ERK activity in combination therapy with classical antitumor compounds might affect the efficiency of such compounds. For example, in MCF-7 human breast adenocarcinoma cell line such combined therapies with: doxorubicin [78], tamoxifen [79], taxol [80] or Δ Raf1 [81] and, TNF α [82] were used. For example, tamoxifen, the most commonly used antiestrogen, exerts its pharmacological action by binding to estrogen receptor alpha (ER α) and blocking the growth promoting action of the estrogen in BC cells. However, the development of antiestrogen resistance has become a major impediment in the treatment of ER-positive BC. It was reported that autophagy plays a critical role in the development of antiestrogen resistance and overexpression of Beclin-1 downregulated estrogenic signaling and growth response [83].

5.1. Autophagic genes silencing

Some studies using gene silencing to receive therapeutic effect via cell death induction could represent genetic therapeutic approaches. For example, the Bcl-2 protooncogene (preventing cells from undergoing apoptosis) is overexpressed in half of all human malignancies and more than 60% of BC. Bcl-2 overexpression not only leads to the resistance of cancer cells towards chemotherapy, radiation and hormone therapy but also causes an aggressive tumor phenotype in patients with a variety of cancers [23]. Recent findings suggested that silencing Bcl-2 expression (by siRNA) in MCF-7 cells led to significant autophagic, not apoptotic, cell death [84]. It has been demonstrated that the knockdown of autophagy genes (e.g. *ATG5* and *BECN1*) significantly inhibited both autophagy and cell death induced by Bcl-2 siRNA after a long-term treatment of up to seven days [84]. MCF-7 cells are known to be caspase 3-deficient providing a higher threshold for the induction of apoptosis potentially rendering the auto-

phagic cell death pathway more important. Furthermore, about 45-75% of tumor tissues from BC patients do not have detectable caspase 3 expression [85]. Akar *et al.* reported that doxorubicin predominantly induced autophagy at low doses and apoptosis at high doses [84]. Furthermore, the combination of Bcl-2 siRNA treatment with a doxorubicin low dose enhanced the autophagic response, tumor growth inhibition and cell death. It was the first evidence that targeted silencing of Bcl-2 induction of autophagic cell death in BC cells so a new path for further research on this type of alternative therapeutic strategies.

There are studies connecting autophagy genes profile with BC prognosis. For example, Gu *et al.* separated BC patients into two groups according to the TP53 mutation status in their study [86]. Then, detected the differential gene expression patterns of autophagy-related genes and investigated the association of autophagy with BC prognosis. Using microarray analysis, they identified a set of eight autophagy genes (*BCL2*, *BIRC5*, *EIF4EBP1*, *ERO1L*, *FOS*, *GAPDH*, *ITPR1* and *VEGFA*), which were significantly associated with overall survival in breast cancer. This classifier could accurately predict the clinical outcome of BC independently of other classical clinical factors such as age, tumor size, grade, status of lymph nodes, ER status, PR status and ERBB2 status.

5.2. Pharmacological approach to the autophagic therapies

In pharmacological approach to the anti-tumor autophagic therapies, the aim is to activate or inhibit autophagy. Many drugs and compounds that modulate autophagy are currently receiving considerable attention [26,35]. For example, autophagy inducers such as rapamycin (mTORC1 inhibitor) [26] and its analogs called rapalogs (such as Everolimus; RAD001) are also often used as tools to study autophagy process [6]. Everolimus was shown to enhance the sensitivity of tumors to radiation by induction of autophagy [6].

Also, natural products are considered as potential anti-cancer candidates being direct or indirect sources of new chemotherapy adjuvants to enhance the efficacy of chemotherapy and/or to ameliorate its side effects [87, 88].

The more challenging issue is the monitoring of autophagic activity in humans, in tissue and blood samples. It seems to be more important to measure autophagic flux than autophagosome number. However, measurements of autophagic flux in paraffin-embedded tissue samples have been unsuccessful till now and even the detection of endogenous LC3-II (commonly used marker for autophagosomes) is problematic in tissue sections [26]

6. Concluding remarks

There has been a tremendous amount of progress in our understanding of the role of autophagy in cancer. But still the molecular mechanisms underlying the regulation of autophagy and the role of autophagy in cancer cells are not fully understood but are progressively revealed. Overall, the data support a dynamic role of autophagy in cancer - both as a tumor suppressor early in progression and later as a pro-tumorigenic process critical for tumor maintenance and

therapeutic resistance. The specification of the autophagic cargo in tumors with increased autophagy is important for understanding the changes in metabolism between normal and malignant cells. Undoubtedly, progress in genomics, proteomics and metabolomics will be helpful in this scope. Induction of autophagic cell death may be an ideal approach in resistant cancers therapies. But most experiments regarding BC are carried out on cell lines *in vitro*. Further, functional investigations of autophagy genes using BC cell lines and animal models will increase our understanding of their roles in determining breast cancer prognosis and could thereby provide clinical strategies for the treatment of breast cancer. The first clinical trials where deliberate autophagy inhibition has been attempted in cancer patients are starting to be reported [89-93]. For instance, autophagy inhibition by HCQ in combination with chemotherapy is currently being evaluated in multiple ongoing clinical trials in patients with solid tumors but we should take into account that autophagic effect is context dependent. While tumor cell susceptibility to autophagy may depend on tumor genotype and the therapeutic agents utilized, data are very limited and it remains unclear whether such new strategies will be clinically beneficial.

Author details

Joanna Magdalena Zarzynska

Address all correspondence to: joanna_zarzynska@sggw.pl

Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland

References

- [1] Frankel LB and Lund AH: MicroRNA regulation of autophagy. *Carcinogenesis*. 2012; 33, no. 11: 2018–2025.
- [2] Awan MU and Deng Y: Role of autophagy and its significance in cellular homeostasis. *Applied Microbiology and Biotechnology*. 2014; 98, no. 12: 5319–5328.
- [3] Andon FT and Fadeel B: Programmed cell death: molecular mechanisms and implications for safety assessment of nanomaterials. *Accounts of Chemical Research*. 2013; 46, no. 3: 733–742.
- [4] Cagnol S and Chambard J C: ERK and cell death: Mechanisms of ERK-induced cell death—apoptosis, autophagy and senescence. *FEBS Journal*. 2010; 277, no. 1: 2–21.
- [5] Kroemer G, Marino G, and Levine B: Autophagy and the integrated stress response. *Molecular Cell*. 2010; 40, no. 2: 280–293.

- [6] Benbrook D M and Long A: Integration of autophagy, proteasomal degradation, unfolded protein response and apoptosis. *Experimental Oncology*. 2012; 34, no. 3: 286–297.
- [7] Chen N and Karanza-Wadsworth V: Role and regulation of autophagy in cancer. *Biochimica et Biophysica Acta*. 2009; 1793, no. 9: 1516–1523.
- [8] Sahni S, Merlot AM, Krishan S, Jansson PJ, and Richardson DR: Gene of the month: BECN1. *Journal of Clinical Pathology*. 2014; 67: 656–660.
- [9] Chen Y, Fu LL, Wen X et al.: Oncogenic and tumor suppressive roles of microRNAs in apoptosis and autophagy. *Apoptosis*. 2014; 19, no. 8: 1177–1189.
- [10] Jung CH, Jun CB, Ro S-H et al.: ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Molecular Biology of the Cell*. 2009; 20, no. 7: 1992–2003.
- [11] Sancak Y, Peterson T R, Shaul YD et al.: The rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science*. 2008; 320, no. 5882:1496–1501.
- [12] Kondo Y, Kanzawa T, Sawaya R, and Kondo S: The role of autophagy in cancer development and response to therapy. *Nature Reviews Cancer*. 2005; 5, no. 9: 726–734.
- [13] Howard J H and Bland K I: Current management and treatment strategies for breast cancer. *Current Opinion in Obstetrics and Gynecology*. 2012; 24, no. 1: 44–48.
- [14] Gomes LR, Terra LF, Wailemann AM, Labriola L and Sogayar: TGF- β 1 modulates the homeostasis between MMPs and MMP inhibitors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells. *BMC Cancer*. 2012; 12, article 26.
- [15] Herranz M and Ruibal A: Optical imaging in breast cancer diagnosis: the next evolution. *Journal of Oncology*. 2012; Article ID 863747.
- [16] Shah NR and Chen H: MicroRNAs in pathogenesis of breast cancer: implications in diagnosis and treatment. *World Journal of Clinical Oncology*. 2014; 5, no. 2: 48–60.
- [17] Porter PL : Global trends in breast cancer incidence and mortality. *Salud Publica de Mexico*. 2009; 51, no. 2: S141–S146.
- [18] DeSantis C, Ma J, Bryan L, Jemal A: Breast cancer statistics, 2013. *CA Cancer J Clin*. 2014; 61:52-62.
- [19] Fang Y, Chen Y, Yu L et al.: Inhibition of breast cancer metastases by a novel inhibitor of TGF β receptor 1. *Journal of the National Cancer Institute*. 2013; 105, no. 1: 47–58.
- [20] Langlands FE, Horgan K, Dodwell DD, and Smith L: Breast cancer subtypes: response to radiotherapy and potential radiosensitisation. *The British Journal of Radiology*. 2013; 86, no. 1023, Article ID 20120601.

- [21] Siegel R, Naishadham D, and Jemal A: Cancer statistics, 2012. *CA Cancer Journal for Clinicians*. 2012; 62, no. 1: 10–29.
- [22] Zhou X, Li B, Zhang Y, et al. : A relative ordering-based predictor for tamoxifen-treated estrogen receptor-positive breast cancer patients: multi-laboratory cohort validation. *Breast Cancer Res Treat*. 2013;142:505–514.
- [23] Dalby KN, Tekedereli I, Lopez-Berestein G, and Ozpolat B. Targeting the prodeath and prosurvival functions of autophagy as novel therapeutic strategies in cancer. *Autophagy*. 2010; 6, no. 3: 322–329.
- [24] Bhardwaj A, Rosen D, Liu M et al.: Suppression of AktmTOR pathway—a novel component of oncogene induced DNA damage response barrier in breast tumorigenesis. *PLoS ONE*. 2014; 9, no. 5, Article ID e97076.
- [25] Levine B and Kroemer G: Autophagy in the pathogenesis of disease. *Cell*. 2008; 132, no. 1: 27–42.
- [26] Jiang P and Mizushima N: Autophagy and human diseases. *Cell Research*. 2014; 24, no. 1: 69–79.
- [27] White E: Deconvoluting the context-dependent role for autophagy in cancer. *Nature Reviews Cancer*. 2012; 12, no. 6: 401–410.
- [28] Lock R, Roy S, Kenific CM et al.: Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Molecular Biology of the Cell*. 2011; 22, no. 2: 165–178.
- [29] Fung C, Lock R, Gao S, Salas E, and Debnath J: Induction of autophagy during extracellular matrix detachment promotes cell survival. *Molecular Biology of the Cell*. 2008; 19, no. 3: 797–806.
- [30] Martinet W, Agostinis P, Vanhoecke B, Dewaele M, and deMeyer GRY: Autophagy in disease: a double-edged sword with therapeutic potential. *Clinical Science*. 2009; 116, no. 9: 697–712.
- [31] Debnath J: The multifaceted roles of autophagy in tumors—implications for breast cancer. *Journal of Mammary Gland Biology and Neoplasia*. 2011; 16, no. 3: 173–187.
- [32] Boyer-Guittaut, Poillet L, Liang Q et al.: The role of GABARAPL1/GEC1 in autophagic flux and mitochondrial quality control in MDA-MB-436 breast cancer cells. *Autophagy*. 2014; 10, no. 6: 986–1003.
- [33] Motyl T, Gajkowska B, Zarzynska J, Gajewska M, and Lamparska-Przybysz M: Apoptosis and autophagy in mammary gland remodeling and breast cancer chemotherapy. *Journal of Physiology and Pharmacology*. 2006; 57, no. 7:17–32.
- [34] Lv Q, Wang W, Xue J, et al: DEDD interacts with PI3KC3 to activate autophagy and attenuate epithelial-mesenchymal transition in human breast cancer. *Cancer Res*. 2012; 72: 3238-3250.

- [35] Cheng Y, Ren X, Hait W N, and Yang J-M: Therapeutic targeting of autophagy in disease: biology and pharmacology. *Pharmacological Reviews*. 2013; 65, no. 4: 1162–1197.
- [36] Botti J, Djavaheri-Mergny M, Pilatte Y, and Codogno P: Autophagy signaling and the cogwheels of cancer. *Autophagy*. 2006; 2, no. 2: 67–73.
- [37] Kadota M, Yang HH, Gomez B et al.: Delineating genetic alterations for tumor progression in the MCF10A series of breast cancer cell lines. *PLoS ONE*. 2010; 5, no. 2, Article ID e9201.
- [38] Kim SH, Miller FR, Tait L, Zheng J, and Novak RF: Proteomic and phosphoproteomic alterations in benign, premalignant and tumor human breast epithelial cells and xenograft lesions: biomarkers of progression. *International Journal of Cancer*. 2009; 124, no. 12: 2813–2828.
- [39] Velde CV, Cizeau J, Dubik D et al.: BNIP3 and genetic control of necrosis-like cell death through the mitochondrial permeability transition pore. *Molecular and Cellular Biology*. 2000; 20, no. 15: 5454–5468.
- [40] Yanagisawa H, Miyashita T, Nakano Y, and Yamamoto D: HSpin1, a transmembrane protein interacting with Bcl-2/Bcl-XL, induces a caspase-independent autophagic cell death. *Cell Death and Differentiation*. 2003; 10, no. 7: 798–807.
- [41] Wei H, Guan JL: Pro-tumorigenic function of autophagy in mammary oncogenesis. *Autophagy*. 2012; 8:129–131.
- [42] Jain V, Paczulla AM, Klonisch T et al.: Interconnections between apoptotic, autophagic and necrotic pathways: implications for cancer therapy development. *Journal of Cellular and Molecular Medicine*. 2013; 17, no. 1: 12–29.
- [43] Gentile M, Ahnstrom M, Schon F, and Wingren S: Candidate tumour suppressor genes at 11q23-q24 in breast cancer: evidence of alterations in PIG8, a gene involved in p53-induced apoptosis. *Oncogene*. 2001; 20, no. 53: 7753–7760.
- [44] Tian Y, Li Z, Hu W et al.: *C. elegans* screen identifies autophagy genes specific to multicellular organisms. *Cell*. 2010; 141, no. 6: 1042–1055.
- [45] Zhao X, Ayer RE, Davis SL et al.: Apoptosis factor EI24/PIG8 is a novel endoplasmic reticulum-localized Bcl-2-binding protein which is associated with suppression of breast cancer invasiveness. *Cancer Research*. 2005; 65, no. 6:2125–2129.
- [46] Tong XP, Chen Y, Zhang SY, Xie T, Tian M, Guo MR, et al.: Key autophagic targets and relevant small-molecule compounds in cancer therapy. *Cell Prolif*. 2015;48:7–16.
- [47] Liang XH, Jackson S, Seaman M, et al.: Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*. 1999; 402:672–676.
- [48] Karantza-Wadsworth and White E.: Role of autophagy in breast cancer. *Autophagy*. 2007; 3, no. 6: 610–613.

- [49] Maycotte P, Gearheart CM, Barnard R, Aryal S, Mulcahy Levy JM, Fosmire SP, et al.: STAT3-mediated autophagy dependence identifies subtypes of breast cancer where autophagy inhibition can be efficacious. *Cancer Res.* 2014;74, no.9: 2579-90.
- [50] Libby EF, Frost AR, Demark-Wahnefried W, and Hurst DR: Linking adiponectin and autophagy in the regulation of breast cancer metastasis. *Journal of Molecular Medicine.* 2014; 92: 1015-23.
- [51] Liu J, Xu A, Lam KS et al.: Cholesterol-induced mammary tumorigenesis is enhanced by adiponectin deficiency: role of LDL receptor upregulation. *Oncotarget.* 2013; 4, no. 10: 1804– 1818.
- [52] Pigati L, Yaddanapudi SCS, Iyengar R et al.: Selective release of MicroRNA species from normal and malignant mammary epithelial cells. *PLoS ONE.* 2010; 5, no.10, Article IDE13515.
- [53] Ciuffreda L, Sanza CD, Incani UC, and Milella M: The mTOR pathway: a new target in cancer therapy. *Current Cancer Drug Targets.* 2010; 10, no. 5: 484–495.
- [54] Zhu H, Wu H, Liu X et al.: Regulation of autophagy by a beclin 1-targeted microRNA, miR-30a, in cancer cells. *Autophagy.* 2009; 5, no. 6: 816–823.
- [55] Zhai H, Fesler A, and Ju J: MicroRNA: a third dimension in autophagy. *Cell Cycle.* 2013; 12, no. 2: 246–250.
- [56] Frankel LB and Lund AH: MicroRNA regulation of autophagy. *Carcinogenesis.* 2012; 33, no. 11: 2018–2025.
- [57] Frankel LB, Wen J, Lees M et al.: microRNA-101 is a potent inhibitor of autophagy. *The EMBO Journal.* 2011; 30, no. 22: 4628–4641.
- [58] Cecconi F.: Autophagy regulation by miRNAs: when cleaning goes out of service. *The EMBO Journal.* 2011; 30,no. 22: 4517– 4519.
- [59] Garofalo M, Quintavalle C, Romano G, Croce CM, and Condorelli G: miR221/222 in cancer: their role in tumor progression and response to therapy. *Current Molecular Medicine.* 2012; 12, no. 1: 27–33.
- [60] Maycotte P, Jones KL, Goodall ML, Thorburn J, Thorburn A: Autophagy Supports Breast Cancer Stem Cell Maintenance by Regulating IL6 Secretion. *Mol Cancer Res.* 2015; Jan 8. pii: molcanres.0487.2014.
- [61] Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell.* 2012;21, no.3:283-96.
- [62] Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS: Stem cells in normal breast development and breast cancer. *Cell Prolif.* 2003;36 Suppl 1:59-72.
- [63] Guan JL, Simon AK, Prescott M, Menendez JA, Liu F, Wang F, et al.: Autophagy in stem cells. *Autophagy.* 2013; 9, no 6 :830-49.

- [64] Lieblein JC, Ball S, Hutzen B, Sasser AK, Lin HJ, Huang TH, et al.: STAT3 can be activated through paracrine signaling in breast epithelial cells. *BMC Cancer*. 2008; 8:302. doi: 10.1186/1471-2407-8-302.
- [65] Iliopoulos D, Hirsch HA, Wang G, Struhl K: Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci U S A*. 2011;108, no 4:1397-402.
- [66] Ojha R, Bhattacharyya S, Singh SK: Autophagy in Cancer Stem Cells: A Potential Link Between Chemoresistance, Recurrence, and Metastasis. *BioResearch Open Access*. 2015;4:97-108.
- [67] Gong C, Bauvy C, Tonelli G, Yue W, Delomenie C, Nicolas V, et al. : Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene*. 2012;32:2261-72.
- [68] Gewirtz DA: An autophagic switch in the response of tumor cells to radiation and chemotherapy. *Biochemical Pharmacology*. 2014; 90, no. 3: 208-211.
- [69] Brech A, Ahlquist T, Lothe RA, and Stenmark H: Autophagy in tumour suppression and promotion. *Molecular Oncology*. 2009; 3, no. 4: 366-375.
- [70] Carew JS, Kelly KR, and Nawrocki ST: Autophagy as a target for cancer therapy: new developments. *Cancer Management and Research*. 2012; 4, no. 1: 357-365.
- [71] Garber K: Inducing indigestion: companies embrace autophagy inhibitors. *Journal of the National Cancer Institute*. 2011; 103, no. 9: 708-710.
- [72] Rzeszowska-Wolny J, Przybyszewski WM, and Widel M: Ionizing radiation-induced bystander effects, potential targets for modulation of radiotherapy. *European Journal of Pharmacology*. 2009; 625, no. 1-3: 156-164.
- [73] Huang YH, Yang PM, Chuah QY et al.: Autophagy promotes radiation-induced senescence but inhibits bystander effects in human breast cancer cells. *Autophagy*. 2014; 10, no. 7: 1212-1228.
- [74] Gewirtz DA: The four faces of autophagy: implications for cancer therapy. *Cancer Research*. 2014; 74, no. 3: 647-651.
- [75] Bristol ML, Di X, Beckman MJ et al.: Dual functions of autophagy in the response of breast tumor cells to radiation: cytoprotective autophagy with radiation alone and cytotoxic autophagy in radiosensitization by vitamin D3. *Autophagy*. 2012; 8, no. 5: 739-753.
- [76] Sui X, Chen R, Wang Z et al.: Autophagy and chemotherapy resistance: a promising therapeutic target for cancer treatment. *Cell Death and Disease*. 2013; 4, no. 10: article e838.

- [77] Ko A, Kanehisa A, Martins I et al.: Autophagy inhibition radiosensitizes *in vitro*, yet reduces radioresponses *in vivo* due to deficient immunogenic signaling. *Cell Death and Differentiation*. 2014; 21, no. 1: 92–99.
- [78] Yeh PY, Chuang SE, Yeh KH, Song YC, Chang LLY, and Cheng AL: Phosphorylation of p53 on Thr55 by ERK2 is necessary for doxorubicin-induced p53 activation and cell death. *Oncogene*. 2004; 23, no. 20: 3580–3588.
- [79] Zheng A, Kallio A, and Harkonen P: Tamoxifen induced rapid death of MCF-7 breast cancer cells is mediated via extracellularly signal-regulated kinase signaling and can be abrogated by estrogen. *Endocrinology*. 2007; 148, no. 6: 2764–2777.
- [80] Bacus SS, Gudkov AV, Lowe M et al.: Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53. *Oncogene*. 2001; 20, no. 2: 147–155.
- [81] El-Ashry D, Miller DL, Kharbanda S, Lippman ME, and Kern FG: Constitutive Raf-1 kinase activity in breast cancer cells induces both estrogen-independent growth and apoptosis. *Oncogene*. 1997; 15, no. 4: 423–435.
- [82] Sivaprasad U and Basu A: Inhibition of ERK attenuates autophagy and potentiates tumour necrosis factor- α -induced cell death in MCF-7 cells. *Journal of Cellular and Molecular Medicine*. 2008; 12, no. 4: 1265–1271.
- [83] Li J-L, Han S-H, and Fan X: Moderating of autophagy new strategy for anticancer therapy. *Chinese Journal of Cancer*. 2011; 30: 655–668.
- [84] Akar U, Chaves-Reyez A, Barria M et al.: Silencing of Bcl-2 expression by small interfering RNA induces autophagic cell death in MCF-7 breast cancer cells. *Autophagy*. 2008; 4, no. 5: 669–679.
- [85] Devarajan E, Chen J, Multani AS, Pathak S, Sahin AA, and Mehta K: Human breast cancer MCF-7 cell line contains inherently drug-resistant subclones with distinct genotypic and phenotypic features. *International Journal of Oncology*. 2002; 20, no. 5: 913–920.
- [86] Gu Y, Li P, Peng F, Zhang M, Zhang Y, Liang H, Zhao W, Qi L, Wang H, Wang C, Guo Z: Autophagy-related prognostic signature for breast cancer. *Mol Carcinog*. 2015; Jan 25. doi: 10.1002/mc.22278
- [87] Lu J, Sun D, Gao S, Gao Y, Ye J, and Liu P: Cyclovirobuxine D induces autophagy-associated cell death via the Akt/mTOR pathway in MCF-7 human breast cancer cells. *Journal of Pharmacological Sciences*. 2014; 125, no. 1: 74–82.
- [88] Lu C, Wang W, Jia Y, Liu X, Tong Z, and Li B: Inhibition of AMPK/autophagy potentiates parthenolide-induced apoptosis in human breast cancer cells. *Journal of Cellular Biochemistry*. 2014; 115, no. 8: 1458–1466.
- [89] Rosenfeld MR, Ye X, Supko JG, Desideri S, Grossman SA, Brem S, et al.: A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent

and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy*.2014;10(8):1359-68.

- [90] Rangwala R, Chang YC, Hu J, Algazy KM, Evans TL, Fecher LA, et al.: Combined MTOR and autophagy inhibition: Phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. *Autophagy*. 2014;10(8):1391-402.
- [91] Rangwala R, Leone R, Chang YC, Fecher LA, Schuchter LM, Kramer A, et al. Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. *Autophagy*. 2014;10(8):1369-79.
- [92] Vogl DT, Stadtmauer EA, Tan KS, Heitjan DF, Davis LE, Pontiggia L, et al.: Combined autophagy and proteasome inhibition: A phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy*. 2014;10(8):1380-90.
- [93] Mahalingam D, Mita M, Sarantopoulos J, Wood L, Amaravadi RK, Davis LE, et al.: Combined autophagy and HDAC inhibition: A phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. *Autophagy*. 2014;10(8):1403-14.

