8 Breast Cancer MicroRNAs: Clinical Biomarkers for the Diagnosis and Treatment Strategies

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Abstract

Breast cancer is the second most common cancer in females that accounts for the highest cancer-specific deaths worldwide. Although recent advances in clinical management significantly reduced the mortality rate in breast cancer patients, the success rate of the effective therapy remains largely dependent on early detection. It has been demonstrated that gene expression profile may be a useful tool to define the signature of breast cancer as well as to predict the prognosis or response to treatment. The microRNA expression profile is gaining lots of attention to define various types of cancers since they play critical roles in many different cellular processes including metabolism, apoptosis, differentiation, and development. Several studies have shown that microRNA's signatures are associated with the staging, progression, and response to treatment in breast cancer. In addition to this microRNA has been shown to act as oncogenes and tumor suppressor genes.

Continued efforts to delineate the microRNA function in mammary physiological and pathological conditions will reveal novel insights into normal cells and breast cancer biology and ultimately provide a new molecular target for alternate therapy. The book chapter covers the role of microRNAs in the diagnosis, staging, progression, prognosis, and response to treatment of breast cancer.

Keywords

MicroRNA • Breast cancer • Diagnosis • Prognosis • Breast cancer staging • Treatment strategies

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Introduction

MicroRNAs (miRNAs) are small noncoding RNAs (ncRNAs), usually 20–25 nucleotides long and capable of regulating the gene expressions at the post-transcriptional level. miRNA regulates gene expression by translational repression, mRNA cleavage, and mRNA decay, which have been found to control cell division, differentiation, and death. Their regulatory activity brings about by their binding to the coding region as well as 3′ and 5′ untranslated regions (UTRs) of messenger RNAs (mRNAs). Such bindings result in either inhibition of translation or degradation of mRNAs [1–3]. As estimation, the human genome encodes about 1,500 miRNAs. It is believed that they regulate more than 30 % of protein-coding genes. Like one gene multiple polypeptide and multiple gene single polypeptide story, an individual miRNA can target multiple genes, and each protein-coding gene can be regulated by several miRNAs [4]. Their involvement has been reported in several biological processes, such as apoptosis, proliferation, differentiation, and metastasis [5, 6].

Breast cancer is the most common form of cancer in women and the second most common cause of cancer death for women worldwide [7]. The tools available for breast cancer diagnosis and prognosis are not yet satisfactory at the molecular level and require further improvements. The miRNA expression profiling of human breast cancer has led to the identification of signatures correlated with the diagnosis, staging, progression, prognosis, and response to treatment. MicroRNA fingerprinting can therefore be added to the diagnostic and prognostic tools in diseases including breast cancer used by medical experts.

MicroRNAs: The Discovery

MicroRNAs were first discovered in 1993 by the joint efforts of Ambros and Ruvkun's laboratories [8]. They discovered that lin-4 in *C. elegans* does not code for a protein, but instead produced a pair of short RNA transcripts that each regulates the timing of larval development by translational repression of lin-14, which encodes for a nuclear protein. Hence, it was postulated that the regulation was due in part to sequence complementarity between lin-4 and unique repeats within the 3′ UTR of the lin-14 mRNA. The downregulation of lin-14 at the end of the first larval stage initiates the developmental progression into the second larval stage [9, 10]. It was only in 2000 when let-7 [11] was discovered to repress lin-41, lin-14, lin-28, lin-42, and daf-12 mRNA during transition in developmental stages in *C. elegans*. This function was phylogenetically conserved in species beyond nematodes, and it became apparent that the short noncoding RNA identified in 1993 was part of a wider phenomenon.

Since then, thousands of miRNAs have been identified in different organisms through random cloning and sequencing or computational prediction [12]. However, about 1,500 miRNAs are reported in the human genome. The identified miRNAs and their associated data are currently curated at the miRBase database. miRBase is hosted by the Sanger Institute as a publicly available repository (http://microrna.sanger.ac.uk/). Due to their abundant presence and far-reaching potential, miRNAs have all sorts of functions in physiology, from cell differentiation, proliferation, and apoptosis to the endocrine system, hematopoiesis, morphogenesis, etc. They display different expression profiles from tissue to tissue, reflecting the diversity in cellular phenotypes and as such suggest a role in tissue differentiation and maintenance. Figure 8.1 emphasizes the revolutionary studies that have significantly contributed to the history of miRNAs.

MicroRNAs: The Biogenesis

Various approaches have provided a basic understanding of the molecular details of miRNA biogenesis (Fig. 8.2) and it has long been viewed as linear and universal to all mammalian miRNAs. To understand miRNA biogenesis at a molecular level, we have classified miRNA biogenesis into the following three subheadings:

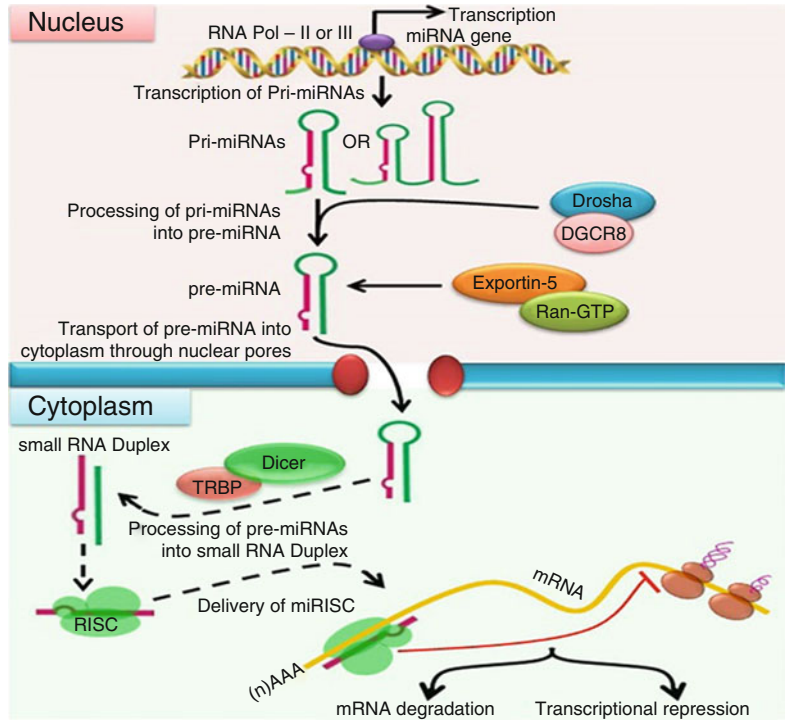

Fig. 8.1 Historical perspective of selected hallmarks on the evolution of microRNA history

1969	Non-protein coding transcripts (activator RNAs) regulate gene activity (Britten and Davidson)
1993	Recognition of *lin-4* as non coding small RNA(Lee, Feinbaum, and Ambros)
2000	Discovery of *let-7* (Reinhart et al.) RNAi "unit": 21-23 nt (Zamore et al.)
2001	Large class of small RNA (miRNA) co-express and regulates the gene expression (Lau et al.; Lagos-Quintana et al.; Lee and Ambros)
2002	Discovery of miRNA and targets of miRNA in plants (Reinhart et al.; Rhoades et al.) Downregulation of miRNA (miR15 and miR16) in blood cancer cells (Calin et al.)
2004	Majority of miRNA genes are located in cancer-associated genomic regions (Calin et al.) miRNA as diagnostic/prognostic biomarker(Takamizawa et al.) Co-expression of miRNAs and their host genes (Rodriguez et al.)
2005	miRNA-target interaction relevant to cancer (Johnson et al.) Altered expression of miRNAs affects tumor formation/growth *in vivo* (He et al.) Association between miRNAs and the MYC oncogene (O'Donnell et al.) Inhibition of miRNA by antagomirs in mammals (Krützfeldt et al.)
2006	Epigenetic regulation of miRNAs (Saito et al.) molecule of the year -hsa-mir-155 and hsa-let-7a-2 (Yanaihara et al.)
2007	5'-UTR also may the target for miRNA (Lytle et al.) miRNAs are deregulated in cancer metastasis (Ma et al.) miRNAs can up-regulate mRNA expression and translation of proteins (Vasudevan et al.) miRNAs can reactivate of silenced tumor suppressor genes by affecting epigenetics (Fabbri et al.) miRNAs can regulate ncRNAs from the category of long ultraconserved genes (UCGs) (Calin et al.) miRNAs carrying hexanucleotide terminal motifs are enriched in the nucleus (Hwang et al.)
2008	miRNAs can transcriptionally silence gene expression (Kim et al.) Functional single nucleotide polymorphism (SNP) in the miRNA seed region (Shen et al.) miRNA binding sites located within mRNA-coding sequence (Tay et al.) Expression of miRNA in serum/plasma (Chim et al.)
2009	Proof of concept of miRNA delivery as cancer therapy (Kota et al.)
2010	miRNA as molecular decoys (Eiring et al.) miRNAs predominantly cause mRNA destabilization (Guo et al.) Overexpression of a single miRNA is sufficient to cause cancer (Medina et al.)
2011	Competing endogenous RNA (ceRNA) communicate with and regulate other RNA transcripts by competing for shared miRNAs(Salmena et al.)

Fig. 8.2 MicroRNA processing and activity. Depicts the formation of long primary microRNA (pri-miRNA) in the nucleus which is processed by the microprocessor complex (Drosha, an RNAse III enzyme, and Pasha, a double-stranded RNA-binding protein) into precursor microRNA (pre-miRNA) (70 nt stem-loop structure) and transported to the cytoplasm by Exportin-5-mediated export, where Dicer, an RNase II enzyme, cleaves it to 20–25 nt mature miRNA that integrates it into the miRNA-inducing silencing complex (miRISC), a complex of proteins that is responsible for regulation of gene expression either by translational inhibition or by target mRNA degradation

Nuclear Processing by Drosha

This canonical maturation includes the production of the primary miRNA transcript (pri-miRNA) by RNA polymerase II or III and cleavage of the pri-miRNA by the microprocessor complex Drosha–DGCR8 (Pasha) in the nucleus [13]. A primary transcript RNA (pri-miRNA) transcribed from a miRNA gene by RNA polymerase II or III is first processed into a stem-loop structure of about 70–80 nucleotides known as precursor miRNA (pre-miRNA) by a microprocessor enzyme comprising of a double-strand (ds)-RNA-specific ribonuclease, Drosha, with the help of its binding partner DGCR8.

Nuclear Export of Pre-miRNAs

The resulting precursor hairpin, the pre-miRNA, is exported from the nucleus by Exportin-5–Ran-GTP [13]. Exportin-5 recognizes the pre-miRNA independently of its sequence or the loop structure. A defined length of the double-stranded stem and the 3′ overhangs are important for the successful binding to Exportin-5, ensuring the export of only correctly processed pre-miRNAs [13].

Cytoplasmic Processing by Dicer

In the cytoplasm, the RNase Dicer in complex with the double-stranded RNA-binding protein TRBP cleaves the pre-miRNA hairpin to its mature length. The functional strand of the mature miRNA is loaded together with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where it guides RISC to silence target mRNAs through mRNA cleavage, translational repression, or deadenylation, whereas the passenger strand is degraded [13].

MicroRNA Genes and Their Transcription

MicroRNA genes reside in regions of the genome as distinct transcriptional units as well as in clusters of polycistronic units—carrying the information of several microRNAs [10, 14–16]. Studies suggest that approximately half of known microRNA reside in non-protein-coding RNAs (intron) or within the intron of protein-coding genes [17].

The understanding of microRNA transcription is very important for determining their regulators as well as the specific role they may play in signaling cascades. The understanding of microRNA transcriptional regulation has great public health significance. The ability to understand how these post-transcriptional gene regulators function in cellular networks may provide new molecular targets for cures or therapies to a variety of human diseases.

Transcription of miRNA Genes

Little is known about the transcriptional regulation of these intergenic miRNAs, although RNA polymerase II appears to be involved in the process [18]. This suggests that miRNAs may have active promoter regions that contain cis-regulator elements similar to coding genes. miRNA genes are currently believed to be transcribed by RNA polymerase II (Pol II), [18] although a few may be transcribed by RNA polymerase III [19]. RNA polymerase II transcribes miRNA genes, generating long primary transcripts (pri-miRNAs) [20]. Subsequently, the process to yield mature miRNAs involves two steps involving RNase III enzymes and companion double-stranded RNA-binding domain (dsRBD) proteins. There are two different classes of miRNAs with respect to transcription mechanism—those found within annotated genes (intronic miRNAs) and those found in intergenic regions of the genome (intergenic miRNAs). It is presently believed that all intronic miRNAs are co-transcribed along with their host gene; this has been shown in both expression correlation studies [21] as well as PCR-based biochemical verification [17]. Intergenic miRNAs have been postulated to come from transcripts of up to 50 kb in length, allowing for the co-transcription of neighboring miRNAs (polycistronic miRNA clusters) [21]. Identification of the method by which miRNA genes are transcribed can lead to

the identification of the factors that are responsible for their regulation.

MicroRNAs in Breast Cancer

MicroRNAs have been concerned with an increasing number of neoplasia and biological process. The latest studies have shown a contribution for these regulatory molecules in breast cancer. For instance, microRNA profiling studies have identified microRNAs that are deregulated in breast cancer. Moreover, functional studies have revealed their roles in breast cancer as both oncogenes (e.g., hsa-*miR-21*) and cancer suppressor genes (e.g., hsa-*miR-335*). MicroRNAs deregulated in breast cancer control the translational regulation of entrenched regulative molecules, such as estrogen receptor-α, which are regulated by novel cancer-related molecules and *miR-206* whose functions are not yet completely understood.

MicroRNA in Cancer in General

miRNAs have been associated with the regulation of differentiation, proliferation, apoptosis, and even exocytosis [22]. Volinia et al. has demonstrated that the predicted targets for the differentially expressed miRNAs are significantly enriched for protein-coding tumor suppressors and oncogenes [22]. There is also confirmation to suggest that these miRNAs function in concert with classical tumor suppressors and oncoproteins to regulate key pathways involved in cellular growth control [23, 24]. miRNA profiling has the potential not only to classify tumors but also to augur patient outcome with high accuracy, but this approach needs more validation and detailed studies by using clinical samples [25]. Jian et al. showed that profile analysis with a probe set of 201 miRNAs achieved the similar discriminative potential as traditional gene array with 8,000 mRNA probes [26]. Consequently, this would mean that classification of tumors can be achieved with a more manageable amount of data and could potentially diminish the disparity that is often seen with mRNA-based classifier systems.

MicroRNAs Study in Breast Cells and Tissues

Since the study of mRNA, various technologies exist that allow the investigation of the expression of either profiling of a large number of miRNAs or individual miRNAs simultaneously. In general, these observational approaches have implicated individual or groups of miRNAs in pathological or physiological processes, as a result of the detection of changes in their expression, while additional functional experiments are required to gain further knowledge of their current roles. A few miRNA profiles have been developed using a large number of single miRNA detection experiments, such as Northern blotting [14], and these technologies remain the standard against which newer profiling methods are primarily compared. Nevertheless, oligonucleotide microarray-based detection platforms, with their associated ease of use and high-throughput nature, have largely supplanted this technique [27]. Microarrays have been used for miRNAs profiling from a wide range of breast tissue types and cell lines, including formalin-fixed paraffin-embedded (FFPE) clinical samples. It is essential to note that, due to the small size of miRNAs, they are comparatively insensitive to the damage that typifies mRNAs within FFPE. Accordingly, miRNAs present an invaluable new target for studies using archival clinical samples, which can often be linked to extensive clinical background and, more importantly, follow-up data or meta-analysis [28]. Multiplex real-time RT-PCR and liquid bead-based technologies are current alternative strategies for miRNA profiling, and it is claimed that they may have a higher sensitivity and specificity [29, 30]. Methodologies based on deep sequencing of small RNA libraries obtained from tissues may also allow miRNA profiling, with the supplementary advantage that these techniques are unbiased with respect to target sequences and may permit detection of novel miRNAs [31].

Profiling Data of miRNA in Breast Cancer

The expression of miRNAs has been investigated in an extensive range of breast cancer cell lines, tissues, and clinical normal. These metadata hint toward functional roles of various miRNAs by association with cellular behavior or particular molecular markers. To gain the best insight into breast cancer, it is also essential to understand miRNA function in normal mammary gland development, and work is underway to address this question in detail [32]. A potentially powerful empirical approach is to compare miRNA expression in normal breast versus breast cancer and thereby to differentiate those miRNAs expressed at different levels. Table 8.1 lists miRNAs identified as playing a role in breast cancer and their potential targets. Iorio et al. reported for 76 breast samples diagnosed for the expression of 246 miRNAs, out of which 29 miRNAs expression levels were found to be significantly different (i.e., p <0.05) in cancer versus normal clinical tissue. The majority consistently downregulated were has-*miR-10b*, has-*miR-125b*, and has-miR-145, while has-*miR-21* and has-*miR-155* were upregulated, suggesting that these may act as oncogenes or tumor suppressor genes, respectively [32]. They went on to examine whether the expression profile varied according to conventional clinical aspects: ER+ /ER−, PR+ /PR−, HER2+ /HER2−, positive and negative lymph node status, presence and absence of vascular invasion, high and the low proliferation index, and ductal/lobular histopathological subtype. The majority of comparisons discovered a small number of differentially expressed miRNAs, indicating that miRNAs may have roles in defining the differences between these pathological and molecular profiles. Yet, comparison between ductal/lobular carcinomas and HER2+ and HER2− tumors did not reveal differentially expressed miRNAs.

Similarly, specific miRNA profiles have been associated with breast cancer subgroups of distinct patterns of molecular marker expression. Profiling of 204 miRNAs has been shown sufficient to allow unsupervised clustering to be used to distinguish HER2+ /ER−, HER2+ /ER−, and HER2−/ER+ breast cancers within a cohort of 20 tumors. While this in itself is not an advance, since these cancers are routinely defined using immunohistochemistry, further supervised analysis of the profiles allowed distinct miRNA subsets to be identified that distinguished HER2+ from HER2− and ER+ from ER− breast cancers, independent of other clinically important parameters. Restricted subsets of miRNAs specific to HER2 status (*let-7f*, *let-7g*, *miR-107*, *miR-10b*, *miR-126*, *miR-154*, and *miR-195*) and specific to

Table 8.1 miRNAs whose expression is deregulated in breast cancer and their potential targets

MicroRNA	Expression pattern	Target
hsa-let-7 family	Down	IL-6, ESR1 (ER)
hsa-miR-101	Down	EZH2
hsa-miR-10b	Down	HOXD10, Tiam1
hsa-miR-1226	Up	MUC1
hsa-miR-122a	Up	
hsa-miR-125	Down	HuR, ERBB2, ERBB3, BAK1, BMPR1B, CYP24, MUC1
hsa-miR-126	Down	IRS1
hsa-miR-128	Up	TGFβR1
hsa-miR-136	Up	
hsa-miR-143	Down	
hsa-miR-145	Down	MUC1, RTKN, ESR1
hsa-miR-146a		BRCA1, BRCA2
hsa-miR-146		IRAK1, TRAF6
hsa-miR-149	Up	
hsa-miR-150	Up	c-MYB
hsa-miR-155	Up	FOXO3, SOCS1, RHOA
hsa-miR-16	Down	MYB, WIP1
hsa-miR-17/92 cluster	Deleted	BRCA1, IL-8, CCND1, HBP1, AIB1, ESR1 (ER), ESR2 (ER), HIF1, STAT3
hsa-miR-185	Down	SIX1
hsa-miR-191	UP	
hsa-miR-196	UP	ANXA1
hsa-miR-200	Down	ZEB1, ZEB2, FTH1, PLCG1, BMI1, FN1, NTRK2, QKI
hsa-miR-202	Up	
hsa-miR-203	Up	
hsa-miR-204	Down	
hsa-miR-205	Down	ERBB3, ZEB1, HER3, VEGF-A

Table 8.2 Oncogenic miRNAs involved in breast cancer and their potential targets

MicroRNA	Targets identified
hsa-miR-21	Bcl-2, *PDCD4*, PTEN, *TPM1*, *TIMP3, HER2,* maspin
hsa-miR-155	Caspase 3, *SOCS1,* RhoA, FOXO3a
hsa-miR-27a	*ZBTB10*, FOXO1
hsa-miR-96	FOXO1
hsa-miR-182	FOXO1, CBS7, DOK4, NMT2, EGR1
hsa-miR-128a	TGFβR1
hsa-miR-10b	*RhoC*, HOXD10
hsa-miR-373	*CD44*
hsa-miR-520c	*CD44*
hsa-miR-221	TRPS1
hsa-miR-222	TRPS1
hsa-miR-375	RASD1
hsa-miR-224	RKIP
hsa-miR-135a	HOXA10
hsa-miR-183	CBS7, DOK4, NMT2, EGR1

ER/PR status (*miR-142-5p*, *miR-200a*, *miR-205*, and *miR-25*) have been also established [33].

Functional Studies: miRNA in Breast Cancer

The functional activity of only a few miRNAs has been practically modeled in the perspective of breast cancer, and it is clear that most of this type of work remains to be done. Potential functions of miRNAs in carcinogenesis into potential oncogenes and tumor suppressor genes have been established in regulating immune system, cell proliferation, differentiation and development, cancer and cell cycle.

Breast Cancer Oncomirs

Table 8.2 summarizes examples of miRNAs that have apparent oncogenic activity in breast cancer. Oncogenic miRNAs, commonly known as oncomirs, may act by hindering the expression of tumor suppressor genes and/or genes responsible for apoptosis and differentiation. Recent studies have identified functional oncogenic role(s) for miRNAs, their individual manipulation in breast cancer cell line models, and subsequent assessment of associated phenotypic changes. Iorio et al. showed that miRNAs aberrantly expressed in human breast cancer compared with normal breast tissue, with the most significantly downregulated miRNAs being miR-10b, miR-125b, and miR-145, whereas the most significantly upregulated miRNAs being miR-21 and miR-155 [33]. Si et al. found that the anti-miR-21-mediated cell growth inhibition associated with increased apoptosis and decreased cell proliferation [34]. Their results suggested that miR-21 functioned as an oncogene and modulated tumorigenesis through regulation of genes such as Bcl-2. miR-301 has been identified as a novel oncomir in human breast cancer, which promotes growth, proliferation, invasion, and metastases, mediated at least by FOXF2, BBC3, and PTEN genes [35]. miR-221/miR-222 activate β-catenin and contributed to estrogen-independent growth, whereas TGFβ-mediated growth inhibition was repressed by the two miRNAs [36]. miR-21, miR-210, and miR-221 expressions play a significant role in triple-negative primary breast cancers [37].

Breast Cancer Tumor Suppressor miRNAs

In contrast to oncomirs, if the expression of an miRNA is lowered in cancer cells compared to normal cells, it is regarded as a tumor suppressor (oncosuppressor). Such miRNAs are associated with tumor-suppressive activity, because they operate by inhibiting genes that promote tumorigenesis (oncogenes) and control cellular differentiation and/or apoptosis. Accordingly, the dysfunction of an oncosuppressor may ultimately lead to the development of malignant cells. Table 8.3 summarizes examples of such miRNAs, which support their role as tumor suppressors in breast cancer. The expression of *miR-125b*, *miR-145*, *miR-21*, and *miR-155* has been shown significantly reduced in breast cancer tissues [33]. Studies have shown that miR-204 exerts its function by targeting genes involved in tumorigenesis and the genomic loci encoding miR-204 are frequently lost in multiple cancers, including

Table 8.3 Tumor-suppressive miRNAs involved in breast cancer and their potential targets

microRNA	Targets identified
hsa-miR-125a	HER2, HER3, HuR
hsa-miR-125b	HER2 HER3, c-Raf
hsa-miR-205	HER2, HER3, VEGF-A
hsa-miR-27b	CYP1B1
hsa-miR-17–5p	AIB1, CCND1, E2F1
hsa-miR-17/20	Cyclin D1
hsa-miR-206	ESR1
hsa-miR-145	RTKN, ER-alpha
hsa-miR-200	ZEB1, ZEB2, PLCG1, BMI1
hsa-miR-146	NF-κB
hsa-miR-335	*SOX4, TNC*
hsa-miR-126	–
hsa-miR-206	–
hsa-miR-224	*CDC42, CXCR4*
hsa-miR-31	*FZD3*, *ITGA5*, *M-RIP*, *MMP16*, *RDX*, *RhoA*
hsa-miR-34a	*Bcl-2*, *SIRT1*, CCND1, CDK6, E2F3, MYC
hsa-miR-342	HER2Δ16
let-7	LIN28, HMGA2, H-RAS, PEBP1
hsa-miR-98	–
hsa-miR-375	MTDH
hsa-miR-203	BIRC5, LASP1
hsa-miR-30a	Vimentin
hsa-miR-7	Pak1

breast cancers, ovarian cancers, and pediatric renal tumors [38]. miR-34b is recognized as an oncosuppressor that targets cyclin D1 and Jagged-1 (JAG1) in an ER+/wild-type p53 breast cancer cell line (MCF-7), as well as in ovarian and endometrial cells, but not in ER-negative or mutant p53 breast cancer cell lines (T47D, MBA-MB-361, and MDA-MB-435) [39].

MetastamiRS Implicated in Breast Cancer Invasion and Metastasis

The current breast cancer management strategies mainly focus on early detection, tumor resection, and neoadjuvant or adjuvant treatment with radiation, chemotherapy, and/or new targeted agents. Despite advancements in the treatment of this disease, breast cancer still remains a leading cause of cancer death. Metastasis is the primary reason for high cancer death rates. Therefore, to successfully contain breast cancer, there is an urgent need to define therapeutic cocktails that could effectively target a breast tumor before it metastasizes. As discussed above, several recent research investigations have established the presence of aberrant expression of miRNAs with the potential of either promoting or suppressing tumorigenesis in breast cancer compared to normal breast tissue. The possibility that miRNAs specifically contribute to metastasis has only recently been explored. Several miRNAs have now been described as potentially promoting or suppressing metastasis (metastamiRs) and are summarized in Table 8.4.

Massimo et al. highlighted a series of recent studies that proved the involvement of miRNAs in breast cancer metastases [40]. They found (a) *miR-10b* indirectly activates the pro-metastatic gene *RhoC* by suppressing *HOXD10* and TIAM1, thus leading to tumor invasion and metastasis [41, 42]; (b) *miR-373* and *miR-520c* can also promote tumor invasion and metastasis, at least in part by regulating the gene *CD44* [43]; and (c) *miR-335, miR-206,* and *miR-126* as a suppressors of breast cancer metastasis miRNAs. The loss of *miR-335* leads to the activation of *SOX4* and *TNC* (encoding tenascin C), which are responsible for the acquisition of metastatic properties [44]. E-cadherin (CDH1) is a tumor suppressor protein that is used as a prognostic marker for breast cancer [45]. There are several studies demonstrating that the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) inhibits EMT and the initiating steps of metastasis by maintaining the epithelial phenotype of cells [46–50]. Tavazoie et al. [44] reported a set of eight miRNAs (miR-335, miR-199a, miR-122a, miR-126, miR-206, miR-203, miR-489, and miR-127) which had a lower expression in metastatic breast cancer cells as compared to their non-metastatic counterparts. Interestingly, concurrent re-expression of ITGA5, RDX, and RHOA abrogated miR-31-imposed metastasis suppression [51], indicating that these three genes were the main mediators of miR-31 effects.

In total, these significant findings are important for our understanding of malignant

Table 8.4 Role of microRNAs in breast cancer metastasis and their potential targets

miRNA	Targets identified
hsa-miR-10b	*RHOC, E-cad, HOXD10, Tiam1*
hsa-miR-373	*CD44*
hsa-miR-502c	*CD44*
hsa-miR-21	HER, TIMP3, PDCD4, TPM1, maspin PTEN, BCL-2, RHOB, MMPs
hsa-miR-200 family	ZEB1, PLCG1, BMI1, TGFβ2 FAP-1, Suz12
hsa-miR-146	NF-κB TRAF6, IRAK1, ROCK1 CXCR4, EGFR
hsa-miR-335	*SOX4, TNC, PTPRN2, MERTK*
hsa-miR-126	–
hsa-miR-206	NOTCH3, SRC-1, SRC-3, GATA-3, ER-alpha Estrogen receptor-alpha
hsa-miR-224	*CDC42*, *CXCR4*
hsa-miR-31	ITGA5, RDX, RhoA
hsa-miR-12b	STARD13
hsa-miR-30a	*Vim*
hsa-miR-34a/c	*Fra-1*
hsa-miR-9	E-cad
hsa-miR-29a	TTP
hsa-miR-103/107	Dicer
hsa-miR-210	–
hsa-miR-132	p120, Ras, GAP
hsa-miR-155	RhoA
hsa-miR-7	Pak1, EGFR
hsa-miR-17/20	Cytokines, cyclin, D1, IL-8
hsa-miR-22	CDK6, SIRT1, Sp1, ERBB3, CDC25C, EVI-1, ER-alpha
hsa-miR-126	Crk
hsa-miR-miR-127, miR-197, miR-222, miR-223	CXCL12
hsa-miR-145	IRS-1, mucin-1, c-Myc, JAM-A, fascin
hsa-miR-193b	uPA
hsa-miR-205	ZEB, VEGF, HER3
hsa-miR-448	ATB1
hsa-miR-661	MTA1, Nectin-1, StarD10
hsa-let-7	RAS, HMGA2, MYC

transformation in the breast and may have implications for the management of patients with advanced breast cancer. The use of miRNAs as anticancer therapeutic agents is promising, and such fine molecular studies definitely help in getting miRNAs closer to clinical practice.

Clinical Potential of miRNAs in Breast Cancer

miRNAs are suitable biomarkers for early cancer detection as they are present and stable in human serum or plasma. Furthermore, they appear to be differentially expressed in cancer patients compared to healthy donors. Several studies have successfully identified miRNAs linked to breast cancer subtypes and clinical–pathological features. Several miRNAs appeared to be specifically expressed in each of the clinicopathological groups. The assessment of the presence of ER, PR, and/or HER2 on breast cancer specimens is currently a routine procedure. All these markers have been independently associated with breast cancer prognosis. Actually, breast cancers presenting as triple negative (ER–/PR–/HER2–) are characterized by more aggressive behavior and poor prognosis. It is therefore of great importance to identify other factors, specially miRNAs, which play a role in receptor regulation that could be used to influence the therapeutic management of breast cancer patients. Identification of miRNAs is associated with ER and PR status, followed by others including also HER2 status evaluation and the functional role of specific miRNAs in ER, PR, or HER2 regulation.

In addition, the identification of patients who can benefit from treatment with chemotherapeutic agents in terms of quality of life and/or probability of survival is of great importance in oncology. Further ineffective chemotherapy may increase patient mortality. miRNAs have proven to be useful in predicting breast cancer cell sensitivity to chemotherapy.

MicroRNAs and Drug Response in Breast Cancer

Breast cancer patients may have a different susceptibility to anticancer drugs, due to their genetic and epigenetic background, or cancerous cells may become resistant during tumor progression. Progression of breast cancer and resistance to therapies have been attributed to the

Table 8.5 Single nucleotide variation in breast cancer microRNA genes

Genetic variation	MicroRNA	Clinical outcome
rs2910164 (G–>C)	hsa-miR-146a	Down
rs11614913 (T–>C)	hsa-miR-196-a2	Up
rs3746444 (A–>G)	hsa-miR-499	Down/up
Germ line G–>T 8nt	hsa-miR-125a	Down
rs895819 (A–>G)	hsa-miR-27a	Down
rs895819 (C/T)	hsa-miR-27a	–
ESR1 3′ UTR (target site)	hsa-miR-453	Down
SET8 3′ UTR (target site)	hsa-miR-502	Up
BMPR1B 3′ UTR (target site)	hsa-miR-125	Up

possibility of miRNAs involved in the regulation of certain signaling pathways. Several recent studies have focused on the identification of miRNAs linked to the acquisition of the resistant phenotype in breast cancer. Salter [52] and others examined the full mRNA and miRNA profile on the NCI-60 panel of cell line to identify signatures linked to sensitivity to paclitaxal, 5-fluorouracil, Adriamycin, and cyclophosphamide (TFAC): a miRNA signature linked to each drug response was identified.

Recently, several single-nucleotide polymorphisms (SNPs) have been described in precursor or mature cancer-related miRNAs or in miRNA target sites, and some of them have been linked to increased cancer risk. Table 8.5 summarizes a list of genetic alterations in miRNAs in breast cancer. Mishra et al. [53] identified SNP in human dihydrofolate reductate 3′ UTR and found it was responsible for methotrexate resistance in cancer cells due to lack of inhibition by miR-24, whose binding site is located near the polymorphism.

miRNAs differentially expressed in breast cancer not only play a key role in the regulation of apoptosis and invasion, but it appears to confer poor prognosis and drug resistance by sensitizing cells and modulating drug response. Thus, studies suggest the importance of integrating information derived from the miRNA profile with currently used markers.

Methylation of Breast Cancer miRs Genes

Epigenetics including DNA methylation plays a key role in the regulation of miRNA expression, and a number of reports explain silencing of miRNA expression linked to the aberrant DNA methylation of individual miRNA genes in breast cancer. miRNA genes can be epigenetically deactivated by aberrant DNA methylation in a manner similar to that of classical tumor genes. The involvement of epigenetic mechanisms in the regulation of miRNA gene expression in breast cancer was first addressed by Scott et al. [54] in an in vitro model system. Saito et al. [55] provide in their pioneering study evidence for the involvement of DNA methylation in the regulation of microRNA genes, suggesting epigenetic reactivation of microRNA expression as a promising novel strategy for cancer therapy.

The extensive and frequent hypermethylation of microRNA genes in human breast cancer supports the concept that epigenetic instability is an important and also an early event in human tumorigenesis. Considering the high frequency of miRNA gene hypermethylation found in breast cancer, miRNA gene methylation might serve as a sensitive marker for epigenetic instability. Given the fact that the binding specificity of an miRNA is conferred by a very short sequence, the prediction of specific targets remains a major bioinformatics challenge.

Conclusion and Future Perspective

In a nutshell, miRNAs have rewritten the rules about our understanding of molecular cancer biology since their initial discovery in 1993, followed by their association with cancer in 2002, and the subsequent identification of their presence in the systemic circulation in 2008. Both oncogenic and tumor-suppressive roles of miRNAs in breast cancers have encouraged numerous investigations regarding quantity of miRNAs that could be used as biomarkers and possibly manipulated for clinical benefit. The panorama of circulating miRNAs may be useful as diagnostic,

prognostic, and/or predictive biomarkers, some of which may also have relevance as new therapeutic targets, and this looks promising and very exciting.

Further investigations are warranted that will fully characterize miRNAs, their functional targets, and the phenotypic effects associated with their targeted manipulation, to harness the power and potential of miRNAs and translate this information to the clinic in the interest of breast cancer patients. Since their discovery, miRNAs have shown great potential, both as tumor biomarkers and potential as therapeutic targets in a comparatively short period, and are important contributors to the future management and therapeutic strategies of cancers in general and breast cancers in particular.

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