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REVIEW

Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes

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MicroRNAs (miRNAs) are short non-coding RNAs that regulate the function of target genes at the post-transcriptional phase. miRNAs are considered to have roles in the development, progression and metastasis of cancer. Recent studies have indicated that particular miRNA signatures are correlated with tumor aggressiveness, response to drug therapy and patient outcome in breast cancer. On the other hand, in routine clinical practice, the treatment regimens for breast cancer are determined based on the intrinsic subtype of the primary tumor. Previous studies have shown that miRNA expression profiles of each intrinsic subtypes of breast cancer differ. In hormone receptor-positive/human epidermal growth factor receptor 2 (HER2)-negative breast cancer, miRNA expressions are found to be correlated with endocrine therapy resistance, progesterone receptor expression and heat shock protein activity. Some miRNAs are associated with resistance to HER2-targeted therapy and HER3 expression in HER2-positive breast cancer. In triple-negative breast cancer, miRNA expressions are found to be associated with BRCA mutations, immune system, epithelial–mesenchymal transition, cancer stem cell properties and androgen receptor expression. As it has been clarified that the expression levels and functions of miRNA differ among the various subtypes of breast cancer, and it is necessary to take account of the characteristics of each breast cancer subtype during research into the roles of miRNA in breast cancer. In addition, the discovery of the roles played by miRNAs in breast cancer might provide new opportunities for the development of novel strategies for diagnosing and treating breast cancer.
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INTRODUCTION

Breast cancer exhibits biological heterogeneity in terms of its prognosis and sensitivity to anti-cancer agents. Numerous studies are currently being conducted in an attempt to identify markers of cell growth and differentiation, which are involved in tumor formation and progression, in breast cancer.[1]

Breast cancer has recently been classified into several intrinsic subtypes, such as the luminal A, luminal B, human epidermal growth factor receptor type 2 (HER2)-enriched, basal-like, claudin-low and normal-like subtypes, based on semi-unsupervised gene expression array analyses.[2] In routine clinical practice, these intrinsic subtypes are identified based on a combination of immunohistochemical analyses of estrogen receptor (ER), progesterone receptor (PgR) and HER2 expression, and the Ki67 labeling index (Figure 1), and the following practical classification of intrinsic subtypes was proposed at the St Gallen consensus meeting of breast cancer: luminal A-like type (ER-positive and/or PgR-positive, HER2-negative, low proliferation and low tumor burden), luminal B-like type (ER-positive and/or PgR-positive, HER2-negative, high proliferation and high tumor burden), hormone receptor-positive and HER2-positive type, hormone receptor-negative and HER2-postive type and triple-negative (TN) type (hormone receptor-negative and HER2-negative)[3,4] (Table 1). This classification has provided valuable information about the tumor biology of each subtype and facilitates the appropriate selection of hormonal, chemotherapeutic and HER2-targeting agents during the treatment of breast cancer. The Cancer Genome Atlas Network indicated that the biologic finding of breast cancer subtypes caused by different subsets of genetic and epigenetic abnormalities.[1]

On the other hand, microRNAs (miRNAs) are small (19–22 bases in length) non-coding RNAs, and negatively regulate protein-coding gene expressions by promotion of mRNA degradation or inhibition of translation.[5] In breast cancer, various miRNAs have been shown to be deleted or to exhibit downregulated or upregulated expression. Recently, it has been demonstrated that aberrational miRNAs targeting to several cancer-related genes induce cancer initiation, progression, metastasis or drug resistance. In breast cancer, some miRNAs have been shown to upregulate the functions of oncogenes while others stimulate tumor suppressors.[6–11] Previous studies have demonstrated that the miRNA expression profiles of each intrinsic subtype of breast cancer differ.[12,13] In the present review, reports of miRNA research as

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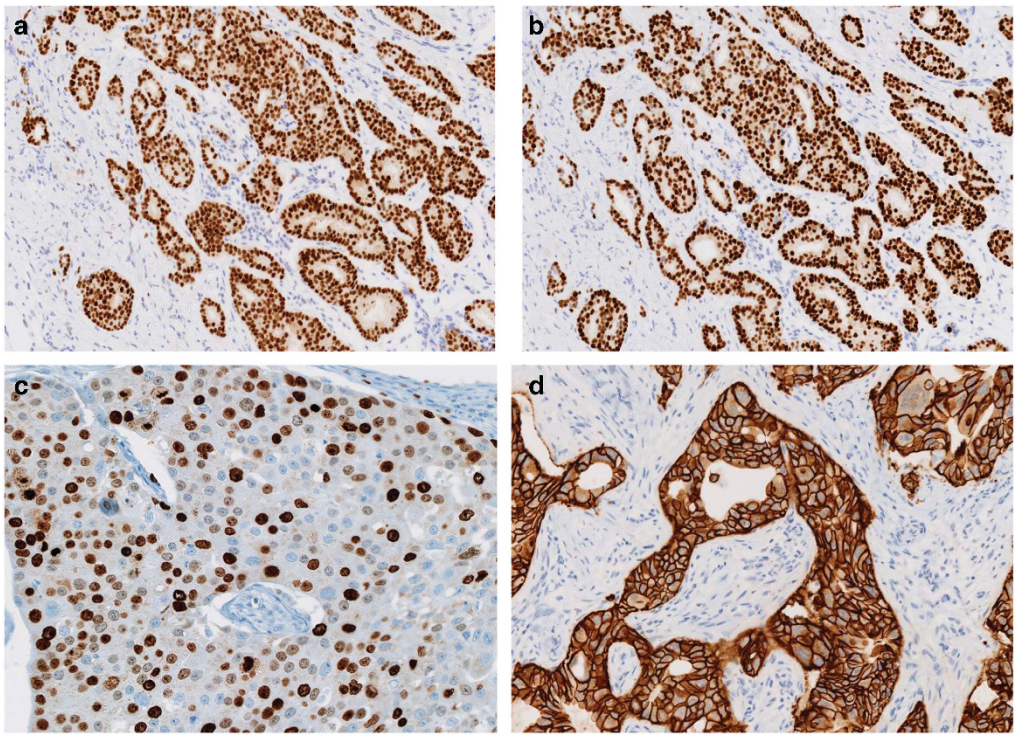

Figure 1 Immunohistochemical expression levels of the ER, PgR, HER2 and Ki67. (**a**) Strong ER staining was detected in the cancer cell nuclei. (**b**) Strong PgR staining was detected in the cancer cell nuclei. (**c**) The tumor cells exhibited a high Ki67-labeling index. (**d**) Strong HER2 staining was detected on the cancer cell membrane.

Table 1 Treatment-oriented classification of sub-groups of breast cancer and combined definitions proposed in St Gallen consensus meeting of 2013 and 2015

Clinical subtypes	*Definitions*
Luminal A-like	ER and/or PgR positive (⩾1%), multiparameter molecular marker 'favorable prognosis' if available. High ER/PgR and clearly low Ki67. Low or absent nodal involvement, smaller tumor size
Luminal B-like	ER and/or PgR positive (⩾1%), multiparameter molecular marker 'unfavorable prognosis' if available. Lower ER/PgR with clearly high Ki67. More extensive nodal involvement, histological grade 3, extensive lymphovascular invasion, larger tumor size
HR-positive and HER2-positive	ER and/or PgR positive (⩾1%), ASCO/CAP HER2 guidelines
HR-negative and HER2-positive	ER and PgR negative (<1%), ASCO/CAP HER2 guidelines
Triple negative	Negative ER, PgR and HER2

Abbreviations: ASCO/CAP, The American Society of Clinical Oncology and College of American Pathologists; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; PgR, progesterone receptor.

to the relationship between miRNA expressions and breast cancer subtypes were searched using MEDLINE system and we focus on recent findings regarding the associations between miRNA expressions and the various subtypes of breast cancer.

BIOGENESIS AND FUNCTIONS OF miRNA IN BREAST CANCER

miRNA was initially reported by Lee *et al.* in 1993,[14] and it was identified as a short non-coding RNA with a function of mediating post-transcriptional gene silencing. More than 2500 human miRNAs have subsequently been recorded in the miRBase, a searchable database of published miRNAs.[15] miRNAs mediate mRNA degradation and inhibit translation. Most miRNA genes are transcribed by RNA polymerase II in the nucleus, and primary miRNAs (pri-miRNAs) are capped, spliced and polyadenylated.[16,17] Pri-miRNAs are cleaved by a microprocessor composed of the double-stranded RNase III enzyme Drosha and the double-stranded RNA-binding protein DiGeorge syndrome critical region 8 (DGCR8).[17–19] Hairpin-shaped precursor miRNAs (pre-miRNAs) [20,21] are produced from pri-miRNAs by cleavage using Drosha, and they are exported to cytoplasm from nucleus by exportin 5,[22–24] before being further processed by Dicer, an RNase III enzyme that interacts with the 5′ and 3′ ends of pre-miRNA.[25,26] In order to exert their effects, mature miRNAs require ribonucleoprotein complexes, such as RNA-induced silencing complexes, to be assembled.[27] The mature single-stranded miRNAs that interact with the Argonaute proteins (AGO1, AGO2, AGO3 and AGO4) in RNA-induced silencing

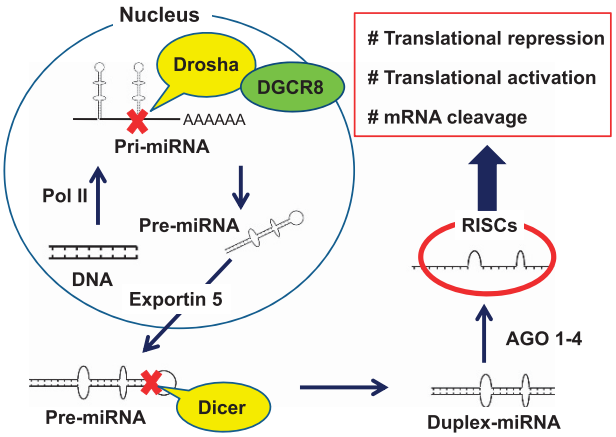

Figure 2 Biogenesis and function of microRNA (miRNA). miRNA genes are transcribed by RNA polymerase II (Pol II) in the nucleus to generate primary miRNA (pri-miRNA). Pri-miRNAs are cleaved by a microprocessor composed of a double-stranded RNase III enzyme (Drosha) and the double-stranded RNA-binding protein DiGeorge syndrome critical region 8 (DGCR8). Drosha cleaves pri-miRNA into hairpin-shaped precursor miRNA (pre-miRNA). The pre-miRNAs are then exported from the nucleus to the cytoplasm by exportin 5, before being further processed by Dicer, an RNase III enzyme that interacts with the 5′ and 3′ ends of pre-miRNA. In order to exert their effects, mature miRNA require ribonucleoprotein complexes, such as RNA-induced silencing complexes (RISCs), to be constructed. Mature single-stranded miRNA interact with the Argonaute proteins (AGO) in RISCs to regulate their target genes.

complexes typically bind to the 3′-untranslated regions of their cytosolic mRNA targets. These miRNAs inhibit the translation or promote the deadenylation and degradation of mRNA transcripts.[28–30] This miRNA processing pathway is shown in Figure 2. Overexpressions of oncogenic miRNAs that inhibit tumor suppressor genes are associated with cancer development. On the other hand, reduction or loss of expression of tumor-suppressive miRNAs induce upregulated expression of their target oncogenes.[5] In breast cancer, a number of miRNAs have been found to have oncogenic or tumor-suppressive effects, and miRNAs have important roles in tumor initiation, drug resistance and metastasis.[6–11] The function of miRNAs associated with tumor development in breast cancer are shown in Table 2.

miRNA AND INTRINSIC SUBTYPES OF BREAST CANCER

Previous studies have reported that the various breast cancer subtypes exhibit different molecular miRNA signatures.[6,12,13,31] Blenkiron *et al.*[13] profiled 309 miRNAs in 93 breast tumors. As a result, they detected different miRNA expression levels between the basal and luminal subtypes. In addition, De Rinaldis *et al.*[32] identified a 46-miRNA signature that could be used to differentiate between breast cancer subtypes. Dvinge *et al.*[33] also obtained similar findings in their research. In a meta-analysis of independent trials, van Schooneveld *et al.* described various subtype-specific miRNAs. Specifically, let-7c, miR-10a and let-7f were found to be associated with the luminal A type; miR-18a, miR-135b, miR-93 and miR-155 were shown to be associated with the basal type; and miR-142-3p and miR-150 were demonstrated to be associated with the HER2 type.[12] Moreover, 453 miRNAs in 29 early-stage breast cancer tumors were profiled, and signatures that could be used to accurately predict the ER, PgR and HER2 status of breast tumor were identified. In addition, miR-342 was expressed most strongly in the ER-positive/HER2-positive tumors.[34] MiR-342 influences the ER expression level and the response to tamoxifen.[35,36] MiR-10b, miR-26a and miR-153 have been suggested to be potential biomarkers of triple-negative breast cancer (TNBC).[37] miRNAs associated with each subtype of breast cancer are shown in Table 3.

miRNA IN HORMONE RECEPTOR-POSITIVE/HER2-NEGATIVE BREAST CANCER

Relationship between miRNA and PgR expression

Approximately 70% of breast cancers are ER-positive and/or PgR-positive. The ER is known to have important roles in the development and progression of breast cancer. It controls the expression of a wide variety of genes and proteins through genomic and non-genomic pathways. In the genomic pathway, estrogen signals are mediated through the ER, which functions as a transcription factor for the signals' target genes. ER is also activated by signal crosstalk between estrogen and growth factors such as epidermal growth factor and insulin growth factor-1 via transmembrane receptor phosphorylation. PgR expression is induced by the ER, and PgR-related signaling pathways have important roles in the induction, progression and maintenance of neoplastic phenotype in breast cancer. Recent studies have suggested that PgR status needs to be considered when discussing the relative-risk reductions expected from endocrine treatments in individual patients. Prat *et al.*[38] reported that a PgR tumor cell positivity cutoff value of $>20\%$ was a significant predictor of the survival differences within luminal-type breast cancers defined by their molecular classification. In addition, we revealed that the extent of PgR expression is a potent prognostic indicator that can aid evaluations of the long-term prognosis of ER-positive/HER2-negative breast cancer.[39] PgR and Stat5a are potent prognostic factors of breast cancer and predict the responsiveness of tamoxifen therapy. Finlay-Schultz *et al.*[40] suggested a mechanism by which the progesterone-triggered loss of miR-141 facilitates breast cancer cell de-differentiation through the deregulation of PgR and Stat5a. Furthermore, Lowery *et al.*[34] revealed that four miRNAs (miR-520g, miR-377, miR-527-518a and miR-520f-520c) have the ability to predict PgR status with great accuracy in breast cancer. MiR-29 and miR-513a-5p also influence PgR expression in breast cancer.[41]

Efficacy of endocrine therapy and miRNA in hormone receptor-positive/HER2-negative breast cancer

miRNAs have important roles in endocrine resistance, and some studies have attempted to identify miRNAs that contribute to the clinical benefits of hormonal therapies. The expression of miR-221 in breast cancer has been identified as a good prognostic marker and is associated with ER positivity and lymph node negativity.[42] However, the miR-221/222 cluster is associated with tamoxifen resistance in breast cancer cells.[43,44] Miller *et al.*[45] reported that miR-221/222 expressions were upregulated in endocrine therapy-resistant luminal-type breast cancer cells. MiR-221/222 are negative regulators of $p27^{kip1}$, a cell cycle inhibitor and tumor suppressor,[46–50] and upregulated expressions of these miRNAs and significant reductions in $p27^{kip1}$ levels have been reported in tamoxifen-resistant breast cancer cells; therefore, miR-221/222 might regulate tamoxifen sensitivity via the direct targeting of $p27^{kip1}$.[51,52] Pichiorri *et al.*[53] found that miR-221/222 expressions were modulated by nucleolin at the post-transcriptional level. Recently, it has been indicated that these miRNAs induce resistance to the selective ER downregulator, and this was caused by the activation of β-catenin and the repression of transforming growth factor-β-mediated growth inhibition.[54] Lu *et al.*[55] demonstrated that miR-221, miR-222 and miR-181b directly target tissue inhibitor of metalloproteinases (TIMP)3, and MCF7 cells that had been subjected to TIMP3 knockdown were found to be able to

Table 2 The microRNAs associated with tumor development in breast cancer

miRNAs	*Targets*	*Ref.*	*miRNAs*	*Targets*	*Ref.*
Cellular proliferation			*Drug resistance*		
miR-146a	NF-kB	154	miR-328	ABCG2	155
miR-146b	NF-kB	154	miR-451	ABCB1	156
miR-106b	p21, CDKN1A	157	miR-326	ABCC1	158
miR-128	Bmi-1	159	miR-487a	ABCG2	160
miR-17-5p	CCND1	7	miR-221	p27Kip1	45
miR-20a	CCND1	7	miR-222	p27Kip1	45
miR-21	PTEN	7	miR-30c	TWF1, IL-11	161
let-7	Ras	7	miR-31	PKCepsilon	162
Invasion and metastasis					
miR-146a	EGFR	163	miR-206	CyclinD2	164
miR-146b	EGFR	163	miR-335	SOX4, TNC	165
miR-125a	HuR	166	miR-96	FOXO1	167
miR-125b	HuR	166	miR-29b	VEGFA, ANGPTL4, LOX	168
miR-182	FOXO1	167	miR-27a	FOXO1	167
miR-10b	HOXD10	169	miR-708	NNAT	170
miR-126	VEGF	165			

Abbreviation: Ref., References.

Table 3 The microRNAs associated with each molecular subtypes of breast cancer

	Micro RNAs			
Clinical subtypes	*Over-expression*	*Ref.*	*Under-expression*	*Ref.*
Luminal A-like (HR-positive and HER2-negative and low proliferation)	let-7c, let-7f	13	miR-206	164
	miR-10a	13	miR-15b	171
	miR-191	164	miR-107	171
	miR-26	164	miR-103	171
	miR-190b	172		
	miR-99a	171		
	miR-130	171		
	miR-126	171		
	miR-136	171		
	miR-146b	171		
	miR-100	171		
Luminal B-like (HR-positive and HER2-negative and high proliferation)	miR-342	34	miR-100	171
	miR-15b	171	miR-99a	171
	miR-107	171	miR-130	171
	miR-103	171	miR-126	171
			miR-136	171
			miR-146b	171
HR-negative and HER2-positive	miR-142-3p	13	miR-125a/b	171
	miR-150	13		
Triple negative	miR18a/b	13	miR-29	172
	miR-135b	13	miR-190b	172
	miR-93	13		
	miR-155	13		
	miR-17-92	172		

Abbreviations: HR, hormone receptor; Ref., References.

grow in the presence of tamoxifen. Furthermore, miR-30c has been identified as an independent predictor of the response to tamoxifen treatment and has been shown to have a role in increasing progression-free survival.[56] MiR-301 expression was recently found to be stronger in tumors than in normal tissue, and patients who suffered recurrence after tamoxifen treatment exhibited higher miR-301 levels than those who did not suffer recurrence.[57] MiR-375 influences the response to tamoxifen treatment by directly targeting metadherin. The loss of metadherin has been shown to restore sensitivity to endocrine therapy and is correlated with disease-free survival in ER-positive patients.[58] He *et al.*[36] demonstrated that miR-342 expression was positively correlated with ER expression and also found that the introduction of miR-342 into estrogen-dependent breast cancer cell lines enhanced their sensitivity to tamoxifen-induced apoptosis. In agreement with this, Cittelly *et al.*[59] reported that the downregulation of miR-342 expression was associated with tamoxifen resistance. miRNAs are also associated with resistance to aromatase inhibitors.[60] Masri *et al.*[61] suggested that miR-128a modulates the transforming growth factor-β signaling and survival of letrozole-resistant cell lines. miRNAs expression profiling before and after letrozole treatment reveal post-treatment increases in let-7f expression in both the preclinical and clinical settings.[62]

miRNA and heat shock proteins in hormone receptor-positive/HER2-negative breast cancer

Heat shock protein (HSP) reveals various effects on multiple oncogenic signaling pathways. The function of HSP is generally considered to be ATP-dependent protein chaperoning. In addition, HSP also has critical roles in post-translational level, which maintains proteins in their correct configurations to ensure their stability and protect carcinoma cells from their apoptosis.[63] Overexpressed HSP70 increases levels of unfolded and denatured proteins in stressful cellular conditions. Therefore, HSP70 is considered to be important to maintain the functions of several housekeeping genes. Yiu *et al.*[64] suggested that the downregulation of HSP70 expression was correlated with the treatment response to neoadjuvant endocrine therapy in ER-positive postmenopausal breast cancer patients. The carboxyl terminus of the Hsc70-interacting protein (CHIP) was originally identified as a co-chaperone of E3 ligase, which ubiquitinates misfolded or abnormal proteins presented by molecular chaperones such as HSP70.[65] This protein is considered to be a U-box-type ubiquitin ligase that induces the ubiquitination and degradation of its substrates, which include several oncogenic proteins.[66,67] We previously demonstrated that strong CHIP expression is correlated with ER positivity, PgR positivity and HER2 negativity, and identified CHIP expression as a potent prognostic factor in postmenopausal patients with invasive breast cancer.[68] Ozgur *et al.*[69] reported that two miRNAs, miR-29a and miR-193b, are associated with breast cancer through their contact with HSP70.

On the other hand, HSP90 is correlated with breast carcinogenesis. HSP90 induces protein folding and refolding under stressful cellular conditions.[70] In aromatase inhibitor-resistant breast cancer, growth factor signaling pathways such as insulin-like growth factor-I receptor and HER2-mediated signaling pathways have important functions in tumor growth. Therefore, these pathways have a key role in resistance to aromatase inhibitors and also act as HSP90 client proteins.[71] Wong *et al.*[72] suggested that HSP90 inhibitors are effective against aromatase inhibitor-resistant breast cancers. HSP90 is associated with various miRNAs, and miRNA-based inhibition of HSP90 is easier to achieve than miRNA-based HSP70 inhibition.[69]

miRNA IN HER2-POSITIVE BREAST CANCER

Efficacy of HER2-targeted therapy and miRNA in HER2-positive breast cancer

Trastuzumab, a humanized monoclonal antibody to HER2 protein, binds to the extracellular domain of HER2 molecules in the cell membrane of carcinoma cells for suppression of HER2 signaling and inhibition of cell proliferation by arresting the cell cycle during the G1 phase. In addition, antibody binding to HER2 leads to antibody-dependent cell-mediated cytotoxicity triggering the carcinoma cell death by immune cells. The treatment strategies for breast cancer changed markedly after trastuzumab was approved as a treatment for HER2-overexpressing breast cancer by the US Food and Drug Administration in 1998. Moreover, other HER2-targeting therapeutic agents such as lapatinib,[73] pertuzumab[74] and ado-trastuzumab emtansine (T-DM1)[75] have been approved as treatments for HER2-overexpressing breast cancer. Jung *et al.*[76] suggested that the plasma miR-210 level is useful for predicting and/or monitoring the therapeutic response to treatments involving trastuzumab, and the upregulation of miR-21 expression has been reported to be associated with trastuzumab resistance in HER2-positive breast cancer.[77] In agreement with the latter study, Nishida *et al.*[78] suggested that miR-125a-5p directly targets HER2. MiR-125a-5p was previously shown to strongly suppress the proliferation of gastric cancer cells, and these growth inhibitory effects were enhanced when miR-125a-5p was used in combination with trastuzumab. Ichikawa *et al.* also found that miR-26a and miR-30b mediate the effects of trastuzumab.[79,80] Furthermore, Iorio *et al.*[81] demonstrated that miR-205, which targets HER3 and impairs the downstream Akt-mediated survival pathway, not only has an oncosuppressive role in breast cancer, but also increases its responsiveness to lapatinib and gefitinib.

Relationship between miRNA and HER3

HER2 is a member of the ErbB-protein family and contributes to a signaling network that operates in the cellular membrane.[82] HER2-containing dimers have been shown to enhance downstream signaling.[82,83] As the ligand of HER2 is not discovered, HER2 activation is thought to be strictly dependent on trans-interactions with other members of the HER family such as HER3.[81,84] HER3 has an impaired kinase domain that lacks catalytic function. However, when it forms a heterodimer with a signaling-competent HER family member, HER3 is transphosphorylated and acts as a signaling platform.[85]

Several studies have demonstrated that HER3 is frequently co-expressed with HER2 in breast cancer, and HER3 has a role in HER2-mediated breast carcinogenesis.[86,87] Iorio *et al.*[81] suggested that the reintroduction of miR-205 into SKBr3 cell increases the responsiveness of lapatinib (tyrosine kinase inhibitors) avoiding HER3-mediated resistance and restoring potent proapoptotic activity. Wang *et al.*[88] predicted that miR-205 binds to the 3′ untranslated regions of HER3 mRNA, and the upregulation of miR-205 reduced HER3 protein expression. Scott *et al.*[89] suggested that the downregulation of HER2 and HER3 protein expression via the overexpression of miR-125a and miR-125b influences the critical features of the malignant cell phenotype, such as proliferative growth, motility and invasiveness *in vitro*. Wang *et al.* and Lyu *et al.* also demonstrated that the overexpression of HER2 promotes HER3 expression via a mechanism involving miR-125a, miR-125b and miR-205 *in vivo*.[90,91] Bischoff *et al.* found that miR-148b, miR-149, miR-326, and miR-520a-3p directly reduced HER3 mRNA and protein levels.[85] Yan *et al.*[92] provided insights into the role of the miR-143/145 cluster as a tumor suppressor in breast cancer; that is, they suggested that it inhibits HER3 translation *in vivo*. Yu *et al.*[93] suggested that miR-148a attenuates angiogenesis by inhibiting HER3.

miRNA IN TRIPLE-NEGATIVE BREAST CANCER

Molecular subtypes of triple-negative breast cancer

Lehmann *et al.* revealed that TNBC can be classified into at least six distinct molecular subtypes with differing biological characteristics based on mRNA profiling. These subtypes include two basal-like types (BL1 and BL2), an immunomodulatory type (IM), a mesenchymal type (M), a mesenchymal stem-like type (MSL) and a luminal androgen receptor type (LAR). The BL1 subtype exhibits higher levels of the components of cell division and DNA damage response pathways, including the BRCA pathway. The BL2 subtype has a unique genetic background that involves growth factor signaling (epidermal growth factor, nerve growth factor, MET, Wnt/β-catenin and insulin-like growth factor 1 receptor pathways). The IM subtype displays higher immune cell and immune signal transduction pathway activity. The M and MSL subtypes are both characterized by higher expression levels of the genes involved in motility, the extracellular matrix, cell differentiation pathways and epithelial-to-mesenchymal transition (EMT). Furthermore, the MSL subtype demonstrates elevated cancer stem cell (CSC)-associated gene expression. The LAR subtype is associated with high androgen receptor (AR) expression levels.[94,95] The miRNA expression profiles of these molecular subtypes of TNBC might differ.

Relationship between miRNA and BRCA mutations

The BL subtype of breast cancer is characterized by TNBC and the BL identified by expressions of CK5/6, CK14 and EGFR. Garcia *et al.* reported that the highest levels of miR-146a and miR-146b-5p were found in BL *in vitro* and TNBC patients.[96] On the other hand, the BRCA1/2 gene is a well-characterized cancer susceptibility gene that is associated with hereditary breast and ovarian cancer.[97,98] Shen *et al.*[99] suggested that genetic polymorphisms in the miR-146a gene might be associated with young age in familial cases of breast or ovarian cancer. The basal phenotype have a strong relationship to BRCA1 mutations, and 80–90% of BRCA1-abnomality expressing cancers exhibiting this phenotype.[100] Yan *et al.*[101] reported that sporadic and BRCA1-positive BL subtype cancers demonstrate grade-independent miRNA expression profiles. Furthermore, Shen *et al.*[102] showed that miR-17 binds to BRCA1 mRNA. Chang *et al.*[103,104] discovered that BRCA1 has a role in the epigenetic control of the oncogenic miRNA miR-155. Crippa *et al.*[105] reported that miR-342 regulates BRCA1 expression by modulating the expression of inhibitor of differentiation 4, which in turn negatively regulates BRCA1 expression in breast cancer. Moskwa *et al.*[106] suggested that miR-182 downregulates BRCA1 expression and found that the manipulation of miR-182 expression in breast cell lines affected their sensitivity to poly-ADP ribose polymerase (PARP) 1 inhibition. Tanic *et al.*[107] recently investigated miRNA classifiers in an attempt to predict the BRCA germline mutation status of routinely available formalin-fixed, paraffin-embedded breast tumor biopsy samples based on 6 types of miRNA (miR-142-3p, miR-505, miR-1248, miR-181a-2, miR-25 and miR-340).

Relationship between miRNA and the tumor-associated immune system

The immunity affects all phases of tumor growth from initiation to progression and dissemination. Tumor-infiltrating lymphocytes (TILs) are mononuclear immune cells that infiltrate tumor tissue.[108] Several retrospective studies have suggested the possibility of TILs as prognostic factor as well as predict factor of chemotherapy in a part

of breast cancer.[109] In the Breast International Group 2–98 trial, retrospective–prospective analyses detected a positive correlation between the number of TILs and survival in TNBC.[110] The presence of TILs is also associated with increased pathological complete response (pCR) rates. The first randomized controlled trial indicated that a relationship exists between increased numbers of TILs and pCR rates.[111] Podshivalova *et al.*[112] suggested that miRNA have an important role in T-lymphocyte activation and also described a mechanism for regulating the impact of miRNA. Jasinski-Bergner reported that natural killer cell and CD8-positive T-lymphocyte ligands are both regulated by the number of miRNAs.[113] Therefore, miRNA might contribute to the immune system in breast cancer. Rodriguez *et al.*[114] suggested that bic/miR-155 has a role in regulating homeostasis in the immune system in cancer patients. Zonari *et al.* reported that miR-155 increases tumor growth by the activation of tumor-associated macrophages in breast cancer. They also found that miR-155 reveals antitumoral effect by acting as an integral effector of immunosurveillance, thereby inhibiting the early stages of breast cancer development.[115]

On the other hand, programmed cell-death protein 1 and programmed cell death 1 ligand 1 (PD-1/PD-L1) eliminate T-cell activation in various forms of cancer.[116] The prospective trials to evaluate the efficacy of antibodies to PD-1/PD-L1 are undergoing in patients with TNBC. These studies may suggest the potential of immune checkpoint inhibitors targeting PD-1/PD-L1 axis in patients with TNBC.[117,118] Iliopoulos *et al.*[119] demonstrated that miR-21 expression was upregulated by ovalbumin stimulation in T cells and also that the inhibition of PD-1 increased miR-21 expression. Chen *et al.*[120] suggested that a relationship exists between miR-200 and PD-L1 expression in human lung cancer. Furthermore, previous studies have indicated that a relationship exists between these immune checkpoints and a number of miRNA; however, these relationships have not yet been elucidated in detail. Further studies are needed in order to determine the relationships between miRNA and the immune system in breast cancer.

Relationship between miRNA and EMT

miRNA might control tumor cell migration caused by EMT and suppress the metastatic potential of breast cancer. Smad and Twist have recently been shown to favor the metastatic dissemination of cancer cells through their abilities to induce EMT. Twist is thought to increase the invasiveness of cancer cell and upregulate miR-10b expression *in vitro*.[121,122] Moreover, the repression of miR-10b decreases the presence of Twist in the bone metastasis of breast cancer.[123] These findings suggest that Twist induces the formation of bone metastasis through a miR-10b-dependent mechanism in breast cancer. Snail is a zinc finger transcriptional repressor, the pathological expression of which has been linked to cancer cell EMT programs and the induction of tissue invasive activity.[124–127] MiR-34a reduces the invasiveness of breast cancer cells by repressing EMT through the Snail pathway.[128] Gregory *et al.* found that all five members of the miRNA-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 was markedly downregulated in cells that had undergone EMT in response to ectopic protein tyrosine phosphatase expression. The enforced expression of members of the miR-200 family were also sufficient to prevent transforming growth factor-β-induced EMT.[129] At the cellular level, one of the key events associated with miR-103/107 is the induction of EMT via the downregulation of miR-200 expression.[130] Song *et al.* found that miR-22 triggered EMT, enhanced invasiveness, and promoted metastasis in mouse xenografts. They also demonstrated that miR-22 induced metastatic potential by silencing miR-200 through the targeting of ten-eleven translocation proteins, which are a family of methylcytosine dioxygenases.[131] Moreover, Park *et al.*[132] showed that these miRNA cooperatively regulated the expression of the E-cadherin transcriptional repressors ZEB1 and ZEB2, which have previously been implicated in EMT and tumor metastasis. Jiang *et al.* reported that miR-29 mediated EMT and promoted metastasis in breast cancer.[133,134]

Cancer stem cell-related miRNA

Recent studies have suggested that miRNA contribute to tumor initiation by regulating the properties of CSC, including their self-renewal, de-differentiation and drug resistance.[5,9,135] Han *et al.*[136] demonstrated that the formation of CSC-like cells that were undergoing EMT was associated with the overexpression of hypoxia-inducible factor 1α and that this process was regulated by miR-21. Let-7 has an important role to regulate the function of CSC, because the reduction of let-7 expression inhibits differentiation and maintains proliferation. Therefore, let-7 is a potential molecular marker of CSC and might be a therapeutic target for anti-cancer therapy.[137,138] Several recent studies have suggested that miR-200 family members and their target mRNAs are associated with the properties of CSC. Shimono *et al.*[139] revealed that the expression levels of miRNA-200c-141, miR-200b-200a-429 and miR-183-96-182 were consistently downregulated in CSC of breast cancer. Lim *et al.*[140] suggested that the downregulation of miR-200 may induce the conversion of mammary epithelial cell to a stem cell-like phenotype in breast cancer. Song *et al.*[131] reported that miR-22 modified a crucial epigenetic change, promoted EMT, and induced breast cancer stemness. Takahashi *et al.*[141] identified miR-27b as a key regulator of the generation of breast cancer cells with CSC properties.

Relationship between miRNA and the LAR type

The AR is a nuclear receptor that has a role in the complex network of signaling pathways that regulate cell proliferation in breast cancer.[142,143] In breast cancer, some studies demonstrated that androgenic effects mediated by AR stimulate tumor growth, while other studies suggested that AR-mediated effects inhibit the growth of breast cancer cells.[144,145] Therefore, the mechanisms responsible for the loss of AR expression during breast carcinogenesis remain unclear. Although the loss of AR expression is associated with high nuclear grade and a negative ER, PR and HER2 expression status in breast cancer, the significance of AR expression in human breast cancer has been examined in recent studies into TNBC.[146,147] A number of studies have proposed that a correlation exists between specific miRNA and AR expression. The expression levels of several androgen-inducible miRNA (miR-21, miR-101, miR-125b, miR-221 and miR-222) have been well-characterized in prostate cancer cells, and these miRNA have been found to be involved in the progression to androgen independence.[148–152] MiR-21 has an androgen-responsive element, and is directly upregulated by androgens in prostate cancer cell lines. In addition, miR-21 was expressed at higher levels in AR-positive than in AR-negative prostate cancer cells.[148,149,151] The roles of miRNA in the regulation of AR expression in breast cancer have been investigated. Nakano *et al.*[153] demonstrated that miR-363 is an androgen-inducible miRNA in breast cancer. However, at present there is limited evidence that a relationship exists between the AR and miRNA in breast cancer.

CONCLUSIONS

The present review highlighted recent findings regarding the role of miRNA in breast cancer. Previous studies have revealed that breast

cancer comprises several intrinsic subtypes with different molecular profiles, and several miRNAs have important roles to determine and regulate such subtypes. The profiling of miRNA expressions in breast cancer and clarifying molecular mechanisms of breast cancer-specific miRNAs are important future topics for basic and clinical research of breast cancer. The elucidation of the roles played by miRNA in breast cancer has provided new opportunities for the development of strategies for the diagnosis and treatment of cancer. Further biological research into the ability of novel agents to regulate miRNA expression is warranted, and miRNA are expected to become a therapeutic target of treatments for breast cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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