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Review:

MicroRNAs in breast cancer: oncogene and tumor suppressors with clinical potential*

Wei WANG, Yun-ping LUO†‡

(*Department of Immunology, Institute of Basic Medical Science, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, China*)

†E-mail: yunpingluo@hotmail.com

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Abstract: MicroRNAs (miRs) are small single-stranded RNA molecules, which function as key negative regulators of post-transcriptional modulation in almost all biological processes. Abnormal expression of microRNAs has been observed in various types of cancer including breast cancer. Great efforts have been made to identify an association between microRNA expression profiles and breast cancer, and to understand the functional role and molecular mechanism of aberrant-expressed microRNAs. As research progressed, 'oncogenic microRNAs' and 'tumor suppressive microRNAs' became a focus of interest. The potential of candidate microRNAs from both intercellular (tissue) and extracellular (serum) sources for clinical diagnosis and prognosis was revealed, and treatments involving microRNA achieved some amazing curative effects in cancer disease models. In this review, advances from the most recent studies of microRNAs in one of the most common cancers, breast cancer, are highlighted, especially the functions of specifically selected microRNAs. We also assess the potential value of these microRNAs as diagnostic and prognostic markers, and discuss the possible development of microRNA-based therapies.

Key words: Breast cancer, MicroRNA, Oncogene, Tumor suppressors, Diagnosis marker, MicroRNA-based therapy
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1 Introduction

Cancer has long been the gravest challenge to human health, which not only reduces the quality of life, but also increases mortality. Breast cancer is the most common malignancy and the leading cause of death by cancer in women, with more than 500000 cases globally per year (Iorio and Croce, 2009; Zoon *et al.*, 2009). Under this complex neoplasma, the scenario consists of tumor initiation and growth, metastasis and invasion, angiogenesis and possible relapse. All of these stages may be affected by changes in genetic information and maintenance of the tumor microenvironment (TME) (Al-Hajj *et al.*, 2003; Brabletz *et al.*, 2005; Sica *et al.*, 2008). As breast cancer is one kind of highly-heterogeneous tumor, a number of expression signatures have been developed to classify its molecular subtypes. According to the St. Gallen symposium, five breast tumor subtypes are well defined: luminal A, luminal B, basal-like, $HER2^+$ (human epidermal growth factor receptor 2 positive), and normal breast-like. ER^+ (estrogen receptor positive) is the main feature of the luminal A/B subtypes, and the others are ER^- subtypes (Sorlie *et al.*, 2001; Bertucci *et al.*, 2005; Blenkiron *et al.*, 2007).

Recently, cancer stem cells (CSCs) have received more attention, especially in breast cancer. Although the CSC theory was always accompanied by scepticism and intense debate, numerous evidence

‡ Corresponding author
* Project supported by the National Basic Research Program (973) of China (No. 2013CB967202) and the National Natural Science Foundation of China (No. 81472654)
ORCID: Yun-ping LUO, http://orcid.org/0000-0001-6905-0768

has proved the existence in tumors of this minority subpopulation with amazing tumor-initiating powers. In some cases, tumor-initiating cells are also called CSCs to represent their CSC characteristics. The issues about how CSCs come into being, why they have the ability to initiate and drive tumor growth, and how they can be resistant to therapeutics remain confused (Shipitsin and Polyak, 2008; Valent *et al.*, 2012; Kreso and Dick, 2014). The activation of epithelial-to-mesenchymal transition (EMT), which plays a crucial role in cell-type interconversions during the normal development of organogenesis, can induce the acquisition of mesenchymal traits and the expression of stemness markers in cancer cells. Understanding this key process in tumor initiation and metastasis may explain how CSCs form and suggest a possible avenue for their therapeutic manipulation (Karnoub *et al.*, 2007; Mani *et al.*, 2008; Scheel *et al.*, 2011). To improve the ability for diagnosis and treatment and reduce the danger of cancer, many scientists have explored the etiological mechanisms at the cellular or molecular level. Increasing evidence supports the view that a change in epigenetic modifications, microenvironment or specific-gene expression occurs in many types of cancer progression (Calin and Croce, 2006; Iorio and Croce, 2009; Hermeking, 2012). Some of the small aberrant effective molecules, like microRNAs, have the potential to be markers for diagnosis and prognosis, or serve as effective targets for therapeutic interventions (Heneghan *et al.*, 2010; Jansson and Lund, 2012).

MicroRNAs are a group of small molecules that are highly conserved evolutionarily and encoded by about 1% of the genome in most species (Bartel, 2004). Since the discovery of *lin-4* (Lee *et al.*, 1993), the exploration of this kind of single-stranded RNA has been the focus of much attention. With the help of polymerase II, a primary microRNA is transcribed from a coding-gene intron or the intergenic region like other regular mRNA. Following further processing with a series of enzymes or functional proteins, such as Drosha, Exportin 5, and Dicer, the mature microRNA can be spliced from its precursor (Kim, 2005). Although existing as mini bodies of only about 22 nt, microRNAs play an important role in post-transcriptional regulation by binding to their targets in an incomplete base-pairing manner, particularly in mRNA 3' untranslated regions, causing translation suppression or mRNA degradation (Bartel, 2004; O'Hara *et al.*, 2009). Many studies have proved that microRNAs are highly specific in their expression in different tissues and development stages (Lagos-Quintana *et al.*, 2002), and play an essential role in diverse biological events, such as cell proliferation, differentiation, and apoptosis (Croce and Calin, 2005). Recent data have demonstrated that microRNAs located in one cluster can work together in mapping a regulatory network by binding their target to fulfill the same mission, which enhances their function in both physiological and pathological processes (Tanzer and Stadler, 2004).

It is well known that some microRNAs are deregulated in numerous kinds of disease, including breast cancer. Abnormal microRNA expression involved in the cancer process provides a suitable entry point to explore its functional role in cancer progression. With the rapid development of sequencing and molecular biology techniques, amplified or decreased microRNA can easily be detected and functional microRNAs readily identified. Up-regulated 'oncogenic microRNAs' inhibit the expression of potential tumor suppressive genes, while down-regulated 'tumor suppressive microRNAs' cause the augmentation of downstream signal pathways responsible for tumor development. Both can promote the development and progression of cancer. These two kinds of population may participate in pathogenic pathways by targeting the same or different molecules within a complicated regulatory network. Different cancers may share common aberrant microRNAs, although cancer-specific microRNAs are the major population. In this review, we focus on recent evidence about the role of oncogenic and tumor suppressive microRNAs in breast cancer and discuss their applications to clinical approaches, especially their potential to be diagnostic and prognostic markers and therapeutic targets (Iorio *et al.*, 2005; Iorio and Croce, 2009; Andorfer *et al.*, 2011; Melo and Esteller, 2011).

2 Oncogenic microRNAs in breast cancer

2.1 miR-21

miR-21 is one of the most well-known tumor-promoting microRNAs in many species of cancer, whose expression is dramatically up-regulated in

breast cancer. Numerous studies have confirmed its association with advanced clinical stages of cancer, tumor metastasis, and poor prognosis. Several genes associated with tumor growth and metastasis, such as tumor suppressor tropomyosin 1 (*TPM1*), programmed cell death 4 (*PDCD4*), TIMP metallopeptidase inhibitor 3 (*TIMP3*), and phosphatase and tensin homolog (*PTEN*), have been proved to be targeted by miR-21 (Meng *et al.*, 2007; Zhu *et al.*, 2007; Frankel *et al.*, 2008; Qi *et al.*, 2013). The overexpression of miR-21 in tumor cells could alleviate their function in 'suppressing' cell apoptosis and death. By regulating target genes and modulating the following downstream pathway, miR-21 aberrant expression results in tumor metastasis and invasion. Knocking down its expression could attenuate its contribution in the cancer process. Recent studies also found high levels of miR-21 in breast cancer patient serum (Wang *et al.*, 2010; Asaga *et al.*, 2011). Its increased expression can also be detected in a variety of other malignancies including glioblastoma, ovarian cancer, lung cancer, and colorectal cancer (Chan *et al.*, 2005; Yanaihara *et al.*, 2006; Iorio *et al.*, 2007; Schee *et al.*, 2012; Wang and Zhang, 2012), indicating that miR-21 may have diagnostic and prognostic values.

2.2 miR-155

miR-155 is frequently up-regulated in breast tumor tissue. Its ectopic expression influences tumor cell survival and chemosensitivity through down-regulating forkhead box O3 (*FOXO3a*), whereas knocking down the expression of miR-155 can enhance cell chemosensitivity and mediate apoptosis (Kong *et al.*, 2010). Other work supports the suggestion that miR-155 plays its oncogenic role by targeting suppressor of cytokine signaling 1 (*SOCS1*). Inflammation stimuli can up-regulate miR-155 in breast cancer cells, leading to the activation of *STAT3* (signal transducer and activator of transcription 3). This indicates a possible functional link between inflammation and cancer mediated by miR-155 (Jiang *et al.*, 2010). In addition, *caspase-3*, a potent suppressor of apoptosis, has been reported to be a target of miR-155 (Ovcharenko *et al.*, 2007).

2.3 miR-182

EMT is the initial event of breast tumor-initial cell (BT-IC)-associated metastasis. β-Catenin, a key regulator in EMT, can bind to the promoter of miR-182 and positively regulate miR-182 expression in breast cancer cells. miR-182 is also overexpressed in human breast tumor tissues. By repressing its target gene reversion-inducing-cysteine-rich protein with kazal motifs (*RECK*), ectopic expression of miR-182 induces matrix metallopeptidase 9 (*MMP-9*) activity, cell invasion, and colony formation, and further increases tumorigenicity and invasiveness (Chiang *et al.*, 2013). Other data also demonstrate that both *MIM* (missing in metastasis, which activates the Ras homolog family member A) and *FOXO1* (forkhead box protein O1, a putative tumor suppressor transcription factor) are the targets of miR-182 and are involved in its promotion of breast cancer metastasis (Guttilla and White, 2009; Lei *et al.*, 2014).

2.4 miR-10b

In metastatic breast cancer, miR-10 has been confirmed as a 'metastasis miR', a crucial downstream effector of twist-related protein 1 (TWIST1) (Ma *et al.*, 2007). By numbing the homeobox D10 (*HOXD10*) tumor suppressor signaling pathway and regulating T lymphoma invasion and metastasis 1 (*Tiam1*)-mediated Rac activation, miR-10b makes a significant contribution to the invasion and migration of breast cancer cells (Moriarty *et al.*, 2010; Haque *et al.*, 2011). The expression level of miR-10b is positively correlated with pathological grading, clinical staging, and lymph node metastasis. A novel E-cadherin-related mechanism has recently been shown to be involved in miR-10b modulation of breast cancer metastasis (Liu *et al.*, 2012).

2.5 miR-27a

miR-27a, which is overexpressed in breast cancer cells, promotes cell viability by promoting cell cycle traverse and inhibiting cell death by targeting *FOXO1* (Guttilla and White, 2009). Tang *et al.* (2014) revealed that increased miR-27a expression is associated with reduced expression of *ZBTB10* (zinc finger and BTB domain containing 10), which has been validated as a target of miR-27a. This results in the promotion of angiogenesis by mediating breast CSC properties. Moreover, the zinc finger protein *ZBTB10* gene, as a putative specificity protein (*Sp*) repressor, which contributes to cancer angiogenic ability, helps miR-27b fulfill its oncogenic function (Mertens-Talcott *et al.*, 2007).

2.6 miR-9

Tumor cell motility and invasiveness is an important reference point of tumor metastasis. miR-9, which is expressed more highly in breast tumor tissue than in normal tissue, promotes the oncogenic process by negatively regulating E-cadherin. Also, miR-9 indirectly induces the expression of vascular endothelial growth factor (VEGF) and contributes to angiogenesis at the same time, promoting cancer development. Thus, miR-9 is an example of a signal microRNA that may regulate one process with different targets (Ma *et al.*, 2010a).

3 Tumor suppressive microRNAs in breast cancer

3.1 let-7 family

The let-7 families are the most ancient and conserved microRNAs. They were discovered in *Caenorhabditis elegans* (Johnson *et al.*, 2003). They function as well-recognized tumor suppressive microRNAs in a heterochronic pathway that is necessary for cancers, including breast cancer, to undergo correct initiation, differentiation, and metastasis at the appropriate time (Nimmo and Slack, 2009). In the initial stage of a breast tumor, let-7a is involved in BT-IC stem cell-like activities by silencing its target genes *H-Ras* (transforming protein p21) and *HMGA2* (high-mobility group AT-hook 2). Overexpression of let-7 effectively delays the development of breast cancer (Yu *et al.*, 2007). Sun *et al.* (2012) claimed that down-regulation of let-7b/c in breast CSCs caused let-7b/c to lose its ability to restrain Ras mRNA, resulting in the activation of p-Ras and p-ErK. This revealed an important role of let-7 in maintaining breast cancer cell stemness. let-7b is often down-regulated in lymph node metastases of breast cancer cells. By silencing four target genes related to the actin cytoskeleton pathway, *PAK1* (serine/threonine-protein kinase 1), *DIAPH2* (also known as protein diaphanous homolog 2), *RDX* (radixin), and *ITGB8* (integrin β-8), breast migration and invasion are greatly inhibited (Hu *et al.*, 2013).

3.2 miR-145

miR-145 exerts its tumor suppressive function by silencing different target genes in stage-specific events. Insulin receptor substrate-1 (*IRS-1*), which is necessary for BT-IC differentiation, has been shown to be a direct target of miR-145 (Rubin *et al.*, 2007). *ER-α* is reported to be negatively regulated by miR-145 at two complementary sites, thereby mediating breast cancer cell growth (Spizzo *et al.*, 2010). Rhotekin (RTKN) protein expression can be significantly reduced by miR-145, resulting in inhibition of cell growth and induced apoptosis (Wang *et al.*, 2009). miR-145 also can directly target mucin 1 (*MUC1*), a metastasis gene, leading to a reduction of β-catenin and cadherin 11 and giving rise to suppressed cell invasion and lung metastasis (Sachdeva and Mo, 2010). Additionally, miR-145 was proved to inhibit breast cancer cell EMT by blocking the expression of octamer-binding transcription factor 4 (*Oct4*) (Hu *et al.*, 2012).

3.3 miR-200 family

miR-200 families have a moderating effect on controlling the transition between CSC-like and non-stem-cell-like phenotypes. Under stem-like phenotypes, through epigenetic modifications, the miR-200b-200a-429 cluster was silenced, followed by the repressed expression of the miR-200c-141 cluster (Lim *et al.*, 2013). miR-200 families also exert their effects in distinct metastatic stages in a moesin-dependent manner. miR-200b can regulate tumor cell plasticity and metastasis as a tumor suppressor (Li X. *et al.*, 2013). However, Dykxhoorn *et al*. (2009) found that while miR-200b/c promotes mesenchymal-to-epithelial cell transition (MET) by inhibiting *ZEB2* (zinc finger E-box-binding homeobox 2) expression, it also promotes macroscopic metastases in breast cancer cell lines. Therefore, the whole story of the miR-200 family needs further investigation.

3.4 miR-205

miR-205, which is significantly down-regulated in human breast tumor tissue, negatively regulates EMT by targeting *ZEB1* and *ZEB2*. It is also expressed at low levels in mesenchymal breast cancer cell lines and triple negative breast cancers (Gregory *et al.*, 2008; Radojicic *et al.*, 2011). HER2 is a hallmark of aggressive breast tumors, and is driven largely through phosphorylation of HER3. By targeting *HER3*, miR-205 could interfere with the HER receptor family-mediated proliferative pathway (Iorio *et al.*, 2009). In *in vitro* experiments, ectopic expression of miR-205 in breast cancer cells suppressed cell proliferation,

growth, and invasion by directly targeting *HER3* and *VEGF-A* (Wu *et al.*, 2009; Wang *et al.*, 2013). Most recently, Chao *et al*. (2014) indicated that miR-205 secreted from the tumor stroma can help promote a cancer-associated stem cell phenotype, suggesting that targeting-miR-205 may have a potential therapeutic value.

3.5 miR-335

Deletion or epigenetic silencing of miR-335 is a common event in breast cancer metastasis (Png *et al.*, 2011). Tavazoie *et al*. (2008) reported that miR-335 can repress breast cancer migration and invasion through targeting SRY-related HMG-box 4 (*SOX4*) and *tenascin C*. By simultaneously regulating the known breast cancer 1 (*BRCA1*) activators, *ER-α*, *IGF1* and *Sp1*, and the repressor *ID4*, miR-335 exerts its tumor-suppressive function by decreasing cell viability and inducing apoptosis (Heyn *et al.*, 2011).

3.6 miR-19a

The TME exerts a dominant role in the cancer process. Tumor associated-macrophages (TAMs) are the major components of the TME comprising about 40%. The transition of TAMs from a pro-immune (M1-like) phenotype to an immune-suppressive (M2-like) phenotype is one of the hallmarks of malignancy (Mukhtar *et al.*, 2011). Down-regulated miR-19a in TAMs, induced by the TME, is able to maintain the phenotype of TAMs by targeting Fos-related antigen 1 (*Fra-1*) and other genes in its downstream signaling pathway. The reintroduction of miR-19a to this kind of macrophage can promote transformation of M1 to M2, resulting in the enhancement of migration and invasion of breast cancer cells (Yang *et al.*, 2014).

4 Other breast cancer-associated microRNAs

There is an increasing body of data identifying the involvement of microRNAs in the development and progression of breast cancer. The most recent investigations of breast cancer-associated microRNAs with identified targets, including those mentioned above, are listed in Tables 1 and 2.

Table 1 Oncogenic microRNAs associated with breast cancer

MicroRNA (family)	Identified target	Associated event	Reference
miR-21	*TPM1*, *PDCD4*, *TIMP3*, *PTEN*	Cancer metastasis	Meng *et al.*, 2007; Zhu *et al.*, 2007; Frankel *et al.*, 2008; Li J. *et al.*, 2013
miR-155	*FOXO3a*, *SOCS1*, *caspase-3*, *TP53INP1*	Cell proliferation and apoptosis	Ovcharenko *et al.*, 2007; Jiang *et al.*, 2010; Kong *et al.*, 2010; Zhang *et al.*, 2013
miR-182	*RECK*, *MIM*, *FOXO1*	Cell invasion, colony formation	Guttilla and White, 2009; Chiang *et al.*, 2013; Lei *et al.*, 2014
miR-10b	*HOXD10*, *Tiam1*	Cell invasion, migration	Moriarty *et al.*, 2010; Haque *et al.*, 2011
miR-27a	*HOXO1*, *ZBTB10*	Cell viability, angiogenesis	Mertens-Talcott *et al.*, 2007; Guttilla and White, 2009; Tang *et al.*, 2014
miR-9	*E-cadherin*	Cell motility and invasiveness, angiogenesis	Ma *et al.*, 2010a
miR-22	*TET* family	EMT	Song *et al.*, 2013
miR-181a	*Bim*	EMT, cancer metastasis	Taylor *et al.*, 2013
miR-373, miR-520c	*CD44*	Cell migration and invasion	Huang *et al.*, 2008
miR-375	*RASD1*	Cell proliferation	de Souza Rocha Simonini *et al.*, 2010
miR-221/222	*TRPS1*, *ADIPOR1*, *p27Kip1*	Cancer metastasis, tumor growth, EMT	Stinson *et al.*, 2011; Hwang *et al.*, 2013; Nassirpour *et al.*, 2013
miR-632	*DNAJB6*	Cancer metastasis	Mitra *et al.*, 2012
miR-7, miR-218	*HoxB3*	Cell cycle, colony formation	Li *et al.*, 2012
miR-374a	*WIF1*, *PTEN*, *WNT5A*	Cancer metastasis	Cai *et al.*, 2013

Table 2 Tumor suppressive microRNAs associated with breast cancer

MicroRNA (family)	Identified target	Associated event	Reference
let-7 family	*H-Ras*, *HMGA2*, *PAK1*, *DIAPH2*, *RDX*, *ITGB8*	Tumor initiation, cell differentiation and metastasis, cell stemness maintenance	Yu *et al.*, 2007; Nimmo and Slack, 2009; Sun *et al.*, 2012; Hu *et al.*, 2013
miR-145	*IRS-1*, *ER-α*, *RTKN*, *MUC1*, *OCT4*, *N-Ras*, *VEGF-A*	Tumor growth, cell differentiation, invasion and metastasis, angiogenesis	Rubin *et al.*, 2007; Wang *et al.*, 2009; Sachdeva and Mo, 2010; Spizzo *et al.*, 2010; Hu *et al.*, 2012; Zou *et al.*, 2012
miR-200 family	*ZEB1*, *ZEB2*, *HER3*, *Sec23a*, *SIRT1*	EMT, tumor growth and metastasis	Dykxhoorn *et al.*, 2009; Ahmad *et al.*, 2011; Eades *et al.*, 2011; Korpal *et al.*, 2011; Li X. *et al.*, 2013; Lim *et al.*, 2013
miR-205	*ZEB1*, *ZEB2*, *HER3*, *VEGF-A*	EMT, cell proliferation and invasion, CSC stemness promotion	Gregory *et al.*, 2008; Iorio *et al.*, 2009; Wu *et al.*, 2009; Wang *et al.*, 2013; Chao *et al.*, 2014
miR-335	*SOX4*, *tenascin C*, *ER-α*, *IGF1*, *RSP1*, *ID4*	Tumor migration and invasion, cell viability and apoptosis	Tavazoie *et al.*, 2008; Heyn *et al.*, 2011; Png *et al.*, 2011
miR-126	*IGFBP2*, *MERTK*, *PITPNC1*	Metastatic angiogenesis	Png *et al.*, 2012
miR-30 family	*Ubc9*, *TWF1*, *Vimentin*, *KRAS*, *MTDH*	CSC self-renewal, cell apoptosis, EMT	Yu *et al.*, 2010; Cheng *et al.*, 2012; Tanic *et al.*, 2012; Bockhorn *et al.*, 2013
miR-146a/b	*IL-1-RSK*, *NFRSF-6*	Cancer metastasis	Bhaumik *et al.*, 2008
miR17-20 cluster	*Cyclin D1*	Cell proliferation	Yu *et al.*, 2008
miR-26b	*SLC7A11*	Cell apoptosis	Liu *et al.*, 2011
miR-290	*Arid4b*	Cell apoptosis	Bhaumik *et al.*, 2008
miR-27b	*CYP1B1*	Tumor growth	Tsuchiya *et al.*, 2006
miR-31	*Integrin-α5*, *radixin*, *RhoA*, *WAVE3*, *PRKCE*	Cancer metastasis, cell apoptosis	Sossey-Alaoui *et al.*, 2011; Valastyan *et al.*, 2011; Korner *et al.*, 2013
miR-125a/b	*HER2*, *HER3*	Cell invasion	Wang *et al.*, 2013
miR-203	*SNAI2*	EMT, cell invasion	Ding *et al.*, 2013
miR-224	*CDC42*, *CXCR4*	Cancer metastasis	Zhu *et al.*, 2010
miR-20b	*HIF-1*, *STAT3*	Angiogenesis	Cascio *et al.*, 2010
miR-206	*Cyclin D2*	Cell proliferation	Zhou *et al.*, 2013
miR-342	*HER2*	Cell apoptosis	Cittelly *et al.*, 2010
miR-519c	*HIF-1α*	Angiogenesis	Cha *et al.*, 2010
miR-16	*Cyclin E*	Tumor growth	Rivas *et al.*, 2012
miR-290	*Arid4b*	Tumor growth, cell apoptosis	Goldberger *et al.*, 2013
miR-497	*Cyclin E1*	Cell proliferation and invasion	Luo *et al.*, 2013
miR-133a	*EGFR*	Cell cycle and proliferation	Cui *et al.*, 2013
miR-26a	*MCL-1*	Cell proliferation and apoptosis	Gao *et al.*, 2013
miR-720	*TWIST1*	Cell invasion and migration	Li *et al.*, 2014
miR-7	*KLF4*	Cancer metastasis	Okuda *et al.*, 2013
miR-98	*MMP1*, *ALK4*	Angiogenesis	Zou *et al.*, 2012
miR-542-3p	*Angiopoietin-2*	Angiogenesis	He *et al.*, 2014
miR-148a/152	*IGF-IR*, *IRS1*	Angiogenesis	Xu *et al.*, 2013

5 MicroRNAs as possible markers for diagnosis and prognosis in breast cancer

With the rapid development of gene microarrays and experimental technologies, an increasing and encouraging number of studies are demonstrating the contribution of microRNAs to the pathogenesis and progression of breast cancer. Indeed, abnormal microRNA expression patterns are closely related to specific tumor stages, lymph node metastasis, poor survival, disease outcomes and responses to specific therapies in many types of cancer. Apart from those with traditional intercellular functions, diverse forms of microRNA have been found in the past decade: serum, saliva, urine, and milk all contain microRNAs, which are packaged by microvesicles or exosomes, or exist as compounds with protective modifications (Gilad *et al.*, 2008; Mitchell *et al.*, 2008; Park *et al.*, 2009; Chen *et al.*, 2010; Hanke *et al.*, 2010).

Considering that the profiling of microRNAs correlates with biological processes more precisely than gene expression profiling, profiling of microRNAs has been used to diagnose breast cancer at an early stage and determine the prognosis of therapy in breast cancer patients. Blenkiron *et al*. (2007) first analyzed miRNA expression and genomic changes in human breast cancer. They used the distinct miRNA signatures of different molecular breast tumor subtypes (Luminal A, Luminal B, basal-like, $HER2^+$, and normal-like) for characterization in prognosis. Soon after, Farazi *et al.* (2011) accomplished deep sequencing of microRNA in breast tumors and showed that the microRNA 17-92 cluster has a high level in triple-negative breast carcinomas, distinct from other tumor subtypes. Integrated mRNA and microRNA expression profiling in breast cancer brings us more information about microRNAs associated with prognosis. High levels of miR-767-3p, miR-128a, and miR-769-3p are associated with a poor prognosis, the same as miR-27b, miR-144, and/or miR-210 in ER-negative cases (Buffa *et al.*, 2011). let-7 family members are down-modulated in metastasis lymph node or breast cancer samples with a high proliferation index, suggesting that a deficiency of let-7 family microRNAs is associated with a poor prognosis (Iorio *et al.*, 2005). Also, the miR-106b-25 cluster is available to predict relapse more quickly (Smith *et al.*, 2012). miR-181a (Taylor *et al.*, 2013) and the miR-221/miR-222 cluster (Chen *et al.*, 2013) are valuable diagnostic and prognostic candidates because of their positive correlation with tumor development. Above all, this indicates that microRNAs have the potential to be diagnostic and prognostic markers. Moreover, several microRNAs have been identified that are specifically deregulated in the blood plasma of breast cancer patients compared to healthy subjects (Cookson *et al.*, 2012; Cuk *et al.*, 2013). The expression of miR-451, miR-21, and miR-16 in the serum of breast cancer patients was amplified compared to that of healthy individuals (Nguyen *et al.*, 2014). MicroRNA aberrant expression has also been quantified in breast cancer patient serum. miR-21, miR-106a, and miR-155 were significantly overexpressed, whereas the expression of miR-126, miR-199a, and miR-335 in tumor specimens was opposite to that of normal specimens (Wang *et al.*, 2010). Interestingly, the above elevated microRNA levels were drastically lowered in postoperative compared with preoperative cases. All of these findings support the suggestion that these circulating microRNAs in serum can serve as diagnostic markers for breast cancer (Wang *et al.*, 2010; Cuk *et al.*, 2013; Ng *et al.*, 2013).

6 Analysis of the prospects and challenges for microRNA-targeted therapy in breast cancer

Despite advances in detection and therapy, breast cancer is still a major challenge for our medical workers. Traditional treatments such as surgery, chemotherapy, and radiotherapy, inevitably have side effects, although they have been undeniably effective. Accompanied by the emerging evidence for the participation of microRNAs in cancer, the potential usefulness of microRNA-based therapy in cancer is now being exploited. By using microRNAs as both targets and tools, microRNA-based therapy has proved to be feasible and efficacious in preclinical models. The inhibition of oncogenic miR-21 reduces tumor development and metastatic potential by way of pro-apoptotic and anti-proliferative effects (Si *et al.*, 2007). Re-introduction of miR-205 improves the responsiveness to tyrosine kinase inhibitors through numbing HER3 in breast cancer cells (Meng *et al.*,

2006). Knocking down miR-34 has a radiosensitizing effect in p53-mutant breast cancer (Weidhaas *et al.*, 2007). Systemic treatment of tumor-bearing mice with miR-10b antagomirs also produced satisfactory curative effects in suppressing breast cancer metastasis (Ma *et al.*, 2010b). Additionally, in ER-positive metastatic breast cancer, let-7 administration has proved to be an effective method against mouse-model breast cancer by regulating apoptosis and CSC differentiation (Barh *et al.*, 2010). There are many impressive cases suggesting that microRNAs may be a viable approach to augment current cancer therapies.

Results to date provide the experimental bases for the use of microRNAs as both targets and tools in anti-cancer therapy, but there are at least three essential issues to address to translate microRNA-based therapy advances from fundamental experiments into medical practice: (1) engineered animal models need to be explored to study cancer-associated microRNAs, fully controlling all their effects on every tumor event; (2) the delivery efficiency of miRNAs/anti-miRNAs *in vivo* needs to be improved by solving the problems of degradation and instability; (3) the specificity and targeted ability of the delivery system need to be enhanced, avoiding damage to normal tissues. To overcome these issues, modified microRNAs and suitable carriers need ongoing development. Adenovirus (Esquela-Kerscher *et al.*, 2008) and adeno-associated virus (AVV) (Kota *et al.*, 2009), which are used for expressing microRNA, are more useful than synthetic double-stranded hairpins for overcoming the vulnerability of unmodified dsRNAs. To achieve loss-of-function in microRNAs, 2′-*O*-methyl oligonucleotides (Meister *et al.*, 2004), antagomirs (intravenous administration of cholesterol-conjugated AMOs) (Krützfeldt *et al.*, 2005), locked nucleic acid (LNA)-oligonucleotides (Ørom *et al.*, 2006), and microRNA sponges (microRNA inhibitory transgenes) (Ebert *et al.*, 2007) have been employed to inhibit exogenously introduced microRNAs with high specificity. Also, active small molecule clinical compounds and peptide nucleic acids (PNAs) (Brognara *et al.*, 2012), such as 'azobenzene' (Gumireddy *et al.*, 2008), have been tested for their ability to inhibit the expression of specific microRNAs. To improve cellular delivery, the methods for short-interfering RNA (siRNA) or short heteroduplex RNA (shRNA) could also be applied to microRNAs (Dykxhoorn *et al.*, 2006), although excessive shRNA may increase the probability of off-target silencing and elicit non-specific effects. Furthermore, a highly specific ligand-targeting of liposomal nanoparticles (NPs) (Liao *et al.*, 2011) to solid tumors has been shown to improve tumor selectivity and drug sensitivity in a drug test, by enhancing solid-tumor penetration and uptake of tumor cells. This kind of delivery system eliminates accumulation in normal tissues and circulation, which could also help to avoid systemic toxicity induced of microRNA delivery.

7 Conclusions

In recent decades, increasing efforts have been made to elucidate the molecular mechanisms involved in breast cancer. The results from this work support the crucial role played by dysregulation of specific microRNAs in breast cancer progression. The molecular mechanisms underlying the pathological process mediated by microRNAs have largely been demonstrated. Based on the details discussed above, microRNAs have tremendous potential for clinical diagnosis and prognosis. Defining the functional network connecting microRNAs and their targets will contribute to their usefulness as a therapeutic target. Although there are infinite possibilities for using microRNAs in clinical treatments, some puzzling issues remain to be addressed. For instance, among the numerous deregulated microRNAs in breast cancer, we do not know which is the most representative for each cancer stage. We do not know whether the microRNAs found at high levels in the serum of breast cancer patients are released from the tumor, and what are their major functions. Single microRNAs could target multi-genes, and mRNA might also bind to different microRNAs, so it is possible that one microRNA could participate in various events in both cancer progression and normal tissue development, creating uncertainty in microRNA-based therapy. In the future, under-explored problems such as how to use microRNAs as markers for diagnosis and prognosis, and how to apply miRNA-based therapy in clinics to treat human cancers, may be the biggest challenges to be solved.

Compliance with ethics guidelines

Wei WANG and Yun-ping LUO declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：MicroRNA 在乳腺癌发展过程中的作用机制及临床应用分析

概　要：搜集已报道的在乳腺癌发生发展过程中有重要作用的 microRNA 信息，结合相关肿瘤模型的实验数据，评估 microRNA 在乳腺癌诊断和治疗方面的应用前景。以“癌基因”和“癌抑制基因”为分类依据，全面地总结归纳乳腺癌相关 microRNA 的功能和作用机制，进一步从临床诊断标记物和治疗靶点的层面分析 microRNA 的潜在临床应用价值。表 1 和 2 总结了 microRNA 参与乳腺癌发展过程的具体事件及其调控的靶基因。同时，本文还深入探讨 microRNA 作为临床诊断标记物及治疗靶点的可行性，揭示已有研究的不足之处，为今后的相关工作方向提供一些建议。

关键词：MicroRNA；乳腺癌；癌基因；抑癌基因；诊断标记物；治疗靶点