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Review

Role of miR-21 as an authentic oncogene in mediating drug resistance in breast cancer

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Research highlights

1. Breast cancer (BC) is the most common cancer among women worldwide.
2. miR-21 is an oncogene that has a crucial role in the regulation of drug resistance.
3. Overexpression of miR-21 is linked with the development and progression of MDR in BC.

Abbreviations list:

BC: Breast cancer

HNSCC: Head and Neck Squamous Cell Carcinoma

MiRNA: microRNA

MDR: Multi Drug Resistance

ABC: ATP-binding cassette

PDCD4: programmed cell death 4 gene

PTEN: phosphatase and tensin homolog

TPM1: tropomyosin 1

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Abstract

Breast cancer (BC) is the most common cancer among women that is responsible for the most of the cancer-related death in worldwide. Drug resistance is remaining as a significant clinical obstacle to treat BC patients effectively. Therefore, to help overcome this problem, it is necessary to understand the mechanisms of drug resistance. microRNAs classify as highly conserved non-coding RNAs (~22 nucleotides) and interact with mRNAs-coding genes for direct post-transcriptional repression. It has been reported that miR-21 is overexpressed and also acts as oncomiR in many human malignancies by targeting of several tumor suppressor genes– associated with apoptosis, proliferation and metastasis. Specifically, it has been reported that miR-21 is responsible for the drug resistance and its overexpression is related to the development of Multi Drug Resistance (MDR) in breast cancer. In this review, we discussed about the role of miR-21 on the drug resistance of breast cancer.

Keywords: Breast cancer, Drug resistance, microRNA, miR-21

1. Introduction

Breast cancer (BC) is responsible for the death of approximately 500,000 women per year in the world. Also, more than one million new case of BC is diagnosed across the world per year. The occurrence of BC accounts for 7-8% of the entire number of malignant tumors. Chemotherapy is known as a principal strategy for cancer treatment; however, its application is limited due to drug resistance. Resistance to chemotherapeutics is mainly divided into two extensive categories: (i) intrinsic and (ii) acquired. Intrinsic resistance shows the presence of resistance-mediating factors in the tumor before starting of treatment process that makes chemotherapy ineffective. Acquired drug resistance, however, will be developed during of the treatment [1,2]. Drug resistance brings serious clinical obstacles to the prosperous treatment of BC patients. To overcome these problems, a well understanding of the drug resistance mechanisms is definitely required. The molecular mechanism of chemotherapeutic resistance in BC cells is completely complicated, and comprises multiple processes, including epigenetic changes, gene mutation, gene amplification and microRNA expression. miRNAs are short non-coding RNAs (~22 nucleotides) that could be found in all eukaryotic cells. They have a significant role in the progression of cancer by binding to the 3' untranslated region (UTR) of the target genes, leading to the target mRNA degradation and inhibition of translation. miRNAs firstly were detected in 1993 as post-transcriptional regulators that are connected with cell differentiation, survival, and cell death. Abnormal mRNA post-transcriptional regulation reduces apoptosis, increases proliferation, and metastatic capacity of the affected cells. MiR-21 has been reported to be overexpressed as an oncomiR in many human malignancies and is connected with proliferation, apoptosis, and metastasis. In addition, in many studies, miR-21 has been reported as a clinical diagnostic and prognostic marker for breast cancer [3].

2. Breast cancer

BC is the most widespread malignancy that takes place in any of the mammary gland cells and is categorized by histological or molecular characteristics that each affect response to treatment. There are numerous types of breast cancer, comprising HER2-positive (HER2+), HER2-negative (HER2-), triple-negative breast cancer (TNBC), invasive ductal carcinoma (IDC), inflammatory breast cancer (IBC), and etc. miR-21 is one of the well-known and most studied microRNAs in BC. Poor prognosis has been demonstrated in patients with overexpression of miR-21 that acts as an oncogene through inhibiting tumor suppressor genes. miR-21, one of the main miRNAs in HER2+ breast cancer, enhances epithelial-mesenchymal transition (EMT) and promotes early disease [4]. miR-21 induces invasion of HER2+ breast cancer via regulating the MAPK pathway. The upregulation of miR-21 also increases HER2 expression and decreases PDCD4 expression, one of the targets of miR-21 in inhibiting breast cancer invasion. Moreover, overexpression of miR-21 induces trastuzumab (Herceptin) resistance of HER2+ breast cancer [5]. The high expression of miR-21 also induces therapeutic resistance in HER2- breast cancer. Overexpression of miR-21 reduces PTEN regulation, another target of miR-21, thereby causing drug resistance to doxorubicin in HER2- breast cancer [6]. Moreover, various studies have recognized the overexpression of miR-21 in TNBC compared to non-TNBC, which is associated with poor outcomes. miR-21 also regulates the PTEN gene and leads to an anti-apoptotic effect in TNBC [7]. Another study has shown that reducing miR-21 expression decreases cell growth in TNBC. According to the results of these studies, miR-21 could be suggested as a therapeutic target in TNBC [8]. In addition, in a study by Qian et al., it was found that overexpression of miR-21 is connected with poor disease-free survival (DFS) in early-stage BC [9]. High expression of miR-21 is also associated with a higher grade of disease in patients with inflammatory breast cancer (IBC) and advanced stages in non-IBC as well as unfavorable molecular subgroups [10]. miR-21 is also a powerful biomarker in invasive ductal carcinoma (IDC). According to studies, the expression level of miR-21 is high in IDC compared with normal tissue and is related to tumor size and stage of the disease [11].

3. Drug resistance

Drug resistance as a multifactorial event comprises redistribution of the intracellular gathering of drugs, improved DNA damage and repair, alteration of drug target molecules, suppression of drug-induced apoptosis and so on. Recent studies have shown that microRNAs have the ability to regulate protein expression. They also have a remarkable role in tumor growth and chemoresistance [12]. The effect of miRNAs on chemotherapy was systematically considered by Blower et al. [13]. Some miRNAs, such as miR-10a , miR-21 , miR-22 , miR-29a, miR-30c, miR-31 , miR-34a , miR-93 , miR-125b and so on have been linked to drug resistance in BC [14,15] . miR-21 is the most importantly upregulated miRNAs in human breast cancer, and its expression is associated with tumor progression and poor prognosis [16]. In specific, miR-21 has been exposed to function in the oncogenesis and also drug resistance in BC patients. Knowledge of drug resistance mechanisms is indispensable to advance the present chemotherapy regimens. A number of developing papers have shown that miRNAs may play a fundamental role in Multi-Drug Resistance (MDR) by targeting multiple signaling pathways. for instance, hundreds of cancer-associated gene transcripts may be targeted by one miRNA; therefore, a clear target can be regulated by multiple miRNAs [17]. In MDR, resistance to a drug is followed by resistance to several drugs. The ATP-binding cassette (ABC) family has the most common MDR proteins, including P-glycoprotein (or P-gp), MDR-associated protein (or MRP1) and also breast cancer-resistant protein (known as BCRP or ABCG2). These proteins have analogous transmembrane domains and through pumping out of the cell, they prevent the penetration of harmful drugs into tumor cells [18,19].

4. miRNA biogenesis

miRNAs (19–25 nucleotides) are short non-coding endogenous RNA molecules that control the gene expression mainly by degradation of target mRNAs or prohibition of protein translation. Complex multistep processes of the miRNAs biogenesis start inside the cell nucleus and finishes in the cytoplasm. Through the activities of RNA polymerase II, most miRNAs are transcribed as early transcription units, called primary miRNA. Then, 7-methylguanosine cap (m7G) is added to the 5' end, and promoted splicing of this miRNA. Consequently, it is polyadenylated at the 3' end. Before the processing by a microprocessor complex that consists of numerous proteins comprising RNase III enzyme, Drosha and its co-factors, the primary microRNA forms a typical hairpin-loop structures. Following that, a member of a family of the Ran transport receptor that is well-known as Exportin 5, transports the precursor-miRNA to the cytoplasm. Here, Dicer (endoribonuclease Dicer) which is another ribonuclease (RNAse) III enzyme cleaves the stem-loop and forms short double-stranded microRNA molecule. Mature miRNA is elected upon thermodynamic specifications, loaded on an Ago (Argonaute) protein., which is the main component of the RNA-Induced Silencing Complex (RISC) and passenger strand is damaged [20].

5. miR-21 in BC

Chromosomal location of miR-21 in *Homo sapiens* is on 17q23.2, where intersects with VMP1 or TMEM49, which is the protein-coding gene and human homolog of rat vacuole membrane protein. miR-21 overexpression has been exhibited in many human malignancies, which confirms its oncogenic function related to proliferation, metastasis and apoptosis. Pursuant to studies, miR-21 acts as an oncogene in human cancers and its inhibition is the cause of cell cycle arrest, enhanced cell death and also improved chemosensitivity to anticancer factors. Recently, it has been reported that miR-21 is associated with the protection of tumor cells from apoptosis. The involvement of down-regulation of miR-21 expression can not only successfully increase the apoptotic cell numbers, but also it can decrease the invasiveness of BC cells. Huang and colleagues showed that the higher levels of miR-21 can lead to a more violent phenotype for BC patients. Furthermore, miR-21 can affect metastasis and invasion of BC [21].

6. Expression of miR-21 in cancer tissues and cells

miR-21 is now one of the most studied miRNAs due to its involvement in cancer progression. The recognition of numerous miRNAs targets as classical oncogenes or tumor suppressors, has directed to the accepted belief that miRNAs play crucial parts in cancer initiation, progression and metastasis. miR-21 was noted firstly as an apoptotic suppressor in several cell lines. In a subsequent large-scale study from 540 human samples, it was observed that miR-21 is the only miRNA that is overexpressed in six solid cancers, including breast, colon, lung, pancreas, prostate and stomach. Latest researches, has revealed miR-21 to be an oncogene that is overexpressed in most types of cancer [22]. The prognosis of cancer patients is meaningfully correlated with the miR-21 expression levels. It has been proposed that it may act as a prognostic marker in several cancers, chiefly in HNSCC (Head and Neck Squamous Cell Carcinoma) and digestive system cancers [23]. Furthermore, the higher expressions of miR-21 are associated with poorer survival rate in these two cancers, representing the significance of miR-21 in cancer diagnosis and treatment. Higher levels of miRNA-21 expression in BC show a characteristic signifying violent disease that is associated with the negative hormone receptor position and high tumor grade [24].

7. Role of miR-21 as a mediator in drug resistance in BC

Overexpression of miR-21 is linked with the development and progression of MDR in BC. An overview to the main chemotherapy drugs that are used for BC treatment and their mechanism of actions are listed in Table 1. The therapeutic efficacy of primary prophylactic granulocyte, a multifunctional nano complex in BC can be improved by miR-21 downregulation. Moreover, the downregulation of miR-21 could also improve the chemotherapeutic effect of Taxol in BC cells [25]. One study has shown that treatment of transfected cells with miR-21 inhibitor remarkably reduced cell survival and cellular invasion compared to the control group. Hence, the incorporation of miR-21 inhibitors with Taxol could be considered as a promising and novel therapeutic approach for the treatment of BC. Trastuzumab resistance has been appeared to be the main subject in anti-human epidermal growth factor receptor 2 (HER2) therapy for BC and miR-21 upregulation could confer resistance to Trastuzumab therapy [26]. Moreover, studies have exposed that blocking miR-21 activity by antisense oligonucleotides (ASOs) can re-sensitize breast cancer cells to Trastuzumab (Herceptin) treatment by inhibiting growth of cancer cells and cell cycle arrest. [27]. Other researches have also revealed that over-expression of miR-21 provide Trastuzumab resistance in Her-2 positive breast cancer via directly targeting the PTEN gene and suppressing its expression [26]. Wang et al. have examined the relationship between miR-21 expression and the sensitivity of BC cells to doxorubicin treatment. As the expression of miR-21 increased, expression of PTEN was inhibited in the doxorubicin-resistant cells. PTEN was post-transcriptionally regulated by miR-21 via directly targeting of the 3'-UTR. Their studies also indicated that inhibition of miR-21 expression is responsible for PTEN expression and re-sensitivity to doxorubicin via increased caspase-related apoptosis [28]. miR-21 may inhibit the expression of PTEN, which act as a tumor suppressor and programmed cell death 4 gene (known as PDCD4) in breast tumors. In recent years, Si et al. have revealed that by utilizing Topotecan (TPT) for BC treatment, miR-21 inhibitor reduced MCF-7 cells growth up to 40%. Also, the inhibition of miR-21 expression was sensitized in MCF-7 cells to Topotecan by causing an enhanced apoptotic response, partially caused by down-regulation of the anti-apoptotic protein (Bcl-2) [29]. Although many studies have been performed on miR-21 and it has been found that its expression is increased in different types of cancer, especially BC, the relationship between the expression of this miRNA and therapeutic responses is still controversial. For example, in a study by Müller et al., 2014, miR-21 level was studied in patients with HER2 positive breast cancer, and the results illustrated that miR-21 expression was high before and after chemotherapy compared to healthy cases [30]. However, in 2016, Yadav et al. displayed that miR-21 expression level in BC patients after chemotherapy were lower than before treatment [31]. In contrast with two previous studies, another study by Yoruker and colleagues found no difference between miR-21 expression levels before and after chemotherapy [32]. Moreover, in a study by Nielsen and colleagues, they claimed that clinical results did not show a significant association between miR-21 expression and resistance to Trastuzumab treatment in primary BC [33]. Therefore, these studies indicate that further investigations are required to ascertain the association between miR-21 expression level and response to chemotherapeutic agents in BC as well as other cancers.

8. Upstream pathway of miR-21

Realizing the regulatory network of miR-21 could be helpful to develop our knowledge about its importance in cancer advancement and also drug resistance. miR-21 could inhibit the expression of PTEN, PDCD4 and activate PI3K/Akt, MEK/ERK signaling pathways (Fig.1) , subsequently leads to the chemoresistance [34].

PDCD4 (tumor suppressor gene) is primarily explained as a cellular transformation inhibitor in a mouse cell culture model. PDCD4 expression is lost or down-regulated in several tumor types, and the ectopic expression of PDCD4 causes the reduction of tumor formation in a mouse skin cancer model. Consequently, as a promising molecular target in cancer treatment, PDCD4 has been

considered in several studies [35]. PDCD4 binds to a translation initiation factor called eukaryotic initiation factor 4a (elF4A) and inhibits it at the molecular level and thereby affects protein translation. In addition, it has been noticed that PDCD4 inhibits transactivation mediated by Activating protein-1 (AP-1) and also is responsible for the expression of cyclin-dependent kinase inhibitor p21. Based on the reports, it has been shown that the durability of PDCD4 is managed by mTOR pathway. During of the mitogen stimulation, S6K1 kinase phosphorylates PDCD4 and marks it for deterioration by the proteasome [36]. According to studies, PDCD4 repression by miR-21 could affect PI3K/AKT/mTOR pathway; subsequently, it leads to the downstream of mTOR signaling pathway in BC cells. In addition, miR-21 has been revealed to regulate PTEN, which is a PI3K antagonist in hepatocellular carcinoma cells [37]. Therefore, miR-21 also targets diverse negative regulators of PI3K/AKT/mTOR survival pathways in various cell types. Consequently, the inhibition of miR-21 could knockdown the tumor suppressor protein p53 partially, leading to the reduction of MCF-7 cells proliferation. These results suggested that there is a functional link between miR-21 over-expression and a the tumor suppressing pathways [38]. There is an amassing evidence of widespread cross-talk between p53 and PI3K/AKT/mTOR pathways. PTEN, known as a tumor suppressor gene, can regulate activation of the PI3K-Akt-mTOR pathway and is a major target for miR-21. In addition, PI3K activation is responsible for the phosphorylation and activation of Akt-mTOR, a pivotal downstream PI3K / AKT kinase that regulates tumor autophagy, proliferation, and cell survival. Repression of the pro-apoptotic proteins and activation of the anti-apoptotic proteins can be occurred by the activation of this pathway. PI3Ks are also involved in the repression of autophagy through upstream activation of target of rapamycin (TOR), a pivotal factor that strongly suppresses autophagy [39]. TOR also controls the activity of Atg1 negatively, which is vital for autophagy, and employment of microtubule-associated protein light chain 3 (LC3). In accordance with one study, they noted that the activation of PI3K-AKT-mTOR could be inhibited by miR-21 silencing, and the amplification of the repression of AKT and mTOR phosphorylation induced Tamoxifen (TAM) and Fulvestrant (FUL) resistance in BC cell line. In addition, knockdown of miR-21 was shown to have a negligible effect on the phosphorylation of ERK and JNK. Thus, by inhibiting the PI3K-AKT-mTOR pathway, miR-21 regulated TAM / FUL-induced apoptosis and autophagic cell death via targeting PTEN in ER BC cells [40].

NF-κB3 is a transcription factor that regulates the expression of diverse genes and have a pivotal role in cellular proliferation and also stress response. Aberrant function of NF-κB is related to the inflammatory/ autoimmune disorders, especially cancer. Moreover, upon radiation and chemotherapy, the anti-apoptotic function of NF-κB has been revealed to promote resistance in tumor cells [41]. Consistent with a previous report , expression of IL-6 in MDA-MB-231 cell line is meaningfully increased by doxorubicin treatment [42]. Remarkably, two main cytokines of senescence-associated secretory phenotype (or SASP) can be induced via DNA damaging that recognized in senescent cells (IL-6 and IL-8). Studies have proposed that senescent cells can induce the tumorigenesis as well as the proliferation of epithelial cells, start an epithelial-mesenchymal transition through paracrine effects of SASP, stimulate angiogenesis, and speeds up the invasion of malignant cells. It has been suggested that NF-κB is a pivotal transcription factor that regulates the expression of numerous SASP components [43]. Moreover, according to the recent study, IL-6 is able to enhance NF-κB activation; subsequently, it results in a positive feedback loop that also contributes to tumor cells growth . In addition, NF-κB-related up-regulation of miR-21 and IL-6 may intensify the therapeutic resistance and invasion in BC subtypes, like basal-like TNBC which is illustrated by sharp-rising chemo-resistance and also aggressive invasion in tumor cells. It was found that the ability of BC cells to the metastasis can be increased by chemotherapy [44].

Findings have been suggested that the transcription of miR-21 in BC cells upon genotoxic stress occurs via IL-6-dependent STAT3 activation. NF-κB is not only essential for inducing IL-6 upon DNA damage, but also for promoting miR-21 together with STAT3, which creates a feed-forward loop to direct miR-21 transcription. Moreover, it has been revealed that numerous transcriptional factors could modulate miR-21 transcription in concert of different types of cancers upon diverse stimuli. For instance, NF-κB together with STAT3 activates miR-21 transcription in IFN-treated prostate cancer cells, where NF-κB /p65 recruitment to the promoter region of miR-21 is STAT3- dependent [45].

Another study displayed that miR-21 is involved probably in epithelial-mesenchymal transition (EMT) of BCcells through both p-AKT and p-ERK pathways by PTEN inhibition. Also, up-regulation of Her2/neu receptors starts MAPK signaling; consequently, it up-regulates miR-21 to promote metastasis [46].

9. Potential targets of miR-21

miR-21 is one of the first miRNAs that known to be transcribed by RNA polymerase II. Then, it has been identified as the main driver of miRNA transcription. It has been reported that miR-21 could increase the cellular proliferation, colony formation, invasion and also prevent cell death in a wide variety of cancerous cells by regulation of various targets (Fig.2) [47]. Targets of miR-21 are mRNA-encoding genes that are known to regulate the cell cycle, proliferation, and also cell death, such as PTEN. , TIMP3 as a tissue suppressor of metalloproteinase 3 , tropomyosin 1(TPM1) , and also programmed cell death 4 known as PDCD4 [48]. In a study, it was shown that tumor invasion and metastasis were impressed by miRNA-21 through targeting of tumor suppressor genes including tropomyosin 1 (TPM1) that contains a putative miRNA-21 binding site in its 3'UTRs. TIMP3 protein has also been displayed to regulate cell death and extracellular matrix (ECM) remodeling. The obstruction of translation of TIMP3 mRNA into a protein, allows matrix metalloproteinases (MMPs) to reduce ECM as a physical barrier on the basement of the membrane. This allows the invasive cancer cells to expand via the surrounding tissue. [49]. Another tumor suppressor is PDCD4 that could be repressed by miR-21. It could regulate the cell growth, proliferation and motility by affecting in mTOR pathway. PDCD4 was discovered as a potential target for doxorubicin (Adriamycin) treatment of BC. It has been reported that the levels of PDCD4 can be reduced in MDA-MB-231 cell line upon doxorubicin treatment; however, its down-regulation was exposed to be inverted by miR-21 expression. It was also shown that miR-21 can repress the expression of PDCD4 gene in Dox-treated MDA-MB-231 cell line [42]. A tumor suppressor gene, phosphatase and tensin homolog (PTEN), is also targeted by miR-21. In one study, it was shown that both PTEN and Smad7 were downregulated in BC. By inhibition of PTEN, miR-21 caused to the activation of fibroblast proteins that aid to the growth and proliferation of tumor cells, while the inhibition of Smad7 by miR-21 could enhance the expression of alpha-smooth muscle actin (alpha-SMA) and fibroblasts-myofibroblasts transition; thereby, increased proliferation and invasion [50]. It has been shown that the reduced PTEN expression is associated with lymph node metastasis, estrogen receptor status, tumor grade, tumor-node metastasis (TNM) stage and microvessel density (MVD). It is also a valuable prognostic marker in BC. It is also found that PTEN is involved in the resistance of Doxorubicin [51] and Trastuzumab-treatment in BC. The up-regulation of miR-21 is related to the PTEN reduction and Trastuzumab resistance in BC. Conforming to the miR-21 up-regulation, the expression level of PTEN, a miR-21 target, was remarkably lower in Trastuzumab-resistant tumors than the sensitive ones [26].

10. Conclusion and future perspectives

Overexpression of miR-21 has been revealed in various types of leukemia and solid malignancies. The evidence proposes that miR-21 is an authentic oncogene that enhances tumor growth and invasion by regulating signaling pathways involved in apoptosis and tumor suppressor genes including PDCD4 and PTEN [52]. Moreover, miR-21 plays a role in anticancer therapeutic resistance through regulating apoptosis, cell cycle, repair of DNA damage as well as the NF-κB signaling pathway [27]. Increasing researches have shown that miRNAs are novel biomarkers for the prognosis and diagnosis of cancer as well as can be used as therapeutic targets in different types of cancer. Therapeutic methods based on targeting oncogenic miRNAs, not only prevent cancer metastasis, but it can also prevent tumor recurrence and drug resistance. The suppression of miR-21 is able to sensitize BC cells to anticancer agents, such as Taxol, Trastuzumab, Topotecan, and Doxorubicin. In addition, miR-21 can be used as a potent marker in BC, especially in drug resistance and as a targeted therapy in this cancer. Expression of miR-21 as an oncogene in the development of resistance to anticancer agents highlights the evaluation of the clinical application of miR-21 inhibition to reduce therapeutic resistance in a variety of cancers, especially BC. Although the function of miR-21 has been extensively studied, the detailed molecular mechanisms of miR-21 deregulation and its role in MDR in various cancers is still unknown. So, further studies and efforts to evaluate the function of miR-21 can help to elucidate its role in the development of cancers including BC.

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Drug Class	Drug	Cancer	Mechanism of action
Anthracyclines	Doxorubicin	Leukaemias, Hodgkin's Lymphoma, breast, bladder, stomach, lung, Kaposi's sarcoma, soft tissue sarcomas, ovarian, thyroid, multiple myeloma and etc.	Acts by intercalating itself into the DNA, with the inhibition of both DNA and RNA polymerase. Triggers DNA cleavage by topoisomerase II resulting in apoptosis.
Taxanes	Paclitaxel	Ovarian cancer and metastatic breast cancer, lung, bladder, prostate, melanoma, esophageal and Kaposi's sarcoma.	Mitotic inhibitor; Promotes polymerization and stabilization of the polymer leading to the accumulation of microtubules and induces apoptosis of the cancerous cells.
	Docetaxel	Breast, lung, prostate, gastric, head and neck, and ovarian cancer	Promotes polymerization and stabilization of the polymer

Fig.1 The upstream pathway of miR-21 in breast cancer, stimulating its expression.

Fig.2 The function of miR-21 through regulating its targets in BC

			leading to the accumulation of microtubules
Vinca Alkaloids	Vinblastine	Hodgkin's Lymphoma, lung, breast, head and neck and metastatic testicular	It's binding to the microtubular protein, tubulin, blocks the ability of tubulin to polymerize to form microtubules and inhibits the assembly of microtubules.
Anti-metabolites	5-Fluorouracil	Breast, head and neck, gastric, ovarian, colorectal, and some skin cancers	Metabolizes to cytotoxic metabolites which are next incorporated into DNA and RNA, eventually causing cell cycle arrest and apoptosis by inhibiting the cells ability to synthesize DNA.
	Methotrexate	Breast, head and neck, leukemia, lymphoma, lung, skin, bladder, and trophoblastic neoplasms	Inhibition of dihydrofolate Reductase enzyme which is important in the synthesis of thymidine and purines. Inhibits the synthesis of DNA, RNA and proteins and also it is cytotoxic during the S-phase of the cell cycle.
Anthracenediones	Mitoxantrone	Metastatic breast cancer, leukemia, and Non-Hodgkin's Lymphoma and Prostate	Type II topoisomerase inhibitor; Disrupts DNA synthesis and DNA repair by intercalation between DNA bases.

Table 1. Chemotherapy agents used for BC treatment subjected to MDR.