REVIEW

Functional miRNAs in breast cancer drug resistance

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Abstract: Owing to improved early surveillance and advanced therapy strategies, the current death rate due to breast cancer has decreased; nevertheless, drug resistance and relapse remain obstacles on the path to successful systematic treatment. Multiple mechanisms responsible for drug resistance have been elucidated, and miRNAs seem to play a major part in almost every aspect of cancer progression, including tumorigenesis, metastasis, and drug resistance. In recent years, exosomes have emerged as novel modes of intercellular signaling vehicles, initiating cell–cell communication through their fusion with target cell membranes, delivering functional molecules including miRNAs and proteins. This review particularly focuses on enumerating functional miRNAs involved in breast cancer drug resistance as well as their targets and related mechanisms. Subsequently, we discuss the prospects and challenges of miRNA function in drug resistance and highlight valuable approaches for the investigation of the role of exosomal miRNAs in breast cancer progression and drug resistance.

Keywords: microRNA, exosome, breast cancer, drug resistance

Introduction

Breast cancer (BCa) is the most common invasive malignancy and the second leading cause of tumor-related death among women worldwide.[1] Due to the improved early diagnosis and advanced therapeutic strategies, the mortality of BCa has been appreciably decreased.[2] However, although the majority of patients were responsive to the initial treatment for certain time, the patients eventually developed more aggressive tumor forms that were generally resistant to the chemotherapy and radiotherapy.[3,4] Thus, as a major challenge, metastasized recurrence leading to a poor prognosis remains to be conquered in BCa therapeutics. Current therapeutic strategies for treating BCa usually combine surgery with multiple adjuvant therapies, such as chemotherapy, radiotherapy, hormonal therapy, targeted therapy, or combination thereof.[5] However, multiple drug resistance (MDR) is a major obstacle for successful systemic therapeutics. Multiple mechanisms involved in drug resistance have been elucidated in BCa treatment, including somatic mutations or epigenetic modifications within drug targets, deregulated apoptosis or survival, adapted signaling pathways or metabolic reprogramming, as well as drug reaching to the targets or drug transport modification within the tumor microenvironment.[6–9] In this context, abnormal expression of miRNA leading to deregulation of gene expression has been implicated in the therapeutic resistance.[9]

miRNAs, a group of 20–25 nucleotides noncoding RNA that specifically bind to the 3′-untranslated region of mRNA of the target genes, negatively regulate gene expression at post-transcriptional level.[10] It has been well recognized that miRNA-mediated downregulation of gene expression is involved in tumorigenesis, metastasis, and tumor response to treatment. Particularly, increasing evidences have demonstrated that the

dysfunction of miRNA contributes to therapeutic resistance in BCa. As shown in Tables 1–4, a number of miRNAs are deregulated in BCa, which is associated with radioresistance and MDR including chemotherapy, endocrine therapy, and targeted therapy.[9] However, individual miRNA can repress several hundred target genes, and single target gene is often regulated by multiple miRNAs. Importantly, exosomes function as vehicles delivering miRNAs from donating cells to accepting cells in the tumor microenvironment, which plays a pivotal role in the regulation of miRNA functions.[11]

Exosomes, extracellular vesicles secreted from various types of cells, carrying small molecules including miRNAs and proteins.[12] Initially exosome was identified as cell debris containing metabolic products. However, the recent studies have verified that exosome is critical for cell–cell communication.[13] As key components in the tumor microenvironment, exosomes shuttle bioactive molecules and lead

to the exchange of genetic information and metabolic reprogramming of the recipient cells.[14] Thus, exosome is thought to be critical for signal transduction between cells, and the function of exosome directly contributes to the promotion of proliferation, invasiveness, and metastasis of recipient cells, as well as transferring drug resistance, thereby enhancing tumor recurrence and metastasis.[15] Our previous studies showed that tumor-derived exosomes transfer doxorubicin resistance to the susceptible BCa cells through exosomes shuttling miR-222.[16]

In this review, we focus on specific miRNAs associated with BCa drug resistance. Since substantial evidences predict that exosome-mediated dynamic and reciprocal cross-talks are essential for sustaining specific tumor microenvironment, we particularly highlight the recent novel findings that revealed the aspects of exosomal miRNAs implicated in drug resistance, which may be beneficial for seeking

Table 1 miRNAs involved in the regulation of chemotherapeutic resistance in breast cancer

miRNA	Drug	Target(s)	Expression level*	Mechanism	Reference
miR-505	Doxorubicin	Akt3 (indirect)	↓	Not investigated	19
miR-181a	Doxorubicin	Bcl-2	↓	Increase apoptosis	24
miR-663	Doxorubicin	HSPG2	↑	Inhibit apoptosis	7
miR-25	Doxorubicin	ULK1	↑	Inhibit autophagy	6
miR-145	Doxorubicin	MRP1	↓	Induce intracellular doxorubicin accumulation	8
miR-644a	Doxorubicin	CTBP1	↓	Inhibit EMT	95
miR-128	Doxorubicin	Bmi-1, ABCC5	↓	Increase apoptosis	23
miR-30c	Doxorubicin Paclitaxel	TWF1 (PTK9) VIM, IL-11	↓	Reverse EMT	32
miR-326	Doxorubicin	MDR-1	↓	Downregulate MRP-1	36
miR-181a	Doxorubicin	Bax	↑	Inhibit apoptosis	86
miR-106b~25 cluster	Doxorubicin	EP300	↑	Activate EMT	25
miR-34a	Doxorubicin Cisplatin	HDAC1, HDAC7	↑	Inhibit autophagic cell death	30
miR-345 miR-7	Cisplatin	MRP1	↓	Uncharacterized	27
miR-302b	Cisplatin	E2F1 (direct) ATM (indirect)	↓	Inhibit cell cycle progression	28
miR-24	Cisplatin	BimL, FIH1	↑	Promote EMT and CSCs	79
miR-129-3p	Docetaxel	CP100	↑	Reduce cell cycle arrest and apoptosis	33
miR-34a	Docetaxel	BCL-2, CCND1	↑	Inhibit apoptosis	87
miR-125b	Paclitaxel	Sema4C	↓	Reverse EMT	35
miR-100	Paclitaxel	mTOR	↓	Enhance cell cycle arrest and apoptosis	31
miR-125b	Taxol	Bak1	↑	Inhibit apoptosis	34
miR-484	Gemcitabine	CDA	↓	Promote proliferation and cell-cycle redistribution	37
miR-200	Carboplatin	Zeb	↓	Reverse EMT	99
miR-218	MDR	Survivin	↓	Enhance apoptosis	38
miR-205	Doc, Dox plus CTX	VEGFA, FGF2	↓	Inhibit PI3K/Akt/mTOR pathway and CSCs	37

Note: *Upregulation (↑) or downregulation (↓) of miRNAs in drug-resistant cell lines/tissues.
Abbreviations: EMT, epithelial–mesenchymal transition; CSC, cancer stem cell.

Table 2 miRNAs involved in the regulation of endocrine resistance in breast cancer

miRNA	Drug	Target(s)	Expression level*	Mechanism	Reference
miR-451	Tamoxifen	14-3-3ζ	↓	Inhibit PI3K/Akt/mTOR pathway and enhance apoptosis	77
miR-451a	Tamoxifen	14-3-3ζ, ERα	↓	Inhibit autophagy	47
miR-320a	Tamoxifen	ARPP-19, ERRc, c-Myc, Cyclin	↓	Activation of the c-Myc and Cyclin D1 pathways	46
miR-15a miR-16	Tamoxifen	Cyclin E1, Bcl-2	↓	Promote cell growth arrest and apoptosis	44, 91
miR-378a-3p	Tamoxifen	GOLT1A	↓	Uncharacterized	51
miR-342	Tamoxifen	Uncharacterized	↓	Promote apoptosis and cell cycle progression	49
miR-574-3p	Tamoxifen	CLTC	↓	Not investigated	52
miR-873	Tamoxifen	CDK3	↓	Inhibit ERα activity; restore anti-proliferative effect	43
miR-375	Tamoxifen	MTDH	↓	Reverse EMT	50
miR-214	Tamoxifen Fulvestrant	UCP2	↓	Inhibit inhibiting PI3K/Akt/mTOR pathway and enhance apoptosis	48
miR-519a	Tamoxifen	PTEN, RB1, CDKN1A	↑	Promote proliferation, inhibit apoptosis	17
miR-101	Tamoxifen	MAGI-2	↑	Promote PI3K pathway	53
miR-301	Tamoxifen	FOXF3, BBC3, PTEN, COL2A1	↑	Uncharacterized	56
miR-221/222	Fulvestrant	β-Catenin, TGF-β	↑	Deregulate multiple signaling pathways including β-catenin and TGF-β	78
miR-221/222	4-hydroxytamoxifen	P27kip1	↑	Promote cell cycle arrest	42

Note: *Upregulation (↑) or downregulation (↓) of miRNAs in drug-resistant cell lines/tissues.
Abbreviations: EMT, epithelial–mesenchymal transition; CSC, cancer stem cell; TGF-β transforming growth factor-β.

more effective therapeutic targets for the improvement of BCa treatment.

miRNAs involved in BCa drug resistance

The function of miRNA influences a variety of physiological and pathological processes by regulation of the target genes expression, which is generally implicated in tumor progression and resistance to treatment. A number of miRNAs were identified to be involved in BCa drug resistance by screening the established resistant cell or tissue models using either high-throughput sequencing or miRNA microarray.[17] Subsequently, functional validation was conducted to assess the role of miRNA in drug resistance by ascertaining the expression of target genes and the alteration of related pathways. Finally, the selected candidate miRNAs were evaluated by using animal experimental models and/or clinical samples, which provided promising approaches to discover the

Table 3 miRNAs involved in regulation of target therapy resistance in breast cancer

miRNA	Drug	Target(s)	Expression level*	Mechanism	Reference
miR-21	Trastuzumab	PTEN PDCD4	↑	Promote PI3K/Akt pathway and IL6/STAT3/NFκB pathway	71–73
miR-221	Trastuzumab	PTEN	↑	Promote PI3K/Akt pathway	75
miR-210	Trastuzumab	Uncharacterized	↑	Uncharacterized	76
miR-205-5p	Trastuzumab	P63	↑	Promote EGFR pathway	78
miR-200c	Trastuzumab	ZNF17 and ZEB1	↓	Inhibit TGF-β signaling	79
miR-542-3p	Trastuzumab	AKT	↓	Inhibit PI3K pathway	80
miR-375	Trastuzumab	IGF1R, AKT	↓	Inhibit PI3K/Akt pathway	81
miR-630	Lapatinib	IGF1R	↓	Uncharacterized	82
miR-16	Trastuzumab Lapatinib	Cyclin and FUBP1	↓	Enhance chromatin accessibility	83
miR-7	Trastuzumab	EGFR	↓	Suppress EGFR expression	84
miR-515	Trastuzumab	CD44	↓	Inhibit CSC marker	103

Note: *Upregulation (↑) or downregulation (↓) of miRNAs in drug-resistant cell lines/tissues.
Abbreviations: EGFR, epithelial growth factor receptor; CSC, cancer stem cell; TGF-β, transforming growth factor-β.

Table 4 Exosomal miRNA involved in cancer resistance

Exosomal miRNA	Drug	Expression level*	Cancer type	Donor cell	Recipient cell	Target(s)/mechanism	Reference
miR-222	Doxorubicin	↑	BCa	MCF-7/R	MCF-7/S	Uncharacterized	16
miR-221/222	Tamoxifen	↑	BCa	MCF-7/R	MCF-7/S	P27, ERα/unknown	136
miR-155	Multidrug	↑	NBL	NBL	Monocyte	TERF1 – increase telomerase activity	138
miR-155	Gemcitabine	↑	PDA	Panc1/R	Panc1/S	TP53INP1/enhance apoptosis	139
miR-222-3p	Gemcitabine	↑	NSCLC	A549/R	A549/R	SOCS3 – enhance Jak2/Stat3 and Bcl-2	141
miR-9	Temozolomide	↑	GBM	MSC	GBM	Efflux drug	146
miR-21	Paclitaxel	↑	OC	CAA/CAF	OC cell	APAF1/inhibit apoptosis	13
miR-21	Cisplatin	↑	GC	TAM	GC cell	PTEN/enhance PI3K/AKT	144
miR-100-5p	Cisplatin	↓	NSCLC	A549/R	A549/S	mTOR	142
miR-134	17-AAG and PU-H71	↓	BCa	MCF-7/R	MCF-7/S	STAT5B, Hsp90/unknown	137
miR-122	Multidrug	↓	HCC	AMSC	HCC	CCNG1, IGF1R, and ADAM10/unknown	145
miR-34a	Docetaxel	↓	PC	PC3/R	PC3/S	Bcl-2/promote apoptosis	143
miR-146a-5p	Cisplatin	↓	NSCLC	A549/R	A549/R	Atg12/inhibit autophagy	147

Note: *Upregulation (↑) or downregulation (↓) of miRNAs in drug resistant cell lines/tissues.
Abbreviations: R, resistant; S, sensitive; BCa, breast cancer; PC, prostate cancer; OC, ovarian cancer; HCC, hepatocellular carcinoma; GC, gastric cancer; NBL, neuroblastoma; PDA, pancreatic ductal adenocarcinoma; NSCLC, non-small cell lung cancer; GBM, glioblastoma multiforme; TAM, tumor-associated macrophage; AMSC, adipose tissue-derived mesenchymal stem cell; MSC, mesenchymal stem cell.

potential drug targets for improving BCa therapeutics and prognosis. Clinically used drugs for treating BCa mainly involve in chemotherapy, hormone therapy, and targeted therapy.[18] In this study, we summarize drug resistance and relevant miRNA-mediated mechanisms in BCa.

Chemotherapy resistance

BCa patients with high risk of recurrence are generally recommended for adjuvant chemotherapy after definitive surgery.[18] Doxorubicin (adriamycin) was the first-line anti-cancer drug to treat BCa which blocks DNA replication. Correspondingly, miR-505, miR-128, and miR-145 function as tumor suppressors, which are downregulated in doxorubicin-resistant BCa, and upregulation of these miRNAs led to reversal of doxorubicin resistance.[8,19–23] In contrast, miR-663, miR-181a, and miR-106b~25 clusters serve as oncogenic miRNAs; downregulation of them resulted in enhancement of the sensitivity of doxorubicin-resistant cells.[7,24,25]

Similar to doxorubicin, cisplatin is used to treat multiple types of cancer by inhibiting DNA replication.[26] A microarray screening for miRNA expression signatures in cisplatin-resistant BCa cells versus sensitive BCa cells has shown upregulation of 46 miRNAs but downregulation of 57 miRNAs.[27] In particular, miR-146a, miR-10a, miR-221/222, miR-345, miR-200b, and miR-200c were significantly deregulated in the resistant cells.[27] Functional validation experiments indicated that miR-345 and miR-7 endow the cisplatin-resistant phenotype, while miR-302b can sensitize BCa cells to cisplatin.[27,28]

In addition, docetaxel and paclitaxel are often used to treat cancers by interfering the function of microtubules during cell division.[29] It has been reported that miR-34a, miR-100, and miR-30c were downregulated in paclitaxel-resistant BCa cells.[30–32] By contrast, miR-129-3p was upregulated in docetaxel-resistant BCa cells and contributed to docetaxel resistance.[33] However, two studies present controversial results regarding the role of miR-125b in the regulation of paclitaxel resistance in BCa. Zhou et al primarily demonstrated that miR-125b was increased in paclitaxel-resistant BCa cells as an oncogenic miRNA.[34] While Yang et al reported that miR-125b presumably serves as a tumor suppressor miRNA[35] (Table 1).

Moreover, it is recognized that miR-326 and miR-484 can reverse MDR phenotype; nevertheless, miR-218 and miR-205 contribute to the acquisition of MDR in BCa cells.[36–39]

Hormone therapy resistance

Since ~70% of BCa patients exhibit high levels of estrogen receptor-α (ER-α),[40] targeting ER-α seems to be the most valuable endocrine-based therapy. Several modulators of ER were selected to treat ER-positive BCa patients.[41] In particular, tamoxifen, a well-documented hormone therapeutic drug, is able to bind to ER and block the interaction between estrogen and ER.[42,43] Unfortunately, resistance and disease relapse have limited its long-term therapeutic efficacy.[44] Manavalan et al initially screened miRNA expression profiles and identified multiple miRNAs deregulated in tamoxifen-resistant (TamR) cells versus the sensitive control cells.[42]

The downregulation of miRNAs in TamR BCa cells was further confirmed to consist of miR-15a, miR-16, miR-320, miR-451, miR-214, miR-342, miR-873, miRNA-375, miR-378a-3p, and miR-574-3p. Subsequently, mechanistic studies have ascertained that these miRNAs play the roles of tumor suppressors due to the inhibition of multiple cell survival signaling pathways.[43,45–52] Conversely, miR-101, miR-221/222, miR-301, and miRNAs-C19MC clusters were found to highly express in TamR BCa cells.[17,53–56] (Table 2).

Targeted therapy resistance

Overexpression of human epidermal growth factor receptor 2 (HER2) is highly correlated to BCa metastasis and poor prognosis.[57] HER2 targeted therapy has successfully achieved excellent results for treating HER2-positive BCa.[58] Trastuzumab, a humanized monoclonal antibody targeting HER2, lapatinib, a small-molecule tyrosine kinase inhibitor dually targeting HER2 and epithelial growth factor receptor (EGFR), have largely improved therapy outcome and prognosis.[59–65] However, despite their initial efficacy achieved for the early patient response, most of the patients eventually developed resistance after 1 year.[66–70] Several mechanistic studies have revealed that deregulation of miRNA is involved in BCa trastuzumab resistance. Correlation of upregulation of miR-21 with trastuzumab resistance was validated in vitro and in vivo.[71–73] However, one study asserted that the results from clinical research did not display the association between miR-21 expression and recurrent disease after adjuvant trastuzumab treatment.[74]

In addition to miR-21, miR-221 has been reported to promote trastuzumab resistance and metastasis of HER2-positive BCa.[75] Also, circulating miR-210 levels in plasma from HER2-positive and trastuzumab-resistant BCa patients were determined to be correlated to BCa growth and metastases, suggesting that plasma miR-210 may serve as a predictive biomarker in surveillance of the therapeutic responsiveness.[76] Furthermore, miR-205 was identified as a tumor suppressor, whose expression is downregulated in BCa cells, but is upregulated in patient-derived cancer stem cells (CSCs).[77,78] miR-200c seemed to restore trastuzumab sensitivity and suppress invasion of BCa cells.[79] Downregulation of miR-542-3p and miR-375 was also reported to contribute to trastuzumab resistance.[80,81] In addition, miR-630 was found to be downregulated in lapatinib-resistant cell lines,[82] while miR-16 expression was upregulated by trastuzumab and lapatinib.[83] In the case of HER2Δ16, an oncogenic isoform of HER2 has been reported to prompt metastasis and MDR including trastuzumab and tamoxifen.[84] The results from miRNA array have indicated that 16 miRNAs were abnormally expressed in HER2Δ16-expressed cells, particularly miR-7 appeared to have a 4.8-fold reduction.[84]

Mechanisms involved in miRNA-mediated therapeutic resistance of BCa

The function of miRNA in drug resistance has been ascribed to the alteration of drug transporters leading to efflux of anticancer agents,[113,114,118] modification of autophagy/apoptosis to enhance survival,[24,28,47,48,85,86] promotion of growth factors to disturb associated signal pathways[47,71,77,87–88] and activation of epithelial–mesenchymal transition (EMT) process and augment of CSC populations to promote metastasis[25,32,50,79,80] (Figure 1).

Autophagy/apoptosis

Autophagy is a lysosome-mediated intracellular self-catabolic degradation process for maintaining cellular homeostasis.[91] Recent studies suggest that miRNA affects drug resistance by the regulation of autophagy that is considered to be a surviving mechanism.[92,93] miR-451a is involved in BCa resistance to tamoxifen through significantly reduced autophagosomes.[47] A novel compound isoliquiritigenin can reverse chemoresistance of BCa cells by negatively regulating miR-25, and overexpression of miR-25 was validated to stop isoliquiritigenin-induced autophagy and trigger chemosensitization by targeting ULK1.[6]

Nevertheless, as the procedure of programmed cell death, apoptosis is considered to be involved in sensitization of cancers to treatment, which is regulated by pro-apoptotic regulators like caspases and/or antiapoptotic regulators including Bcl-2 family.[94,95] As a tumor suppressor, miR-505 induces cell apoptosis via targeting Akt3 that participates in metastasis and drug resistance.[19–22] miR-100 sensitizes BCa cells to paclitaxel by inducing cell apoptosis mainly by targeting mTOR.[31] Bak1 was validated to be a direct target of miR-125b, which enhanced docetaxel-induced apoptosis.[34] In addition, NF-κB-dependent STAT3 activation contributes to the upregulation of miR-181a to suppress BAX, resulting in decreased apoptosis and increased invasion of triple-negative breast cancer (TNBC).[95] Inhibition of miR-181a significantly enhanced the sensitivity of TNBC to doxorubicin.[96] Upregulation of miR-34a converted docetaxel resistance of BCa cells by downregulation of Bcl-2.[97] In addition to miR-34a, miR-15a, miR-16, and miR-214 were also found to target Bcl-2 family in TamR cells.[45,48,49] Moreover, miR-218 was found to contribute to the development of MDR in BCa cells by targeting survivin.[38]
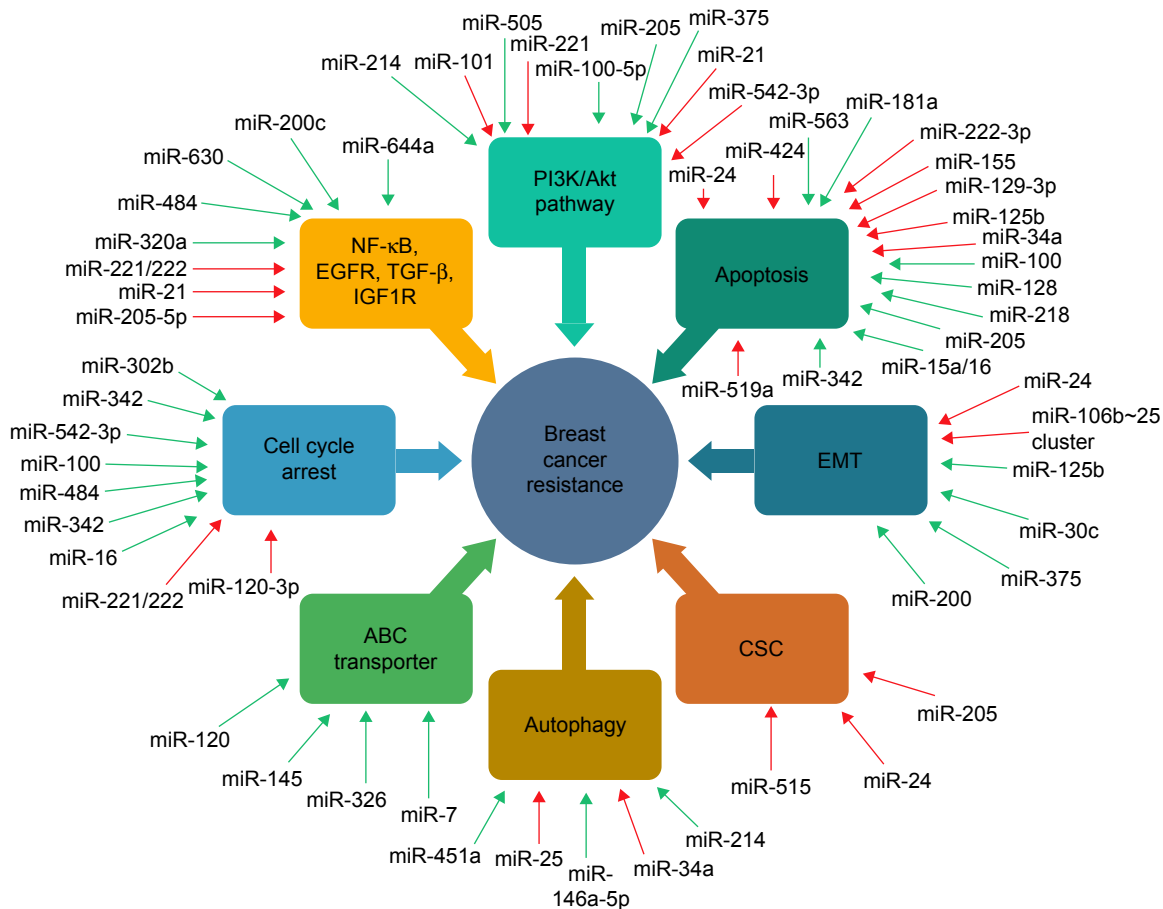
Figure 1 miRNA-mediated mechanisms involved in the regulation of BCa drug resistance. Red arrows indicate oncogenic miRNAs and green arrows represent cancer suppressor miRNAs.
Abbreviations: BCa, breast cancer; EGFR, epithelial growth factor receptor; CSC, cancer stem cell; TGF-β, transforming growth factor-β; EMT, epithelial–mesenchymal transition.

Cell cycle arrest

It has been shown that there is a balance between cell cycle arrest and cell death in cancer therapy.[98] CDKs act as dominators in mammalian for the regulation of cell cycle through phosphorylation of their selected cyclins by maintaining exit or entry to the different phases of the cell cycle.[99,100] Several miRNAs participate in cell cycle regulation by targeting CDKs, cyclins, and E2F. Overexpression of miR-302b can sensitize BCa cells to cisplatin by targeting E2F1, a major regulator of the G1/S transition.[28] In TamR BCa cells, miR-221/222 has been shown to target cell cycle inhibitor p27Kip1 that contributes to antagonizing cell death and promoting hormone-independent cell growth.[42] Restoration of miR-320a expression is able to sensitize TamR cells to tamoxifen through the downregulation of c-Myc and Cyclin D1.[46] In addition, the forced expression of miR-16 reduced BCa resistance to trastuzumab and lapatinib, and the underlying mechanism involves inhibition of c-Myc-enhanced chromatin assessibility.[83]

In contrast, several miRNAs were identified as tumor suppressors, miR-15a/16 inhibits Cyclin E1 and promotes tamoxifen-induced cell cycle arrest.[101] Elevating miR-873 in TamR cells restored tamoxifen sensitivity by targeting CDK3.[43] In addition to directly targeting BCL-2, miR-34a also negatively regulates Cyclin-D1-regulated G1 arrest in response to docetaxel-induced apoptosis.[97] Furthermore, miR-342 seems to regulate the expression of genes involved in tamoxifen-induced cell cycle arrest, but the specific targets for miR-342 remain uncharacterized.[49]

CSCs and EMT

The intractable tumor relapses suggest that not all cells have been targeted, for example, CSCs may be a typical "escaping cells" that are capable of regenerating the tumor and do not respond to therapy drugs.[77] CSCs are thought to be major contributors for drug resistance by activating HIF1α under hypoxic condition.[89] It has been reported that miR-24 increases the expression of CSC markers, conferring cisplatin

resistance.[89] Although miR-205 was identified as a tumor suppressor, it has appeared to be upregulated in patient-derived CSCs.[77,78] Similarly, miR-205 suppresses ErbB3 expression in BCa cells, whereas it inhibits EFGR by targeting p63 in BCa CSCs.[78,102] In addition, CD44 is a putative marker of CSCs, highly expressed in trastuzumab-resistant HER2-positive BCa. Knockdown of CD44 can efficiently restore the trastuzumab sensitivity of the resistant cells.[103] Several miRNAs have been shown to regulate CD44 expression in CSCs, particularly miR-515 is essential for the regulation of CD44 in response to trastuzumab resistance.[103]

Increasing evidences suggest that the function of miRNA is particularly involved in the EMT process that plays a crucial role in MDR by the promotion of tumor metastasis.[104] E-cadherin transcriptional activator EP300 is suppressed by miR-106b~25 cluster, leading to acquired doxorubicin resistance by activating EMT.[25] In addition, miR-24 confers cisplatin resistance by enhancing EMT process by targeting BimL and F1H1.[89] In contrast, miR-644a suppresses a transcription repressor CTBP1, resulting in enhanced p53-mediated inhibition of EMT.[105] Furthermore, miR-30c increases chemoresistance by targeting EMT-related cytokines in BCa, such as vimentin, stress fiber F-actin, twinfilin 1, Interleukin-6, and interleukin-11.[32] Loss of miRNA-375 in TamR cells resulted in the activation of EMT, and ectopic expression of miRNA-375 in TamR cells was able to restore tamoxifen sensitivity by reversing EMT.[50] Intriguingly, miR-125b serves as a tumor suppressor miRNA through the inhibition of EMT by targeting Sema4C, regardless of its effect on apoptosis.[35]

It is worth noting that cells undergoing EMT often obtain properties associated with CSCs.[106] Thus, EMT can cause drug resistance by inducing CSCs. TNBC accounts for ~15%–20% of all BCa cases, which is often documented as clinical chemoresistance due to the lack of molecular targets, particularly claudin-low tumors with extremely rich CSCs and EMT markers.[90,107,108] As a central regulator of EMT, miR-200 has been shown to inhibit self-renewal capabilities of CSCs and keep cells in an epithelial state by suppressing the function of Zeb transcription factors.[109–112] Induction of miR-200 in claudin-low tumors resulted in the suppression of tumor growth by inhibiting the functions of CSCs and reversal of EMT.[90]

Growth factor-related pathways

Numerous studies have revealed multiple signaling pathways involved in therapeutic resistance of BCa and suggested many important molecular targets for the development of new drugs to treat aggressive BCa that are resistant to transitional chemotherapy and radiotherapy. In particular, growth factors, cytokines including chemokines, serve as major contributors for promoting BCa metastasis and leading to therapeutic resistance.

PI3K/Akt/mTOR

PI3K/Akt/mTOR-mediated amplification of growth factors is well recognized to play a pivotal role in BCa metastasis and drug resistance.[47,48,113] Multiple miRNAs were identified to participate in BCa MDR through the regulation of PI3K/Akt/mTOR signaling pathway. For example, miR-205 and miR-214 enhance chemoresistance and TamR by inhibiting PI3K/Akt/mTOR pathway.[39,48] However, phosphorylation of Akt and mTOR are reduced by miR-451, resulting in restored sensitivity to tamoxifen.[47] Downregulation of miR-542-3p contributes to trastuzumab resistance in BCa partially through inhibiting PI3K.[80] In addition, PTEN, an inhibitor of PI3K, is a key regulator for PI3K/Akt/mTOR pathway. Reduction or loss of PTEN has been involved in BCa progression and drug resistance.[66,72] PTEN was verified to be a target gene of miR-21 and miR-221.[73,75] In addition, miR-101 and miRNA-519a confer TamR and chemoresistance by targeting PTEN.[17,53] Reducing miR-301 expression was validated to increase sensitivity to tamoxifen.[56]

NF-κB

NF-κB, stress responsible transcription factor, plays a crucial role in cancer progression and therapeutic resistance.[114–117] In addition to IKK family identified as standard upstream signaling to activate NF-κB, PI3K and STAT3 can also trigger NF-κB activation.[118] Through NF-κB transcriptional activation, miR-21 is uniquely highly expressed in HER2-positive BCa and is responsible for the resistance to trastuzumab.[73] In addition to PTEN, PDCD4 was found as another direct target of miR-21.[73] In contrast, miR-31 represses NF-κB activation and increases apoptosis in TNBC cells by targeting protein kinase C epsilon.[119]

EGFR

Overexpression of EGFR family containing ErbB1, HER1, HER2, HER3, and HER4 imperatively increases drug resistance of BCa.[120–124] Notably, the activation of EGFR finally triggers PI3K/Akt/mTOR and STAT3 signaling pathways, resulting in increased cell survival by activating antiapoptotic proteins.[120,125] miR-451 was reported to specifically target 14-3-3ζ, leading to enhanced apoptosis and sensitized BCa cells to endocrine drugs via inactivation of EGFR.[87] miR-7 was identified to suppress EGFR expression and sensitize

HER2Δ16 to the targeted therapy.[84] In addition, miR-205 was also found as a tumor suppressor, sensitizing targeted therapy by suppressing HER3 and Zeb.[78] However, it is upregulated in BCa CSCs and increases the therapeutic resistance through the regulation of P63-mediated EGFR activation.[78,102]

Cytokines

Cytokines including chemokines are important inflammatory factors and highly related to BCa progression and drug resistance. Several miRNAs have been involved in cytokine-mediated drug resistance of BCa due to the activation of EMT. Overexpression of miR-221/222 contributes to drug resistance through deregulation of multiple signaling pathways including β-catenin and transforming growth factor-β (TGF-β).[88,126] In addition, miR-200c suppresses TGF-β signaling and counteracts trastuzumab resistance of BCa by targeting ZNF217 and ZEB1.[79] Both miR-630 and miR-375 have been found to enhance patient responses to HER-targeting drugs by targeting IGF1R.[81,82] Furthermore, miR-30c inhibits chemoresistance of BCa by targeting TWF1, an activator of EMT, leading to suppression of IL-6 and IL-11 production.[32]

ATP binding cassette (ABC) transporter

Overexpression of ABC transporters in cancer cells is considered to be a common mechanism of MDR due to efflux of drug substrates to maintain their concentrations below effective levels.[127] Human ABC transporters are a large group of membrane protein complexes, including MDR1(P-gp) and MRP1 (ABCC1).[127,128] ABCC5, another member in ABC family, was targeted by miR-128, leading to sensitization of BCa to doxorubicin.[23] In addition, miR-145 was able to inverse doxorubicin resistance in BCa by suppressing MRP1 and triggering drug efflux transporter.[8] Targeting MRP1 by miR-326 was also validated to reverse MDR phenotype.[36]

Commentary

However, it is intriguing that there are some controversial results reported in response to the regulation of different sets of target genes. For example, downregulation of miR-181a was previously reported to inhibit BCa cell apoptosis induced by doxorubicin through the upregulation of Bcl-2,[24] while miR-181a was upregulated in TNBC cells in response to chemotherapy, which was activated by STAT3 to target BAX.[96] Similarly, miR-125b was upregulated in paclitaxel-resistant BCa cells and target Bak1.[34,129] Nevertheless, miR-125b was also shown to be downregulated in paclitaxel-resistant MCF-7 cells, leading to reverse EMT by targeting Sema 4C.[35]

Despite one miRNA targets different genes which may exert the same or opposite effects, the roles of miRNAs in drug resistance of BCa finally depend on their targets for achieved dominant effects in particular cellular and tissue types corresponding to the particular treatment. In addition, it also needs to be noted that multiple miRNAs may interact with one another or may interplay on gene expression through either cooperative effects or antagonist competition, which forms a complicated network to regulate all the target genes in cancers. However, to date, most of studies cited in this review described only the role of miRNA in drug resistance dependent upon the effect of single miRNA, but the concert action of multiple miRNAs contributes to the resistance remains obscure.

Function of exosome in BCa chemoresistance

Exosome is widely recognized to be an important transfer vehicle for cell–cell communication within the tumor microenvironment. Emerging evidences have demonstrated that exosome-mediated miRNA transfer between host–tumor cells plays a pivotal role in tumor progression and resistance to treatment.[130]

Exosomal miRNA

Expression profiles of miRNA in the established drug-resistant BCa cells have indicated that 22 miRNAs were concentrated in exosomes and correlated to resistance.[131] We and others have reported that exosome-derived miRNAs can transfer doxorubicin-resistant capacity from the resistant cells to their parent sensitive cells.[132] Subsequently, we found that miR-222 in exosomes of doxorubicin-resistant cells was able to transfer into the sensitive cells and conferred resistance capacity to the recipient cells by inhibiting PTEN. To confirm the effect of exosome-derived miRNA transferring on drug resistance transmitting, we further enlarged the significance of the miRNA delivered by exosomes. The results suggested that the miRNA regulated drug resistance due to exosomes involving more infused microenvironment within transmitting resistant capacity through miRNA-mediated alteration of gene expression.[16,132–135]

In addition to doxorubicin, Wei et al[136] have demonstrated distinct differences in the exosome concentration and size distribution between TamR cells and the sensitive cells. TamR cells seem to have an increased amount of exosomal RNA and proteins. Exosome secreted from TamR cells was able to enter into the sensitive cells and release miR-221/222, leading to TamR by targeting P27 and Era.[136] Nevertheless,

TNBC cells secrete miRNA-134-enriched exosomes and contribute to increased sensitivity to anti-Hsp90 drugs through the reduction of STAT5B and Hsp90, suggesting that TNBC-derived exosomes may have therapeutic potential as miRNAs delivery vesicles.[137]

In addition to BCa, exosomal miRNAs involving drug resistance have been studied in other types of cancer. Exosomal miR-155 contributes to chemoresistance of neuroblastoma by targeting TERF1.[138] Increase of exosomal miR-155 was also found to enhance gemcitabine resistance in pancreatic ductal adenocarcinoma cells through the enhancement of anti-apoptotic activity.[139] Exosomal miRNAs from lung cancer A549 cells were increased after exposure to cisplatin, which can endow the resistant capacity of the untreated cells.[140] Exosomal miR-222-3p has been shown to enhance gemcitabine resistance by targeting SOCS3 in A549 cells.[141] While exosomal miR-100-5p was decreased in cisplatin-resistant A549, leading to increase in cisplatin resistance through reverse regulation of mTOR.[142] Exosomal miRNAs including miR-598, miR-34a, miR-146a, and miR-148a were significantly increased in docetaxel-resistant prostate cancer cells.[143] Exosomal transfer of stromal-derived miR-21 can transmit paclitaxel resistance into ovarian cancer cells by targeting APAF1.[13] In addition, exosomes derived from tumor-associated macrophages can mediate cisplatin resistance by directly transferring miR-21 to target PTEN in gastric cancer cells.[144] Interestingly, Lou et al successfully sensitized hepatocellular carcinoma to chemotherapy by using exosomes from adipose tissue-derived mesenchymal stem cells for packaging miR-122.[145] In addition, Munoz et al transferred exosomes carrying anti-miR-9 into glioblastoma cells and achieved an enhanced therapeutic efficacy through the suppression of P-gp.[146] Furthermore, the low levels of serum exosomal miR-146a-5p were found in advanced non-small cell lung cancer patients with higher recurrence rates, leading to sensitized cisplatin resistance by targeting Atg12 for inhibiting autophagy.[147]

Other exosomal contents

Except for miRNAs, other exosomal contents have been noted to participate in exosome-mediated drug resistance in BCa. P-gp exists in exosomes and contributes to drug resistance by pumping drugs out to maintain cellular levels beyond effective concentration. We have reported that exosome-mediated cell–cell transferring P-gp to be a main mechanism by which exosomes contribute to docetaxel resistance in BCa cells.[127] Since TrpC5 can stimulate the formation of P-gp, TrpC5 was also accumulated in exosomes and promoted exosomes to trap P-gp for doxorubicin resistance in BCa.[148]

Within the tumor microenvironment, cross-talk between stromal cells and cancer cells plays an important role in transfer of drug resistance through exosomal vehicles. It has been noted that exosomes transferred from stromal cells to BCa cells can activate antiviral pathway as well as increased interferon-related DNA damage resistance signature including STAT1, MX1, ISG15, OAS1, IFIT1, IFIT3, and IFI44, which has been validated to impact chemoresistance of BCa.[149] In addition, Wang et al recently reported that bone marrow stromal cell-derived exosomes can transfer bortezomib resistance in multiple myeloma cells through the delivery of growth factors and cytokines.[150]

Exosome in the tumor microenvironment

Tumor microenvironment is often likened to the soil where tumor cells grow, which refers to the variety of normal cells, signaling molecules, blood vessels, and extracellular matrix surrounding the tumor cells.[151] As a key component in the tumor microenvironment, exosomes shuttle bioactive molecules and mediate cellular communication in the tumor microenvironment, facilitating a more malignant and refractory tumor environment. The major function of exosome in the tumor microenvironment in response to drug resistance is considered to be a molecular shuttle. As described above, exosomes can enrich and transfer a variety of small molecules including miRNAs between donor cells and recipient cells. Recent studies suggest two main categories, namely from resistant cells to sensitive cells for transferring the resistant capacity as well as from stromal cells to their surrounded cancer cells for survival and resistance.[13,132,136,140] The relevant stromal cells have been defined as cancer-associated fibroblasts, mesenchymal stem/stroma, and adipocytes.

Beyond its encapsulated abundant functional molecules, exosomes also participate in the regulation of drug resistance by acting as an efflux pump. The drugs and their metabolites can be encapsulated and exported by exosomes.[152–154] Therefore, it is very promising to apply exosomes for targeted therapeutics for their unique advantages, because they have a good tolerance for their wide distribution in various human body fluids. Overall, exosomes can serve as an optimal carrier of drugs and functional molecules including diagnostic biomarkers and therapeutic targets, which may provide a promising approach for the improvement of anticancer therapy.

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Disclosure
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