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Opportunities and Challenges in Breast Cancer

Sarjana Raikwar*[1] and Sanjay K. Jain[1]

[1]Pharmaceutics Research Projects Laboratory, Department of Pharmaceutical Sciences,

Dr. Harisingh Gour Vishwavidyalaya, Sagar (M.P.) 470 003, INDIA

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Abstract

Breast cancer is a leading cause of death in women worldwide. Literature review assembles and compares available data on breast cancer clinical stage, time intervals to care and access barriers in different countries. It provides evidence that while more than 70% of breast cancer patients in most high-income countries are diagnosed in stages I and II, only 20%-50% patients in the majority of low- and middle income countries are diagnosed in these earlier stages. Recent improvement in the understanding of the molecular and genetic alterations underlying breast cancer progression and development has provided a platform to develop novel therapeutic strategies for breast cancer. According to new hypothesis, the development of novel treatment for breast cancer will result from identification of specific molecular targets that are exposed in studies designed to illuminate the gene and molecules involved in breast tumorigenesis. This review highlights the basic, pathophysiology and classification of breast cancer, challenges including multidrug resistance (by ABC transporter, P-glycoprotein, MDR-associated Protein (MRP1), breast cancer resistance protein (BCRP), drug resistance in breast cancer, multidrug resistance in breast cancer cells *in vitro*), microtubules alteration, altered enzyme, p-53 tumor suppressor gene and cell cycle, alteration in DNA repair processes, cell death response, difficulty to treat metastatic stage, unable to reach the target site. It also deals with opportunities and advance imaging techniques in breast cancer treatment.

Keywords: Breast cancer, Multidrug resistance (MDR), Human epidermal growth factor-2 (HER-2), Therapeutics, Imaging techniques.

1. Introduction

An improvement in the understanding of breast cancer development and progression and the development in the targeted approaches, breast cancer remains challenging problem and become a second leading cause of cancer related death in women. According to recent reports the breast cancer–related deaths are mainly due to the "incurable" nature of metastatic breast cancer (MBC). It is estimated that about 6% of patient have metastatic disease at the time of diagnosis and 20-50% patient first diagnosed with primary breast cancer will eventually develop metastatic disease. Even with the remarkable advances in research and clinical management, the current treatment strategies for breast cancer metastasis still largely rely on the use of systemic cytotoxic agents, which frequently deteriorate the patient's life quality due to severe side effects and, in many cases, have limited long-term success. Only 26% of patient are survive for 5-years, due to poor diagnosis for MBC patients. So, MBC remains the most challenging problem facing both cancer researcher and oncologist.[1]

***Corresponding Author**
E-mail: sarjanaraikwar@gmail.com

1.1 Breast Cancer

According to the last Global Cancer Statistics (GLOBOCAN 2018), breast cancer represented 11.6% of all cancers, which places this disease as the second most commonly diagnosed cancer after lung cancer and caused 6.6% of the total cancer deaths in 2018[2]. Among women, incidence rates for breast cancer significantly exceeded those for other cancers in both transitioned and transitioning countries, it remaining as a remaining as most commonly diagnosed cancer and the prominent cause of cancer death in women worldwide[2]. Although for the majority of breast cancer patients it is not possible to identify a specific risk factor, these are diverse and well documented and include obesity, physical inactivity, alcohol consumption, use of hormone therapy, high breast density and hereditary susceptibility due to mutations in autosomal dominant genes, which represents between 5–10% of all breast cancer cases in women. Among these genetic alterations, mutations affecting BRCA1 and BRCA2 genes, which control DNA repair and transcriptional regulation in response to DNA damage, can lead to the accumulation of genetic alterations and greatly increase lifetime risk to develop different types of malignancies, including breast cancer[3]. Indeed, mutations in BRCA1 and BRCA2 genes are associated with an increased risk of inherited breast and ovarian cancer, representing the strongest susceptibility markers that have been identified for breast cancer worldwide, with an estimated 45–80% lifetime risk of breast cancer for BRCA1-BRCA2 mutation carriers[3]. In a similar manner, mutations affecting TP53 are also related to triple negative breast cancer. As with other types of cancer, early diagnosis greatly increases the chances for successful treatment, allowing for a 20% reduction in overall mortality rates[4]. In this regard, despite reported handicaps of screening programs like high over diagnosis rates and costs, risks that are derived from ionizing radiation, or false positive biopsy recommendations, both mammography, breast self-examinations, clinical breast examinations, digital breast tomosynthesis, ultrasonography, magnetic resonance imaging and oncogene identification represent the main tools for early diagnosis, sorting out and prevention of risk factors as well as timely treatment to lessen breast cancer morbidity[4]. Besides screening programs, adjuvant chemotherapy has also had a significant impact on the prognosis of breast cancer patients, having significantly improved their overall survival, disease-free survival and death rates related to breast-cancer since the early 1990s[5].

1.2 Pathophysiology of Breast Cancer

The mechanism of breast cancer initiation remains unknown. But many efforts have been made to molecularly illustrate breast cancer and describe its formation and progression. The clonal evolution model (in which mutation accumulate, epigenetic changes in tumor cells takes place and the survival of 'fittest' cells) and cancer cell stem model (where precursor cancer cell initiate and sustain progression) are both implicated and further the cancer stem cells may also evolve in a clonal fashion[6]. At morphological level, there is a variety of lesions and genetic modifications from normal glands to cancer. Next to molecular level, there is evidence showing that breast cancer evolves along two molecular divergent molecular pathway of progression, i.e. ER expression and tumor grade and proliferation. Additionally, the identification of breast cancer liability genes has shed the light on some characteristics of diagnosis of both sporadic and inherited breast cancer. According to molecular pathway, first pathway is low-grade-like pathway, which is identified by gain1q, loss 16, infrequent amplification of 17q12 and a gene expression signature (GES) with a majority of genes associated with ER phenotypes, diploid or near diploid karyotypes and low tumor grade. This pathway involves in luminal A group and to luminal B type of breast cancer. The second pathway i.e. the high-grade-like pathway which is identified by loss of 13q, gain of chromosomal region 11q13, amplification of 17q12 (containing ERBB2, encoding HER2) and an expression signature of genes involved in the cell cycle and cellular proliferation[7]. Tumor composed of intermediate to high grade, including HER2-positive tumors and TNBC, fall into this pathway.

1.3 Classification of breast cancer on the molecular basis

Several reports recognized that the complexity and heterogeneity of breast cancer. Subdivision of primary breast cancer into distinct subtype, but

varies in diagnosis and treatment strategies[8]. Commonly, such alterations are made by pathologist through a sub-classification on the basis of tumor stage (size, invasiveness and metastatic status), grade (differentiation state), origins (ductal or lobular) and immuno histochemical staining. Standard immune-histochemical staining consists of progesterone receptor (PR), estrogen receptor (ER) and human epidermal growth factor receptor (HER2) levels. A year ago, advance technological expression array analysis, provide a new tool for cancer research. Microarray-based expression profiling of breast tumors demonstrated that tumor subtypes can be differentiated according to their gene expression profiles. These different molecular subtypes include normal breast-like, basal, luminal A, luminal B and HER2/ERBB2 amplified/overexpressing breast cancers[9]. The existing pathological classification in combination with molecular classification has proven to be an important source of information. Luminal breast cancers (comprising about 70% of the total population) are typically ER-positive and lower grade tumors. The gene expression patterns of these cancers are related to normal cells that line the breast ducts and glands, referred to as the lumen[9]. The 'molecular classification' shown that there are two subclasses of ER-positive tumors. The luminal A subtype is a more aggressive luminal B subtype, although these subtypes likely represent the different ends of the ER-positive spectrum of tumors. Another type is HER2/ERBB2 tumors which are characterized by significant increase in expression of ERBB2 oncogene, usually due to amplification of the chr17q21 genomic region. These tumors are typically faster growing than the luminal cancers, have a higher grade and a worse diagnosis[10]. A neutralizing antibody that targets HER2 (trastuzumab) has provided substantial benefit to patients with HER2-positive tumors. Approximately 50% of HER2 overexpressing tumors are ER positive with both ER-negative and ER-positive tumors possessing a similar clinical benefit on trastuzumab treatment but with minimal response to tamoxifen or other endocrine treatments[11]. However, therapy is often combined, circumventing intrinsic resistance. The basal subtype tumors, a.k.a. triple negative (negative for ER, PR and HER2) are often high grade and have an unfavorable diagnosis. BRCA1 tumors are more frequently triple negative, even though not all triple-negative tumors are basal tumors and not all triple-negative or basal tumors harbor BRCA1 mutations. A fifth molecular subtype has recently emerged, the 'low claudin-like', most commonly found in metaplastic tumors that exhibit a more epithelial to mesenchymal phenotype and stem cell-like properties. The use of gene expression microarrays to discriminate different tumor subtypes has led to a number of prognostic tools based on subsets of specific genes, although the validity of these warrants additional investigations. These studies have suggested that different treatment strategies might be optimal for each tumor subgroup. Indeed, the ERBB2/HER2 amplified subgroup responds favorably to targeted therapies that inhibit the HER2 protein, improving overall disease-free survival[12]. Luminal breast cancers are typically ER positive and are, as a consequence, more likely to respond to endocrine treatment, while patients with triple-negative basal-like tumors lack a single-defining drug-able target and are generally treated with chemotherapeutic agents. Despite the fact that several endocrine therapies exist for luminal tumors, ER-positive breast cancers can relapse. Interestingly, tumors that were resistant to one endocrine treatment can still potentially respond to alternative agents[13]. This recommends that each tumor requires its own unique pallet of cellular modifications in order to attain resistance to that specific compound. This seems to be the case, both in primary breast tumors and in tissue culture-based experiments. The resistance mechanism remains obscure for most of endocrine treatment. In the case of tamoxifen treatment, multiple mechanisms of resistance have thus far been defined. These mechanisms tend to include a deregulation of certain kinase pathways, leading to a phosphorylation of the receptor itself or its cofactors, which appear to play a causal role in drug resistance[14].

1.4 Diagnosis of Breast Cancer

Standard diagnostic factors including age, stage, tumor grade, tumor type and lymphovascular status. Breast cancer before 35 years of age is rare (75 years of age) experience 17% higher disease-specific mortality than younger patients[15]. With

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the extent of mammography screening, the stage at diagnosis has decreased and, concomitantly, the natural history of breast cancer has changed; prognostication, therefore, relies on tumor biology (histological type, grade, lymphovascular invasion and theranostic marker status). For ER-negative, HER2-negative breast cancers and for HER2-positive breast cancers, the presence of tumour-infiltrating lymphocytes is associated with good diagnosis[16]. The combination of chemotherapy and targeted therapy are used in the treatment of HER2-positive breast cancer and TNBCs. Breast cancer before 35 years of age is rare (75 years of age) experience 17% higher disease-specific mortality than younger patients[15]. With the extent of mammography screening, the stage at diagnosis has decreased and, concomitantly, the natural history of breast cancer has changed; prognostication, therefore, relies on tumor biology (histological type, grade, lymphovascular invasion and theranostic marker status). For ER-negative, HER2-negative breast cancers and for HER2-positive breast cancers, the presence of tumor-infiltrating lymphocytes is linked with good diagnosis[16]. TNBCs and HER2-positive breast cancers are generally treated with chemotherapy with or without targeted therapy.

2. Challenges in breast cancer treatment

Several challenges are occur during the treatment of breast cancer. These challenges lead to reduce the therapeutic outcomes of therapy. It also reduces the efficacy and enhances the side effects associated with drugs. Several challenges during the therapy includes, multidrug resistance (by ABC transporter, P-glycoprotein, MDR-associated Protein (MRP1), breast cancer resistance Protein (BCRP), drug resistance in breast cancer, multidrug resistance in breast cancer cells *in vitro*), microtubules alteration, altered enzyme, p-53 tumor suppressor gene and cell cycle, alteration in DNA repair processes, cell death response, difficulty to treat metastatic stage, unable to reach the target site. To overcome these challenges, several measures are taken into considerations which are as follow; pharmacogenomics and imaging techniques, drug-induced reversal of tumor resistance, novel antineoplastic agents, new targeted therapies for treatment of metastatic breast cancer, provide a better understanding of breast cancer metastasis at the molecular and cellular level, introduce cutting-edge technologies in metastatic breast cancer detection, including clinicopathologic detection, circulating tumor cells (CTC) detection and advanced imaging and solicit innovative ideas in basic, translational research and clinical patient management. The symposium led to a positive consensus notion that we will be able to prevent and to a lesser degree, treat metastasis and ultimately save most patients from metastatic deaths in the foreseeable future. So, these methods could use to overcome these challenges and provide a promising strategy for the treatment of breast cancer[17].

2.1 Multidrug resistance (MDR)

The management of breast cancer associated with major problem of resistance during chemotherapy. It includes many of the initially responsive tumors relapse and developed resistance to many anticancer agents of different structure and mechanism of action[18]. This is known as multidrug resistance (MDR). The mechanism of resistance is still unclear, but it may be due to precise nature of resistance and potential role of drug resistance genes involved in passage of anticancer drugs. To overcome drug resistance, a well understanding of the underlying molecular mechanisms of chemotherapy resistance is essential in order to produce successful therapeutic strategies. Drug resistances can be mediated by a number of different mechanisms. It may be due to an increase in the activity of ATP-dependent efflux pumps resulting in reduced intracellular drug concentrations. Agents commonly associated with this type of resistance include doxorubicin, daunorubicin, vinblastine, vincristine and paclitaxel. It can also be caused by a reduction of cellular drug uptake. Water-soluble drugs may attach to transporters carrying nutrients and therefore fail to accumulate within the cell. Resistance to drugs like cisplatin, 8-azaguanine and 5-fluorouracil is mediated by this mechanism[19]. Another general mechanism of resistance involves the activation of regulated detoxifying systems such as the cytochrome P450 mixed function oxidases and also of increased DNA repair. In addition, resistance can result from defective apoptotic pathways due to malignant transformation, a change in the apoptotic pathway during exposure to

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chemotherapy, or changes in the cell cycle mechanisms that activate checkpoints and prevent initiation of apoptosis. Other mechanisms involved in drug resistance include lack of drug penetration, modification of the ability to activate prodrugs and alterations in drug targets. The different mechanism of drug resistance are shown in **Fig. 1**

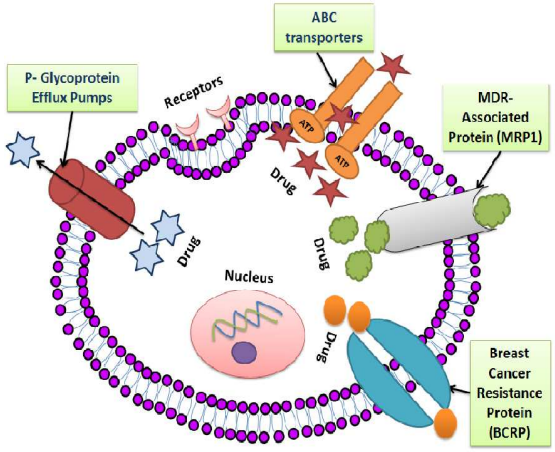

Fig.1: Different mechanisms of drug resistance

2.2 ABC Transporters

ATP-binding cassette transporters (ABC-transporters) are the trans-membrane transporter proteins have been displayed in the resistant cancer cells to anticancer drugs. It utilizes the energy of adenosine triphosphate (ATP) hydrolysis to carry out biological processes. ABC transporters can be divided into three functional categories: Importers mediate the uptake of nutrients into the cell (amino acids, sugars, ions and other hydrophilic molecules). Exporters pump toxins and drugs out of the cell. The final category of ABC proteins are involved in translation and DNA repair processes. Human ABC genes have been identified to date, these have been divided into 7 subfamilies (ABCA-ABCG) based on their sequence homology and domain organization[20]. All proteins in the ABC family are characterized by two distinct domains, the trans-membrane domain (TMD) and also known as the membrane spanning domain or the integral membrane domain and the nucleotide-binding domain (NBD). The TMD recognizes a variety of substrates and undergoes conformational changes to transport these across the membrane. The sequence and structure of TMDs is variable, reflecting the chemical diversity of substrates that can be translocated. The NBD or ATP-binding cassette (ABC) domain is located in the cytoplasm and has a fixed sequence and structure where ATP-binding occurs. The attentions on P-glycoprotein (PGP), multidrug resistance-associated protein 1 (MRP1) and breast cancer resistance protein (BCRP), are the main ABC transporters implicated in the development of multidrug resistance in breast cancer[21].

2.3 P-glycoprotein (PGP)

First ABC transporter recognized as overexpressed protein in breast cancer cell lines displayed MDR and has wide tissue distribution. Mouse PGP, which has 87% sequence morphology to human PGP in a drug-binding state, has recently been described[22]. PGP is a broad spectrum multidrug efflux pump that has 12trans-membrane domains and two ATP-binding sites. It is involved in the transport of neutral and cationic hydrophobic compounds (vinblastine, vincristine, doxorubicin, daunorubicin, etoposide and paclitaxel) out of cells. For transport via PGP, extraction of the drug directly from the cytoplasmic side of the lipid bilayer often occurs. Most PGP substrates readily partition into the plasma membrane and lipids are required for drug stimulated ATPase activity. PGP is a unidirectional lipid flippase that transports phospholipids from the inner to outer sections of the bilayer[22].

2.4 MDR-Associated Protein (MRP1)

Different organ and cell type including breast cancer cell lines displayed expression of MRP1[23]. Several reports demonstrated that overexpression of MRP1 leads to cells becoming resistant to a wide variety of anticancer drugs, for example, doxorubicin[24]. MRP1 is a drug efflux transporter with broad substrate specificity. For many drugs, MRP1-mediated transport is stimulated by the presence of glutathione. Unlike PGP, which tends to be located in the apical membranes of epithelial cells, MRP1 is located basolaterally. MRP1 has a similar structure to PGP and also requires two molecules of ATP as its energy source, but the nucleotide binding sites 1 and 2 (NBD1 and NBD2) differ in their affinity for ATP. The substrate binds to the MRP1 trans-membrane domain causing a conformational change of the protein, which initially induces ATP-binding at

NBD1. Further changes in conformation enhance ATP binding atNBD2. When both NBD1 and NBD2 are occupied, the bound substrate is transported out of the cell. ATP bound at NBD2 is then hydrolyzed and the subsequent release of ADP from NBD2 partially returns MRP1 back to its original conformation, facilitating the release of the ATP bound at NBD1 completing the cycle[25].

2.5 Breast Cancer Resistance Protein (BCRP)

Different anticancer drug such as mitoxantrone, camptothecins, anthracyclines, flavopiridol and anti-folates are associated with the BCRP expression in a variety of tumors which leads to resistance of drugs. Unlike PGP and MRP1, the BCRP protein consist only one trans-membrane domain and one nucleotide binding domain. Two molecules of BCRP are bound by a disulfide bridge to form a functioning homodimer[26]. The mechanism of drug transport facilitated by BCRP has not been investigated in as much detail as that of PGP and MRP1, but it is thought to be similar in some steps, involving a cycle of substrate transport and ATP hydrolysis. Stem cells and tumor cells in a hypoxic surrounding may be protected from anticancer drugs due to an increased expression of BCRP induced by hypoxia[27]. Hoechst 33342 and rhodamine-123 have been utilized to investigate the efflux efficiency of these substrates in mammary stem cells[28]. Hoechst 33342 is a substrate of BCRP and causes BCRP-positive cells to display a unique "side population" phenotype. Stingl *et al*. reported a small proportion of mammary stem cells were found to possess a side population phenotype and only a small minority of cells effluxed Hoechst or rhodamine. The results suggest that in the distinction to haematopoietic stem cells, there is no increase in BCRP in the mammary stemcells[28].

2.6 Drug Resistance in Breast Cancer

Development of drug resistance is the major problem in the treatment of breast cancer. Response rates to first line chemotherapies in metastatic breast cancer, either single or a combination of drugs, are around 30%–70% and the disease-free period following treatment is often only 7–10 months. The role of ABC transporters in breast cancer MDR has been investigated by evaluation of gene and protein expression in tumor samples using RT-PCR, Western blot and immuno-histochemistry. The levels of expression have then been scored and linked to treatment response and outcome. It is difficult to generate accurate overall measures of ABC transporter expression due to the heterogeneity of the tumors and changes inexpression due to therapy. Additionally, the large number of different proteins involved in mediating MDR means that there is significant redundancy in the system. Ultimately, it is the combined activity of the expressed ABC transporters over the course of disease progression that determines the tumor response to therapy[29].

2.7 Multidrug Resistance in Breast Cancer Cells *in vitro*

Recently, *in vitro* models were used for experimental manipulation to determine the complex role of ABC transporters in MDR, which is not permitted in clinical studies. Expression levels of the relevant proteins have been modified in breast cancer cell lines and the resulting alterations in sensitivity to various anticancer drugs assessed. Effects of anticancer drugs on expression levels of the individual ABC transporters have also been determined, alongside functional assays of ABC-mediated drug transport a cross cell monolayers. They describe some examples of *in vitro* approaches that have been utilized to investigate the relationship between expression and activity of ABC transporters and sensitivity to chemotherapeutic agents. Hembruff and colleagues generated a panel of MCF-7cell lines selected for resistance to various chemotherapy drugs and used these to study how expression of drug transporters related to drug uptake and sensitivity[30]. The cell lines were resistant to either paclitaxel (MCF-7tax-2), docetaxel (MCF-7txt), doxorubicin (MCF-7dox-2) or epirubicin (MCF-7epi). Cellular uptake of 3H-paclitaxel, doxorubicin and epirubicin was evaluated to determine any relationship between drug accumulation and resistance. A threshold drug concentration was required for both taxanes and anthracyclins for the cells to acquire drug resistance and there was a significant degree of cross-resistance to drugs of the same class. Taxane-resistant cells exposed for 2 weeks to increasing concentrations of taxanes had significantly reduced 3H-paclitaxel accumulation, with uptake as low as 2% of control in MCF-7tax-

2 cells. Very similar data were observed for anthracyclin-resistant cell lines, anthracyclin resistance was associated with a reduction in drug uptake. However, in both cases there was no clear, dose-dependent correlation between changes in drug accumulation and degree of resistance. Whether the levels of expression of MDR-associated transporters were linked to acquisition of drug resistance was determined by real-time PCR analysis and western blotting. There was a substantial increase in ABCB1/PGP protein levels in MCF-7tax-2, MCF-7txt and MCF-7epi and in ABCC1/MRP1 in MCF-7dox-2 cells, supporting that drug resistance is associated with both modified drug accumulation and increased levels of a subset of ABC transporter proteins. Combining results indicated that there is an association between the onset of drug resistance and reduced drug uptake, additional mechanisms must be involved in determining the sensitivity of the cells to chemo therapeutic agents. One method for determining the functional activity of ABC transporters is by using the Caco-2 cell model of trans-epithelial drug transport[31]. For determination of apical to basolateral drug transport (i.e., absorptive), the drugs are added to the apical side of the cell monolayer and medium added to the basolateral side. At regular time intervals medium is removed from the basolateral side and the concentration of drug determined using high performance liquid chromatography (HPLC). The determination of baso-lateral to apical drug transport (i.e., secretory) is measured in the same system by adding drugs to the opposite side of the monolayer. When this model system was used to study transport of belotecan and topotecan in the presence of PGP, MRP2 and BCRP inhibitors, the inhibitors caused a significant reduction in the secretory flux of both drugs. Consistent with this decrease, the absorptive fluxes of the drugs were considerably increased by the apical presence of the inhibitors of PGP and MRP1, but not by inhibitors of MRP2 or BCRP. These data suggest that BCRP, PGP and MRP2 are all involved in the transport of belotecan and topotecan, supporting that there is considerable redundancy in the MDR system. Other models for investigating trans-epithelial drug transport include the use of MDCKII and LLC-PK cells over expressing one or several of the ABC transporters. A difference between efflux ratios in the transfected cells compared to the parental cells lines indicates transporter-mediated active drug uptake or efflux[17].

2.8 Microtubule alterations

The β-subunit of tubulin in microtubules is the binding site for paclitaxel, which suppresses microtubule dynamic instability and ultimately causes mitotic arrest and cell death. It has been suggested that overexpression of the β-tubulin III isotype induces paclitaxel resistance[32]. In clinical studies, overexpression of β-tubulin III has been identified as a potential biomarker for paclitaxel resistance in patients with advanced breast cancer. Moreover, overexpression of β tubulins type I–IV may be associated with resistance to docetaxel therapy[33]. In addition to the modification of expression levels of the different tubulin isotypes, studies have shown altered levels of polymerized tubulin in anti-microtubule-resistant cells. Several other reports highlighted that the reductions in levels of tubulin may be a mechanism of taxane resistance[34]. Several mutations in β-tubulin have been identified *in vitro* that confer drug resistance to several anti-microtubule agents. However, the significance of these tubulin mutations in the clinical setting has to be corroborated and further investigation is required[35].

2.9 Altered enzymes

The genomic stability is maintained by the topoisomerases enzyme, which is the main enzyme for the DNA replication. The mechanism of action of anthracyclines (anticancer drug) is to intercalate with DNA leading to the inhibition of topoisomerases II enzyme. Other agents that target topoisomerase II are the epipodophyllyotoxins, e.g. etoposide (VP-16) and the anthraquinone, mitoxantrone. Both agents have recently been trialed in MBC patients and have shown activity in this setting[36]. Extensive studies have been performed to clarify mechanisms that result in resistance to topoisomerase II directed therapies and changes in the expression levels of the target enzyme are commonplace findings in such studies. In a breast cancer cell line MDA-MB-231 with acquired resistance to etoposide a marked decrease in both topoisomerase II α and β forms was seen which was seen in the absence of P-gp or MRP over expression[37]. Other enzymes relevant to drug-

resistant breast cancers are those involved in mechanisms that result in a reduced sensitivity to cyclophosphamide. Up-regulation of the enzyme aldehyde dehydrogenase (ALDH) isoforms ALDH1A1 and ALDH3A1 has been shown to detoxify aldophosphamide, the precursor of the active alkylating species phosphamide mustard. Breast adenocarcinoma cells have been shown to express several-fold higher levels of ALDH3A1 compared with normal tissues. Several strategies have been recommended to circumvent the activity of ALDH, such as antisense RNA or by direct inhibition of the catalytic activity of the enzyme. The glutathione S-transferases (GSTs) are cytosolic enzymes involved in drug-metabolising phase II conjugation reactions. They are thought to be particularly relevant to the detoxification of cyclophosphamide. There are multiple genetic polymorphisms of GSTs in humans and there is some evidence to suggest that these may affect the clinical response to cyclophosphamide and contribute to inter individual differences seen in the metabolism of this drug[38]. A recent study describe a microarray based approach to measure tissues taken from breast cancer patients prior to treatment with the taxane (docetaxel) identified a number of redox enzyme genes i.e. thioredoxin, glutathione-s-transferase and peroxiredoxin as predictors of chemo-unresponsiveness. Although the patients in this study had either primary breast cancers or locally recurrent disease these outcomes may also have applicability in the metastatic setting where chemoresistance to taxanes is apparent[39].

2.10 p53 tumor-suppressor gene and cell cycle

The tumor-suppressor gene p53 plays an essential role in inducing apoptosis in response to cellular damage, including DNA damage. In fact, p53 is consistently quoted as being the most frequently mutated gene in human cancers. In a study assessing the National Cancer Institute (NCI) panel of human tumor cell lines, the majority of breast cancer cell lines were mutant for p53. Deletions and point mutations are observed in at least 50% of all tumors with an approximately 25% incidence in sporadic breast cancers. p53 mutation leads to development of resistance to doxorubicin in breast cancer patients[40]. Mutated p53 with consequential loss of function, for example, was shown to abolish trans-activation of p21waf-1 leading to tumor resistance, due to tolerance of the insult and reduced apoptosis. However, the data can sometimes be conflicting; for example, disruption of p53 in epithelial breast cancer-derived MCF-7 cells can give rise to an increase in platinum sensitivity. Scudiero *et al*. reported the doxorubicin resistant line MCF-7/ADR, which is still used widely by the cancer research community, could not have been derived from parental MCF-7 cells and a laboratory error was thus highlighted. Apart from this some reports also show an association between p53 mutations and modified chemo responsiveness[41]. Geisler *et al*. demonstrated an association of p53 mutation with c-erbB2 expression. This study concluded that other factors together with p53 mutations were shown to correlate with resistance to doxorubicin. Interestingly, for breast cancer patients with metastatic disease, tumors harboring wild-type p53 were shown to be less likely to respond to paclitaxel[42]. Deregulation of the cell cycle is recognized to be closely associated loss of the regulatory role of p53 and there are particular scenarios of this defined in the context of breast cancer. For example, over expression of cyclin E, sometimes of a low molecular weight form of cyclin E can lead to enhanced entry into S-phase without checkpoint control and regulatory arrest that would otherwise be controlled by wild type p53 function. 96 The result is unregulated cell cycle that is devoid of arrest or commitment to cell death a process fundamental to carcinogenesis[43].

2.11 Variations in DNA repair processes

The problem of tumor resistance in breast cancer is particularly related to the DNA repair processes. Mutations in breast cancer susceptibility gene 1 (BRCA1) cause a reduction in homologous recombination repair (HRR) of DNA double-strand break repair and crosslinks, which ultimately leads to genomic instability. Another mechanism that has a broader relevance to the problem of tumor resistance in breast cancer (bearing in mind the small percentage of breast cancers that are associated with BRCA genes) is mismatch repair (MMR). Low or absent expression of hMLH1 and hMSH2 genes involved in MMR – occasionally due to epigenetic silencing – has been displayed to correlate with low response rates to cyclophosphamide,

methotrexate and fluorouracil (CMF) therapy in breast cancers[44]. Deficiencies in MMR are correlated with microsatellite instability in breast carcinomas and this has been shown to be particularly relevant to tumor resistance to topoisomerase poisons such as the anthracyclines, but not to the taxanes. p53 plays an integral role in HRR as BRCA can decrease its trans-activating activity, thus controlling the extent of cell-cycle arrest following DNA damage[44].

2.12 Cell death responses

Programme cell death is mediated by two routes i.e mitochondrial (intrinsic pathway) and cell surface receptor (Fas) mediated (extrinsic pathway). The breast cancer cell line MCF-7 is well cited for its lack of the downstream executioner caspase-3 expression. MCF-7 cells can undergo apoptosis by the sequential activation of caspases-9 (associated with mitochondrial mediated apoptosis), -7 and-6. Recently, a splice variant form of caspase-3 has been shown to be overexpressed in chemo-resistant, locally advanced breast cancers and is particularly associated with response to cyclophosphamide[45]. It is measured that caspase-7 can compensate for the absence of caspase-3, MCF-7 has also emerged as an important model of non-caspase-mediated cell death, sometimes referred to as ‘‘autophagy’’. The significance of autophagic cell death in cancer chemotherapeutic tumor resistance is at present unclear, but is an interesting area of research. In considering anticancer drug resistance the data in terms of number of reports are weighted far more in the direction of studies relating to the intrinsic pathway, rather than the extrinsic pathway. However, the fas receptor has been shown to be down-regulated in breast cancer and many breast cancer cell lines have been shown to be fas resistant. In consideration of the intrinsic pathway, the Bcl-2 family of proteins are pivotal and can be subdivided into two main types, (1) pro-apoptotic i.e. Bax and Bak and Bid, Bim, Bad and PUMA; (2) anti-apoptotic subfamily Bcl-2, Bcl-xl and Mcl-1 which block the release of pro-apoptotic molecules by the formation of heterodimers. Hence, the relative concentrations of pro-apoptotic vs anti-apoptotic Bcl-2 protein family determines whether cells survive or undergo apoptosis and may form the basis for anticancer drug resistance in some instances. The phosphorylation state of the Bcl-2 onco-proteins has been shown to modulate response to taxanes[46]. The survivin gene is a member of the IAP (inhibitor of apoptosis) genes and is expressed in high amounts in a number of tumors – including breast carcinomas where it has been shown to be associated with a poor prognosis. Moreover, a positive relationship was revealed between survivin and Bcl-2 using immunohistochemistry and was associated with an assessable reduction in apoptotic index i.e. reduction in TUNEL-positive compared with total number of breast cancer cells. A positive correlation has also been seen for survivin and COX-2 in recurrent DCIS. There are currently no similar data available for MBC. There is a basis for considering surviving as a therapeutic target in the treatment of chemo-resistant breast cancers, including those in the metastatic setting[47].

3. Strategies used to Overcome Tumor Resistance

3.1 Pharmacogenomics and imaging techniques

The main strategy to overcome drug resistance is screening of factors that are identified to bring or confer resistance to chemotherapy. It is used to avoid subjecting patients to unnecessary, ineffective and potentially toxic treatment. The initiation of DNA microarray analysis with a whole human genome platform there is a faith that breast cancer patients can be stratified according to their “molecular structure”. It is also for those patients who can take advantages from particular therapies that already identified. Additionally gene expression profiles may help to determined treatment failure thus facilitating patient to switch to alternative drugs. The rational design to new therapy on the basis of data that generated in the form of a molecular signature that may help to identified new targets. One report described the clinical use of DNA microarray analysis involved breast cancer patients and “poor diagnosis signature” was characterized and is associated with decreased survival rate and involved gene relating to cell cycle, angiogenesis and invasion. Cleator *et al*. 2018 evaluated the response of breast cancers to particular chemotherapeutic regimens, such as doxorubicin and cyclophosphamide using gene expression profiling. Other reports are utilized to determine the use of microarrays to calculate response to

single chemotherapeutic agents such as docetaxel[48]. In the case of MBC DNA microarray analysis holds promise for identifying a patient's risk of developing metastasis and could be used to help guide the choice of therapies to be given to that individual. The signatures can vary from only 2 genes to in excess of 500 and many of these approaches are still being validated. On the basis of expression arrays using tissue samples from heavily pre-treated breast cancer patients, a number of gene have been recognized as potential new therapeutic targets. The drug development programme initiated with the help of various genes that were shown to be highly amplified including FGFR1, ADAM9, PNMT and NR1D1 and they may also improve the efficiency of breast cancer therapy[49]. Alternative method such as reverse transcription polymerase chain reaction (RT-PCR) and IHC were used for the assessment of messenger ribonucleic acid (mRNA) expression and protein expression respectively to determine resistance to anticancer agents. *In vivo* evidence of the MDR1 phenotype can be identified using a specific and sensitive *in vivo* 99mTc-sestamibi scanning technique. This has been investigated in untreated breast cancer patients and has the ability to identify functional P-gp based on the efflux (wash out) rate of 99mTc-sestamibi from breast tumors. Using this method, tumor-to-background ratios have been shown to be significantly lower in patients with P-gp than in those without and this is strongly correlated with IHC-measured P-gp expression. Studies have shown that tumor-to-noise ratios are useful in the calculation of response to chemotherapy with epirubicin and cyclophosphamide or docetaxel. A novel approach by antibody microarrays has been recently developed that may use to recognized a panel of proteins to differentiate between tumor resistance and tumor sensitivity in breast cancer cell lines. MDA-MB-231 cell line (doxorubicin-resistant cell line), it was found that lower expression of proteins, including MAP kinase and cyclin D2, were related with resistance to doxorubicin[50].

3.2 Drug-induced reversal of tumor resistance

A number of clinical studies in several tumor type, demonstrated that the use of drugs that inhibit P-gp and MRP1 as a method for reversing resistance. First-generation P-gp inhibitors drugs include quinine, cyclosporine and tamoxifen etc. They did not significantly increase response rates and also associated with several toxicities. Second-generation P-gp inhibitors drugs include valspodar and biricodar. They required the dose reductions of the anticancer drug, due to pharmacokinetic interactions leading to excessive toxicities when these drugs were administered in combination, so compromising concentrations of the cytotoxic agents within tumor cells. Third-generation P-gp inhibitors with fewer pharmacokinetic interactions, such as tariquidar, zosuquidar and laniquidar are currently in development. Although, the confirmation of tariquidar demonstrated it is a potent and effective P-gp inhibitor that can be safely administered with chemotherapy. It has display clinical activity in restoring sensitivity to anthracycline or taxane chemotherapy in chemotherapy- resistant, advanced breast cancer[51]. Preformulation studies of Tariquidar displayed that it has no influence on the pharmacokinetics of paclitaxel, vinorelbine and doxorubicin when administered to patient having solid tumor, so there is no need of dose reduction. Very recently, studies with flavonols such as kaempferol have been shown to reduce P-gp expression and consequence in the inhibition of P-gp activity[51]. In the same study isoflavones, described as potential chemopreventive agents (e.g., genistein and daidzein), have also been shown to modulate intra-cellular drug concentrations by inhibiting P-gp function whilst not varying P-gp expression. Other compounds that have recently emerged as novel potential MDR reversing agents include the quinoline derivative dofequidar fumarate which has been shown to achieve well in breast cancer patients when given in combination with cyclophosphamide, doxorubicin and fluorouracil. There was a significant increase in response rate (24.6%) and increase in progression-free survival in favour of dofequidar. Natural diterpenes, triterpenes and carotenoids have also recently been shown to modulate MDR by inhibiting transporters by conformational change of the transporter protein. In addition to new compounds emerging with the potential for overcoming tumor resistance, new techniques are also being developed. One such technique, known as photochemical internalization, has revealed

promise in tackling the problem of MDR by rupturing endocytic vesicles, which can trap chemotherapeutic agents such as doxorubicin[52].

3.3 Novel antineoplastic agents

Newer anticancer drugs that are not subject to these common mechanisms of tumor resistance may provide new opportunities in patients that are currently difficult to treat due to resistance. Agents to overcome specific resistance mechanisms such as BRCA-mutations are being investigated. Poly (ADP) polymerase (PARP) inhibitors may characterize as specific way of targeting BRCA-associated breast cancers. The activity of PARP inhibitors has been shown to be limited in breast cancer, but nevertheless these agents represent an interesting new class of targeted cancer therapy. Overcoming resistance due to P-gp is one of the key requisites for many developmental anticancer drugs. Novel microtubule-destabilizing agents, including pseudolaric acid B, are being investigated due to their ability to circumvent P-gp drug resistance. XRP-9881 (RPR-109881A) is a new taxoid that is under development in an attempt to overcome the problem of taxane resistance. XRP-9881 is minimally recognized by P-gp and has demonstrated preclinical anti-tumor activity *in vitro* and *in vivo* and has also demonstrated activity in a Phase II trial of patients with MBC after previous taxoid chemotherapy[53]. The epothilones are anticancer agents produced from the myxobacterium Sorangium cellulosum and have been shown to induce microtubule bundling, formation of multipolar spindles and, hence, mitotic arrest. The epothiloneB analog, ixabepilone, is the first epothilone analog in this new class of antineoplastic agents, which has been developed to optimize the characteristics of the naturally occurring epothilone B. Ixabepilone and paclitaxel suppress microtubule dynamics in a similar manner, but they are structurally distinct and have different binding modes to tubulin. The epothilones, unlike anthracyclines and taxanes, have low susceptibility to common resistance mechanisms including P-gp efflux and alterations in β-tubulin expression. Ixabepilone also does not induce tumor cells to overexpress P-gp or MRP1. Pre-clinical studies have displayed that ixabepilone is a highly active inducer of tubulin polymerization, which exhibits significant anti-tumor activity in cell lines displaying acquired resistance to currently available drugs[54]. Outcomes of Phase II studies involving patients with MBC who were pretreated with or found to be resistant to taxanes, anthracyclines and capecitabine suggest that ixabepilone has activity in patients with multidrug-resistant breast cancer[55]. Ixabepilone is currently undergoing clinical evaluation in Phase III trials for the treatment of MBC and other types of cancer (including taxane resistant), as monotherapy and in combination with other agents such as capecitabine and trastuzumab. Ixabepilone, therefore, has the potential to be effective in a broad range of tumors and overcome many mechanisms of resistance[55].

3.4 New targeted therapies for the treatment of MBC

Multi-targeting new cancer therapies include Lapatinib which has dual action against both HER1 and HER2. Lapatinib has been displayed an activity in preclinical models of trastuzumab resistance and also in breast cancer patients who have progressed on trastuzumab therapy[56]. There is now good evidence to suggest that lapatinib and trastuzumab have non-cross resistant mechanisms of action. Xia *et al.* have shown that lapatinib shows its anti-tumor activity in a PTEN-independent manner whereas trastuzumab acts via a PTEN-dependent mechanism. PTEN – phosphatase and tensin homologue deleted on chromosome 10 – is a phosphatase that can down-regulate phosphotidylinositol 3-kinase– Akt signalling and for this reason is considered as a tumor suppressor. There are numerous clinical trials involving anti-angiogenic therapies in breast cancer that are currently running. One study demonstrated the activity for the humanized monoclonal antibody therapy bevacizumab directed against VEGF in a number of tumor types including colorectal tumors and breast cancers. Miller *et al.* described a phase III clinical trial in which chemo- native breast cancer patients in the metastatic setting were given paclitaxel with or without bevacizumab with significant improvement in progression free survival, but not overall survival as compared to paclitaxel alone[57]. The dual targeting agent ZD6474, an orally available inhibitor of vascular endothelial growth factor receptor-2 (VEGFR- 2), tyrosine kinase

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with activity against EGFR tyrosine kinase has been trailed in MBC in phase II trials. This therapy was well tolerated and there was no significant activity noted when given as monotherapy. Sunitinib (SU11248) is a multi-targeting tyrosine kinase inhibitor of VEGFR1 and VEGFR2, platelet derived growth factor (PDGFR), c-kit receptor and Flt3 and has shown action in pre-clinical models of breast cancer phase II trial of anthracycline and taxane-resistant MBC is currently ongoing with preliminary data demonstrating evidence of activity[58]. A completed phase II trial in pretreated MBC patients showed disease stabilization and partial responses with some toxicity necessitating dose reduction[58]. Sorafenib is an orally administered multi-kinase inhibitor that targets the Raf/MEK/ERK pathway and the receptor tyrosine kinases VEGFR-2 and PDGFR-b. This agent has been used in Phase I/II trials as a single-agent in MBC where it proved safe and well tolerated with modest stabilization of disease[59].

4. Advanced Imaging Techniques for Breast Cancer

Cutting-edge imaging technology features a combination of multidimensional (e.g., three-dimensional and above) and multimodality (i.e., a combination of various modalities) imaging. Scientist use these imaging technologies for comparison of detection potential in cancer imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and the smaller physical footprint but more ubiquitous ultrasound. For breast cancer metastases, whole body imaging is an important in surveying and potentially targeting all possible sites as well as primary lesions. Presently, only PET intrinsically offers a capabilityin three dimensions, but its clinical limitation to a single tracer (e.g., fluorodeoxyglucose) limits its effectiveness in pinpointing disease type and staging more specifically. SPECT suffers from more physical limitations than PET but its reliance on many more available tracers makes it a viable contender, and even more so in its SPECT/CT manifestation. Other modalities, e.g., magnetic resonance imaging (MRI), are making strides toward whole body imaging as well and could soon become a good alternative or at least a complement to PET/CT for identifying and illustrating metastases. Advanced imaging has become a significant component in the management of MBC. By combining ever improving biological knowledge with sophisticated instruments and advanced computer imaging techniques, breast cancer metastasis will likely be better detected and targeted and potential therapies will be more objectively estimated, all of which should lead to an improved outcome for patients[60, 61].

5. Conclusions

The treatment of cancer offer several challenges and opportunities during chemotherapy. The exact role of ABC transporters in breast cancer MDR has been challenging to pinpoint due to the complexity of the mechanisms involved. Investigations into the expression of these proteins in breast cancer cells and tumor samples have often demonstrated in conclusive and differences in the experiment all techniques have made it difficult to directly compare results between studies. Although a number of clinical studies have reported that high levels of tumor ABC transporters are associated with tumor progression, a clear association between expression levels and tumor sensitivity to chemotherapy or patient outcome has not been identified. Overall, further comprehensive studies are needed to fully explain the role that ABC transporters play in breast cancer multidrug resistance. A better understanding of this complex and dynamic system is essential to enable us to develop therapeutic strategies that by pass MDR and also effective ways of inhibiting MDR components to increase the efficacy of our current extensively used chemotherapies. To overcome these challenges several strategies are developed such as pharmacogenomics and imaging techniques, drug induced the reversal of tumor resistance, novel anti-neoplastic agents and new targeted therapies for the treatment of MBC. Advance imaging techniques used in breast cancer are discussed in this review, which help in the diagnosis and treatment of cancer. This review also provides a base for the designing of novel therapy with better therapeutic effect and devoid of such limitation. This could be employed in better understanding of pathophysiology of breast

cancer along with challenges and opportunities in the treatment.

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