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Autophagy-regulating microRNAs: two-sided coin in the therapies of breast cancer

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Abstract. – Despite recent advances in the treatment of breast cancer (BC), it still remains as a prevalent and deadly cancer in the world. Given that BC is a heterogeneous disease, it is necessary to clarify molecular mechanisms in tumor cells to improve various therapy outcomes and overcome therapy resistance. Autophagy represents one of the most important intracellular degradation pathways involved in diverse biological processes and plays an important bi-directional role in tumor formation and progression. Among the several mechanisms that affect autophagy, microRNAs (miRNAs) play a crucial role as gene regulators. Several *in vivo* and *in vitro* studies have reported multiple miRNAs regulating autophagy in BC that affect tumor initiation, progression, and response to various therapies. In the present review, we highlighted the mechanisms through which miRNAs regulate autophagy in BC and their potential use as therapeutic targets.

Key Words:
Autophagy, MicroRNA, Breast cancer, Chemotherapy, Radiotherapy.

Introduction

Despite all the efforts that have been made in recent years for the prevention and treatment of BC, this disease is currently the most widespread cancer with the highest death rate in the world which indicates a need to explore the molecular details of tumor cells activities in more detail[1].

As a cell recycling mechanism, autophagy plays an important role in maintaining cells' homeostasis and basic activities which have been attracted attention in recent years in cancer research. Considering autophagy's quality control and stress-management roles in cells, any disorder in this process can play a role in cancer initiation and progression. However, according to the results of various studies, an ambiguous relationship between autophagy and carcinogenesis has been observed, which has necessitated further research[2].

MiRNAs are among the factors that have been considered as a tool for detection, treatment, and monitoring cancer patients. These molecules, as regulators of gene expression, affect a wide range of cellular events and are among the vital factors in some events including cancer[3]. In recent years, studies have been shown the regulatory relation between miRNAs and the autophagy process[4].

Given that autophagy and miRNAs have both been linked to tumorigenesis and miRNAs have a regulatory effect on autophagy, it seems plausible to use autophagy-regulating miRNAs as diagnostic and therapeutic targets. In this review, we will provide a comprehensive description of autophagy, followed by a discussion on autophagy-regulating miRNAs in BC, especially those involved in responding to different therapies in BC.

Breast Cancer

BC is the most prevalent and deadliest cancer globally with more than 2 million new cases being diagnosed in 2019. According to statistics, eight out of one woman (13%) will get BC, and 35 out of one woman (3%) will pass away due to the BC during her lifetime[5]. Age, gender, family history, gene mutations, radiation exposure, periodontitis, medical intervention such as hormonal replacement therapy, and microbiota

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are all risk factors for BC[6]. The presence or absence of molecular markers, as well as estrogen or progesterone receptors (hormone receptors) and human epidermal growth factor 2 (EGFR2 or HER2), cause molecular subtypes inclusive of luminal subtypes A (HR+/HER2-), B (HR+/HER2+), HER2-enriched (high expression level of EGFR2) and triple-negative tumors (TNBC) (HR- and HER2-)[7]. These subtypes cause high heterogeneity and make the necessity for various treatment options[8]. Although usage of various therapy options including surgical resection, radiotherapy, chemotherapy, targeted therapy, immunotherapy, and systemic therapy have lowered the mortality rate, there are still many barriers to the treatment of patients with BC, which limits the success of the therapy.

MicroRNAs

MiRNAs as a type of small non-coding RNAs play pivotal roles in regulating gene expression in a target-specific manner based on the extent of complementarity with targeted mRNAs. Their regulatory effects are exerted by mRNA decay or translational repression. Considering their effect on the gene or protein expression, various cellular functions such as metabolism, proliferation, differentiation, apoptosis, survival, and stress responses in different cell types are affected by miRNAs[9-11]. There is a link between dysregulation in miRNAs expression patterns and many malignancies[3,12]. Depending on the mRNA they are targeted during carcinogenesis, they operate as an oncomiR or tumor suppressor[13,14]. All stages of tumorigenesis including initiation, progression, spreading, and even the response to therapies can be affected by changes in miRNA level[15-17].

The first relation between miRNAs and BC was shown in 2005[18] and since then many studies have been conducted and have shown that miRNAs have the potential to be used as diagnostic, prognostic, and therapeutic biomarkers in BC[19].

Autophagy

The cornerstone of biological activities in cells is the maintenance of physiological homeostasis *via* biosynthesis and degradation. Autophagy is a conserved and regulated cellular mechanism that helps to degrade and recycle cytoplasmic components and organelles in organisms ranging from yeast to mammals[2]. For the first time, it was suggested by Christian de Duve in 1963 as the cellular process in which the bilateral membrane vesicle called the autophagosome, engulfs intracellular contents and delivers them to the lysosome for digestion[20]. Due to this action, not only cellular substances and damaged organelles are removed, but also the materials needed by the cell are recycled and energy homeostasis is maintained. Autophagy is active in most cells at a low level regulating cell metabolism by eliminating damaged proteins and organelles, called basal autophagy[2]. Furthermore, stress conditions such as starvation, unfolded protein response, hypoxia, DNA damage, viral infection, growth factor depletion, etc. can initiate autophagy which is called induced autophagy to respond to the needs of the cell[21]. There are several biological functions for which autophagy is critical ranging from embryonic development to cell death in a way it is suggested as programmed cell death type II or autophagic cell death (ACD)[22]. There is a strong association between autophagy and numerous cellular signaling pathways. In response to stimulant or stressful conditions autophagy-related genes (ATGs) are activated and contribute to the formation of autophagosome and fusion with the lysosome[2]. Recent studies have shown what was thought to be a non-selective process of autophagy can be a selective one under nutrient-rich conditions (macroautophagy), and there are different forms of selective autophagy, according to the targets, which are: nucleophagy, ER-phagy, mitophagy, ribophagy, lipophagy, glycophagy, etc. selective autophagy is facilitated by the presence of receptors such as p62 (sequestosome-1 or SQSTM1), OPTN (optineurin), CALCOCO2 (calcium-binding and coiled-coil domain-containing protein 2), and BNIP3L (BCL2-interacting protein 3-like)[23].

There is evidence that mutation in these genes has a relation with human disorders. An altered autophagic process is associated with cardiovascular diseases, autoimmune diseases, neurodegenerative disorders, infections, myopathies, diabetes, and cancer[24].

Types of Autophagy

Eukaryotic cells administer three types of autophagy based on the delivery pathway of targets to lysosomes: macroautophagy (MA), microautophagy (MI), and chaperone-mediated autophagy (CMA)[25]. MA is the most intensively studied type of autophagy in which time-worn proteins and organelles are engulfed by autophagosomes. Subsequently, lysosomes combine with the created autophagosomes and form autophagolysosomes (or autolysosomes) which provide a milieu for the degradation of proteins and organelles[26]. Other types of autophagy differ in the manner in which the material is delivered to the lysosome as in MI, the lysosome itself engulfs abundant, small cytoplasmic cargoes. In CMA, target substrates tagged with special C-terminal KFERQ motifs are recognized and transferred into the lysosomes by Hsc70 (Heat shock cognate protein of 70 kDa) and endosomal sorting complexes are required for transport I and III (ESCRT/III). Delivery of substrates to lysosomes occurs through the binding of Hsc70 to the lysosome-associated membrane glycoprotein type 2A (LAMP2A)[27,28]. MA represents the canonical pathway of autophagy and has been more intensively decoded, we will focus on this type and use the term "autophagy" instead of MA in this review.

Autophagy Mechanisms

The mechanism of MA consists of several stages and the participation of numerous factors. The stages include initiation, nucleation, elongation, autophagosomal formation and maturation, lysosomal fusion, degradation, and recycling[29].

Initiation

Intracellular and extracellular stimuli bring upstream autophagy factors and substrates together in a specific site called pre-autophagosomal structure (PAS) for autophagosomal formation[30]. This stage is governed by the complex of Unc-51-like kinases (ULK) which is recruited and incorporated into PAS. One of the most important regulatory and influential factors in this stage is the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1)[31]. In normal conditions, mTOR activation causes autophagy inhibition through the phosphorylation of ATG13 and blockage of its link to ULK1 to form the ULK complex. In stressful conditions, mTOR is suppressed and causes the activation of ULK1 and ULK2. Ultimately, ATG13 along with ATG101, ATG9A, and the focal adhesion kinase (FAK) family kinase-interacting protein 200 (FIP200) form a ULK complex and anchored it to the PAS[32].

Nucleation

ULK complex causes phosphorylation and activation of class III phosphatidylinositol 3-kinase (PI3K) complex including Beclin1/Vps34/ATG14L/Vps15/UVRAG/AMBRA1 which produce phosphatidylinositol 3-phosphate (PI3P). Following, the isolation bilayer membrane called phagophore is formed and enriched by phosphatidylinositol 3-phosphate (PI3P) and then is extended to a double-membrane vesicle called omegasome[33]. According to studies, these membranes have been found to come from the ER, Golgi apparatus mitochondria, and plasma membrane[34]. Along with the formation of the phagophore, ATG proteins are recruited and help to enlarge the membrane. Bcl-2 and Bcl-XL can bind to Beclin1 and inhibit this step of the autophagy process. This inhibitory effect is only related to the ER-localized Bcl-2, not the mitochondrial Bcl-2[35].

Elongation

As the membrane expands, several ATGs are joining it. Two conjugation systems including ATG12/ATG5/ATG16L1 and microtubule-related protein light chain 3 (LC3) are essential pathways to regulate elongation[36]. ATG7 activates ATG12 which moves towards ATG10 and *via* its help is conjugated with ATG5 and makes ATG12/ATG5 complex. Then, ATG16L1 through non-covalent interaction binds to ATG12/ATG5 complex. Moreover, ATG4 cleaves LC3 at carboxy terminus to produce LC3-I as a cytosolic free agent which is then conjugated to phosphatidylethanolamine (PE) of the membrane of the autophagosome with the help of ATG7 and ATG3. Ultimately, the LC3-II complex is produced which its presence in phagophore helps to autophagosome formation[37].

Autophagosomal Maturation

During the autophagosome maturation ATG5, ATG12, and ATG16 and after maturation LC3-II detaches *via* ATG4[38]. Then, syntaxin17 (STX17) is recruited to the autophagosome membrane and maturation is completed[39].

Lysosomal Fusion, Degradation, and Recycling

For fusion of the autophagosome with lysosome presence of several proteins including soluble N-ethylmaleimide-sensitive factor activating protein receptors (SNARE) complexes (VAMP7, VAMP3, VAMP8, and STX17), Rab proteins, and integral lysosomal proteins (LAMP-2) are required. Among these proteins, STX17 is the most important one in a way that fusion starts when the STX17 presents on the surface of the maturated autophagosome. During fusion, the structure of the inner membrane is degraded and the contents are exposed to lysosomal enzymes, and the degradation process begins[40].

Autophagy Inducers

Autophagy is a mechanism by which cells adapt to environmental and nutritional stresses. Mutually, nutritional starvation and excess nutrient stress can promote autophagy. Energy starvation activates autophagy *via* the AMP-activated protein kinase (AMPK) signaling pathway. In this pathway, starvation activates LKB1 (liver kinase B1)-AMPK which can in turn directly activate the ULK-1 complex[41]. LKB1-AMPK can also suppress mTOR through TSC1-TSC2 (tuberous sclerosis complex 1-2) activation. Both of these routes cause autophagosome formation[42]. Also, DNA damage and hypoxia activate the AMPK pathway which leads to ULK-1 complex activation[41]. It was revealed that elevated concentrations of glucose, also induce autophagy mainly through the reactive oxygen species (ROS) pathway[43]. On the other hand, mTORC1 has a major role in autophagy induction in response to amino acid deprivation[44]. Excessive accumulation of misfolded proteins in the endoplasmic reticulum (ER) can lead to ER stress. ER stress is one of the activating mechanisms of autophagy. This type of stimulation occurs *via* activation of ER membrane-associated proteins; protein kinase R-like kinase (PERK), endoplasmic reticulum inositol-requiring enzyme 1 (IRE1), and activation of transcription factor 6 (ATF6). PERK effects are due to the regulatory effects on LC3, ATG5, and eukaryotic initiation factor2 (eIF2) which finally inhibit the synthesis of unfolded or misfolded proteins[45]. The second mechanism is performed by disassembling BCL2 protein from Beclin1 through activating the IRE1[46]. ATF6 as a transcription factor increases the expression of the ER chaperone, HSPA5 which then activates AKT[47]. Growth factors as other regulators of autophagy, trigger the AKT pathway through activating PI3K which inhibits the TSC1-TSC2 complex. TSC1-TSC2 suppression countermands mTOR inhibition which enhances mTOR activity resulting in autophagy inhibition. Consequently, PTEN (phosphatase and tensin homologue) can disable this pathway *via* blocking PI3K activation and reverse the effect of mTOR inhibition[48].

Autophagy and Cancer

The first finding indicating the relation between autophagy and cancer was the presence of deleterious mutations in the Beclin-1 in breast, prostate, and ovarian cancer patients[49].

In the early stages of cancer, autophagy shows tumor-suppressor effects *via* oncoprotein degradation, oxidative stress elimination, maintaining genomic integrity, defenses against bacterial and viral pathogens, and participation in the development of immune responses. Hence, tumor initiation and progression are inhibited by autophagy at the early stages of cancer[50]. Another tumor suppression effect of autophagy is its role in maintaining genomic integrity. Some disorder in the autophagy process causes DNA damage and increases cancer risk[51]. However, in advanced stages where the hypoxia becomes dominated, autophagy functions providing the necessary materials for cells to deal with the hypoxia and nutrients cause an improvement in tumor cells survival, metastasis, and suppression apoptosis[52,53]. With more details, tumor cells usually have a problem supplying their needful glucose. In this condition AMPK-mediated autophagy is activated in tumor cells, also blockage of glycolysis due to lack of glucose causes ER stress which activates autophagy[54]. In the same way, amino acid depletion activates AMPK-mediated autophagy. The mentioned conditions lead to autophagy-mediated cell survival which promotes tumor growth[55]. Therefore, autophagy functions as a double-edged sword based on type, stage, and genetic context of tumor cells because as mentioned above it can lead tumor cells to autophagy-mediated cell survival and enhance tumor development, in contrast, it can also steer cancer cells to autophagy-mediated cell death and block tumor development[56].

In clinical application, chemotherapeutic agents like 5-fluorouracil (5-FU), gemcitabine, and cisplatin cause DNA damage and induce AMPK-mediated autophagy in cancer cells that lead to cancer cell survival[57-59]. Also, exposing infrared radiation (IR) to tumors activates autophagy in tumor cells and makes them survive *via* DNA damage and mTOR inhibition. However, in some cases usage of conventional cancer treatments causes activation of autophagy and developing drug resistance. In consequence, targeting autophagy was suggested as a therapeutic option in cancer therapies[60]. Using a mixture of chemotherapeutics and autophagy inhibitors such as Bafilomycin, Chloroquine (CQ), and 3-methyladenine (3MA) can overcome drug resistance and block autophagy-mediated cell survival and steer cancer cells to cell death[61].

As indicated previously the regulatory effects of autophagy as both tumor suppression and promotion have also been identified in BC[62]. There is evidence that factors and molecules involved in the onset and progression of BC affect autophagy through different pathways. For example, in the early stages of BC the activity of tumor suppressor genes, p53 and PTEN, trigger autophagy and suppress tumorigenesis while along with tumor progression activation of oncogenes, Bcl-2 and PI3K/AKT, restrain stress responses, and suppress autophagy[63]. Furthermore, studies have been shown that in advanced stages of BC, oncogenic mTOR-activating proteins were upregulated[64]. On the other hand, mutations in genes expressing proteins involved in the early stages of autophagy such as Beclin-1 or BECN1 have been reported in almost all cases of BC[49]. Conventional approaches and even new targeted drugs such as anti-HER2 drugs, PI3K/AKT inhibitors, CDK4/6 inhibitors, and immune checkpoint inhibitors can also affect the autophagy pathway and result in treatment resistance in some cases. In light of these findings, autophagy inhibitors have been suggested as an alternative therapeutic option in BC patients, but more studies are needed[65]. Given that the relationship between autophagy and BC has been thoroughly studied in another review article[64], we will not elaborate further here.

Autophagy and MiRNAs

Numerous studies have shown that autophagy and miRNAs have a bilateral relationship. Autophagy performs an important role in maintaining the homeostasis of miRNAs and miRNAs have regulatory effects on autophagy both *in vivo* and *in vitro* due to their gene expression regulation effects on ATGs and proteins involved in signaling pathways related to autophagy. Depending on whether the tumor cells are under metabolic or therapeutic stress, miRNAs-mediated autophagy can have either pro-survival or pro-death effects. The relation between miRNAs and autophagy not only affects the different stages of tumor growth and development but in the same way affects the response to different cancer therapies such as radiotherapy and chemotherapy[66]. For the first time in 2009, Zhu et al[67] showed that miR-30a downregulates the expression level of Beclin-1 to suppress rapamycin-induced autophagy in tumor cells and further studies have shown that miRNAs can affect all stages of autophagy. This regulation can occur by both types of tumor-suppressor and oncomiRs, leading to activation or inhibition of autophagy[68]. In squamous cell carcinoma miR-885–3p targets ULK2 and inhibits autophagy in response to cisplatin treatment[69]. Under hypoxic conditions of hepatocellular carcinoma (HCC) cells, miR-375 decreases ATG7 and inhibits autophagy *in vitro* and *in vivo*[70]. MiR-30d suppresses Beclin-1 and increases the response to cisplatin in thyroid carcinoma cells[71]. MiR-140-5p by targeting ATG12 causes an inhibition of autophagy which suppresses the survival of colorectal cancer stem cells[72]. In cervical cancer, increased expression of miR-224-3p inhibits autophagy *via* targeting FIP200. MiR-17 can inhibit the formation of autophagolysosome by targeting Rab7 GTPase members[73]. A study by Yuan et al[74] showed that increasing the expression level of tumor-suppressor miR-375 in gastric cancer inhibits autophagy *via* targeting the mTOR pathway. In another study on gastric cancer, increasing the expression level of miR-21 led to inhibition of autophagy *via* the PI3K/Akt/mTOR pathway which decreased cisplatin resistance[75]. These are just a few of the many studies that have been conducted on the first step of autophagy and they are still ongoing revealing other miRNAs regulatory effects on different steps of autophagy.

Autophagy and MiRNAs in BC

Like other cancers, the autophagy process in BC is affected by changes in the expression level of miRNAs and several studies have been reporting various autophagy regulating miRNAs at many points in BC[76].

MiR-20a and miR-20b involvement in autophagy regulation *via* targeting FIP200/ RB1CC1, as an important part of the ULK complex, was shown in MCF-7 and MDA-MB-231 cells by Li et al[77]. The study revealed that overexpression of miR-20a and miR-20b inhibits basal and rapamycin-induced autophagy which causes inhibition in cancer progression. However, in another study, Liu et al[78] showed the ability of miR-20a to inhibit basal and nutrient starvation-induced autophagy *via* targeting Beclin-1, ATG16L1, and SQSTM1 in MDA-MB-231 and MCF-7 cells and unexpectedly improved tumor initiation and growth. MiR-23a is among miRNAs involved in the regulation of autophagy in BC by targeting X-linked inhibitor of apoptosis (XIAP) as one of the autophagy inhibitor proteins in the cell[79]. MiR-92b has been introduced as a tumor suppressor in BC through the inhibition of viability and invasion. Autophagy-inducing stimuli, starvation, and rapamycin cause overexpression of miR-92b that negatively regulates the histone methyltransferase enhancer of zeste homolog 2 (EZH2) which leads to promotion of autophagy[80]. Overexpression of miRNA-96-5p inhibits autophagy and apoptosis and enhances tumorigenesis of human BC cells. Furthermore, upregulation of miR-96-5p suppresses autophagy through inhibiting LC3II production and degradation of p62. This inhibitory effect of miR-96-5p happens through targeting forkhead box protein O1 (FOXO1). Shi et al[81] showed that increased expression of miR965p not only improves proliferation, migration, and invasion but also inhibits basal and starvation-induced autophagy through targeting FOXO1 in MCF7 and MDA-MB-231 cells. Frankel et al[82] conducted an *in vitro* and *in vivo* study and found miR-101 as one of the effective inhibitors of basal-, etoposide- and rapamycin-induced autophagy in BC. They revealed ATG4D, STMN1, and Rab5A as targets of miR-101. Ai et al[83] observed a relation between decreased expression of miR-107 and suppressed autophagy process and tumor cell proliferation *via* targeting high mobility group protein B1 (HMGB1). They suppressed the expression of HMGB1 and detected increased expression of p62 protein and decreased Beclin1 protein in MDA-MB-231 and MDA-MB-453 BC cells.

Decreased expression of miR-124-3p on BC tissues and cell lines was shown in a study by Zhang et al[84]. They showed that miR-124-3p affects the autophagy process *via* targeting Beclin-1. Increased expression of miR-224-5p in metastatic and non-metastatic BC cell lines was reported to inhibit autophagy through suppressing the TGF-β/Smad4 signaling pathway which is one of the autophagy activating pathways that increased ATG5, ATG6, and ATG7 proteins[85]. MiR-372 suppresses autophagy and tumor growth in BC by targeting p62[86]. An *in vitro* study described that down-regulation of miR-486-5p would increase autophagy through targeting PTEN in MCF-7 BC line[87]. MiR-638 as an oncomiR displayed the regulatory effects on the autophagy process *via* targeting ULK1 and DACT3, as a regulator of the Wnt/β-catenin signaling pathway and induced autophagy *via* increasing cellular expression level of LC3-II[88]. Furthermore, miR-638 has been linked to autophagy in controversial ways. In a case-control study, ATG5 was reported to be targeted by miR-638 which suppresses the autophagy process and improves tumor progression[89]. MiR-1275 has been shown that to inhibit the expression of ATG7 and suppression of autophagy and tumor progression. The inhibitory effect of miR-1275 is related to the formation of the mature autophagosome. There is another study that showed miR-1275 could target ULK1 and suppress autophagy[90]. Another miRNA that promotes autophagy in BC [91] is let-7a while exerting the opposite effect in other cancers like lung[92] and gastric[93]. MiR-1910-3p has an oncomiR role in BC and its increased expression causes an improvement in proliferation, metastasis, and autophagy both *in vitro* and *in vivo* in BC. Moreover, delivering exosomes enriched with miR-1910-3p to BC and mammary endothelial cells induced autophagy and inhibited apoptosis[94]. Additionally, there are studies have been shown that miRNAs including miR-25, miR-30a, miR-30c-1, miR-149, miR-221, miR-376b, miR-489, miR-519a, miR-611, miR-615-5p, miR-659, miR-636, miR-659, miR-675, miR-1303, miR-1308, miR-1908, miR-1914, miR-1915, miR-2861, miR-3184, miR-4292, and miR-4259 can target ULK complex components and regulate autophagy in BC[64,95] (Figure 1). In this review, we focused on the numerous studies in the field of autophagy regulating miRNAs in BC that modulate BC responses to therapies and the possibility of using them as a therapeutic target.

Effects of Autophagy and MiRNAs on Hypoxia of BC

Hypoxia is one of the pathologic features that affects various tumor processes such as metastasis, angiogenesis, recurrence, and chemoresistance in BC[96]. In response to hypoxia generated in the tumor microenvironment, tumor cells increase the expression of hypoxia-inducible factor (HIF)-1[97] which regulates genes and intracellular pathways including hypoxia-regulated miRNAs (HRMs)[98] and autophagy[99]. Regarding BC, in a study by Hu et al[100] on breast cancer stem cells (BCSCs), they found HIF-1-mediated down-regulation in the expression level of miR-137 under hypoxia conditions. Following miR-137 restoration, mitophagy/autophagy is inhibited *via* targeting FUN14 domain-containing protein 1 (Fundc1) and promotes tumorigenesis. Fundc1 as a mitochondrion membrane protein promotes autophagy and upregulates the expression of Beclin1, ATG5, and ATG7.

Effects of Autophagy and miRNAs on Chemotherapy of BC

One of the most fundamental therapies for patients with BC is chemotherapy, whether it is used alone or in combination with another treatment method. Different types of chemotherapy drugs with different mechanisms are used in BC including anthracyclines (doxorubicin and epirubicin), taxanes (paclitaxel and docetaxel), platinum (cisplatin), *etc.* Despite the promising results

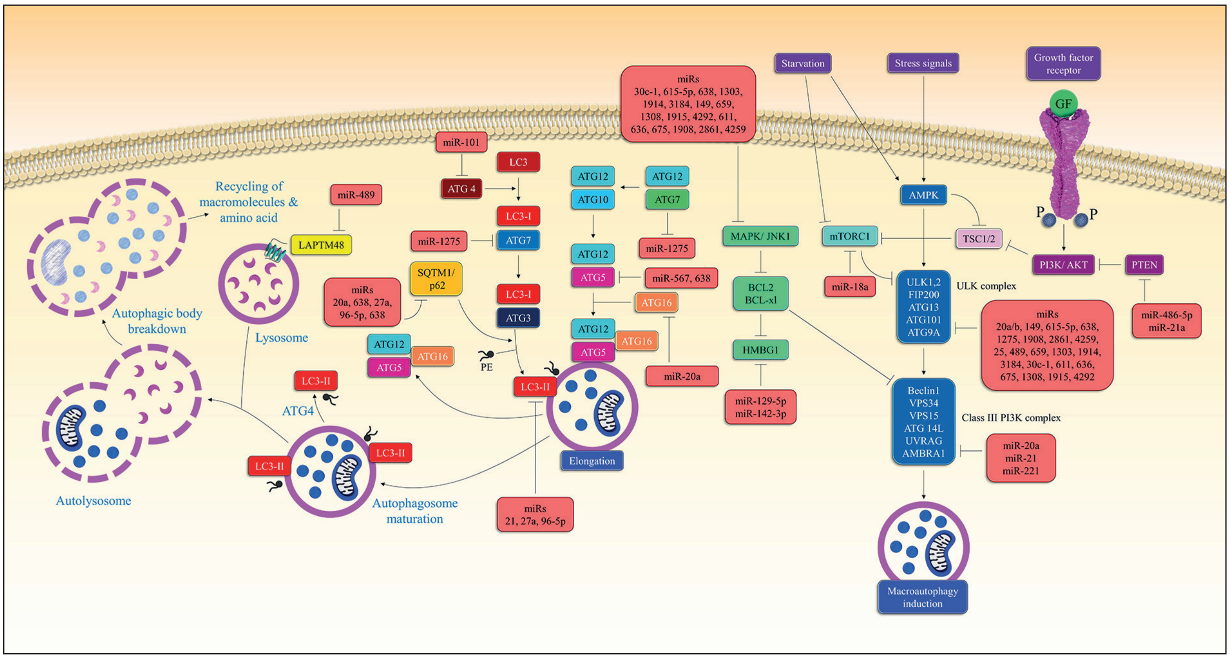

Figure 1. The roles of miRNAs in regulating the autophagy process in breast cancer. Upon the presence of stimuli, the autophagy process is induced and consists of several different stages; initiation, nucleation, elongation, autophagosomal formation and maturation, lysosomal fusion, degradation, and recycling. miRNAs exert their dual regulatory effects by affecting the expression of components involved in different stages of autophagy. In the figure, the proteins involved in the stages of autophagy are shown in different colors, and their regulating miRNAs are shown in pink boxes.

of chemotherapy in reducing the growth and development of tumor cells, resistance to these agents reduces the therapeutic effects and also leads to tumor metastasis and recurrence[1]. The results of various studies revealed an association between autophagy and drug resistance not only in BC but also in different types of other cancers. This includes colorectal, bladder, ovarian, prostate, osteosarcoma, and malignant glioma[101].

Generally, three types of association between autophagy and drug resistance have been observed in BC. First, autophagy by protecting tumor cells against external stressors, such as chemotherapy drugs, develops resistance. Indeed, in some cases inhibition of autophagy reversed the resistance to chemotherapy drugs. This type of autophagy is designated cytoprotective autophagy that acts as an important inhibitory factor in the treatment of BC patients. Unlike the first case, some studies have shown that increased autophagy enhances the sensitivity of BC cells to chemotherapy drugs and promotes different types of cell death. Furthermore, any change in different stages of autophagy can affect the process of drug resistance in BC[102]. MiRNAs are one of the critical factors involved in regulating the sensitivity of tumor cells to several chemotherapy drugs[103]. They can increase the sensitivity or the resistance of BC tumor cells in response to chemotherapeutic agents due to their effects on the proteins involved in the autophagy process[102].

Resistance to paclitaxel (PTX) is among those reasons for death associated with treatment failure in BC patients. Shi et al[104] demonstrated that up-regulation of miR-129-5p increased paclitaxel sensitivity of MCF-7 cells which led to inhibition of autophagy and promoting apoptosis by targeting HMGB1. The presence of BCSCs causes chemoresistance, recurrence, and metastasis of tumor cells[105]. Ueda et al[106] showed that miR-27a acts as a master regulator for BCSCs through regulating autophagy. Overexpression of miR-27a increased the sensitivity of MCF-7 and MDA-MB-231 cells to PTX and doxorubicin (DOX) *via* suppressing autophagy and affecting p62. The involvement of miR-18a in PTX resistance was shown in TNBC cells. PTX resistant cell line, MDA-MB-231/PTX cells, showed a higher level of miR-18a and autophagy in comparison with MDA-MB-231 cells. The study revealed that overexpression of miR-18a inhibited mTOR expression which further increased autophagy and resistance against paclitaxel. Moreover, usage of autophagy inhibitor, Bafilomycin A1, increased apoptosis and sensitivity to PTX[107].

It has been shown that miR-489 has tumor suppressor effects in BC and inhibits the autophagy process[108]. Soni et al[109] showed that suppressing autophagy through the restoration of miR-489 in the BC cell line increased the sensitivity toward DOX *via* targeting lysosomal protein transmembrane 4 beta (LAPTM4B) as one of the important factors in autophagosome maturation *in vivo* and *in vitro*. Furthermore, Liang et al[110] reported that upregulation of miR-142-3p as a tumor suppressor miRNA in BC inhibited autophagy and improved the chemosensitivity toward DOX through the targeting HMGB1 in the MCF-7 cell line. They showed that DOX-resistant cells had low expression of miR-142-3p and a high level of autophagy.

Regarding the second relation between autophagy and drug resistance, in an *in vitro* study on HS578T cell line, overexpression of miR-181a-5p negatively regulated vitamin D receptor (VDR) as an autophagy regulator protein which increased autophagy and then improved sensitivity to cisplatin[111]. In a similar study, the usage of isoliquiritigenin (ISL) as an anti-cancer agent, repressed miR-25 expression. Upregulation of miR-25 suppressed autophagy through targeting ULK1 and improved the chemoresistance in epirubicin-resistant BC cells (MCF-7/ADR)[112]. Furthermore, in a study by Li et al[113] increased expression of miR-125b-5p enhanced autophagy *via* negative regulation of peptidylarginine deiminase 2 (PAD2) enzyme which has an inhibitory effect on mTOR. Increased autophagy further increased the sensitivity to tamoxifen and improved docetaxel effects as combination therapy on the tamoxifen-resistant cell line.

Effects of Autophagy and MiRNAs on Radiotherapy of BC

One of the most important treatment modalities used for BC patients is radiotherapy, which leads to killing tumor cells *via* generating oxidative damage, membrane permeability, chromosome aberrations, metabolic imbalances, and activation of signaling pathways including apoptosis and autophagy. Indeed, DNA damage, ER stress, dysfunctional mitochondria, and elevated Ca2+ levels are among the causes of radiation-induced autophagy in tumor cells[114]. Furthermore, radiotherapy through modulation of the immune system and tumor microenvironment has anti-cancer effects. Recent studies show that radiation-induced autophagy *via* impressing antigen presentation, generating damage-associated molecular patterns (DAMPs), and releasing of IFN-γ improves anti-tumor responses[115]. Depending on the severity and duration of radiotherapy, radiation-induced autophagy shows bi-lateral effects. When the amount of stress is low, autophagy compensates for the complications and makes resistance to radiotherapy (cytoprotective autophagy) and improves tumor cell survival, but with the increase in stress severity autophagic cell death occurs[116].

On the subject of BC, autophagy led to an improvement in tumor cell survival when radiotherapy was applied to the MDA-MB-231 cell line through PI3K-Akt mTOR pathway interaction[117].

Recent studies have indicated the involvement of miRNA in autophagy-mediated radiation resistance of BC tumor cells[116]. Meng et al[118] showed that miR-26b negatively regulated the DNA damage-regulated autophagy modulator 1 (DRAM1) and made a suppression in autophagy process in MCF-7 cells. Following miR-26b over-expression autophagy was inhibited and sensitization to radiotherapy was increased. Decreased expression of miR-129-5p caused an increase in the expression of HMGB1 and induced irradiation-induced autophagy which protects BC tumor cell survival. Along with the overexpression of miR-129-5p, the sensitization of MDA-MD-231 cells against irradiation was improved[119]. In another interesting study, Yi et al[120] found that miR-199a-5p has a dual regulatory effect on irradiation-induced autophagy was inhibited *via* DRAM1 and Beclin1. They showed that by over-expression of miR-199a-5p irradiation-induced autophagy in MCF-7 cells, whereas this miRNA induced irradiation-induced autophagy in MDA-MB-231 cells. Considering the different expression patterns of miR-200c in BC cell lines, Sun et al[121] showed that decreased expression of miR-200c has a relation with radioresistance in MDA-MB-231 cells through targeting ubiquilin 1 (UBQLN1) which plays a role in autophagosome formation. Along with the ectopic expression of miR-200c, autophagy was inhibited and radiosensitivity was increased.

Effects of Autophagy and miRNAs on Endocrine and Targeted therapy of BC

Endocrine therapy (ET) also called hormone therapy is a type of therapy in which hormones especially estrogen amounts are decreased, or their functions are blocked. This therapy is used only in hormone-receptor-positive BC patients and involves a variety of medications like tamoxifen, fulvestrant, aromatase inhibitors, *etc*[122]. ET reduces recurrence and mortality rate in BC patients and not only improves the quality of life but also has the fewest side effects compared to other methods[123]. However, drug resistance limits therapy efficacy. Among the factors that cause ET resistance, changes in intracellular pathways such as autophagy and miRNAs expression patterns are among the important ones[124]. *In vitro* study proposed miR-101 as an autophagy inhibitor through targeting ATG4D, RAB5A, and STMN1. Overexpression of miR-101 restrained resistance toward 4-hydroxytamoxifen (4-OHT) and increased MCF-7 cells sensitivity[82]. Increased expression of miR-451a suppressed autophagy and improved tamoxifen sensitivity in MCF-7 and LCC2 BC cells *via* regulation of 14-3-3ζ protein[125]. By disrupting cell amino acids level *via* targeting an amino acid transporter, Solute Carrier Family 6 Member 14 (SLC6A14), miR-23b-3p induced autophagy and drug resistance in BC against tamoxifen and fulvestrant[126]. Knockdown of miR-21 induced autophagy *via* suppressing PI3K-AKT-mTOR pathway and increasing Beclin-1 and LC3-II expression level which promoted the sensitivity toward tamoxifen and fulvestrant in MCF-7 cells[127]. MiR-214 was found to suppress autophagy through activation of the PI3K-Akt-mTOR pathway and increasing uncoupling protein 2 (UCP2). Inhibited autophagy increased sensitivity toward tamoxifen and fulvestrant in MCF-7 cells[128]. Regarding targeted therapy, trastuzumab or Herceptin is a monoclonal antibody used for HER-2 positive BC patients in early and advanced stages[129]. MiR-567 showed an inhibitory effect on the autophagy process and resistance to trastuzumab *via* targeting ATG5 in BC patients and cell lines. Although its expression level is downregulated in trastuzumab-resistant cells, increased expression of miR-567 suppressed autophagy and enhanced the sensitivity to trastuzumab *in vitro*[130].

Discussion

In light of the high prevalence, heterogeneity, and resistance to therapy observed in BC, a better understanding of the molecular mechanisms involved in tumor progression and response to therapies could remove existing barriers to therapy development and enable new ones to be developed. It has been shown that autophagy is one of the intracellular degradation pathways implicated in tumorigenesis, responsiveness, or resistance to various therapies in BC. Currently, studies are aimed at identifying therapeutic agents that can target multiple pathways, of which miRNAs are one of the best that have the ability to regulate several pathways including autophagy in multiple steps. Targeting autophagy regulating miRNAs is proposed as a treatment procedure that would increase clinical outcomes and reverse therapy resistance (Table I).

However, due to the bilateral effects of miRNAs studied, it seems that targeting them requires consideration of factors which previous studies[131] have shown their regulatory effects on the expression and effects of miRNAs. The factors currently identified are genetic or epigenetic alterations of DNA sequences that code miRNAs, transcriptional regulation, alterations of mRNA target sites. Hence, there is a possibility that these factors directly or indirectly affect the miRNA's effect on the autophagy pathway. It would be helpful to consider these factors in targeting autophagy regulating miRNAs. Here, as discussed in the chemotherapy section of the manuscript, miR-181-5p, miR-25, and miR-125 induced autophagy which resulted in enhanced sensitivity to chemotherapy drugs, whereas other miRNAs by inducing autophagy caused drug resistance. In radiotherapy, while overexpression of miR-199-5p caused inhibition in IR-induced autophagy in MCF7 cells, it improved IR-induced autophagy and basal autophagy in MDA-MB-231 cells. Furthermore, miR-20a has presented different results in terms of inducing autophagy and enhancing BC cell progression and/or death.

Conclusions

The importance and role of autophagy-regulating miRNAs in the growth and development of cancer have been demonstrated in recent years. In order to develop an effective therapy based on miRNAs that regulate autophagy in BC, many factors must be taken into account. MiRNA itself, cell line type and induced autophagy effects are some of the things that have been identified by studies so far and further investigations are needed. Although there have been a few cases where

Table I. Effects of autophagy-regulating miRNAs on treatment responses in breast cancer.

miRNA	Target	Effect on autophagy	Influence on treatment response	Drug(s)	Ref
Chemotherapy					
miR-129-5p	HMGB1	Inhibition	Sensitivity-promoting	Paclitaxel	104
miR-27a	p62	Inhibition	Sensitivity-promoting	Paclitaxel & doxorubicin	106
miR-18a	mTOR	Promotion	Resistant-promoting	Paclitaxel	107
miR-489	LAPTM4B	Inhibition	Sensitivity-promoting	Doxorubicin	109
miR-142-3p	HMGB1	Inhibition	Sensitivity-promoting	Doxorubicin	110
miR-181a-5p	VDR	Promotion	Sensitivity-promoting	Cisplatin	111
miR-25	ULK1	Inhibition	Resistant-promoting	Epirubicin	112
Radiotherapy					
miR 26b	DRAM1	Inhibition	Sensitivity-promoting	-	118
miR-129-5p	HMGB1	Inhibition	Sensitivity-promoting	-	119
miR-199a-5p	DRAM1 Beclin1	Inhibition in MCF7 Promotion in MDA-MB-231	No changes Sensitivity-promoting	-	120
miR-200c	UBQLN1	Inhibition	Sensitivity-promoting	-	121
Endocrine therapy					
miR-101	ATG4D STMN1 RAB5A	Inhibition	Sensitivity-promoting	Tamoxifen	82
miR-451a	14-3-3ζ	Inhibition	Sensitivity-promoting	4-hydroxytamoxifen	125
miR-23b-3p	SLC6A14	Promotion	Resistant-promoting	Tamoxifen and fulvestrant	126
miR-21	PTEN	Promotion	Resistant-promoting	Tamoxifen and fulvestrant	127
miR-214	UCP2	Inhibition	Sensitivity-promoting	Tamoxifen and fulvestrant	128
Chemotherapy & Endocrine therapy					
miR-125b-5p	PAD2	Promotion	Sensitivity-promoting	Docetaxel & tamoxifen	113
Targeted therapy					
miR-567	ATG5	Inhibition	Sensitivity-promoting	Trastuzumab	130

miRNA; microRNAs, HMGB1: high mobility group protein B1, mTOR: mechanistic target of rapamycin, LAPTM4B: lysosomal protein transmembrane 4 beta, VDR: vitamin D receptor, ULK1: Unc-51-like kinases 1, DRAM1: DNA damage regulated autophagy modulator 1, UBQLN1: ubiquilin 1, ATG4: autophagy-related genes 4, SLC6A14: Solute Carrier Family 6 Member 14, PTEN: phosphatase and tensin homologue, UCP2 : uncoupling protein 2, ATG5: autophagy-related genes 5.

the results were unexpected, in general, it seems that focusing on autophagy-regulating miRNAs as biomarkers or therapeutic targets could be an important step toward improving BC patients' therapies.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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