Review Article

Combinational Treatments for Breast Cancer

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Abstract

Heterogenicity is an indispensable element of breast cancer, which manifests itself on clinical, histopathological, and molecular levels. This heterogenicity could be a determinant factor of disease progression and drug resistance in the patients. Common therapies for metastatic breast cancer include surgery, radiotherapy, chemotherapy, and immunotherapy. On the introduction of biologic medicine, target therapy, and gene therapy, a potential has been reached to lower the rate of morbidity and mortality and also to improve the quality of life among patients with breast cancer. Although these treatments have been frequently proved by yielding promising results, a very few number of them have found their way into clinic settings due to progressive nature of these tumors, diversity of cancer populations and their microenvironments, genetic instability, and heterogenicity of breast cancers. As only minor advancements have been made in the case of recurrent metastatic breast cancer, handling this condition is now considered a medical necessity. According to genetic instability and heterogenicity of breast tumors, it is implausible to assume a single-targeted therapy could help treating most solid tumors. So, this review aimed to put together studies focused on combinational treatments targeting growth inhibition and apoptosis induction in breast cancer cells and comparing the results with monotherapies.

Keywords: *breast cancer, combinational treatment, combinatorial treatment*

Introduction

The most commonly used treatment approaches toward metastatic breast cancer include surgery, radiation therapy, hormone therapy, chemotherapy, and immunotherapy. Fortunately, introduction of biological medicines, targeted therapy, and gene therapy has been potentially reducing mortality and increasing life quality of patients with breast cancer. Moreover, the availability of modern diagnostic tools, systemic combination therapies, and gene therapy have all made a significant progress in the treatment of primary breast cancer and therefore an increased survival rate for patients. Although these treatment agents have shown promising results against breast cancer, a very few number of them have actually made it to the drug market. In other words, the majority of key genes responsible for cancer are considered undruggable targets. Therefore, despite the potential of early diagnosis and the targeted therapies developed, the breast cancer mortality rate has decreased only by 1.3% in the last decade due to the progressive nature of the tumor, the diversity in cancer cell populations and their microenvironments, and the genetic instability and heterogeneity of the tumor. Moreover, limited amount of improvements has been achieved in patients with recurrent metastatic breast cancer. Therefore, more thorough research on the subject remains a crucial necessity in the field of breast cancer treatment. However, the translation of this increasing knowledge gained in the research area into clinical settings has been facing certain challenges.[1-5] In addition to the development of some resistance mechanisms in cancer cells, the lack of specific treatments for those breast cancers expressing no hormone receptor is another reason for compromised treatment effectiveness. The other obstacle faced in the treatment is cancer cells not presenting any hormone receptors to be specifically targeted. Therefore, it is strongly suggested to turn to new therapeutic approaches toward overcoming drug resistance and appropriately targeting receptor-free cancer cells.[6,7] Given the genetic instability and heterogenic nature of breast tumor, it seems irrational to focus on only a single target for long-term treatment of solid tumors. As our understanding of tumor biology grows, new targets are being identified capable of interfering with several pathways involved in cancer cell proliferation (such as phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR)) studies of apoptosis in breast cancer,[7-10] cell cycle regulation, heat shock protein (HSP) function,

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and epigenetic pathways. Therefore, applying a combination of targeted therapies could be a more promising treatment option for breast cancer. With this background in mind, this study aimed to address combination therapies used to suppress the growth and increase the apoptosis of breast cancer cells.

Epigenetic-based combination therapies

Molecularly speaking, eukaryotic deoxyribonucleic acid (DNA) binds to histone molecules to form a more complex structure called chromatin. Histone acetylation that occurs at lysine residues of the *N*-terminal tails of histones leads to chromatin relaxation and increased gene expression. Histone-modifying enzymes such as histone deacetylases (HDACs) could therefore catalyze histone deacetylation, leading to chromatin constriction, and finally suppress the expression of certain genes. Through repressing the expression of tumor suppressor genes, HDACs are known faulty for increasing the chance of tumorigenesis. Accordingly, inhibitors of HDAC could be capable of preventing such situation and thus allowing tumor suppressor gene expression to finally restrain tumor cells progression. So, HDAC inhibitors are considered epigenetic drugs that target a part of cancer cell signaling.[11,12]

As a result, some researchers were focused on evaluating the effect of epigenetic modulators (e.g., HDAC inhibitors) on breast cancer cells, which yielded some promising results associated with fewer side effects. A combination of targeted therapies, including HDAC and poly (ADP-ribose) polymerase (PARP) inhibitors, could a promising option for breast cancer, especially for triple-negative breast cancers (TNBC). Despite the effectiveness of HDAC inhibitor monotherapy on hematological malignancies, it has no impact on solid tumors (e.g., TNBC). To explain such digression, Zeng *et al.* reported that as soon as HDAC is inhibited, the cytokine receptor pathways involving signal transducer and activator of transcription (STAT3) are reprogrammed. This reprogramming associates with an antiapoptotic effect, limiting the rate of responses to HDAC inhibitors in breast cancer cells, especially those expressing hormone receptors. Zhang *et al.* then tested a pharmacologic inhibitor of Bromodomain-containing protein 4 (BRD4) in combination with vorinostat and showed that growth was inhibited in breast cancer cells on the inhibition of BRD4 and HDAC. They also examined the response of cancer cells to vorinostat in combination with ruxolitinib, a Janus kinase (JAK1)/2 inhibitor, which resulted in an increase in apoptosis and subsequently decreased proliferation of breast cancer cells. Moreover, there was a significant inhibition of tumor growth in three of the four cases of TNBC obtained from xenograft models. These results clearly show that the mentioned combination therapy of HDAC inhibitors and JAK1/2 inhibitors should be considered in TNBC patients. On the contrary, other inhibitors of PARP (e.g., olaparib) could also have synergetic effects with HDAC inhibitors in the treatment of many cancer types. For instance, a pan-HDAC inhibition could create BRCAness in TNBC cells and cause sensitivity in TNBC cells to PARP inhibition.[11,13-17]

Along with other factors, unfavorable epigenetic changes could be responsible for pathogenesis of various types of cancers. Owing to their epigenetic modulatory properties, medicinal herbs could exert inhibitory effects in the early stages of cancer development. To evaluate such effects, a combination of bioactive nutrients, such as green tea polyphenols (GTPs) and broccoli sprouts powder (BSP), was exploited to neutralize epigenetic disorders occurring during early stages of breast tumor development in breast cancer cell transplantation and rat xenograft models. Such combination treatment with epigallocatechin-3-gallate in GTPs and sulforaphane in BSP has been demonstrated to have a synergistic effect on cell cycle arrest in breast cancer models. Further studies showed that this combination led to an extensive level of epigenetic changes in the tumor cell genome.[18-22]

Abnormal activity of histone lysine demethylase (KDMs) and lysine deacetylases (HDACs) is associated with the expression of abnormal genes involved in the development of breast cancer. However, the precise molecular mechanisms explaining the interactions between KDMs and HDACs and chromatin remodeling or gene transcription regulation are still not fully comprehended. Combination therapy with lysine-specific demethylase (LSD1) and HDAC inhibitors resulted in increased levels of H3K4me2 and AcH3K9, showing a synergism in growth inhibition of breast cancer cells.[23-25]

Neoadjuvant FEC 100 drugs (5-fluorouracil, epirubicin, and cyclophosphamide) are one of the approved options for the treatment of primary or advanced metastatic breast cancers. Later, valproic acid was added to this combination. The results from the initial phase of studies on valproic acid and FEC100 in patients with cancer led researchers to believe that this combination was better than the standard treatment. However, in clinical trial phase 1, researchers realized that although this combination seemed more efficient, it could also bear more complications, compared to chemotherapy.[26,27]

In a study, the combination of resveratrol and pterostilbene at doses close to physiological levels resulted in the inhibition of TNBCs growth. It was shown that this combination could lead to downstream regulation of silent information regulator T1 (SIRT1) and H2A histone family member X (H2AX) and a significant reduction in gamma H2AX protein and telomerase. This in turn increased the growth inhibition, apoptosis, and cell cycle arrest in Cellosaurus cell line (HCC 1806) and MD Anderson –metastatic breast 157 (MD-MB157) cells. Interestingly enough, these compounds significantly decreased DNA methyl transferase (DNMT) enzymes level in breast cancer cells without leaving any significant impact on the expression of the same enzyme in normal MCF10A breast cells.[28-30]

HSP 70–based combination therapies

HSPs are highly protected molecular chaperones, produced by cells in response to various cellular stresses.[31-33] In mammals, HSPs are classified into several categories based on their molecular weight, including HSP90, HSP100, HSP72, and

smaller HSPs such as HSP27.[32,34] HSPs are essential proteins that play an important role in cell survival by being involved in cellular protective mechanisms. In addition, HSPs are often expressed in severe cancers (e.g., breast cancer), and it seems that their expression is accompanied by poor clinical outcomes.[35-38]

Studies have shown that HSP70 is not only cytoprotective but it also effectively counteracts cell death induced by strong stimuli of kinase pathway activated under stress. In addition, it inhibits apoptotic signaling through interacting with signaling intermediates such as JUN, ASK, and SEK1.[31,39,40] This protein is also a negative regulator of mitochondrial apoptosis pathway. functioning majorly during events that occur after mitochondrial membrane disruption.[40,41]

In addition to preventing procaspase-9 association with apoptosome, HSP70 is able to negatively interfere with the formation of apoptosomes through direct interaction with apoptotic protease activating factor 1 (Apaf-1). Furthermore, the activation of caspase-3 and therefore the cleavage of its target molecules (e.g., ICAD [inhibitor of caspase activated DNase] and GATA could be inhibited by HSP70. On the contrary, recent studies have reported that through interfering with upstream events, HSP70 is capable of halting processes (such as Bax activation) that eventually lead to the permeability of the mitochondrial outer membrane.[42-45] Regarding the mechanism of HSP70 action, inhibiting this protein pharmacologically could ultimately trigger apoptosis. Up to now, various studies have reported the application of drugs such as triptolide, quercetin, and KNK434 for HSP70 inhibition.[46,47] However, the clinical use of these small inhibitors, alone or in combination with other chemotherapeutic agents, is still a matter of controversy. In a study, it was shown that Antp-TPR (a hybrid peptide) induced cytotoxic activity in breast cancer cells by reducing the target proteins of HSP90 (e.g., P53, AKT, and cRaf) in the presence of HSP70-targeting peptide.[48] The simultaneous targeting of HSP70 and HSP90 with HSP70 targeting peptide is a smart approach and treatment option toward selectively killing cancer cells.

Quercetin bioflavonoids induce apoptosis in cancerous cells by reducing HSP70 expression. In a research, the combination of quercetin with simultaneous inhibition of GRP78 induction was used to exacerbate the proapoptotic effect of quercetin. The results of this study showed that treating human breast cancer cells with quercetin led to HSP70 downregulation and GRP78 upregulation in a dose-dependent manner.[49] The combined administration of flavonoids (e.g., quercetin and EGCG [epigallocatechin gallate]) could suppress HSP70 and GRP78, and therefore be another novel approach toward cancer treatment and chemoprevention.[49-53]

Nonsteroidal anti-inflammatory drugs–based combination therapies

Several clinical trials have been conducted to evaluate the effects of COX inhibitors alone (nonsteroidal anti-inflammatory drug [NSAID]) and in combination with other agents in breast cancer chemoprevention. In a study, we showed that the simultaneous effect of ibuprofen and ZNF703SIRNA as a combination therapy in the MCF7 cell line yielded a higher proliferation inhibition compared to either of these compounds alone. This combination therapy significantly increased the expression of *BAX* gene in this cell line confirming an increased apoptosis.[54]

A pilot study was performed on 32 patients under the regimen of FEC (5-fluorouracil-epirubicin-cyclophosphamide) along with 400 mg celecoxib as a neoadjuvant compared. In this research, patients were compared in two groups. The first group, consisting of 16 patients, used FEC alone, whereas the second group, with the same number of patients, underwent combination of FEC and celecoxib. The clinical and pathological responses for both combination therapies were 81.03% and 87.5%, respectively. Meanwhile, this rate was approximately 62.5% for single treatments. Phase 2 outcomes of a randomized trial on 111 women with advanced postmenopausal breast cancer treated with exemestane and celecoxib showed slowed down progression course of the disease and a significant reduction of the adverse effects.[55]

High expression level of COX2 in locally ductal carcinoma *in situ* (DCIS) has encouraged researchers to use COX2 inhibitors in clinical settings. Various studies have introduced celecoxib as a selective inhibitor of COX2 and growth-inhibiting apoptosis-promoting agent in breast cancer cells. It has been shown that drug delivery using adenoviral (Ad-mda7) in breast cancer leads to downregulation of AKT. Evidently, the PI3K/Akt pathway is critical in cell survival, and it probably increases cell survival through a positive feedback loop. In a research on two breast cancer cells lines of HER18 and MDA-MB436 with COX2 overexpression, researchers evaluated the simultaneous effect of celecoxib and Ad-mda7, which resulted in an increased antitumor activity in breast cancer. This combination provided a new therapeutic option for the treatment of breast cancer through the inhibition of COX2.[56]

The use of celecoxib is associated with a significant lower risk of breast cancer. However, a long-term use of high-dose celecoxib is not recommended considering the future risk of cardiovascular complications. Using acetylbritannilactone (ABL), a Chinese medicinal plant extract, has been shown to decrease the required celecoxib dose by synergically functioning with this agent to increase the inhibitory effect of growth in breast cancer cells, at the same time limited the risks associated with celecoxib. This combination showed a higher antitumor efficacy compared to either of these two agents alone.[57]

In another study on the breast cancer in rats, it was observed that the simultaneous effect of celecoxib as a COX inhibitor and *N*-(9-fluorenyl-methyloxycarbonyl)-l-leucine (Fl-Leu) as the peroxisome proliferator-activated receptor γ (PPARγ) agonist significantly reduced tumor volume and significantly boosted

apoptosis. As this level of change was more significant than the individual effect of each drug, exploiting the simultaneous effect of these two agents was introduced as an effective strategy to prevent the proliferation and development of breast cells.[58]

siRNA–based combination therapies

Using ribonucleic acid interference (RNAi), it is possible to specifically knockdown genes involved in resistance to chemotherapy.[59] As multiple drug resistance (MDR) has always been a major challenge in cancer chemotherapy, we could expect to overcome this issue by using the combination of RNAi and other therapeutic agents to simultaneously silence genes and increase the efficacy of chemotherapy. So, this synergetic effect of RNAi would be an improvement over chemotherapy alone. Despite this encouraging prospect of RNAi in cancer treatment field, an effective drug delivery system for carrying this factor to cancer site continues to be a major challenge in the path of clinical application of RNAi.[60,61]

Designing specific nanocarriers, for simultaneously delivering the chemotherapeutic agents and RNAi, is one of the hot topics in pharmacy with numerous studies being carried out on the subject.[62-64] Among these drug delivery systems, we could refer to liposome/lipid, as well as organic and polymer nanoplatforms. In a study by Chen *et al.*,[65] the inhibition of GLI1 and SMO by RNAi resulted in inhibited hedgehog signaling pathway and decreased proliferation of cancer cells. The studied cancer tissue showed a decreased resistance to tamoxifen after treatment with RNAi.[65]

Drugs commonly used in the treatment of patients with breast cancer, such as paclitaxel (Taxol) and Herceptin (trastuzumab), are often associated with severe side effects or drug resistance. The impact of a combination therapy containing Plk1 (polo-like kinase 1)-specific small interfering RNAs (siRNAs) was evaluated in a study, and it was introduced as a powerful tool for inducing a "mitotic catastrophe" in cancer cells. The sensitivity for chemotherapy drugs was increased using this combination. The percentage of apoptotic nuclei in MCF-7, MDA-MB435, SK-BR-3, and BT-474 cells increased after being treated and incubated with Plk1-specific siRNA, paclitaxel, and Herceptin. Interestingly, the caspase pathway was reactivated after treatment with the mentioned triple-drug combination. Therefore, it is safe to say that treating breast cancer cells with siRNAs raised the cells sensitivity to paclitaxel and Herceptin by targeting Plk1-related signaling. Accordingly, the combination therapy, including a PlK1-specific siRNA film loaded onto a doxorubicin (DOX)-loaded liposome, increased the effectiveness of DOX by up to four times *in vitro*. According to the results of the aforementioned study, the use of layer-to-layer films to change the structure of liposomal DOX with a synergetic effect from cancer seems to be a rational choice of therapy in clinical trials.[66]

Using siRNA could sensitize resistant cancer cells to chemotherapeutic agents. As already established, chemotherapy is an effective method for halting the proliferation of malignant cells. However, most chemotherapy drugs are quickly losing their efficacy due to the development of drug resistance in tumor cells. In this regard, the silencing potential of siRNA combinations has been evaluated in terms of restraining the growth of drug-resistant breast cancer cells. In such studies, classes of breast cancer cell lines were exposed to chronic DOX treatment to induce drug resistance. After administering drug combinations containing siRNAs and carrying out microarray analysis, apoptosis-associated proteins were shown to be (upregulated) regulated in drug-resistant cells. This method could also be introduced into clinical settings for drug-resistant cancers.[67]

In another study, researchers aimed to determine whether drug sensitivity increases in MDA-MB231, MDA-MB468 cell lines using siRNA and antisense oligonucleotides (ASO) against NEK2. For such purpose, these cells were exposed to different concentrations of paclitaxel and DOX to assess cell viability and apoptosis. Results were indicative of a significant increase in drug sensitivity in transfected cells. In addition, it was observed that (combination of) siRNA or (with) ASO therapy could increase the sensitivity of cancer cells to chemotherapy agents.[68]

Nanoparticle-based combination therapies

Several researches have reported that nanoparticles combined with other therapeutic agents reduce drug resistance and increase the effectiveness of the treatments. The effectiveness of a combination therapy containing tubastatin (TUB-A) and palladium nanoparticles (PDNPs) in breast cancer cell lines has been evaluated showing a decreased resistance to chemotherapy and an increased dose-dependent effectiveness of the treatment. The results of this study also showed the effectiveness of this combination therapy in inhibiting HDAC activity and increasing apoptosis through modifying the biochemical content and cellular status of cells.[69]

In another research, mesoporous silica nanoparticles were used for co-delivery of an siRNA/drug combination to overcome the drug resistance in breast cancer both *in vitro* and *in vivo*. The combination contained DOX as the drug and an siRNA against glycoprotein P administered into a multidrug-resistant breast cancer xenograft. Compared to free DOX or siRNA alone, the dual delivery system led to a synergetic effect in inhibiting tumor growth.[70,71]

Nanoparticles with intrinsic antimetastatic properties have been reported for the targeted delivery of Plk1 siRNA. The net charge of these compounds has been indicated neutral, and their nonspecific cellular toxicity as low. The results of this study indicated that this combination modified the migration and invasion of TNBC cells by adjusting NADPH oxidase 4 (NOX4) and reactive oxygen species (ROS). In addition, the combination reported in this study was able to simultaneously inhibit metastasis markers, which is suggesting a considerable potential for targeting metastatic cells.[72]

In another study, silver nanoparticles coated with different types of polymers were applied as a drug delivery device for DOX. Among the polymers used, polyvinylpyrrolidone (PVP) was detected to result in the highest toxicity level in breast cancer cell lines. According to the researchers of this study, the toxic effect of DOX was increased synergistically by being loaded in such nanoparticles.[73] In another study, a hydrophilic cationic polymer [poly (l-glutamic acid γ-hydrazide)-b-poly (N, N-dimethylamino propyl methacrylamide)] 3-g-poly ethylene glycol ([PGAH-b-PDMAPMA] 3-g-PEG) was designed to minimize the side effects and to increase the effectiveness of chemotherapy, and used for co-delivery of DOX and siRNABcl2 to breast cancer cells. This nanomicelle was able to more effectively accomplish the delivery of the combination to MCF7 cells and showed a higher cellular toxicity compared to nontargeted nanomicelles. These results are clearly indicating that nanomicelles could be quite effective in treating breast cancer.[74]

Immunogenic-based combination therapies

Despite the great advances made in the treatment of malignancies, there is still a need for developing new methods and technologies in the field. The major drawback to the current cancer therapies, such as chemotherapy and radiation therapy, is the severe side effects as these methods eliminate the normal cells as well while killing the cancerous ones. Moreover, the theory on the existence of cancer stem cells has been proposed to explain cells' resistance to chemotherapy and radiation therapy in many patients. In many cases, these challenges have led to discontinuation of the treatment. Therefore, current research has been dedicated to achieving targeted cancer treatments. Fortunately, the basics of the immune system functioning has made it into an ideal platform for designing new malignancy strategies against discontinuation. A successful combination of chemotherapy and radiotherapy along with the activation of the immune system could provide an effective mean of fighting cancer. Up to present, various types of viral and nonviral viruses have been used to transfer genes associated with the immune system to cancer cells, among which we could refer to genes of cytokines, specific ligands, specific tumor antigens, and monoclonal antibodies (combinatorial therapy).[75,76]

Combining different types of factors that sensitize cancer cells with immune drugs has shown promising results in eliminating cancer cells. For instance, Camato *et al.* have stated in their research that simultaneous use of CpG motifs and radiation therapy leads to more than 60% treatment success in mice with cancer. Meanwhile, radiation therapy alone had not yielded an acceptable response due to the resistance of cancer cells to ionization. Their results clearly indicated the activation of specific killer T cells against cancer cells.[77,78]

Regarding breast cancer, monoclonal antibody trastuzumab designed against human epidermal growth factor receptor 2 (HER2) has raised hopes for the treatment of $HER2^+$ breast cancer. However, unfortunately, many patients eventually develop resistance to this treatment. To overcome this challenge,[79] Liu *et al.*[80] have designed an anti-HER2 aptamer attached to a specific siRNA against Bcl-2, an antiapoptotic agent. The results showed that the conjugated RNA molecule entered the cancer cells by interacting with certain surface receptors and effectively induced cell death through reducing Bcl-2 levels. Bcl-2 inhibition also resulted in higher sensitization of target cells cisplatin. Although Romond *et al.*[81] confirmed the role of trastuzumab in increasing the sensitivity of cancer cells to radiation therapy, apoptosis was not induced by trastuzumab alone as cancer cell lines each had a different level of HER2 expression. Meanwhile, prescribing the combination of this antibody and radiotherapy led to an effective induction of apoptosis in a HER2 expression-dependent manner.[80,81]

Slamon *et al.* reported that adding trastuzumab to the treatment regimen of patients with $HER2^+$ breast cancer improved the disease-free and overall survival. The results of this study showed that the combination of docetaxel and carboplatin with trastuzumab (TCH) was associated with a lower risk of cardiovascular toxicity and leukemia.[82] However, the effectiveness of this regimen was similar to that containing DOX and cyclophosphamide (AC-T). Oak *et al.*[83] realized that using the combination of trastuzumab and salinomycin more effectively reduced the expression of surface markers in breast cancer stem cells compared to salinomycin or trastuzumab alone. In a report presented by Yao *et al.*,[84] using a combination of a HER2 inhibitors (such as trastuzumab) and an inhibitor of PI3K signaling pathway was shown to lead to an effective control of cancer properties and a change in the morphology of breast cancer cells cultured on three-dimensional (3D) scaffolds.[85]

Recombinant human interferon-alpha was another immune-based drug used by Wampler *et al.*, 5 days before recombinant therapy with Taxol and radiotherapy. This drug not only increased the sensitivity of the cells to radiation but also increased the effective whole-body radiation dose.[86] The late recurrence of cancer after chemotherapy is a serious obstacle faced after breast cancer treatment, which could be attributed to breast cancer stemlike cells (CSCs). It has been revealed that blocking transforming growth factor beta (TGF-β) signaling, an important mediator of the immune system, using a TGF-β receptor kinase inhibitor reduced the rate of epithelial-to-mesenchymal transition (EMT). The EMT reaction has been known as the main mechanism behind formations of CSCs when the cells are treated with paclitaxel and other drugs of its family.[87] Also, inhibiting TGF-β signaling pathway has been shown to lead to reduced mammosphere-forming efficiency (MSFE), decreased activity of aldehyde dehydrogenase, and reduced CD44+/CD24 ratio and pluripotency regulators, including Oct4, Nanog, Klf4, Myc, and Sox2. In another study, researchers found that the simultaneous subcutaneous administration of interleukin (IL)-12 and systemic administration of paclitaxel effectively reduced tumor growth and increased the immune response to it. In this study, a combination of new drug delivery methods was used in a way that paclitaxel was injected systemically

every 2 months with a combination of HySol (biodegradable solvent) or Cremophor EL. This combination was used along with a plasmid construct containing human IL-12 gene in a water-soluble form injected subcutaneously to the patient every week. Taken together, the findings of this study showed that the combination of novel drug delivery methods and topical gene therapy would more effectively stimulate the local and systemic immune responses and therefore result in greater chemotherapy efficiency. Further studies on a molecular level showed that cell exposure to trastuzumab leads to a decrease in phosphorylation level of two important downstream molecules of Akt and MAPK, and thereby increases the sensitivity of the cells to radiotherapy.[88]

Autophagy, a protective mechanism of cells, might be responsible to regulate the acquired resistance of $HER2^+$ breast carcinomas to trastuzumab (Herceptin). Trastuzumab-resistant cells generally develop an autophagy addiction, as the genetic ablation of autophagy-related genes (*atg5*, *atg8*, *atg12*) significantly decreases the intrinsic resistance to trastuzumab.[89] In a study, it was shown that when chloroquine (CQ), a lysosomotropic antimalarial drug, was added, autophagosomes accumulated in the presence of trastuzumab, and the cells were committed to death through apoptosis. Therefore, the combination therapy with trastuzumab and chloroquine would radically suppress the growth of tumor cells by up to 90%. In tumor xenografts completely resistant to trastuzumab, adding chloroquine to a trastuzumab-based regimen might improve the outcomes of women with autophagy-dependent $HER2^+$ breast cancer.[90,91]

mTOR inhibitor–based combination therapies

The PI3K/Akt/mTOR is a key pathway in transmitting external and intracellular growth and survival signals. This pathway exerts a wide range of effects by being involved in vital processes such as cell growth regulation, aging, and metabolism. Misregulation of this pathway is an important mechanism of resistance induction in cancer cell. TNBC breast cancer cells that lack estrogen, progesterone, and HER2 receptors constitute approximately 15% of patients with breast cancer. Unfortunately, life expectancy is low among patients having this type of breast cancer. The failure in the treatment of these patients is mainly attributed to the absence of a specific therapeutic goal.[10,92] Despite the fact that approximately 50% of these patients show an increase in the expression of the epidermal growth factor receptor (EGFR), the use of the inhibitors of this receptor, especially in metastatic cases, has not been associated with expected results. Fortunately, recent studies have shown that the use of mTOR inhibitors eliminates the resistance of these cells to EGFR inhibitors. A study by Liu *et al.*[93] showed that the combination of lapatinib, as an inhibitor of EGFR, and rapamycin, as an inhibitor of mTOR, increased cell cytotoxicity more significantly compared to using each inhibitor alone. It is notable that these data have been confirmed both *in vivo* and *in vitro*. Molecular analyses indicated that EGFR inhibition prevented the rapamycin-dependent activation of Akt (protein kinase B).[93]

According to the literature, inhibition of tyrosine kinase receptor using various inhibitors is considered as an appropriate approach to cancer management. However, most studies have stated that inhibition of a single metabolic pathway generally inhibits cancer progression only for a short period. In a study conducted by Issa *et al.*[94] to address this issue, the concurrent inhibition of Fibroblast Growth Factor Receptor (FGFR) (dovitinib) and targeting PI3K/mTOR (NVP-BEZ235) signaling pathway was used for controlling cell proliferation in the rat model. According to their results, combination of dovitinib and NVP-BEZ235 resulted in a very potent inhibition of cancer and prevention of metastasis. Evaluations performed in this research showed that the induction of apoptosis and mitosis inhibition using the combination of dovitinib and NVP-BEZ235 was stronger than the treatment combination of AEE788 (ErbB inhibitor) and dovitinib.[94]

The bilateral blockage of mTORC1/2 and HER2 leads to an antitumor activity in preclinical models of breast cancer that is resistant to anti-HER2 treatment. In a research, the survival and apoptosis induction was evaluated in five breast cancer cell lines that are resistant to trastuzumab and lapatinib. In order to do so, inhibition of tumor growth was studied after treatment with lapatinib and INK-128, or a combination of both agents, in three animal models. Two xenograft models based on a cell resistant to both trastuzumab and lapatinib and a xenograft derived from a patient with recurrence were evaluated for a trastuzumab-based treatment. The results showed that simultaneous blockage of PI3K/AKT/mTOR and ERK pathways using the combination of lapatinib and the INK-128 synergistically induces cell death and inhibits tumors growth in breast cancer models that are resistant to anti-HER2 treatment.[95]

Another study indicated that metformin and an mTOR inhibitor (everolimus [RAD001]) sensitized breast cancer cells to cytotoxic effects of chemotherapy drugs *in vitro*. In this study, metformin was assessed alone and in combination with RAD001 and/or chemotherapy agents (e.g., carboplatin, paclitaxel, and DOX) on several cell lines for cell proliferation. It was observed that the administration of metformin with agents of chemotherapy and RAD001 intensified the inhibition of cell proliferation. In addition, the antitumor effects of everolimus, another mTOR inhibitor, in combination with trastuzumab was investigated on breast cancer stem cells both *in vivo* and *in vitro* environments. In this study, the effect of this inhibitor alone or in combination with trastuzumab was evaluated on stem cells from various $HER2^+$ breast cancer cell lines and BT474 cell line *in vitro* and *in vivo*. The mentioned study also identified everolimus plus trastuzumab as a rational combination therapy to be used in clinical trials.[96]

Conclusion

Going over the literature, it was clear that most therapy patterns and methods designed by now for breast cancer involve key genes associating with this disease and genes coding the receptors for estrogen, progesterone, and so on. However,

few patients show genetic defects associated with the targeted genes. Meanwhile, environmental factors have been recently found to strongly affect the development and progression of breast cancer along with many other risk factors, which had not been considered in previous treatment strategies. Reviewing various studies on breast cancer treatment showed the superiority of combinational therapies over monotherapies due to the heterogenicity of the disease and diversity of signals that affect its course. It is highly recommended based on our perception to consider the role of these less-noticed risk factors, including neurotransmitters, patients' lifestyle, diet, and social relations, to design more successful therapy techniques and increase the total survival of the patients.

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Conflicts of interest

There are no conflicts of interest.

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