Chapter

Potential Biomarkers for Therapeutic Monitoring and Clinical Outcome in Breast Cancer

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Abstract

Non-coding RNAs are a species of RNA that are not translated to proteins. These include transfer RNAs and ribosomal RNAs, microRNAs, transfer RNA-derived fragments, and long non-coding RNA. It is known that expression levels of some non-coding RNAs included microRNAs are altered in cancer cells or tumor tissues. Moreover, expression profiles of such non-coding RNAs correlate between tissues and body fluids. Therefore, several non-coding RNAs are being used as diagnostic/prognosis biomarkers or therapeutic targets in cancer. In this chapter, we review about representative non-coding RNAs and introduce especially microRNA as diagnosis/prognosis biomarkers and therapeutic targets.

Keywords: microRNA, isomiR, exosome, biomarker, therapeutics

1. Introduction

Non-coding RNAs (ncRNAs) are generic terms of RNA that are not translated to protein. For example, ribosomal RNA (rRNA) and transfer RNA (tRNA) are included in ncRNAs. In the body, ncRNA does not encode proteins but has important functions.

MicroRNAs (miRNA), one of the ncRNAs, are small non-coding RNAs with an average length of 22 nucleotides. miRNA is transcribed from genomic DNA, the transcribed miRNA is called "primary microRNA (pri-miRNA)," which is long transcripts having stem-loop structures. Pri-miRNA is processed by Drosha and DGCR8 (DiGeorge syndrome critical region 8), one of the microprocessors, to "precursor miRNA (pre-miRNA)." In the next step, there is pre-miRNA transition from nuclei to the cytoplasm through Exportin-5. Cytoplasmic pre-miRNA is processed by Dicer, one of RNaseIII, to double-strand miRNA. The double-strand miRNA binds to AGO (Argonaute) protein and forms RISC (RNA-induced silencing complex). RISC binds 3′ UTR (3′ untranslated region) of target mRNAs, and downregulate gene expression *via* mRNA cleavage, translational repression, or mRNA degradation (**Figure 1**) [1]. Recently, it is known that miRNA also binds to 5′ UTR (5′ untranslated region) and CDS (coding sequence) of target mRNAs [2, 3]. miRNAs may have over hundreds of target genes, regulating various biological phenomena. In cancer patients, many miRNAs are aberrantly expressed, caused by chromosomal aberration, epigenetic regulation, and genomic mutation [4–6]. Therefore, miRNAs

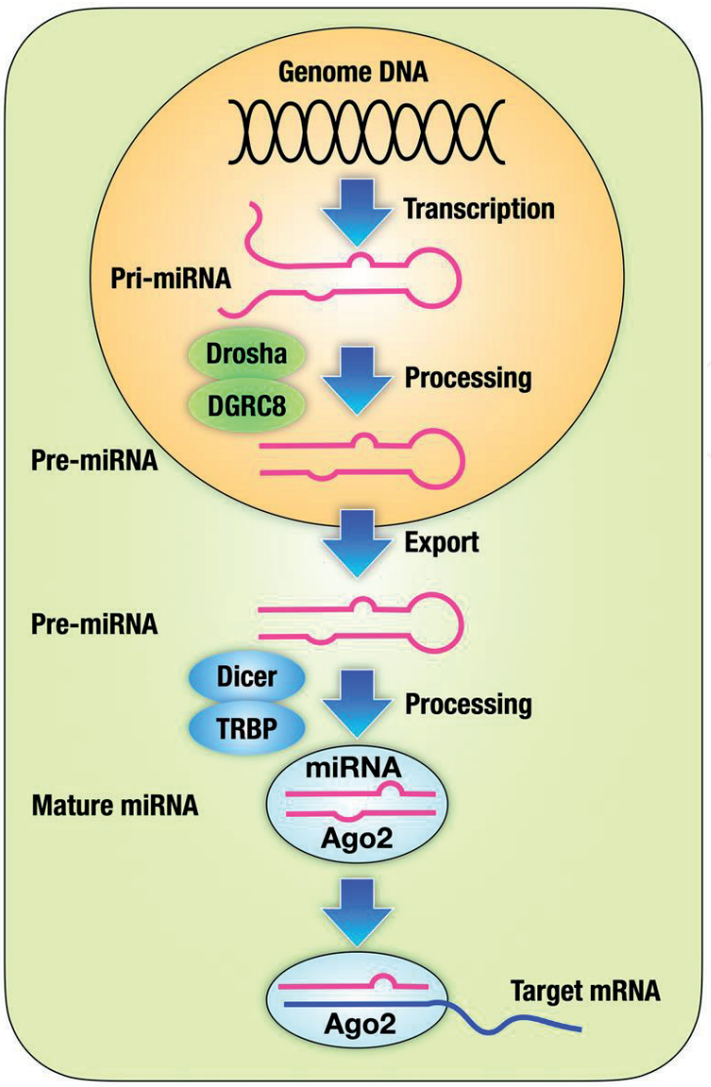

Figure 1.
The overview of miRNA biogenesis. miRNA is transcribed from genomic DNA to primary miRNA (pri-miRNA), which is long transcripts having stem-loop structures. Pri-miRNA is processed by Drosha and DGCR8 (DiGeorge syndrome critical region 8), one of the microprocessors, to precursor miRNA (pre-miRNA). After the transition of pre-miRNA from nuclei to the cytoplasm through Exportin-5, cytoplasmic pre-miRNA is processed by TRBP (transactivation response RNA-binding protein) and dicer, one of RNaseIII, to double-strand miRNA. The double-strand miRNA binds to AGO (Argonaute) protein and forms RISC (RNA-induced silencing complex). RISC binds 3′ UTR (3′ untranslated region) of target mRNAs.

with altered expression under pathological conditions are valuable for biomarkers and targets of therapeutics.

Interestingly, recent studies revealed that miRNA sequences have the variation compared with the reference sequences. As miRNAs are called isomiRs, isomiRs are the miRNA variants that have different sequences and/or lengths [7–10]. These isomiRs are classified as 5′ isomiR, 3′ isomiR, and polymorphic isomiR. isomiRs are generated through the slice site variation by Drosha or Dicer, the nucleotide addition, RNA editing, etc. [11]. Some isomiRs are abnormally expressed in cancer cells caused by chromosomal and/or miRNA processing aberration (**Figure 2**) [12, 13]. Thus, isomiR is also focused on as novel biomarker for cancer detection.

Long non-coding RNA (lncRNA) that is also one of ncRNAs consisting of over 200 nucleotides has multiple functions. LncRNA up- or downregulation has been shown to regulate several biological processes, such as transcription, translation, epigenetic modification, and miRNA expression [14]. It has been shown that lncRNA expressions are altered in various diseases. Therefore, similar to miRNAs, lncRNA has the potential of biomarkers and therapeutics. Moreover, recently it

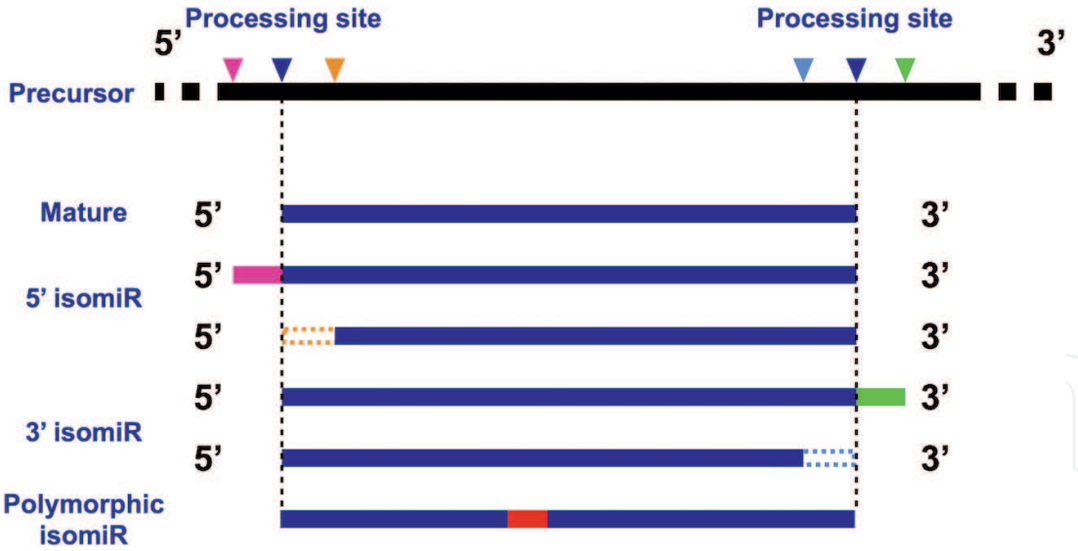

Figure 2.
The classification and generation of isomiRs. The isomiRs have a different length or sequence with mature miRNAs. These isomiRs are generated by a variation of Drosha or dicer variation. The aberrant processing in 5′ site generates 5′ isomiR. On the other hand, 3′ isomiR is generated by the aberrant processing in 3′ site. Polymorphic isomiR is generated by RNA editing. Navy arrow shows the correct processing site. Magenta and green arrows show the aberrant processing sites for isomiRs, which have a longer length. Orange and blue arrows show the aberrant processing sites for isomiRs, which have a shorter length. The red bar indicates the variati of sequence.

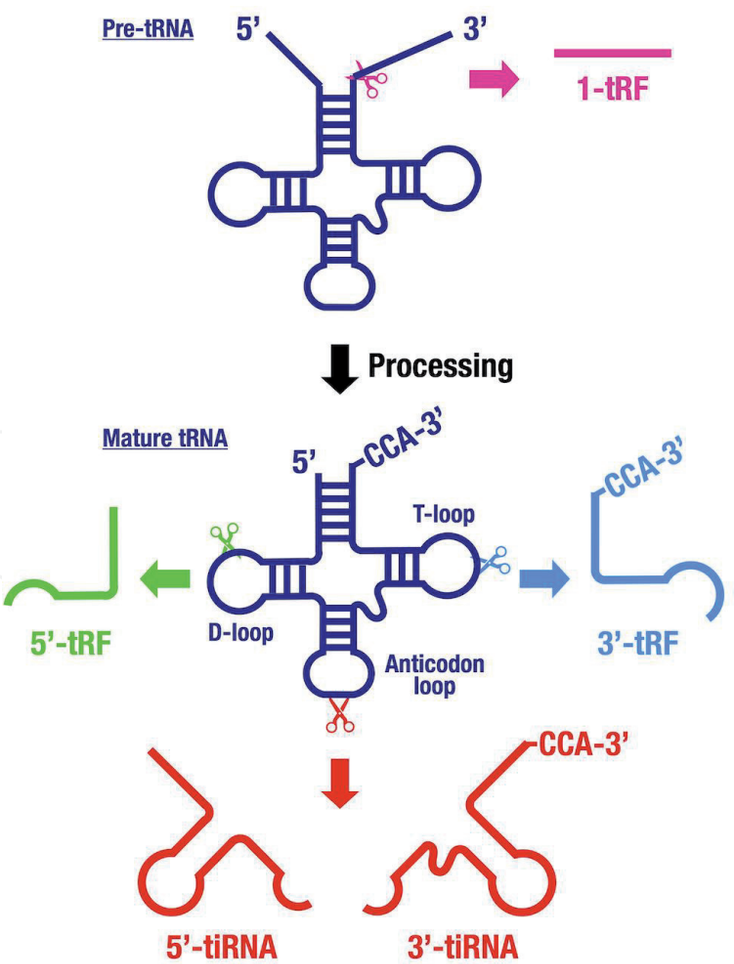

Figure 3.
The classification and various modes of generation of tRFs. tRNA-derived fragments (tRFs) are generated by cleavage of transfer RNA (tRNA). 1-tRFs are cleaved from pre-tRNA. The cleavage of the D-loop generates 5′-tRF. The cleavage of the T-loop generates 3′-tRF. 5′- and 3′-tRNA-derived stress-induced RNAs (tiRNAs) are generated by cleavage of the anticodon loop.

was uncovered that tRNA fragments are functional. Its non-coding RNA is called at tRNA-derived RNA fragment (tRF), and classified as 1-tRF, which is generated from the 3′-end of pre-tRNA, 5′-tRFs, which is generated by the cleavage of 5′ end in D-loop, 3′-tRFs, which is generated by the cleavage of 3′ end in T-loop, and tRNA-derived, stress-induced RNAs (tiRNAs), which is generated by specific cleavage in the anticodon loop. Some tRFs are identified as novel biomarkers for disease diagnosis (**Figure 3**) [15].

2. Liquid biopsy

A biopsy is a method for disease diagnosis using a part of tissues or cells of the lesion. The tissue specimens are sampled by surgery and the cells derived from the lesion are sampled by fine-needle aspiration. The biopsy is useful for diagnosing diseases and malignancies, because it is possible to observe tissues or cells directly.

Generally, to diagnose breast cancer, inspection, palpation, and mammography are performed at first. Then, if the patients are suspected of tumors, the biopsies using tissue or cells derived from the lesion are performed, resulting in diagnosing breast cancer. However, conventional biopsy, surgery, needle biopsy, fine-needle aspiration, etc., are high invasiveness and have the risk of needle tract seeding. As with other problems, it is also concerned that young women are diagnosed as false positive on mammography, caused by high breast density.

Recently, to solve a problem like this, some researchers focus on "liquid biopsy." Liquid biopsy is the method for disease diagnosis using body fluid. Body fluids using in the liquid biopsy are mainly blood, but also saliva, urine, and spinal fluid [16, 17]. Because using body fluid like blood, sampling the specimen is capable of low invasiveness and repetitive. These are one of the features and usefulness of liquid biopsy. In the field of cancer research, circulating tumor cells (CTCs) and circulating cell-free DNAs (cfDNAs) are detected and evaluated, resulting in the diagnosis of cancers. However, a recent study uncovered that circulating miRNA in body fluid is reflected in pathology. Many researchers show that specific miRNAs are aberrantly expressed in each disease. More interestingly, some miRNA expressions are altered from an early stage of cancer. Therefore, investigating the alteration of miRNA expression leads to the early diagnosis of cancer.

2.1 Circulating tumor cells (CTCs)

The cancer cells leak to the bloodstream from the primary tumor in the tumor metastasis phase. In the detection of CTCs, cancer cells that are derived from metastatic tumors are directly evaluated. However, detecting CTCs is not easy, because the number of CTCs is too low. To detect and gather CTCs, cell surface markers are recognized by the specific antibodies. Then, evaluating the shape and gene profile of cancer cells results in the diagnosis of malignancy and specific mutation of each tumor. However, the detection in the early stage of cancer is not useful, because the CTCs are detected in the late stage of cancer.

2.2 Cell-free DNAs (cfDNAs)

In the bloodstream, DNA derived from various dead cells containing hematopoietic cells or other cells derived from tissues are circulating. Additionally, it is known that DNA fragments derived from cancer cells are circulating in the bloodstream of cancer patients. These DNA fragments are generated by apoptosis or clearance by immune cells. In the detection of cfDNAs, the tumor-specific somatic mutation

is detected, resulting in the diagnosis of cancer. However, the detection of cfDNA derived from cancer cells is not useful for the early diagnosis of cancer, because cfDNAs were derived from the dead cells.

2.3 Non-coding RNAs (ncRNAs)

Recent studies have uncovered that the ncRNAs included miRNAs, tRFs, and other ncRNAs circulate in different body fluids [15, 18]. Generally, circulating RNAs are easily degraded by RNase present in plasma. However, ncRNAs secreted to body fluids *via* extracellular vesicles like exosomes, or bound to proteins, are resistant to RNase and therefore can be stable in circulation. Exosomes are a type of extracellular vesicles, ranging in size from 50 to 150 nm. Exosomes that are secreted from donor cells circulate in the body fluids and are transferred to target recipient cells. The uptake of exosomes in recipient cells leads to various physiological functions. These functions are caused by ncRNAs and/or proteins contained in exosomes. In this regard, it is thought that exosomes are required for cell-to-cell communications. For example, it is reported that miRNA contained in exosomes secreted from cancer cells, contributing to the metastasis of cancer [19]. Recently, it is thought that exosomes derided from cancer cells educate metastasis site and help the cancer metastasis *via* the transfer of ncRNA and protein as like this report [20]. Moreover, recent studies uncovered that circulating ncRNA in body fluid is reflected in the pathology. Many researchers show that specific miRNAs are aberrantly expressed in each disease. More interestingly, some miRNA expressions are altered from an early stage of cancer. Therefore, investigating the alteration of miRNA expression leads to the early diagnosis of cancer. In this regard, many researchers focus on the ncRNAs circulating in the body fluids. In particular, miRNAs circulating in the blood are focused on.

3. The method of screening the ncRNAs in the liquid biopsy

In liquid biopsy, there are several methods of screening for the circulating ncRNAs in the body fluids. In the case of using blood, commonly, it is necessary to isolate plasma or serum from blood. Then, RNA purification is performed from plasma or serum. Using purified RNA, the expression profiles of ncRNAs are assessed by real-time PCR or microarray, resulting in the identification of a novel biomarker. Examples are shown below:

3.1 Real-time PCR

In the method using real-time PCR, the specific ncRNA is detected with its primer pair, and the alteration of the ncRNA expression is evaluated. The real-time PCR method is not suited for screening and identification of a novel biomarker, because of using the specific primer pair corresponding to each ncRNAs. On the other hand, this method is available for detecting the already identified ncRNA or the particular ncRNA, because the experimental procedure is easier and the detection sensitivity is more specific than other methods.

3.2 Microarray

In the method using microarray, the alteration of ncRNAs existing on microarray chips is capable of a comprehensive evaluation, because the multiple ncRNAs are detectable at the same time. In the microarray method, the ncRNA expression

is detected by the binding between cDNAs synthesized from RNA and probes anchored to the microarray chip. Therefore, the microarray method is not available for unknown ncRNAs.

3.3 Next-generation sequencing (NGS)

In the NGS, the alteration of ncRNA expression is comprehensively evaluated by direct reading the RNA sequence of whole ncRNAs. In this regard, it is possible to identify and compare the expression pattern of ncRNAs like as microarray method. Additionally, it is also possible to identify unknown ncRNAs because of the directly determining ncRNA sequence. Moreover, isomiRs, that many researchers recently focused on, are also detectable. However, experimental procedures and data analyses are more difficult and complex than the other methods, and the experimental cost is also high.

4. microRNA for breast cancer diagnosis

Some miRNAs are altered in the tumor tissue compared with normal tissue. For example, the expression level of miR-21 is increased in various cancers included breast cancer and associated with tumorigenesis. Additionally, some miRNAs have a specific expression level in breast cancers. Recent studies also reported that the expression of some miRNAs is altered by the difference of subtype or stage and with/without the receptors. Furthermore, it is known that some of these miRNAs are circulating in body fluids. Therefore, such miRNAs are available for the biomarkers in liquid biopsy. Specific ncRNAs are described below, and recent reports are summarized in **Table 1**.

4.1 Circulating miRNAs

Francesca Maria Orlandella and colleagues focused on miR-622 that known to act as a tumor suppressor in several types of cancer. They reported that miR-622 was downregulated in the plasma and tissues of breast cancer patients. Moreover, it was revealed that the expression level of miR-622 correlated with the breast cancer subtypes and the advance of tumor stages. Additionally, miR-622 inhibited the migration of breast cancer cells *via* targeting NUAK family kinase 1 (NUAK1). In this regard, circulating miR-622 is useful for the biomarker of breast cancer [27].

To identify miRNAs that are useful for the early diagnosis of invasive breast cancers, Mio Yoshikawa and colleagues screened exosomal miRNAs of the plasma by microarray analysis. Using exosomal miRNAs of ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC), five miRNAs, miR-223-3p, miR-130-3p, miR-191-5p, miR-146a, and miR-221-3p, were upregulated in IDC compared with healthy control and DCIS. In this study, they revealed that the expression level of miR-223-3p was correlated between the tissue and plasma exosome. Furthermore, the expression level of miR-223-3p was upregulated according to the advance of tumor stages. In these findings, it is suggested that the expression level of exosomal miR-223-3p reflected the tissue pathogenesis, and the miR-223-3p has the potential as a biomarker for early diagnosing of invasive lesions from DCIS patients in liquid biopsy [37].

4.2 isomiR

Yumiko Koi and colleagues investigated the comprehensive expression profile of ncRNA using the NGS method and focused on the expression level of isomiR.

NcRNAs	Source	Alteration	Method	Characters	Reference
Let-7i	Urine	Down	qRT-PCR	Down in breast cancer	[21]
miR-15a	Serum	Down	qRT-PCR	Down in TNBC	[22]
miR-17	Serum	Down	qRT-PCR	Down in TNBC	[22]
miR-18a	Serum Urine	Down	qRT-PCR	Down in TNBC	[22]
miR-19b	Serum	Down	qRT-PCR	Down in TNBC	[22]
miR-30b	Serum Urine	Down	qRT-PCR	Down in TNBC	[22]
miR-92a	Serum	Down	qRT-PCR	Down during tumor progression	[23]
miR-145	Plasma	Down	Microarray	Down in breast cancer	[24]
miR-194-5p	Plasma	Down	NGS	Down in brain metastasis	[25]
miR-195	Plasma	Down	NGS	Down in metastatic breast cancer	[26]
miR-222	Urine	Down	qRT-PCR	Down in TNBC	[22]
miR-320c	Urine	Down	qRT-PCR	Down in TNBC	[22]
miR-423	Urine	Down	qRT-PCR	Down in breast cancer	[21]
miR-622	Plasma	Down	qRT-PCR	Down in breast cancer	[27]
miR-660	Urine	Down	qRT-PCR	Down in breast cancer	[21]
miR-802-5p	Plasma	Down	NGS	Down in brain metastasis	[25]
Let-7a	Serum	Up	qRT-PCR	Up in TNBC	[22]
Let-7e	Serum	Up	qRT-PCR	Up in TNBC	[22]
miR-16	Plasma	Up	Microarray	Up in breast cancer	[24]
miR-21	Serum Plasma	Up	qRT-PCR Microarray	Up during tumor progression Up in TNBC	[22–24, 28]
miR-21-5p (3′ isoRNA)	Serum	Up	NGS	Up in breast cancer	[29]
miR-23a-3p	Serum	Up	NGS	Up in breast cancer	[29]
miR-29c	Serum	Up	qRT-PCR	Up in early breast cancer	[30]
miR-99a-5p	Plasma	Up	qRT-PCR	Up in early breast cancer	[31]
miR-142-5p	Serum	Up	NGS	Up in luminal A breast cancer and TNBC	[32]
miR-150-5p	Plasma Serum	Up	NGS NGS	Prognostic biomarker (recurrence) Up in luminal A breast cancer and TNBC	[32, 33]
miR-155	Serum	Up	qRT-PCR Microarray	Up in breast cancer Prognostic biomarker (drug resistance)	[34, 35]
miR-199a	Serum	Up	qRT-PCR	Up in early breast cancer	[30]
miR-210	Plasma	Up	qRT-PCR	Up in breast cancer	[36]
miR-223-3p	Plasma	Up	Microarray	Up during tumor progression	[37]
miR-331	Plasma	Up	NGS	Up in metastatic breast cancer	[26]
miR-424	Serum Urine	Up Up	qRT-PCR	Up in early breast cancer	[21, 30]

NcRNAs	Source	Alteration	Method	Characters	Reference
miR-451	Plasma	Up	Microarray	Up in breast cancer	[24]
miR-488	Serum	Up	Microarray	Prognostic biomarker (recurrence)	[38]
miR-576-3p	Plasma	Up	NGS	Prognostic biomarker (recurrence)	[33]
miR-1246	Serum	Up	Microarray	Prognostic biomarker (drug resistance)	[35]
miR-1910-3p	Serum	Up	qRT-PCR	Up in breast cancer	[39]
miR-4433b-5p	Serum	Up	NGS	Up in luminal A breast cancer and TNBC	[32]
miR-4665-5p	Plasma	Up	NGS	Prognostic biomarker (recurrence)	[33]

"Up" indicates upregulation in breast cancer and "Down" indicates downregulation in breast cancer. qRT-PCR, quantitative reverse transcription-polymerase chain reaction; NGS, next-generation sequencing; TNBC, triple-negative breast cancer.

Table 1.
The alteration of circulating miRNAs in breast cancer.

They used the exosomal RNA of serum derived from breast cancer patients. At first, they revealed that 11 circulating small RNAs were upregulated in breast cancer serum compared with healthy controls. Then, 3′-isomiR of miR-21-5p was identified as one of the biomarkers for the diagnosis of breast cancer. Additionally, miR-23a-3p and tRF-Lys-TTT were also identified as biomarkers for the diagnosis of breast cancer. Interestingly, they proposed a discriminant model using the expression levels of these small ncRNAs, and this model was more significantly able to diagnose breast cancer than individual small ncRNA. It was revealed that this model was able to diagnose the stage 0 breast cancer. Moreover, the model also could diagnose the breast cancer irrespective of subtypes. In this regard, the model that included the alteration of isomiR is available for the early diagnosis of breast cancer [29].

4.3 tRFs

As mentioned above, it is clear that tRFs are generated in cancers. Yue Huang and colleagues investigated the expression profile of tRFs in normal breast epithelial cell lines and non-triple negative breast cancer (non-TNBC) cells using RNA sequencing. In further investigation, they revealed that the expression level of tDR-7816 (drives from $tRNA^{Gln-CTG-3-1}$), tDR-5334 (derived from $tRNA^{Gly-CCC-5-1}$), and tDR-4733 (derived from $tRNA^{Phe-GAA-2-1}$) is altered in the serum of non-TNBC patients compared with healthy controls. It is suggested that these tRFs are useful for the diagnosis of breast cancer [40].

In addition, Jingyi Wang and colleagues explored the expression profiles of tRFs in plasma derived from breast cancer patients. As the result, six tRFs, tRF-Glu-CTC-003, tRF-Gly-CCC-007, tRF-Gly-CCC-008, tRF-Leu-CAA-003, tRF-Ser-TGA-001, and tRF-Ser-TGA-002, were altered in the early stage of breast cancers compared with healthy controls. These six tRFs are also downregulated in the plasma derived from DCIS. Moreover, they also revealed that these tRFs which excluded tRF-Glu-CTC-003 in HER2+ type and tRF-Gly-CCC-008 in luminal type are downregulated in each subtype. In this regard, it is suggested that these tRFs are useful as a novel biomarker for the diagnosis of early-stage breast cancer [41].

4.4 Urinary miRNAs

In the liquid biopsy, urine as the specimens has the advantage of easy and non-invasive collection. Thalia Erbes and colleagues investigated whether miRNAs in serum/plasma that are already identified as the candidates of biomarkers for breast cancer are altered in also urine [42]. The expression level of miR-155 which is known to be upregulated in serum from the breast cancer patients, and useful for the diagnostic biomarker, was upregulated also in urine from the breast cancer patients [42, 43]. Moreover, they revealed that the expression levels of urinary miR-21, miR-125, and miR-451 were also downregulated in the breast cancer patients like serum miRNAs [24, 42, 44, 45]. In the recent study, it is revealed that the expression level of urinary miR-423, miR-424, miR-660, and let-7i was altered in the breast cancer patients compared with healthy controls. Moreover, this study reported that the combination of these miRNA's alterations more significantly diagnoses the breast cancer than the biomarker using individual miRNA's alteration [21]. In this regard, urinary miRNAs are useful for the diagnostic and prognosis biomarkers.

5. microRNA for breast cancer therapeutics

As mentioned above, the expression level of various miRNAs is dramatically altered in the development and progression of diseases including the tumors. In this regard, it is thought that the alteration of miRNA expression contributes to the development and progression of several diseases. Moreover, the expression profiles of miRNAs are different in each type of diseases. Then, such miRNAs are available for the therapeutic target of the disease. In cancer research, miRNAs that are over-expressed in tumors are called "oncogenic microRNA (OncomiR)", and are associated with tumor development and malignancy. On the other hand, miRNAs that are downregulated in tumors are called at "tumor suppressive microRNA (TS-miRNA)" and are contributed to the suppression of tumor progression *via* targeting genes that are associated with the cancer cell proliferation and survival. It is hoped that miRNA mimics and miRNA inhibitors targeting TS-miRNA or OncomiR are developed as therapeutic drugs. Specific ncRNAs are described below, and recent reports are summarized in **Table 2**.

5.1 miR-22

It is reported that a lot of miRNAs regulate tumor progression and malignancy. Previously, we revealed that miR-22, which induce cellular senescence, suppressed the proliferation of breast tumors. MiR-22 is upregulated during cellular senescence induction. Such miRNAs are called "senescence-associated microRNA (SA-miRNA)." We reported that miR-22 induces cellular senescence *via* targeting CDK6 and SIRT1. Moreover, it was suggested that miR-22 was the therapeutic target of breast cancer, because miR-22 was downregulated in the breast cancer cells. In an additional investigation, it is reported that the replacement of miR-22 expression repressed the breast tumors through the experiment using the xenograft model mouse model [56].

5.2 miR-155

Jiang and colleagues reported that the expression level of miR-155 was upregulated in breast cancer. Then, they suggested that miR-155 functions as OncomiR,

ncRNAs	Types	Targets	Functions	Reference
miR-429	Anti-sense oligonucleotide	VHL	Inhibit the proliferation of $HER2^+$ breast cancer	[46]
miR-302b	inhibitor	RUNX2	Inhibit the proliferation	[47]
miR-532-5p	inhibitor	RERG	Inhibit the proliferation and migration	[48]
miR-1910-3p	inhibitor	MTMR3	Inhibit proliferation and migration	[39]
miR-99a	mimic	FGFR3	Inhibit the tumor proliferation and migration	[49]
miR-128-3p	mimic	NEK2	Inhibit tumorigenicity and tumor growth of breast cancer stem cells	[50]
miR-188-5p	mimic	RAP2C	Induce apoptosis and inhibit the proliferation	[51]
miR-299-5p	mimic	STK39	Inhibit the migration and invasion	[52]
miR-342	mimic	CFL1	Inhibit the migration and invasion	[53]
miR-424-5p	mimic	PD-L1	Induced apoptosis and cell cycle arrest	[54]
miR-590-3p	mimic	SLUG	Inhibit the metastasis in TNBCs	[55]

HER2, human epidermal growth factor receptor 2; TNBCs, triple-negative breast cancers.

Table 2.
The miRNAs as therapeutic targets in breast cancer.

because its target gene is a suppressor of cytokine signaling 1 (SOCS1) known as one of the tumor suppressor genes. This study revealed that the inhibition of the miR-155 expression and function by the antisense oligonucleotide against miR-155 repressed the tumor progression in the investigation using both the breast cancer cells and the xenograft mouse models. In this regard, the inhibition of OncomiR functions is available for the therapeutic targets [57].

5.3 miR-424/503 cluster

It is reported that the miR-424/503 cluster that was coded on X-chromosome was deficient in luminal B breast cancer. It is also clear that the deletion of this locus significantly correlated with the poor prognosis. Therefore, it is suggested that the miR-424/503 cluster functions as TS-miRNA. Moreover, the miR-424/503 cluster targets insulin-like growth factor 1 receptor (IGF1R) and B-cell lymphoma 2 (BCL-2) that contribute to anti-apoptosis. The deletion of this cluster leads to upregulating these gene expressions, resulting in the acquisition of drug resistance [58].

5.4 miR-539

A recent study reported that miR-539 was downregulated in tumor tissues, and it is suggested that miR-539 has the tumor suppressive effects. In the investigation using breast cancer cells, miR-539 mimics repressed the proliferation and migration of cancer cells *via* targeting epithelial growth factor receptor (EGFR). Additionally, miR-539 also repressed the tumor proliferation [59]. Moreover, it is uncovered that miR-539 was downregulated in TNBC, and miR-539 suppressed the proliferation, invasion, and migration *via* targeting Laminin subunit alpha 4 (LAMA4) [60].

5.5 miR-142

It is reported that miR-142 is also downregulated in breast tumor tissue compared with normal tissue and suggested that miR-142 functions as TS-miRNA. It is revealed that miR-142 inhibits the expression of the BTB domain and CNC homolog 1 (BACH1), which is associated with the metastasis of breast cancer, resulting in the suppression of the proliferation, invasion, and migration. Moreover, Mansoori and colleagues reported that miR-142 induced apoptosis *via* targeting estrogen receptor 1 (ESR1) that coded estrogen receptor in the estrogen receptor-positive breast cancer [61, 62].

5.6 miR-34a

miR-34a is the most famous TS-miRNAs that was reported to upregulate in the p53-dependent manner and downregulate in the colorectal cancer patients compared with healthy, and progressed in the development as the nucleic acid drug against cancer. MiR-34a suppressed colorectal cancer progression through the induction of cellular senescence *via* E2F pathway [63]. In further investigation, it is uncovered that miR-34a downregulates the gene expression *via* targeting sirtuin 1 (SIRT1), cyclin D1, cyclin-dependent kinase 4/6 (CDK4/6), and MYC [64–66]. In this regard, the clinical trial of MRX34, the liposomal miR-34a mimic, for various solid tumors included breast cancer was performed in miRNA Therapeutics Inc. Unfortunately, this clinical trial was dropped [67]. However, a recently study reported that miR-34a targets programmed death ligand 1 (PD-L1) in acute myeloid leukemia [68]. Moreover, it is reported that miR-34a expression level is downregulated in TNBC and inversely correlated with PD-L1 expression [69]. Therefore, a novel clinical trial of miR-34a is expected.

6. Conclusions

In this review, we summarized several ncRNAs that are available for the biomarkers diagnosing breast cancer or predicting poor prognosis and the targets of breast cancer therapeutics. The finding and studying of isomiRs or tRFs are leading to the development of highly specific biomarkers, which could lead to early diagnosis of breast cancer. Moreover, it is useful for comparing the alteration of several ncRNA expressions multidimensionally with the comprehensive analysis of the expression profiles of ncRNAs using microarray or NGS method. These approaches and results may lead to highly specific diagnostics of the disease and can correctly predict several different types of breast cancers. In regard to cancer therapeutics, the studies about isomiRs or tRFs may result in the development of novel therapeutic targets for breast cancers. Further research on the ncRNAs will aid to improve the diagnosis and therapeutics of breast cancers.

Conflict of interest

Professor Hidetoshi Tahara is the representative director of a university-originated venture, MiRTeL Co.

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