MicroRNAs in the diagnosis and prognosis of breast cancer and their therapeutic potential (Review)

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Abstract. MicroRNAs (miRNAs) are non-coding single-stranded RNAs in eukaryotes and are involved in the regulation of the post-transcriptional expression of specific genes. Studies have demonstrated that miRNAs play important roles in regulating diverse physiological events such as cell proliferation, differentiation and embryo development. In recent decades, considerable attention has been given to the relationship between miRNA and the pathology of cancers, particularly breast cancer. A large number of miRNAs have been shown to be involved in the pathophysiology of breast cancer. Studies have revealed that some miRNAs might regulate the oncogenesis and growth of breast cancer by acting on breast tumor-initiating cells or other downstream targets. Studies have also demonstrated that some miRNAs act as suppressors of metastasis or promoters of breast cancer. Additionally, certain miRNAs are involved in cancer tissue angiogenesis (one of the most important mechanisms of tumor growth and metastasis). Clinical evidence indicates that some miRNAs can be used as diagnostic and prognostic biomarkers for breast cancer due to their significantly increased or decreased expression in cancer tissue. Moreover, certain miRNAs may have therapeutic potential for targeting ER-α/HER, breast tumor-initiating cells and metastasis as well as multidrug resistance. In this review, we discuss the relationship between miRNAs and the pathogenesis of breast cancer as well as the progress of current research on the miRNA-specific diagnosis, prognosis and treatment of breast cancer.

Contents

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1. Introduction

Breast cancer is one of the most common malignant cancers in women, and it has become a worldwide public health issue, with reports of more than 500,000 deaths per year (1). The pathological progression of breast cancer is multistep and complicated, consisting of oncogenesis, which is primarily due to the self-renewal and differentiation of breast tumor-initiating cells (BT-ICs), tumor growth (proliferation and apoptosis), invasion, metastasis, angiogenesis and possible post-treatment relapse (Fig. 1). As breast tissue is steroid responsive, extensive effort has been directed at identifying steroids and their receptors as well as the nuclear receptor coactivators (2). Endocrine therapy using the estrogen receptor antagonist tamoxifen and aromatase inhibitors for the treatment of breast cancer has been clinically applied. However, many problems, such as drug resistance and the adverse effects of these drugs, still exist. Therefore, novel and effective therapeutic methods for breast cancer are urgently needed.

MicroRNAs (miRNAs) are non-coding single-stranded RNAs with a length of approximately 22 nucleotides, and they function as key post-transcriptional regulators of eukaryotic gene expression through inhibiting translation or targeting mRNAs for degradation (3). Recent studies have uncovered the important roles of miRNAs in diverse physiological and pathological events such as cell proliferation, differentiation, embryo development and cancer progression (4). Accumulating evidence indicates that the putative functions of miRNAs might have important clinical significance. For example, they might be regarded as tumor suppressors and/or promoters (5,6), and their abnormal expression is highly correlated with the progression and pathology of breast cancer (7,8), supporting their diagnostic, prognostic and therapeutic potentials in breast cancer (4).

2. miRNAs in the pathogenesis and progression of breast cancer

miRNAs and breast tumor-initiating cells. Studies have shown that breast tumor-initiating cells (BT-ICs) are the key contribu-

tors to the oncogenesis of breast cancer; thus, understanding the regulation of BT-ICs is of great importance. As BT-ICs have gained mutations causing them to be more tumorigenic, they possess stem-like properties, such as self-renewal, unlimited replication potential, differentiation and metastasis.

At present, approximately 40 miRNAs have been found to be differentially expressed between human BT-ICs and non-tumorigenic breast cancer cells. Some of them, such as Let-7 (9), miR-16 (10), miR-34c (11), miR-200c (12), miR-183 and miR-203 (13), have been shown to promote the self-renewal of BT-ICs by targeting different signaling pathways. Let-7 miRNAs are members of the heterochronic pathway, and they are necessary for BT-ICs to undergo the correct progression of stage-specific events at the correct time, including differentiation (9). In contrast, other miRNAs such as miR-30 have been shown to inhibit BT-IC self-renewal by reducing Ubc9 and inducing apoptosis through silencing ITGB3 (14). Additionally, three genomic clusters, miR-183-96-182, miR-200b-200a-429 and miR-200c-141, are downregulated in BT-ICs, suggesting that they may play key roles in the regulation of self-renewal (12). Moreover, miR-145 has been reported to inhibit the differentiation of BT-ICs via the 3'-UTR of insulin receptor substrate-1 (IRS-1) (15).

Furthermore, miRNAs might also participate in the metastasis of breast cancers mediated by BT-ICs. The miR-200 family has been shown to play pivotal roles in the process of the epithelial-mesenchymal transition (EMT), which is the initial event of BT-IC-associated metastasis. Two pathways, the p53-miR-200c pathway (16) and the ZEB1-miR-200c-BMI1 pathway (17), regulate both EMT and BT-IC-associated metastasis. Furthermore, the downregulation of miR-34c (11) and the overexpression of the miR-106b-25 cluster (18) are also sufficient to induce EMT. Recently, miR-30a (19) and miR-30c (20), targeting the cytoskeleton network genes encoding twinfilin 1 (TWF1) and vimentin (VIM), have also been shown to be involved in EMT.

miRNAs and breast cancer oncogenesis. Studies have revealed that miRNAs associated with genomic changes can act as either breast cancer oncogenes (oncomiRs) or suppressors (5). The oncomiRs are upregulated in breast cancer and may hinder the expression of many genes related to tumor suppression, cell cycle regulation, apoptosis and differentiation. For example, the overexpression of miR-21 in breast cancers influences several targets, including the gene expression of tumor suppressor tropomyosin 1 (TPM1) (21), programmed cell death 4 (PDCD4) (22), phosphatase and tensin homolog (PTEN) (23) and TIMP metallopeptidase inhibitor 3 (TIMP3) (24), in breast cancer. Additionally, miR-27a, miR-96 and miR-182 can target the 3'-UTR of the mRNA that encodes the putative tumor suppressor transcription factor FOXO1 (25). Another miRNA, miR-155, has been reported to act as an oncomiR, targeting caspase-3 as a potent suppressor of apoptosis (26).

Compared to the oncomiRs, cancer-suppressing miRNAs have been reported to target and inhibit oncogenes, and their dysfunction may lead to the development of cancerous cells (27). Both *in vitro* (from MCF-7 cells) and *in vivo* (from analysis of cyclin D1-transgenic mice) analyses have shown that the miR17-5p (miR-91)/miR-20a cluster can inhibit breast cancer cell proliferation by suppressing the proliferative effect of cyclin D1 (28). miR-27b has been reported to play a suppressive role in breast cancer cells and to post-transcriptionally regulate cytochrome P4501B1 (CYP1B1), a key enzyme in the metabolism of 17β-estradiol, which promotes the growth of breast cancer (29). Recently, HER2 and HER3 (erbB2 and erbB3), which significantly correlate with breast cancer and poor prognosis, have been shown to be suppressed by miR-125a, miR-125b and miR-205 in breast cancer cells, leading to a reduction in cell proliferation and migration and increased apoptosis (30). Moreover, the suppressive role of miR-205 is mediated through the direct targeting of oncogenes such as Zeb1 (31). It has been shown that the overexpression of miR-145 inhibits estrogen receptor-α (ER-α) protein expression (32) and reduces RTKN protein expression (33), thus reducing breast cancer cell growth and inducing apoptosis, indicating its tumor-suppressive functions. Similarly, miR-22 (34), miR-206 (35) and miR-375 (36) negatively affect the expression of ER-α.

miRNAs and breast cancer growth. Studies have recently uncovered the roles of miRNAs in cancer cell proliferation and apoptosis, the dysregulation of which is the most important mechanism of cancer growth. For example, oncomiRs, including miR-21 (24), miR-27a, miR-96, miR-182 (25) and miR-155 (37), can target the 3'-UTR of mRNAs that encode tumor suppressor genes. Moreover, cell growth suppression is achieved by the overexpression of cancer suppressor miRNAs such as miR17-5p (miR-91)/miR-20a (28), miR-27b (29), miR-125a, miR-125b and miR-205 (30), suggesting their roles in growth suppression. Furthermore, the upregulation of ER-α accounts for the abnormal cell proliferation observed in approximately two-thirds of breast cancer cases. miR-22 (34), miR-145 (32), miR-206 (35) and miR-375 (36) are reported to negatively regulate ER-α expression, thus inhibiting breast cancer cell proliferation.

Several studies have addressed the roles that miRNAs play in the modulation of apoptosis in breast cancer cells. The overexpression of miR-145 has been demonstrated to induce apoptosis by directly binding to the 3'-UTR of RTKN (33). miR-26b can also impair the viability and trigger the apoptosis of human MCF-7 cells by directly targeting SLC7A11 (38). Moreover, miR-290 was shown to target Arid4b while simultaneously enhancing ER signaling and inducing apoptosis, thereby suppressing breast cancer growth (46). The overexpression of miR-335 decreases cell viability and increases apoptosis by simultaneously regulating the known BRCA1 activators ER-α, IGF1R and SP1 and the repressor ID4 (39).

miRNAs and breast cancer invasion and metastasis. Invasion and metastasis are the major factors underlying the poor prognosis or even death of breast cancer patients. In the past few years, many types of miRNAs have been identified, and their roles have been explored in cancer invasion and metastasis; some of these miRNAs function as metastasis promoters, whereas others function as suppressors (Fig. 2).

miRNAs as suppressors of metastasis. miR-23b has been shown to be a metastatic suppressor miRNA that directly inhibits a number of genes implicated in cytoskeletal remodeling, focal adhesion, cell spreading, cell-cell junction formation and the

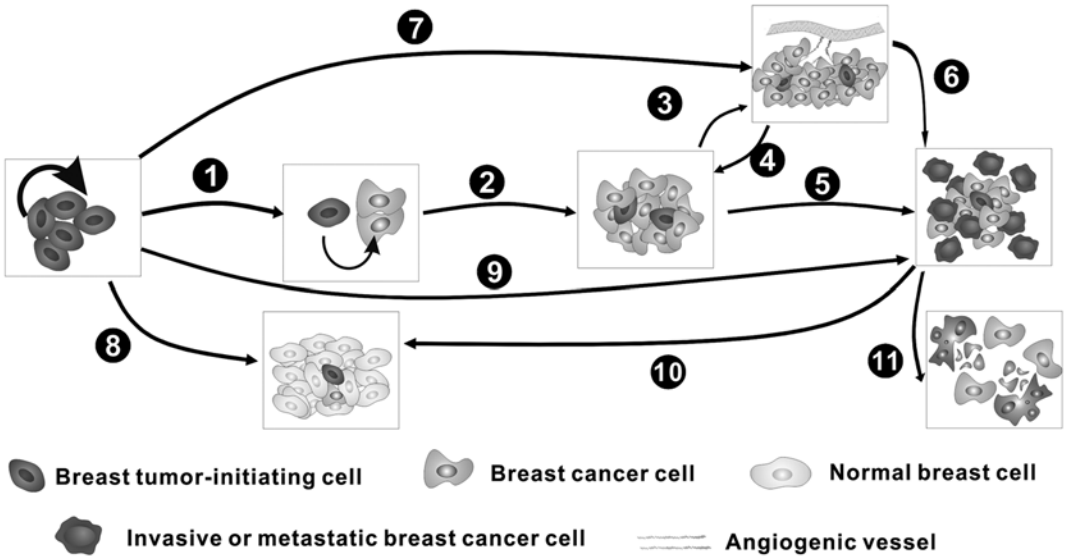

Figure 1. Schematic illustration of the pathogenesis and progression of breast cancer. (1) The initial development of breast cancer. Breast tumor-initiating cells (BT-ICs) self-renew and differentiate, resulting in a large number of breast cancer cells. (2) Breast cancer growth. The differentiated breast cancer cells proliferate to form a larger tumor colony. (3) Tumor angiogenesis. When the tumor colony reaches a certain size, the nutrients and oxygen permeating from the nearby vessels may not be sufficient to meet the needs of the tumor colony; therefore, new blood vessels form within the tumor tissue following stimulation by complex factors such as hypoxia. (4) After new vessel generation, the breast cancer cells obtain more nutrients and oxygen, contributing to the further growth of the breast cancer colony. (5) Breast cancer metastasis. In the advanced stage of breast cancer, it is very likely to undergo a multistep process to form secondary tumors in other distant organs. (6) The angiogenic vessels help the metastatic cells move and support their growth, forming secondary tumors in new organ environments. (7) BT-ICs are reported to be responsible for one of the mechanisms underlying tumor angiogenesis. (8) BT-IC-associated relapse. (9) BT-IC-associated metastasis. (10) Tumor metastasis is a major cause of breast cancer relapse. (11) Cell apoptosis.

formation of lamellipodia in breast cancer cells (40). miR-31 has also been shown to trigger metastatic regression in the lungs by eliciting cell cycle arrest and apoptosis, which can be explained by the miR-31-mediated suppression of integrin-α5, radixin, RhoA (41), the actin cytoskeleton remodeling protein WAVE3 (42) and protein kinase C epsilon (PKC-ε) (43). Other cancer-suppressing miRNAs such as miR-125a, miR-125b and miR-205 significantly inhibit breast cancer cell invasion by directly targeting HER2 and HER3 (30), whereas miR-205 can target Zeb1 (31). Additionally, miR-145 significantly suppresses cell invasion and lung metastasis by directly targeting the metastasis gene mucin 1 (MUC1), leading to a reduction of β-catenin and adherin 11 (44). Moreover, miR-146 may be involved in the inhibition of the breast cancer metastasis suppressor 1 (BRMS1), a predominant nuclear protein, leading to metastasis suppression (45); miR-146a/b functions to negatively regulate NF-κB activity through targeting IL-1 receptor-associated kinase (IL-1-RSK) and TNF receptor-associated factor 6 (TNFRSF-6), thus functioning as a suppressor of breast cancer metastasis (46).

The initiation of metastasis, EMT, has been shown to be significantly regulated by the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429), the members of which are all dramatically downregulated in breast cancer cells that have undergone the EMT induced by tumor growth factor-β (TGF-β), and the E-cadherin transcriptional repressors ZEB1 and ZEB2 have been established as their targets (47). In addition, the miR-200 family also enhances metastatic colonization, partially through directly targeting Sec23a, which is involved in the secretion of metastasis suppressor proteins (48). Furthermore, the miR-200b/c/429 cluster is a stronger regulator of EGF-driven invasion than the miR-200a/141 cluster, and phospholipase C gamma 1 (PLCG1) might be a potential candidate contributing to these differences (49). Moreover, the overexpression of miR-200a has been reported to decrease anchorage-independent growth and migration in breast cancer, targeting the oncogene silent information regulator 1 (SIRT1) (50); miR-200c greatly impacts the regulation of several EMT-related processes in breast cancer by directly targeting the actin cytoskeleton (51).

Studies have reported that in highly metastatic breast cancer cells, the expression of miR-203 is significantly downregulated and that its upregulation inhibits tumor cell invasion and metastatic colonization through the SNAI2 and miR-203 regulatory loop (52). Moreover, miR-335 has been identified as the first selective metastasis suppressor in human breast cancer by targeting SOX4 and tenascin-C (53). Additionally, miR-206 suppresses the invasion and migration of MDA-MB-231 cells *in vitro*, partially via regulating the remodeling of the actin cytoskeleton, with CDC42 as its potential target (54). Another study has shown that the overexpression of miR-224 inhibits cell division cycle 42 (CDC42) and chemokine receptor 4 (CXCR4), accounting for the inhibition of Ubc9-mediated invasion (55). Furthermore, let-7b and the miR-17-5p/miR-20a cluster are significantly decreased in lymph node metastases of breast cancer cells. The forced expression of let-7b significantly inhibits breast cancer cell migration by targeting four genes that are correlated with the actin cytoskeleton pathway, including PAK1, DIAPH2, RDX and ITGB8 (56). The miR-17-5p/miR-20a cluster plays a suppressive role in metastatic breast cancer through heterotypic secreted signaling (57).

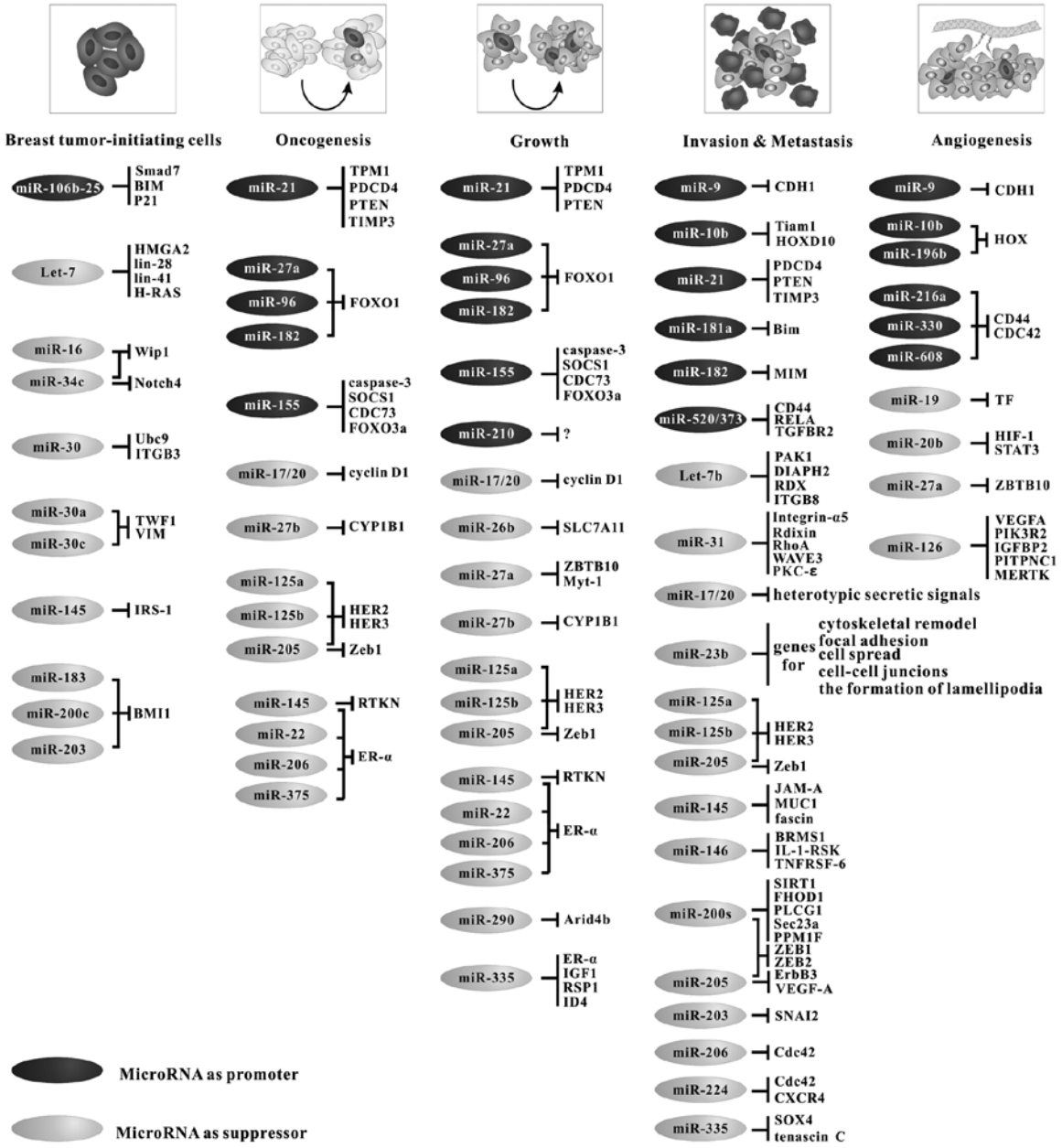

Figure 2. Representative miRNAs involved in the pathogenesis and progression of breast cancer and their targets. The miRNAs in the grey rectangles function as suppressors at each stage of breast cancer progression, while those in the dark rectangles function as promoters. For more detailed information, please see the text.

miRNAs as metastasis promoters. miR-9 is upregulated in breast cancer and increases cell motility and invasiveness by directly targeting CDH1 and E-cadherin-encoding mRNAs (58). miR-10b expression is increased in metastatic breast cancer cells, leading to cell migration, invasion and metastasis through indirectly activating the prometastatic gene RHOC by suppressing the homeobox D10 (HOXD10) tumor suppressor signaling pathway (59). The regulation of T lymphoma invasion and metastasis 1 (Tiam1)-mediated Rac activation in breast cancer cells is a novel target for miR-10b to regulate the invasion and migration of breast cancer cells (60). miR-21, as an oncogenic miRNA, plays important roles in breast cancer invasion and metastasis by inhibiting multiple metastasis suppressor genes. The upregulated expression of miR-181a in metastatic breast cancer cells has been shown to enhance breast cancer metastasis by promoting EMT and migratory and invasive phenotypes, targeting the proapoptotic molecule Bim, which is involved in metastatic cancer cell anoikis (61). Similarly, miR-182 promotes breast cancer metastasis by suppressing the missing in metastasis (MIM), which activates the Ras homolog family member A (RhoA) (62). miR-373 and miR-520c can also promote the invasion and migration of breast cancer cells both *in vitro* and *in vivo* (63) by directly targeting and inhibiting the expression of RELA, TGFBR2 (63) and CD44 (64).

miRNAs and breast cancer angiogenesis. Angiogenesis is one of the most important mechanisms of tumor growth and metastasis

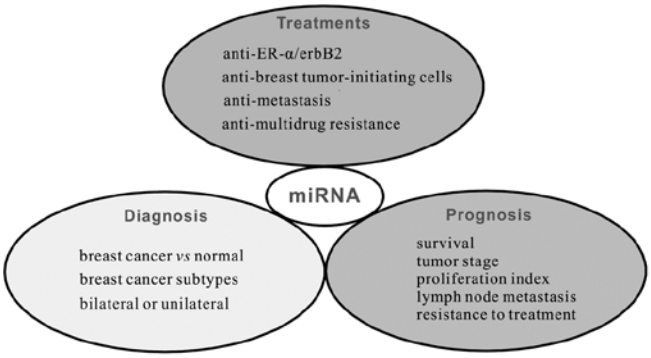

Figure 3. Schematic illustration of potential miRNA-based strategies with clinical implications for breast cancer.

(Fig. 1). Much effort has been directed toward the development of anti-angiogenic tumor therapies. miRNAs appear to have considerable potential as the gene resource for the gene therapy of breast cancer. Endothelial cells (ECs) are one of the key components of the tunica intima, playing vital roles in tumor angiogenesis. It has been shown that many miRNAs such as miR-216a, miR-330 and miR-608, which can bind to both the CD44 and CDC42 (CD44 downstream target mRNA) 3'-UTRs, can modulate EC activities (3). miR-126, which is particularly expressed in vascular endothelial cells, is reported to play a vital role in suppressing breast cancer angiogenesis through regulating the VEGF/PI3K/AKT signaling cascade (65) and targeting IGFBP2, PITPNC1 and MERTK (66).

Moreover, miR-9 contributes to angiogenesis through the upregulation of VEGF expression in breast cancer by directly targeting CDH1 (58). Another report has demonstrated that VEGF expression in breast cancer cells is mediated by hypoxia inducible factor 1 (HIF-1) and signal transducer and activator of transcription 3 (STAT3) in a miR-20b-dependent manner (67). The overexpression of miR-10b and miR-196b has been detected in high-grade breast cancer vasculature and, notably, to be responsive to VEGF stimulation (68). Moreover, miR-27a suppresses breast cancer angiogenesis by inhibiting specificity protein (Sp)-dependent angiogenic gene expression, including that of survivin, VEGF and VEGFR1, as it targets the zinc finger ZBTB10 gene (69). The overexpression of miR-19 downregulates tissue factor (TF) expression, which is an important factor in the regulation of tumor angiogenesis, suggesting the potential of miR-19 for regulating breast cancer angiogenesis (70).

Taken together, the involvement of these miRNAs in the pathogenesis and progression of breast cancer has been elucidated, with some of them acting as suppressors and others as tumor promoters, as summarized in Fig. 2. These studies are crucial to evaluating their use as clinical biomarkers and their therapeutic potential.

3. Clinical implications of miRNAs in breast cancer

Many miRNAs have been shown to contribute to the pathogenesis and progression of breast cancer, which supports the potential of miRNA-specific strategies for the treatment of breast cancer (Fig. 3).

Diagnostic and prognostic biomarkers of breast cancer. The increasing number of reports indicating the significance of miRNAs in the pathogenesis and progression of breast cancer has revealed the potential relevance of aberrantly expressed miRNAs as biomarkers for the detection, diagnosis, classification and therapy of breast cancer. First, the expression profile of miRNAs can be used to distinguish breast cancer tissue from normal tissue. For example, the significant overexpression of miR-21, miR-106a and miR-155 and the decreased expression of miR-126, miR-199a and miR-335 have been reported in breast cancer tissue when compared with normal tissue (71). A study by Iyevleva *et al* has reported higher levels of miR-21, miR-10b and miR-31 in bilateral patients than in unilateral patients (72). Additionally, a number of miRNAs are differentially expressed in the luminal A, luminal B, basal-like, $HER2^+$ and normal-like breast cancer subtypes. For example, the upregulation of miR-17-92 clusters has been shown to account for the great distinction of triple-negative breast cancer from other subtypes (6).

The miRNA expression profile has also been correlated with pathological features such as tumor stage, proliferation index, lymph node metastasis, resistance to breast cancer treatment and poor survival, suggesting their prognostic potential. The most promising prognostic miRNAs for breast cancer are miR-21 (24), miR-181a (61) and the miR-221/miR-222 cluster (73) because their overexpression has been correlated with advanced tumor stage, lymph node metastasis and poor patient survival. Moreover, a high level of the miR-106b-25 cluster in breast cancer can significantly predict a shortened time to relapse (18). Additionally, in ER-positive cases, high miR-767-3p, miR-128a and/or miR-769-3p expression is associated with a poor prognosis, whereas high miR-135a expression is associated with a good prognosis; in contrast, in ER-negative cases, high levels of miR-27b, miR-144 and/or miR-210 are associated with a poor prognosis, and high miR-342, miR-150 and/or miR-30c expression is associated with a good prognosis (74). Moreover, miR-342, miR-27b and miR-150 are prognostic in triple receptor-negative breast cancers (74).

Therapeutic miRNAs. The traditional therapies for breast cancer, such as surgery, chemotherapy and/or radiotherapy, appear to be powerful; however, regardless of the level of damage directly caused to the human body, problems such as drug resistance and adverse effects still exist, making the treatment of breast cancer more difficult than expected. Therefore, novel, feasible and effective therapeutic methods for breast cancer are urgently needed. Because of the distinct roles that miRNAs play in the pathology of breast cancer, many studies have investigated their therapeutic potential in its treatment.

Anti-BT-ICs. One of the greatest challenges for breast cancer research is relapses that occur in patients undergoing chemotherapy and radiotherapy. Recent studies have shown that targeting BT-ICs is promising to prevent relapse and provides a new strategy for breast cancer prevention. Many types of miRNAs are involved in regulating the self-renewal and differentiation of BT-IC as well as BT-IC-induced relapse (Fig. 2). As mentioned above, one attractive candidate for this purpose is let-7 because the administration of let-7 has been found to be effective in mouse models of breast cancer (75).

Table I. Selected miRNAs and their clinical applications in breast cancer.

Application	microRNA	Putative clinical significance	Refs.
Diagnosis	miR-21↑, miR-106a↑, miR-155↑, miR-199a↓, miR-126↓, miR-335↓	Potential biomarkers for breast cancer diagnosis, grading and prognosis	(70)
	miR-21↑, miR-10b↑, miR-31↑	Biomarkers for the diagnosis bilateral or unilateral breast cancer	(71)
	miR-17-92 cluster↑	Identifies the subtype of breast cancer: distinction of triple-negative breast cancer from other subtypes	(72)
Biomarker			
Prognosis	miR-21↑, miR-181a↑, miR-221/miR 222 cluster↑	The overexpression is correlated with advanced tumor stage, lymph node metastasis and poor survival of the patients	(6,23,60)
	miR-767-3p, miR-128a, miR-769-3p, miR-135a	Associated with prognosis in ER-positive cases: poor, miR-767-3p↑, miR-128a↑, miR-769-3p↑; good, miR-135a↑	(73)
	miR-27b, miR-144, miR-210 miR-342, miR-150, miR-30c	Associated with prognosis in ER-negative cases: poor, miR-27b↑, miR-144↑, miR-210↑; good, miR-342↑, miR-150↑, miR-30c↑	(73)
	miR-27b, miR-150, miR-342	Prognostic also in triple receptor-negative breast cancers	(73)
	miR-106b-25 cluster↑	Significantly predicted shortened time to relapse	(17)
	miR-22↓, miR-145↓, miR-206↓ and miR-375↓	Significantly decreased in ER-α-positive human breast cancer tissues and is inversely correlated with ER-α mRNA expression in breast cancer tissues in a dose- and time-dependent manner	(31,33-35)
Anti-ER-α/HER	miR-125a↓, miR-125b↓ miR-205↓	Directly suppressed the HER2 and HER3 (erbB2 and erbB3)	(29)
Anti-BT-IC	let-7↑	Effective in suppressing metastatic breast cancer	(74)
Therapy			
Anti-metastasis	miR-21↓	Significantly reduced invasion and lung metastasis ability	(21-23)
	miR-31↑	Elicits the regression of metastasis and prolongs the survival of patients	(40)
	miR-145↑	Ad-miR-145 suppressed breast cancer cell motility and invasiveness; combining Ad-miR-145 with 5-FU significantly showed anti-tumor effects	(2)
	miR-146a/b↑	Inhibited both invasion and migration, and suppressed experimental lung metastasis	(44)
Anti-MDR	miR-21↑	miR-21 inhibitor gene therapy combining with taxol chemotherapy	(81)
	miR-24-2↓	Combining with an anticancer drug such as cisplatin provides a new avenue for overcoming drug resistance	(86)
	miR-155↑	Knockdown of it renders breast cancer cells to apoptosis and enhance chemosensitivity	(76)
	miR-326↑	Downregulated MRP-1 expression and sensitize cancer cells to VP-16 and doxorubicin	(78)
	miR-328↑	Increased mitoxantrone sensitivity	(79)
	miR-451↑	Increased sensitivity of cancer cells to DOX	(80)

This table shows only some representative miRNAs and their pathological and clinical significance in breast cancer. MDR, multidrug resistance; ↑, increased expression; ↓, decreased expression.

Anti-ER-α/HER. As breast cancer is steroid-responsive, much attention has been given to steroids (e.g., estradiol and progesterone) and their receptors [e.g., ER-α, ER-β and the progesterone receptor (PR)] as well as the nuclear receptor coactivators, particularly steroid receptor coactivator-1 (SRC-1) and SRC-3 (2). It has been shown that ER-α upregulation is responsible for the abnormal cell proliferation observed in approximately two-thirds of breast cancer cases, and anti-ER-α therapy may be of great value for breast cancer treatments. Studies have revealed that the levels of miR-22 (34), miR-145 (32), miR-206 (35) and miR-375 (36) are significantly decreased in ER-α-positive human breast cancer tissues and inversely correlated with ER-α mRNA expression in breast cancer tissues; thus, they have been suggested as novel therapeutic agents for anti-multidrug resistance (MDR) therapies that target only ER-α in breast cancer. Additionally, the overexpression of HER2 and HER3 (erbB2 and erbB3) is significantly correlated with breast cancer grade and poor prognosis, and this overexpression can be suppressed by miR-125a, miR-125b and miR-205 (30), thus resulting in the abrogation of HER2- and HER3-mediated resistance and restoring potent proapoptotic activity.

Anti-metastasis. miR-21 plays a role in invasion and metastasis by targeting multiple tumor/metastasis suppressor genes such as PDCD4 (22), PTEN (23) and TIMP3 (24), suggesting its potential for anti-metastasis treatments. miR-31 has also been shown to regulate a number of metastasis-related genes in breast cancer cells and tissues, and its expression is inversely correlated with the cell's ability to invade and metastasize. Activation of miR-31 in established metastases elicits metastatic regression and prolongs patient survival (41). The overexpression of miR-145 has been shown to suppress breast cancer cell growth and motility, and a treatment combining Ad-miR-145 with 5-FU has shown significant antitumor effects (76). miR-146 is also involved in suppressing metastasis, and miR-146a/b-expressing cells show markedly impaired invasion and migration capacities when compared with controls (45).

Anti-multidrug resistance (MDR). Drug resistance and multidrug resistance (MDR) are the main causes of treatment failure, and these are the most challenging problems in the treatment of breast cancer. Studies have demonstrated that some miRNAs might be involved in the occurrence of MDR, and therefore, targeting these miRNAs might be of great significance regarding the development of novel strategies against breast cancer. For example, the expression of miR-155 (77) and miR-663 (78) has been demonstrated to induce breast cancer cell survival and MDR, whereas their knockdown renders breast cancer cells susceptible to apoptosis and enhances their chemosensitivity. miR-326 is downregulated in advanced breast cancer tissues and is inversely correlated with the expression of multidrug resistance-associated protein-1 (MRP-1); its upregulation in VP-16-resistant MCF-7 cell lines can downregulate the expression of MRP-1, making them sensitive to VP-16 and doxorubicin (DOX) (79). These findings indicate that miR-326 may be an efficient and powerful agent for preventing and reversing MDR in breast cancer cells. Moreover, the miR-328-mediated downregulation of ABCG2 (a molecular determinant of the pharmacokinetic properties of many drugs in humans) in MCF-7/MX100 cells may result in increased mitoxantrone sensitivity (80). Recently, the multidrug resistance 1 gene has been identified as a novel target of miR-451, and transfection of MCF-7/DOX-resistant cells with miR-451 results in increased sensitivity to DOX, indicating that increased miR-451 expression may have significant implications for overcoming the MDR of breast cancer cells (81). The above results are summarized in Fig. 3 and Table I.

4. Conclusions

In the past few years, the correlations between miRNAs and breast cancer have been widely investigated, contributing greatly to studies of breast cancer pathogenesis and their clinical implications. These small molecules play significant roles in the oncogenesis, growth, invasion, metastasis and angiogenesis of breast cancer; thus, altered expression of miRNAs can be regarded as a target for the diagnosis and/or treatment of breast cancer based on the miRNA expression profile. However, many of the remaining problems urgently require further exploration. For example, we know little about the upstream, intrinsic factors that regulate these specific miRNAs; there are tens or hundreds of miRNAs within a cell, and which is the most prominent indicator for the diagnosis and treatment as well as the prognosis of cancer remains to be elucidated. Future work should aim to identify the limited miRNAs involved in each pathological stage as well as the therapeutic targets of breast cancers. These under-explored issues may be the greatest challenges in the future.

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