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Review article

Breast cancer and possible mechanisms of therapy resistance

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ABSTRACT

Breast cancer represents one of the most common cancers in women and is a major life threatening illness found all over the world. Therapy approaches include irradiation and surgery, with chemotherapy considered an important strategy to treat breast cancer. Platinum based anticancer drugs, such as cisplatin (cis-di-amino-dichloride-platin, CDDP), carboplatin, orthoplatin, etc., have been successfully used in breast cancer therapy because they activate multiple mechanisms to induce apoptosis in tumor cells. Nevertheless, during chemotherapy, drug resistance frequently develops; this impairs the successful treatment of breast cancer and often leads to patients' decease. While combinations of anticancer drugs used in chemotherapy regimens reduced the occurrence of drug resistance (e.g. doxorubicin + docetaxel, doxorubicin + cyclophosphamide, docetaxel + herceptin + carboplatin) the molecular mechanism of those effects are not completely understood. Here we review possible mechanisms related to breast cancer treatment and resistance to current therapies as well as possible new therapeutic targets (e.g. calcium signaling) which could be used in the future.

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1. THE BREAST CANCER: OCCURRENCE, RISK, HETEROGENEITY

Cancer is a major public health problem. In particular, breast cancer is the most common cancer in women.[1–4] Worldwide, breast cancer represents the second leading cause of cancer death among women.[4] According to the World Health Organization breast cancer accounts for 16% of all types of cancer deaths globally (total deaths of cancer amount to 7,600,000, whereas total breast cancer deaths are 460,000).[5] Surprisingly, its etiology is still poorly understood. Multiple risk factors have been identified and include but are not limited to: age, geographic location, socioeconomic status, reproductive events, hormones, lifestyle, familial history of breast cancer, mammographic density, previous benign breast disease, ionizing radiation, bone density, height, IGF- 1 and prolactin levels as well as the use of chemopreventive agents. In addition, breast cancer is histologically diverse and has a large molecular heterogeneity. Therefore, achieving successful therapy is challenging.[6–10]

Multiple breast cancer subtypes have been identified. Recently Perou and colleagues as well as Sorlie and colleagues reviewed the molecular classification of breast cancers and discuss their principal features.[11,12] For each tumor-subtype these authors list the principal clinic-pathological characteristics. In brief, the Luminal A subtype is estrogen receptor (ER)-positive, with high amounts of luminal cytokeratins and shows genetic markers of luminal epithelial cells of normal breast tissue with a good differentiation and a low proliferative index. The prognosis to treat this breast cancer subtype is generally good. In contrast, the Luminal B subtype is similar to Luminal A tumors but has poor differentiation, high proliferative index and the prognosis is by far not as good when compared to the Luminal A type. The human epidermal growth factor receptor (HER)-2 subtype has an amplification of *HER2* genes, a high expression of the HER2 and *HER2*-related genes, a poor differentiation, and an overall poor prognosis. *Basal-like tumors* are ER negative, progesterone receptor (PR) negative, HER2 negative, have a high expression of basal cytokeratins and growth factor receptors, and have poor differentiation as well as a poor prognosis.[11,12]

The most important findings to understand the differentiation and development of breast cancer have been made over the past years, most likely due to the development of microarray technologies. By analyzing microarrays of breast tumor specimens, scientists not only confirmed a distinct expression profile of molecular subtypes of breast carcinomas but also have demonstrated a strong prognostic value of specific genes, for example the gene signatures that strongly correlate with the prognostic outcome of breast cancer patients. Furthermore, the genetic signatures found upon bioinformatics analysis are useful predictors for the success of chemotherapy and they might also identify oncogenic pathways that could represent targets for molecular therapy (see the review of Perou et al.[11–14]).

In this context, it has been shown that the risk to develop breast cancer is associated with: (i) susceptibility of high-penetrance genes (e.g. *BRCA1*, *BRCA2*, *p53*, *PTEN*), (ii) low-penetrance genes such as cytochrome P450 genes (e.g. *CYP1A1*, *CYP2D6*, *CYP19*), (iii) glutathione S-transferase family (e.g. *GSTM1*, *GSTP1*), (iv) alcohol and one-carbon metabolism genes (e.g. *ADH1C*, *MTHFR*), (v) DNA repair genes (e.g. *XRCC1*, *XRCC3*, *ERCC4/XPF*) and (vi) genes encoding cell signaling molecules (e.g. PR, ER, TNF-alpha, HSP70).[6,8] Furthermore, the epigenetic modifications in breast cancer also shows heterogeneity of breast tumors. DNA methylation signatures can stratify patients in terms of prognosis[7] while the expression of small non-coding RNAs, microRNAs (miRNAs), has been linked to multiple human diseases including breast cancer (for review see: Iorio et al.[8]). Overall, all these different factors influence the development of breast cancer.[9,10]

The publication of several miRNA or cDNA microarrays studies using breast cancer specimens or cell lines treated with anticancer drugs have enabled the access of scientists and physicians to free databases such as: Cancer Genome Anatomy Project; Cancer Genome Atlas; Stanford Microarray Database; **G**ene **E**xpression **O**mnibus; Array Express; ONCOMINE, Cancer Profiling Database; UNC-Chapel Hill Microarray; GOBO; Oncogenomics etc. (details see in Figure 1). The use of this knowledge is a powerful tool for further research and opens the possibility for further improvements in clinical applications.

2. TREATMENT OF BREAST CANCER

The treatment of breast cancer comprises surgery, systemic treatment and radiotherapy.

Generally, breast cancer therapy depends on the stage of the cancer at discovery and diagnosis. Moreover, typical breast cancer treatment is based on its histology and expressed biomarkers. This

Cancer Genome Anatomy Project	**http://www.ncbi.nlm.nih.gov/SAGE**
Cancer Genome Atlas (TCGA)	http://tcga-data.nci.nih.gov/tcga/)
Stanford Microarray Database	http://smd.stanford.edu/
Gene Expression Omnibus	http://www.ncbi.nlm.nih.gov/geo/
Array Express	http://www.ebi.ac.uk/arrayexpress/
ONCOMINE, Cancer Profiling Database	http://www.oncomine.org/main/index.jsp
UNC-Chapel Hill Microarray	https://genome.unc.edu/
GOBO	http://co.bmc.lu.se/gobo/coexpressed_genes.pl
Oncogenomics	http://pob.abcc.ncifcrf.gov/cgi-bin/JK)

Figure 1. Internet links for the public data basis containing microarrays studies.

underlines that precise prognostic and predictive markers will be helpful in selecting adjuvant therapies.[14–20]

Furthermore, patients who are diagnosed with breast cancer at the same tumor stage may have quite different responses to the treatment; therefore treatment outcomes might be very heterogeneous. Currently, the aim is to develop a more individualized therapy.[21] One example of such therapy would be HER2-directed therapies. HER2 overexpression or amplification occurs in about 20% of all breast cancers and results in a worse prognosis. Anti-HER2 treatments result in improvements in the clinical outcome of patients with HER2-positive breast cancer. For instance Trastuzumab is efficient in early and advanced breast cancer treatment while lapatinib is approved for treatment of advanced disease.[22,23] Although these treatments have improved the outlook for patients diagnosed with the HER2-positive early stage breast cancer, a proportion of these patients still relapse and die of breast cancer. In this regard, new approaches are considered including the use of monoclonal antibodies and small-molecule tyrosine kinase inhibitors with the goal of targeting HER2 or other HER family members, the use of antibodies linked to cytotoxic moieties or modified to improve their immunological function, immunostimulatory peptides, and targeting the PI3K and IGF-1R pathways.[22,23]

Approximately 60% of all patients with early breast cancer receive chemotherapy but only a minority will benefit from it.[14] While adjuvant systemic therapies are employed after surgery for early-stage cancers, their management remains challenging in clinical practice.[15–17,20] It is worth mentioning that chemotherapy or hormonal therapy reduces the risk of distant metastases by approximately one-third.[24] With DNA microarray and bioinformatics analysis of primary breast tumors, gene expression signatures were found to be strongly predictive of a short interval to distant metastases ('poor prognosis' signature).[24] Cytotoxic drugs, such as doxorubicin, paclitaxel, and cisplatin, interfere with the genes and thereby with the ability of cancer cells to divide resulting in induction of cell cycle arrest and eventually apoptosis.[9,10,25] For example, the platinum complexes are clinically used as adjuvant therapy of cancers (breast, testicular, ovarian, bladder, head and neck, esophageal, small and non-small cell lung, cervical, stomach, prostate, Hodgkin's and non-Hodgkin's lymphomas, neuroblastoma, sarcomas, multiple myeloma, melanoma, and mesothelioma) aiming to induce tumor cell death. Depending on cell type and concentration, cisplatin induces cytotoxicity, e.g., by interference with transcription and/or DNA replication mechanisms. Additionally, cisplatin damages tumors through induction of apoptosis mediated by the activation of various signal transduction pathways, including calcium signaling, death receptor signaling, and the activation of mitochondrial pathways.[9,10]

3. DRUG RESISTANCE

Drug resistance is a major complication in cancer chemotherapy and frequently accounts for the failure of chemotherapy. It exists in two forms: *acquired resistance*, where the drug is initially efficient but becomes ineffective over time, while *intrinsic resistance* occurs when a drug is ineffective from the beginning of the treatment (for review see Florea & Büsselberg[9]). For instance, the intracellular mechanisms of acquired resistance to cisplatin are: (a) increased detoxification with the help of

glutathione and metallothionein; (b) improved repair of and/or tolerance to nuclear lesions, and (c) diminished accumulation of anticancer drug within the cell. Moreover, the presence of cellular defects in many cancers has contributed to an acquired resistance to apoptotic cell death thus lowering the effectiveness of chemo- and radiotherapies.[25,26]

While different cytotoxic therapies are available for patients with metastatic breast cancer, response rates are low and acquired resistance is ubiquitous. Since chemo-resistance often overcomes the successful treatment of breast cancer, therapies that improve overall survival of patients with anthracycline- and taxane-resistant metastatic breast cancer are needed.[19,27] Generally, the following mechanisms could be involved in chemo-resistance (for more detail see Kutanzi et al.[25] or Florea & Büsselberg[9,10]):

(i) decreased intracellular drug concentrations (drug transporters, metabolic enzymes);
(ii) disturbances affecting the cell cycle arrest, apoptosis and DNA repair;
(iii) activation of signaling pathways related to the progression of cancer;
(iv) epigenetic modifications; and
(v) alterations in the availability of drug targets.

For example, to minimize the resistance to cisplatin, combinatorial therapies were developed and have been proven to have an increased efficiency in defeating cancers. Nevertheless, the challenge remains and cancer cells could still become cisplatin-resistant due to changes in cellular uptake, drug efflux, increased detoxification, inhibition of apoptosis and increased DNA repair. Thus, a better understanding of the multiple cellular mechanisms in breast cancer that are modified by cisplatin may lead to the development of more efficient platinum derivatives, other drugs, new chemotherapeutic combinations and new therapeutic strategies with reduced side effects.[9,10]

4. GENETIC AND EPIGENETIC FACTORS REGULATING ACQUIRED DRUG RESISTANCE OF BREAST CANCER AND THEIR IMPLICATIONS ON CALCIUM SIGNALING AND APOPTOSIS

4.1 Aberrant gene expression and breast cancers

Variation in transcriptional programs accounts for much of the biological diversity of human cells and tumors. In each cell, signal transduction and regulatory systems transduce information from the cell's identity to its environmental status, thereby controlling the level of expression of every gene in the genome.[11] Gene expression profiling has led to the development of a breast cancer molecular classification and the development of biomarkers that are able to predict outcome and response to chemotherapy.[12,14,28] For example, gene expression signatures are predictive of distant metastases ('poor prognosis') in patients and consist of genes regulating cell cycle, invasion, metastasis and angiogenesis. In addition, unsupervised cluster analysis distinguishes between estrogen receptor (ER) -positive and ER-negative tumors.[24] The gene signature included genes such as keratin 18, *BCL2*, *ERBB3* and *ERBB4* genes which optimally reports the dominant pattern associated with ER status.[24] Furthermore, the extended gene list in the study of van 't Veer and collegues shows differential expression of calcium signaling related genes, *ABC transporter family* of proteins, *apoptosis* related genes, *epigenetic regulators*, *potassium and chloride channels*, *glutathione S-transferase*, *Ras-oncogene family*, *keratins*, *claudins*, etc.[24] All these genes could play an important role in the effects observed in tumors. Furthermore, the expression of several genes, such as *PACSIN3*, *OGG1*, *KCNN4*, *CALB2*, *etc*., correlates with a poor prognosis of patients with breast cancer.[24]

4.2 Calcium signaling and drug resistance

Intracellular calcium concentration ($[Ca^{2+}]_i$) is a ubiquitous cellular signal in tumor and non-tumor cells. Molecular mechanisms leading to the development of cancer as well as the calcium treatment using anti-cancer drugs could alter calcium signaling. For example, altering the expression of Ca^{2+}-selective channels, pumps or calcium binding proteins, such as S100 family, calpains, etc., in turn could modify the ability of $[Ca^{2+}]_i$ to regulate both cell death and proliferation.[24] This is an important scientific aspect, when combined with the potential for pharmacological modulation, offers the opportunity to use $[Ca^{2+}]_i$ – regulating proteins as new drug targets in cancer.[28]

There are three major classes of membrane-associated proteins that are directly involved in $[Ca^{2+}]_i$ homeostasis: channels, ATPases (pumps) and exchangers. Isoforms of these proteins (e.g. kinases and calmodulin) have different cellular and tissue distribution while the regulation might be done through

different signaling pathways.[28] Calcium signaling controls tumorigenesis and influences cell motility, angiogenesis, genotoxicity, transcription, telomerase, differentiation, cell cycle and apoptosis (for review see Monteith et al.[28]). Thus, the ability of $[Ca^{2+}]_i$ signaling to regulate pathways, such as proliferation and apoptosis, suggests that therapies that modulate $[Ca^{2+}]_i$ signaling in cancer cells are definitely a therapeutic option.

The area of research focusing on molecules related to calcium signaling is particularly significant. The identified Ca^{2+}-channels, pumps and exchangers involved in the regulation or deregulation by anti-cancer drugs could be potentially promising targets for drug development. Nevertheless, while altered expression of a Ca^{2+}-channel or pump has been described in multiple cancers, many studies lack proof of whether is activity has been changed as well (for examples, see Monteith et al.[28]).

Specifically for cisplatin and arsenic trioxide (As_2O_3), we have shown that these two drugs are able to interact with calcium signaling *in vitro*.[29–32] Low concentrations (clinically relevant) of As_2O_3 (0.1 nM to 1 μM) and/or cisplatin (1 nM to 10 μM) were effective in modulating $[Ca^{2+}]_i$ homeostasis of tumor cells (SH SY-5Y, HeLa-S3; U2-OS) as well as non-tumor cells (HEK-293). In addition, using specific inhibitors we demonstrated that calcium regulating proteins such as IP3R and RyR were involved in calcium regulation provoked by anticancer drugs. The observed $[Ca^{2+}]_i$ increase is derived from different sources; As_2O_3 triggers the release of Ca^{2+} from intracellular stores,[33–36] cisplatin induces a Ca^{2+}-influx from the extracellular space,[29,35,36] and calcium currents through voltage activated channels are reduced.[35–37]

Most importantly, the increase of $[Ca^{2+}]_i$ was directly related to the induction of apoptosis. Whereas, the combination of As_2O_3 and cisplatin resulted in an additional increase of $[Ca^{2+}]_i$,[32] this $[Ca^{2+}]_i$ increase was directly accompanied with increased cytotoxicity and apoptosis.[29,32–36] This increase in cytotoxicity, which simultaneously modulates the opening of a calcium conductance at the intracellular stores and the cell membrane, suggests that co-administration of these drugs clinically may be a more effective anti-cancer therapy than either one alone.[36]

Other anticancer drugs used in breast cancer therapy have been proven to interact with calcium homeostasis. For example, tamoxifen directly reduces cell proliferation in some tumors and induce apoptosis by modifying $[Ca^{2+}]_i$ signaling in MCF-7 breast cancer cells but had no significant effect on $[Ca^{2+}]_i$ signaling in cultures of primary cells.[38,39] It was also suggested that Ca^{2+}-channel modulators could decrease the concentration of tamoxifen administered to patients without reducing the therapeutic effects.[39] Moreover, combinations of anticancer drugs could be more efficient in breast cancer treatment. Docetaxel-trastuzumab is an effective therapy for HER2-amplified metastatic breast cancer,[20] while the combination of carboplatin and gemcitabine is used for anthracycline/taxane pretreated metastatic breast cancer patients.[40]

4.3 The multidrug-resistance genes

Development of multidrug resistance (MDR) is a major deterrent for the effective treatment of metastatic cancers by chemotherapy. Different mechanisms of drug resistance are responsible for failure of tumor-treatment.[9,10,41,42] Even though MDR and cancer invasiveness have been correlated, the molecular basis of this link remains unclear. Treatment with chemotherapeutic drugs increases the expression of several ATP binding cassette transporters (ABC transporters) associated with MDR.[42] ATP-binding cassette (ABC) membrane proteins comprise a "superfamily" of transporters with a wide variety of substrates. In humans, this superfamily consists of 49 members; some of them, such as ABCB1 and ABCC1, have been attributed to MDR when they were over-expressed.[43] The expression of the ABC transporter gene family was studied in different MDR cell-lines, including MCF-7.[41] Compared with their drug-sensitive parental lines, the MDR cells clearly overexpressed ABC transporter genes.[41]

4.4 Epigenetic factors in drug resistance

Epigenetic mechanisms such as DNA methylation, chromatin modification and miRNA regulation have significant functions in cancer cells from the silencing of tumor suppressors to the activation of oncogenes, drug resistance and the promotion of metastasis. Therefore, it is not surprising that these mechanisms are employed in anti-cancer therapies.[7–9,44,45] For example, the use of histone deacetylase inhibitors or demethylation agents is an emerging area of therapeutic targeting in a number of ontological entities, particularly in the setting of aggressive therapy-resistant diseases. For the HDAC inhibitor, trichostatin A (TSA), it was demonstrated that the suppression of *in vitro*

clonogenicity, in the previously described apoptosis-resistant MCF-7TN-R breast carcinoma cell line, could alter the expression profile of miRNA signatures.[46]

The general loss of genomic methylation as well as regional hyper- or hypomethylation of genes, which are involved in cell signaling, proliferation, or apoptosis, is thought to favor cell survival and tumor progression.[47] But, the down-regulation of genes related to breast cancer could also be the result of an epigenetic modification. Overall, the investigation of epigenetic mechanisms involved in down-regulation of cancer specific genes could give hints on drug resistance and for options in cancer therapy.[45]

DNA methylation is a covalent modification that occurs at cytosine nucleotides, in particular at cytosines that precede a guanine (CpGs). While the DNA methyltransferases (DNMT1; DNMT3a and DNMT3b) catalyze this process, the areas of high CpG content, called CpG islands, are found in approximately 40% of mammalian promoters and are usually unmethylated. The methylation state of CpG islands in a gene's promoter controls gene expression by blocking gene transcription and could hamper the expression of tumor suppressor genes, therefore leading to cancer development.[7,44] Similarly, miRNAs could contribute in the development of cancer. miRNAs are virtually involved in almost every biological process, including cell cycle regulation, cell growth, apoptosis, cell differentiation and stress response. Aberrant miRNA expression has been found in different tumor types; this demonstrate the causal role of these small molecules in the tumorigenic process, and their possible role as biomarkers or therapeutic tools (reviewed in Iorio et al.[8,9,25]). miRNAs modulate oncogenic or tumor suppressor pathways, while their expression can be regulated by oncogenes or tumor suppressor genes. Thus these molecules could represent intriguing and promising perspective of a possible use in therapy also for breast cancer (reviewed in Iorio et al.[8,9,25]).

Overall, epigenetic investigations could uncover biomarkers that predict and diagnose acquired resistance while the elucidation of epigenetic mechanisms may lead to the development of new treatments that specifically target epigenetic abnormalities or vulnerabilities in cancer cells.[45] Some inhibitors of DNA methylation and histone deacetylation are already approved by the US Food and Drug Administration as anti-cancer drugs. Hopefully, the use of epigenetic targets will have a favorable outcome in treating breast cancer.[44]

4.5 The role of genetic and epigenetic changes in drug sensitivity/resistance

The deregulation of gene expression in breast cancer is well recognized. Several genes are potential biomarkers for breast cancer. With the expression profile of other genes the outcome of the diseases or the development of drug resistance is predictable. As mentioned above, gene expression profiling indicates an involvement of calcium regulating structures (calcium channels and receptors, calcium binding proteins, calcium activated proteins), MDR proteins (ABC transporter family of proteins), as well as apoptosis related genes and epigenetic regulators.[11,12,14,24,28]

In addition to comprehensive publications with complex bioinformatics analysis, the present gene lists show: (i) differential expressed genes in breast cancer (breast cancer vs. normal tissue, cell lines), (ii) predictive markers for disease and therapy outcome, differential expression of genes before and after treatment with anticancer drugs. For example, Perou and colleagues studied gene expression patterns in 65 surgical specimens of human breast tumors from 42 different individuals.[11] The tumors could be classified using gene signatures into multiple subtypes. Among these genes, calcium signaling related genes were found thus, they might play a major role in the pathology of breast cancer (e.g. calcium/calmodulin-dependent protein kinase; calpain, s100 calcium-binding protein p and beta; S100A2, S100A1; S100A8 (calgranulin a); S100A11 (calgizzarin); S100A13; diacylglycerol kinase; calcium channel, voltage-dependent, beta 2 subunit; annexin, caspase 1; phospholipase c, beta 2; protein kinase c, beta 1; inositol 1,4,5-triphosphate receptor, type 3; calcium channel, voltage-dependent, beta 3 subunit; calcium channel, voltage-dependent, alpha 2/delta subunit 2 and others).[11]

Genes deregulated in breast cancer patients treated with doxorubicin included: glutathione s-transferase theta 1, 2, m1 and m4; h2a histone family, member l; h2b histone family, member q; cytochrome p450, subfamily iia; glutathione peroxidase 3, glutamate decarboxylase 1; protein kinase, camp-dependent, catalytic, beta; calmodulin 1 phosphorylase kinase, delta; calmodulin 1 phosphorylase kinase, delta; s100 calcium-binding protein p, a1; s100 calcium-binding protein a8 (calgranulin a); potassium channel, subfamily k, member 1 twik-1; calcium/calmodulin-dependent protein kinase cam kinase ii gamma; cytochrome c oxidase subunit vic; etc.[11] This was confirmed by a study of van 't Veer et al.[24] In addition, gene signatures associated with cisplatin response were

found.[48] Such genes were related to ABC transporters (ABCB1, ABCB2, ABCC4); calcium signaling (GCLM, PRKCD, CARD8) apoptosis AIFM1, BAG1, BAK1, BARD1, BAX, BCL2, CARD8 CASP3, CASP9, CFLAR, FASLG, TNFSF10); cisplatin resistance (CROP), glutathione, EGFR and DNA repair (ERCC1-6).[48]

Breast cancer cell lines have been widely used to investigate breast cancer pathobiology and new therapies. Transcriptional profiling of breast cancer cell lines identified one luminal and two basal-like (A and B) subtypes. Luminal lines displayed an ER signature and resembled luminal-A/B tumors; basal-A lines were associated with ETS-pathway and BRCA1 signatures and resembled basal-like tumors; and basal-B lines displayed mesenchymal and stem/progenitor-cell characteristics.[49]

Recent research shows that epigenetic changes such as DNA methylation, miRNA and histone modifications play a prominent role in regulating the expression of genes involved in breast cancer progression and drug resistance (reviewed in Kutanzi et al.[25,45]). The concept of global DNA hypomethylation and promoter hypermethylation in breast cancer is now better understood. Epigenetic profiling of tumor DNAs revealed targets to improve prognosis and treatment of advanced cancer patients (reviewed in Kutanzi et al.[25,45]). For example, a genome-wide profile of DNA methylation in sporadic breast tumors identified 264 hypermethylated loci/genes located in genomic CpG islands.[50] Methylated genes were related to tumorigenesis: cell adhesion, regulation of cell proliferation, negative regulation of cell death, cell migration, cell to cell adhesion, regulation of cell cycle, and tumor suppression.[50] Similarly, Fan and collegues investigated DNA methylation in two breast cancer cell lines, which represented models of acquired endocrine resistance upon long-term culturing with the anti-estrogens tamoxifen or fulvestrant.[51] Although promoter hypermethylation was also observed, methylation analysis revealed that acquisition of endocrine resistance was associated predominantly with global promoter hypomethylation relative to the parental line.[51]

The deregulation of similar pathways in cancer cells, which exhibit resistance to a wide range of drugs, may explain the existence and mechanism of cross-resistance to different types of chemotherapeutic agents and could represent potential therapeutic targets for reversing miRNA-mediated drug resistance (reviewed in Kutanzi et al.[25]).

For example, miRNA expression was investigated in three cisplatin resistant sublines derived from paternal cisplatin sensitive germ cell tumor cell lines (NTERA-2-R, NCCIT-R, 2102EP-R). Altogether 72 of 738 (9.8%) miRNAs were differentially expressed between sensitive and resistant germ cell line pairs.[52] From the deregulated miRNAs: hsa-miR-10b (involved in breast cancer invasion and metastasis), hsa-miR-512-3p, hsa-miR-371–373 cluster (counteracting cellular senescence and linked with differentiation potency), hsa-miR-520c/-520 h (inhibiting the tumor suppressor p21) and several new non-referenced micro-RNA species, hsa-miR-512-3p/-515/-517/-518/-525 and hsa-miR-99a/-100/-145 (associated with cisplatin resistance), could be identified.[52]

5. OUTLOOK

The hallmarks of drug resistance in cancer include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[9,10,47] Modifications in gene expression as well as epigenetic changes have been associated with drug resistance. In particular, calcium signaling related genes, MDR genes as well as the genes controlling apoptosis were shown to play a major role in acquired drug resistance. Furthermore, changes in the expression or activity of Ca^{2+}-channels and pumps could have a causal or promoting role in cancer. For example, increased expression of plasma membrane Ca^{2+}-channels could increase Ca^{2+} influx and promote $[Ca^{2+}]_i$-dependent proliferative pathways.[28] Alternatively, altered activity or expression of specific Ca^{2+}-channels and pumps might be an adaptive response and might offer a survival advantage, such as resistance to apoptosis.[28] Therefore, targeting specific Ca^{2+}-channels or pumps with restricted tissue distribution, altered expression in cancer and/or a role in the regulation of tumorigenic pathways could specifically disrupt $[Ca^{2+}]_i$ homeostasis in cancer cells. Some of the issues relating to Ca^{2+}-channels and pumps as drug targets in cancer are pertinent to many potential drug targets. Consequently, both activators as well as inhibitors of channels or pumps are potential anti-neoplastic agents.

Overall, a deep understanding of the mechanism of drug mediated calcium signaling, drug resistance and toxicity of cancer cells could dramatically improve the knowledge regarding the biology of tumor cells. In addition, understanding how specific drug combinations result in enhanced effects on breast cancer cell death might represent a new therapeutic strategy for breast cancer as well as ways to overcome drug resistance.

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