Review

Molecular Cancer Research

Genomic and Epigenomic Cross-talks in the Regulatory Landscape of miRNAs in Breast Cancer

Devyani Samantarrai[1], Subhra Dash[2], Bini Chhetri[1], and Bibekanand Mallick[1]

Abstract

MicroRNAs (miRNA) are a class of endogenous, small noncoding RNAs found in animals, plants, and viruses that control their target gene expression posttranscriptionally. They are involved in a wide array of biological processes including cell differentiation, development, cell death and homeostasis, and fine-tune the regulation of these pathways. Their aberrant expressions have been associated with different diseases. These small RNAs are also known to function as oncogenes, oncosupressor genes, modulators of metastatic spread, and regulators of cancer stem cells. Their deregulation is a hallmark of different cancers types including breast cancer. Despite the growing evidence for their involvement in breast cancer, understanding the interplay between miRNAs and their targets leading to the disease remains largely unknown. Here, we provide a comprehensive story on miRNA signatures of breast cancer, miRNAs in breast cancer stem cells, metastamirs (i.e., metastasis regulatory miRNAs), circulating miRNAs as invasive blood-based biomarkers, and oncomiRs and oncosupressor miRNAs associated with breast cancer. Furthermore, we provide biological insights on their regulation by various mechanisms including genomic alterations and demonstration of a complicated feedback network between miRNAs and epigenetic regulators forming an epigenetics–miRNA regulatory circuit whose disruption may underlie the cause of breast cancer. *Mol Cancer Res; 11(4); 315–28. ©2013 AACR.*

Introduction

MicroRNAs (miRNA) are short, approximately 19 to 24 nucleotides (nts) long, single-stranded endogenous noncoding RNAs (ncRNA) that posttranscriptionally modulate gene expression (1) and thereby regulate a wide array of biological processes including cell differentiation, development, cell death, and homeostasis (2–3). The first miRNA gene, lin-4, was discovered in *Caenorhabditis elegans* in 1993 (4) while studying the genes involved in regulating the timing of larval development in nematode. Lin-4 was found to negatively regulate translation of lin-14 mRNA by binding to the complementary sites within the 3′untranslated region (3′UTR) of mRNA through imperfect base pairing during the first larval stage (L1) of the nematode (5). The miRNAs generally bind to target mRNAs through Watson and Crick complementary base pairing between 2 through 7/8 nts of miRNA (known as seed region) at 3′UTR (6). These reduce levels of target transcripts and inhibit protein translation, sometimes via noncomplete complementation with one or more mismatches in sequence complementarity (7, 8). Moreover, the binding regions have now been extended to any region of the target including the coding region (CDS) and 5′UTR (6). Seven years after the discovery of the first miRNA, another miRNA family member, let-7, was identified from the same species by Ruvkun and colleagues (9). Since then, there has been an explosion in the number of miRNAs discovered every year. As of now, 21,264 miRNAs have been identified in various organisms, out of which 1,600 precursor miRNAs encoding 2,042 mature miRNAs are in humans [miRBase (http://www.mirbase.org/), Release 19, August 2012].

These small regulatory RNAs are also known to be involved in various pathophysiological processes, including cancer etiology, progression, and prognosis (10–11). The expression of miRNAs is altered in cancer cells and many seminal studies have unraveled the differences in expression of miRNAs in tumor and normal tissues and between tumor types (12–13). The aberrant expression of miRNAs in cancers can lead to the altered expression of target mRNAs. miRNAs themselves are also regulated, especially by genetic or epigenetic mechanisms or by aberrant expression of transcription factors. Furthermore, miRNAs can also modulate multiple genes epigenetically by controlling the levels of primary epigenetic regulators, DNA methyltransferases (*DNMT*), or histone deacetylases (*HDAC*; refs. 14, 15). There is interplay between miRNAs and target mRNAs that determines the fate of the cell, either normal or diseased.

Breast cancer is one of the leading causes of cancer-related death among women of all races. Aberrant miRNA expression in human breast cancer was first shown in 2005 (16).

Authors' Affiliations: [1]RNA Biology and Functional Genomics Laboratory, Department of Life Science, National Institute of Technology, Rourkela; and [2]KIIT School of Biotechnology, KIIT University, Bhubaneswar, Orissa, India

Corresponding Author: Bibekanand Mallick, RNA Biology and Functional Genomics Laboratory, Department of Life Science, National Institute of Technology, Rourkela 769008, India. Phone: 91-661-2462685; Fax: 91-661-2472926; E-mail: mallickb@nitrkl.ac.in, vivek.iitian@gmail.com

doi: 10.1158/1541-7786.MCR-12-0649

Over the last few years, studies on involvement of miRNA in breast cancer have accelerated and its role as critical biomarkers for classification, diagnosis, prognosis, and treatment are being established (17). Many studies have shown that miRNAs, in addition to regulating the cellular levels of various oncogenes or tumor suppressor genes (TSG; ref. 11, 14), also act as oncogenes, termed as oncomiRs (18) and tumor suppressors, termed as oncosuppressor miRNAs (19). In this review, we aim to provide a comprehensive story on the miRNA signatures of cancers, especially in breast cancer, their role as oncomiRs or oncosuppressors, how they regulate the genes and how they are being regulated (genomic and epigenomic alterations) to understand the interplay between miRNAs and mRNAs leading to breast cancer.

Biogenetic Pathways of miRNAs

The mature miRNAs are generated from miRNA genes through a multistep event (Fig. 1). The genes are transcribed into a fragment of 1 to 3 kb long primary transcripts (pri-miRNA), usually by RNA polymerase II (20), similar to protein coding genes in the genome or by RNA polymerase III (21). One pri-miRNA can contain one or several miRNAs. This pri-miRNA present in the nucleus is then recognized and processed by a microprocessor complex formed by the RNaseIII enzyme Drosha and DGCR8 (DiGeorge critical region 8) protein, also known as Pasha (Partner of Drosha; ref. 4). The processing results in the formation of a hairpin intermediate of about 60 to 100 nts with a stem loop structure called pre-miRNA. Drosha is a dsRNA-specific endonuclease present in the nucleus. It is a multidomain protein and mainly contains 2 RNaseIII domains, a dsRNA-binding domain, an amino terminal segment of unknown function, and generates 2 nts long 3′ over hangs at the cleavage site (4). DGCR8/Pasha protein of the microprocessor complex contains 2 dsRNA-binding domains and is essential for miRNA processing in all organisms (22). It stably interacts with pri-miRNA and acts as a molecular ruler to determine the specific cleavage site (23). The pre-miRNA formed is then transported from the nucleus to the cytoplasm via a nuclear export factor Exportin-5 in complex with RanGTP (24). A defined length of the double-stranded stem and the 3′ overhangs of the pre-miRNA are important for successful binding to Exportin-5, which ensures the export of only correctly processed pre-miRNAs (25).

Once the pre-miRNA gets transported into the cytoplasm, its further processing and RISC (RNA-inducing silencing complex) assembly is carried out by RLC (RISC loading complex). RLC is a multiprotein complex composed of the RNase III enzyme Dicer, dsRNA-binding domain proteins TRBP (Tar RNA-binding protein), PACT (protein activator of PKR), and the core component Argonaute-2 (Ago2; ref. 26). The pre-miRNA is cleaved by Dicer and generates approximately 19 to 24 nts long miRNA duplex molecule (27). TRBP mediates cleavage by Dicer and also stablizes it. Among the 2 strands of pre-miRNA, one is called the functional guide strand and the other is called the passenger strand. The guide strand gets incorporated into the RISC and guides the RISC to its target, whereas the passenger strand gets degraded. Ago2 protein facilitates the unwinding of pre-miRNA duplexes and RISC activation by cleaving the passenger strand. The biogenesis process completes with the assembly of the functional guide strand with the RISC complex. The miRNA along with RISC effector Ago2 mediates mRNA degradation, destabilization, or translational inhibition (reviewed in ref. 28). Apart from this, it has also been shown that some miRNAs can actually increase the

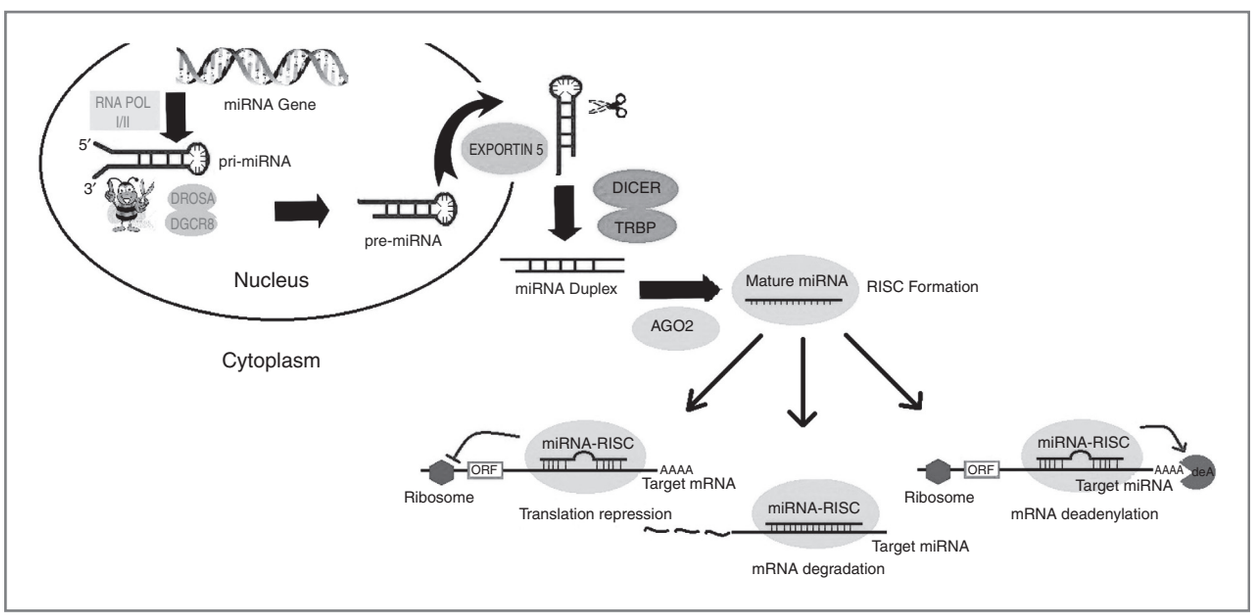

Figure 1. Biogenesis pathway of miRNA in animals and their function.

expression of a target mRNA in humans, termed as small-activating RNAs (29).

There are also reports of noncanonical alternative pathways of miRNA biogenesis. This includes the mirtron pathway in which pri-miRNAs get processed to pre-miRNAs with the help of the splicing machinery, but without the involvement of Drosha. This pathway has been discovered in mammals, *Drosophila*, and nematodes (30–32). Other major Drosha-independent pathways include tailed mirtrons (33), small nucleolar RNA-derived miRNAs (34), RNaseZ-mediated miRNA biogenesis (35), and integrator-mediated miRNA biogenesis (36). The dicer-independent pathway of processing of pre-miRNA has also been reported recently in vertebrate miRNA, mir-451, which requires Ago2 slicing activity (37).

miRNA in Oncogenesis

MiRNAs have been shown to be associated with many of the classical hallmarks of cancer, including defects in proliferation, differentiation, and apoptosis. With their widespread range of influence on biological pathways and implications as either oncogenes or TSGs, their dysregulation justify their significant role in tumorigenesis leading to cancer. This has been established through comparative genomics studies of cancer and normal tissues by adopting various high-throughput technologies, such as digital gene expression, next-generation sequencing, and so on. Until now, nearly 200 miRNAs are reported to be involved in the progression of human cancers (38). The first evidence of involvement of miRNAs in human cancer was noted in 2002 by Calin and colleagues (39) who showed that *miRNA-15a* and *miRNA-16-1* genes are located in a region deleted in more than half (69%) of B-cell chronic lymphocytic leukemia (B-CLL) cases. The same group mapped all the known human miRNA genes and found that many of them are frequently located at fragile sites of the genome, which are usually either amplified or deleted in human cancer (40). Genome-wide profiling has shown that miRNA expression signatures (miRNome, defined as the full spectrum of miRNAs for a specific genome) allowed different types of cancer to be discriminated with high accuracy (41). These profiling studies also help in identification of tissue of origin of poorly differentiated tumors. miRNAs being more stable as compared with long mRNAs, due to their small size, allow expression profiling from fixed tissues or other biological materials (14). This supports their possible use as novel, minimally invasive, and robust biomarkers for cancer.

Several miRNAs have been associated with cancers due to genomic changes, implying that miRNAs can act either as oncomiRs or oncosuppressor miRNAs (Fig. 2). In some cancers, there is amplification of chromosomal regions of miRNAs involved in the negative regulation of a transcript encoding a known TSG. These miRNAs would then silence the TSG leading to the development of cancer. Such miRNAs are called oncomiRs. On the contrary, oncosuppressor miRNAs genes are frequently located in fragile loci where deletions, mutations, promoter methylation, or any other abnormalities occur and have targets that include oncogenes. This can result in reduced miRNA levels and thus overexpression of target oncogenes occurs. These alternations of miRNA lead to tumor formation by inducing cell

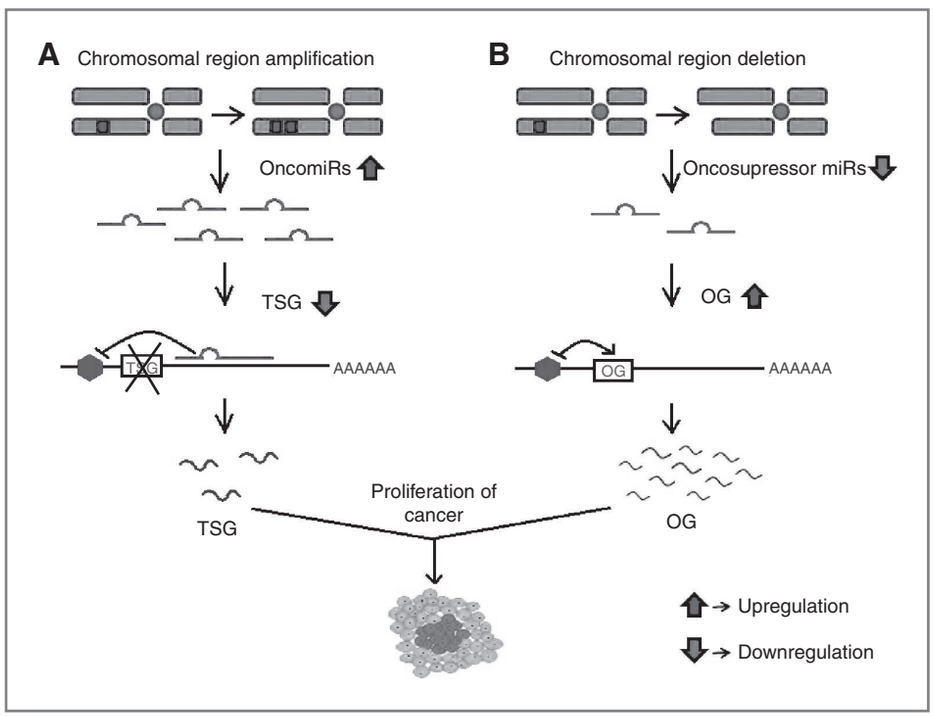

Figure 2. Role of oncomiRs and oncosupressor miRNAs in proliferation of cancer. A, amplification of chromosomal regions of miRNAs encoding oncomiRs leads to their upregulation. OncomiRs are involved in the negative regulation of a transcript encoding a known TSG, thus leading to TSG downregulation and proliferation of cancer cells. B, deletion of chromosomal regions of miRNAs encoding oncosupressor miRNAs leads to their downregulation. Downregulation of oncosupressor miRNAs results in upregulation of oncogenes and thus proliferation of cancer cells. OG, oncogene; TSG, tumor suppressor gene.

proliferation, invasion, loss of apoptosis, and angiogenesis. Thus, in short, miRNAs can act both as oncogenes as well as TSGs (1).

OncomiRs

OncomiRs are usually upregulated in cancer and target TSGs or antioncogenes, genes regulating the growth of cells (cell-cycle regulation genes, *p53*, etc). The best-characterized example of an oncogenic cluster is miR-17-92 polycistron (also known as OncomiR-1) because it was the first miRNA found to be acting as a mammalian oncogenes (42). It is located in chromosome 13 and amplified in human B-cell lymphomas (43), malignant lymphoma cell lines (44), lung cancer (45), and human breast cancer (41). It functions as an antiapoptotic miR cluster by targeting intrinsic apoptotic protein Bim in B-cell lymphoma subtypes (46). Another prominent oncomiR is miR-21, which is upregulated in most types of cancer. Studies by Yan and colleagues on overexpression of miR-21 in breast cancer and its target prediction revealed that the putative target genes of miR-21 include the oncogenes v-ski sarcoma viral oncogene homolog (*SKI*), *RAB6A* and *RAB6C* (members RAS oncogene family), RAS homolog gene family member B (*RHOB*), TGF-β–induced protein (*TGFβI*), TGF-β receptor II (*TGFβR2*), RAS p21 protein activator (*RASA1*), B-cell CLL/lymphoma 2 (*BCL-2*); and the apoptosis-related gene, programmed cell death 4 (*PDCD4*; ref. 47). Furthermore, miR-21 targets different TSGs in glioblastoma, including *p53* and *TGF-β* (48). Other targets of miR-21 includes *PTEN* in hepatocellular carcinoma (49); tumor suppressor genes *ANP32A* (nuclear phosphoprotein 32 family, member A/alias pp32/LANP) in human prostate carcinoma cells, and *SMARCA4* (alias *BRG1*) in B-cell lymphoma (50); maspin, a tumor suppressor in invasion and metastasis (51), and tropomyosin 1 (*TPM1*) in breast cancer MCF-7 cells (52). Another miRNA acting as an oncogene is miR-155 whose role in breast cancer progression has been studied recently (53). It is highly upregulated in breast cancer where it targets tumor suppressor gene *SOCS1* (54).

Oncosuppressor miRNAs

Oncosuppressor miRNAs are frequently lost or downregulated in cancer. The first evidence of miRNAs as tumor suppressors is the downregulation of miR-15a and miR-16-1 cluster, which leads to B-CLL (39). These miRNAs potentiate the normal apoptotic response by targeting the antiapoptotic gene *BCL-2* (55) and their loss thereby leads to tumor formation. Similarly, loss of mir-29b-1/mir-29 a cluster located in *7q32* leads to acute myeloid leukemia. The loss of both mir-15a-16-1 and mir-29b-1/mir-29a results in upregulation of target oncoproteins like *BCL-2*, *MCL1*, and *CDK6* and so on, thereby facilitating cancer progression (20). Another miRNA acting as an oncosuppressor is miR-34a, which is frequently seen to be absent in pancreatic cancer cells (56). It was shown that this miRNA was directly transactivated by *p53*, and is an important component of the *p53* transcriptional regulatory network. Let-7 is also widely viewed as a tumor suppressor as the expression of let-7 is downregulated in many cancer types (57–58). Figure 3 shows different targets of oncomiR miR-21 and oncosupressor miRNA let-7 in breast cancer and their putative functions.

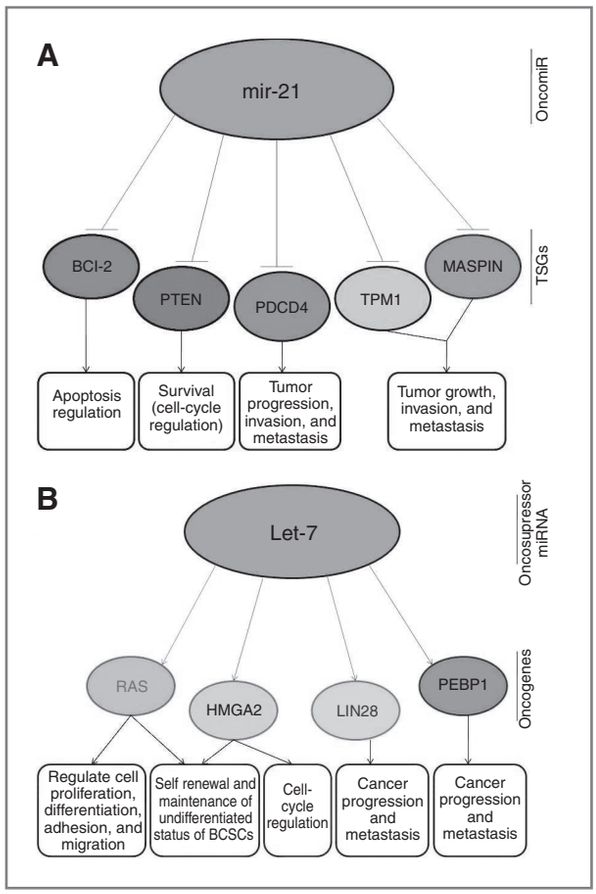

Figure 3. Different targets of oncomiR, miR-21 and oncosupressor miRNA, let-7 in breast cancer and their putative functions. A, oncomiR miR-21 represses the expression of the TSG genes. B, the downregulation or absence of oncosuppressor miRNA let-7 results in upregulation of the oncogenes in breast cancer.

miRNA in Breast Cancer

Breast cancer is considered as one of top 5 most prevailing cancers in the world with highest incidence among women (http://www.who.int/cancer/detection/breastcancer/en/). This disease is classified in many different ways including grade, stage, histopathological types, receptor status, etc. Depending upon the size and characteristics of the tumor, various therapies have been developed for its treatment. Some of the commonly available therapies for treating breast cancer are mastectomy, breast conserving surgery/partial mastectomy, hormone therapy, chemotherapy, and radiotherapy. These classical treatment procedures have many side effects (59). With so many side effects, the development of targeted therapies for breast cancer is urgently needed. The discovery of small RNA as a specific regulator of gene

expression was the most important discoveries of the last decade with some being targeted in therapies (60).

The evidence of involvement of miRNAs in the pathogenesis of human breast cancer gave a new direction to treat and detect cancer in a better and safer way. They are likely to yield a new class of targeted anticancer therapeutics either alone or in amalgamation with current targeted therapies, with the goal to improve disease response and increase cure rates (20). The advantage of using miRNA-based therapies is that they function by subtle repression of gene expression on a large number of oncogenic factors and are, therefore, predicted to be highly efficacious (61). There are 2 approaches for developing miRNA-based therapies, (i) introduction of miRNA mimics, which mimic the expression of protective miRNAs downregulated in cancer and (ii) introduction of antagomiRs, which are synthetic miRNAs complementary to the miRNAs of interest to inhibit oncomiRs overexpressed in tumor cells reviewed in ref. 20).

miRNAs in breast cancer stem cells

Recent studies have suggested key roles of miRNAs in regulating cancer stem cells (CSC; ref. 62). CSCs in contrast to normal stem cells are tumorigenic and metastatic by gained mutations. They have intrinsic mechanisms to evade traditional chemotherapeutic agents, which target more differentiated tumor cells (63). The necessity of miRNAs for proper maintenance of stem cells has been shown earlier (64). It is possible that alterations in regulation of miRNA expression elicited in a stem cell by chromosomal changes, such as amplification or deletion, or epigenetic changes, might induce the transformation of a lineage-restricted stem cell to a CSC (65).

Human breast cancer stem cells (BCSC) were first isolated in 2003 by Al-Hajj and colleagues (66). They identified a different set of cell surface markers $CD44^{+}/CD24^{-/low}$ of BCSCs. These BCSCs are critical progenitors of initiation and progression of breast cancer and they most likely arise from mammary stem cells (67). Many recent studies have shown the link between miRNA and BCSCs with a role of miRNA in phenotype of BCSCs (68–72). A study by Yu and colleagues in 2007 has shown the role of let-7 in regulating the self-renewal property and tumorigenicity of breast cancer cells. They have shown that let-7 inhibited stem cell self-renewal in both normal and CSCs of breast and the downregulation of let-7 was the reason for the phenotype formation of BCSCs, such as the maintenance of self-renewal capacity and undifferentiated status. Let-7 is known to target many oncogenes like *k-Ras, c-Myc, HMGA2, cyclin D*, and *CDC25A* (69) and *MYCN* proto-oncogene, which codes for the transcription factor N-myc (73). Of these oncogenes, *HMGA2* and *k-Ras* are known to be associated with self-renewal and maintenance of undifferentiated status of BCSCs by being highly expressed in them (74). In a recent study in 2012, downregulation of let-7 in side- populations of BCSCs was observed, and this led to the activation of *p-Ras* and *p-ERK*, which play an important role in maintaining the characteristics of this CSCs (72). Comparative studies on miRNA expression profiles in BCSCs and non-BCSC have reported a set of miRNAs differentially expressed in BCSCs (71). MiR-30 showed a 30-fold decrease in expression in BCSCs when compared with differentiated breast cancer cells (70) and is considered as one of the important miRNAs in regulating the stem-like features of BCSCs. Many studies revealed that expression of *BMI1* (a known regulator of stem cell self-renewal) is repressed by miR-200c by direct binding to the 3′UTR of *BMI1* (71). Wellner and colleagues in 2009 showed that epithelial-to-mesenchymal transition (EMT) activator *ZEB1*, a transcription factor promotes tumorigenicity by repressing stemness-inhibiting miRNA, such as miR-200 via repression of the promoter (68). This provided a positive correlation between the levels of *ZEB1* and *BMI1*. Thus *ZEB1*-miR-200-*BMI1* coordinately manipulated the events in the formation of cancer stem cell phenotypes indicating multiple roles of miR-200 in the development and metastasis of breast cancer (71).

Aberrant expression of miRNAs in breast cancer

Many microarray and functional studies have identified that specific miRNAs are repressed or activated in human breast cancer compared with normal breast tissues (refer to Table 1 for miRNA expression profile in various types of breast cancer). A study on miRNA expression in MCF-7 and T47-D breast cancer cell lines reported that miR-143, miR-145, miR-16, and let-7a-1 are downregulated in these cell lines (75), suggesting that change of specific miRNA expression is associated with malignant transformation.

Table 1. miRNA expression profile in different breast cancer types

Breast cancer types	miRNAs upregulated	miRNAs downregulated	References
Ductal carcinoma *in situ*	miR-21, miR-200c, miR-361-5p, miR-374a, miR-93, miR-182, miR-183	let-7d, miR-210, miR-221, miR-7b, miR-125b, miR-127-3p, miR-320	(82–83)
Invasive ductal carcinoma	let-7d, miR-210, miR-221	miR-10b, miR-126, miR-143, miR-143, miR-218, miR-335-5p	(82)
Lobular carcinoma *in situ*	miR-375, miR-182, miR-183, miR-96, miR-203, miR-425-5p, miR -565	—	(84)
Invasive lobular carcinoma	miR-9, miR-375, miR-182, miR-183	—	(85) (84)

Tumor-specific miRNA expression differences are useful as both prognostic and predictive factors. One of the most important prognostic marker in breast cancer is ErB2/ER status. ER-positive (ER^+) and ER-negative (ER^-) breast cancer are shown to have different mRNAs (76) and miRNA expression profile. Iorio and colleagues in 2005 correlated a set of miRNA expression with specific breast cancer biopathological features, such as ER and progesterone receptor (PR) expression, tumor stage, vascular invasion, and proliferation index (16). They found that miR-206 was highly expressed in ER^-, but not in ER^+ breast cancers. Adams and colleagues in 2007 showed that miR-206 targets ERα receptor and represses its mRNA and protein expression in breast cancer cell lines (77). In addition to miR-206, miR-221 and miR-222 negatively regulate ERα receptor (78). Many other miRNAs as biomarkers for breast cancer have also been reported recently. Higher expression of miR-9 has been significantly associated with breast cancer local recurrence and in ER^+ cases (79). Expression of miR-210 was also associated with tumor proliferation and differentiation of breast cancer (80). They reported that miR-210 was a strong potential biomarker of clinical outcome in patients with both ER^+ and ER^- breast cancer. Further functional analyses by them in cell lines revealed that miR-210 is involved in cell proliferation, migration, and invasion. miRNA profile can also be used to classify into a specific tumor pathological phenotype (i.e., PR status), proliferation, tumor stage, metastatic state, HER2 status (16, 81), as well as the tumor subtype (Luminal A, Luminal B, Basal-like, HER2+ and normal-like; ref. 12).

Given that the biological significance of aberrant expression of miRNA relies on the effect they have on their cognate protein-coding gene (PCG) targets, analysis of predicted targets of the miRNAs, which are most significantly upregulated or downregulated in breast cancer have been conducted by different groups working in this field. Table 2 summarizes some of these miRNAs, both oncomiRs and oncosuppressors, associated with breast cancer pathogenesis and their target gene(s).

Circulating miRNAs as blood-based biomarkers for breast cancer

Recent reports on significant levels of miRNAs in serum and other body fluids raised the possibility of circulating miRNAs serving as useful clinical biomarkers (114). The origin and functions of these extracellular miRNAs is poorly understood. These miRNAs were found to be stable and have different expression profiles for different fluids. As serum and other body fluids contain ribonucleases, miRNAs are thought to be shielded from degradation by being packed in lipid vesicles, in complexes with RNA-binding proteins, or both (115). In a pilot study of circulating miRNAs as potential biomarkers for early-stage breast cancer, 48 serum miRNAs were found to be differentially expressed in patients when compared with controls (116). Of these, 22 were upregulated and 26 were downregulated. In another study on patients with primary and metastatic breast cancer for blood-based markers, increased expression of miR-10b and miR-34a (117) and decreased expression of miR-195 and let-7a (118) in serum was observed. A recent study revealed that decreased serum miR-181a levels may represent a novel biomarker for primary breast cancer as well as for early-stage breast cancer diagnosis (119). Similarly, miR-202 was found to be significantly upregulated in early-stage breast cancer whole blood (120). MiR-202 has the highest number of targets in many significant biological pathways and gene ontology categories (121), and its role in influencing a plethora of cancer-relevant biological pathways and involvement in development of breast cancer might be established. Wu and colleagues have shown a connection between serum- and tissue-based miRNA of breast cancer by identifying coupregulated miRNA in serum and tissue using SOLiD sequencing-based miRNA expression profiling (122). Seven upregulated miRNAs (miR-103, miR-23a, miR-29a, miR-222, miR-23b, miR-24, and miR-25) were identified to be counregulated in serum and tissues. They also found a novel miRNA, miR-BS1, in the circulation, which is present on chromosome 3. Thus, blood-based markers can be thought to be of cellular or tissue origin. Moreover, tumor cells are thought to communicate with immune cells through exosomes leading to immune suppression (123). So, it can be speculated that cancer cells might reprogram the tumor microenvironment by altering the expression of surrounding immune cells through miRNA contained in exosomes. With several advantages over protein-based markers, circulating miRNA can serve as novel, minimally invasive blood-based biomarkers. A recent report by Madhavan and colleagues suggested circulating miRNAs as surrogate markers for circulating tumor cells (CTC) and prognostic markers in metastatic breast cancer (MBC; ref. 124). They showed that CTC-positive MBC had significantly higher levels of miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-210, miR-375, and miR-801 than CTC-negative MBC and controls. They also showed a cocktail of miRNAs or miR-200b alone were efficient markers for distinguishing CTC-positive from CTC-negative patient and MBC prognosis.

Metastamirs in Breast Cancer

Similar to oncomiRs and oncosuppressor miRNAs, miRNAs are also involved in regulating the migration and metastasis of cancer. Metastasis is a multistep process, which includes breaking away from primary tumors, invading through basement membrane barriers and extracellular matrix (ECM), intravasating into the circulation, extravasating into distant tissues, and finally establishing secondary tumors. The term "metastamir" was coined recently by Hurst and colleagues to refer to these metastasis regulatory miRNAs (125). Metastamirs have been shown to have pro- and antimetastatic effects and regulate key steps in the metastatic program and processes such as epithelial–mesenchymal transition (EMT), apoptosis, and angiogenesis. Some miRNAs have been associated with an invasive and metastatic phenotype of breast cancer cell lines or identified in metastatic tumor tissues and lymph nodes. These

Table 2. OncomiRs and oncosuppressor miRNAs associated with breast cancer

miRNA	OncomiRs/ oncosuppressor	Targets	Cellular function\mechanism	References
miR-9	OncomiRs	*CDH1*	Increased cell motility and invasion	(86)
miR-10b	OncomiRs	*HOXD10*	Favors cancer cell migration and invasion	(87)
miR-21	OncomiRs	*PTEN, TPM1, PDCD4, Maspin, BCL2*	Invasion, metastasis, and apoptosis	(51–52, 88–89)
miR-27a	OncomiRs	*Zinc finger, ZBTB10, Myt-1*	Cell-cycle progression (G_2–M checkpoint regulation)	(90–91)
miR-92	OncomiRs	*ERβ1*	Downregulated in many breast cancers; inhibition of miR-92 in MCF-7 cells increased ERβ1 expression	(92)
miR-96	OncomiRs	*FOXO3a*	Cell proliferation	(93)
miR-103/107	OncomiRs	*Dicer*	High levels of miR-103/107 were associated with metastasis	(94)
miR-155	OncomiRs	*RHOA SOCS1*	TGF-β signaling	(54, 95)
miR-204 and miR-510	OncomiRs	*PDEF*	Overexpression leads to metastasis progression	(96)
miR-221/222	OncomiRs	*ERα*	Reduce the expression of tumor suppressors	(97)
miR-373/520c	OncomiRs	*CD44*	Metastasis	(98)
miR-661	OncomiRs	*Nectin-1, Star-D16*	Required for efficient invasion of breast cancer cells	(99)
Let-7 family	Oncosuppressor	*H-RAS, HMGA2, LIN28, PEBP1*	Repress cell proliferation and growth; Let-7f promotes angiogenesis	(43, 100–101)
miR-17-5p	Oncosuppressor	*AIB1, CyclinD, E2F*	Estrogen and E2F1-mediated growth, proliferation	(102–103)
miR-18a/b	Oncosuppressor	*ERα*	Inhibition of ER-mediated cell growth	(104–105)
miR-17/20	Oncosuppressor	*AIB1, cyclin D1, E2F, Myc*	Coordinates the timing of cell cycle by targeting multiple cell-cycle regulators	(106)
miR-30	Oncosuppressor	*Ubc9, ITGβ3*	Constitutive expression of miR-30 in breast tumor–initiating cells inhibits their self-renewal capacity	(70)
miR-31	Oncosuppressor	*FZD3, ITGA5, M-RIP, MMP16*	Metastasis	(107)
miR-34a	Oncosuppressor	*CCND1, CDK6, E2F3, MYC*	DNA damage, proliferation	(108)
miR-125a,b	Oncosuppressor	*HER2, HER3*	Anchorage-dependent growth	(81, 91)
miR-145	Oncosuppressor	*RTNK*	Plays an important role in human stem cell growth and differentiation by targeting the pluripotency factors	(109)
miR-200	Oncosuppressor	*BIM1, ZEB1, ZEB2*	TGF-β signaling	(71, 110–111)
miR-205	Onco-suppressor	*HER3* receptor	Oncosuppressor gene that is able to interfere with the proliferative pathway mediated by HER receptor family	(112)
miR-206	Oncosuppressor	*ERα*	ER signaling	(16, 77)
miR-497	Oncosuppressor	*Bcl-w*	Apoptosis	(113)

miRNAs target important proteins involved in signaling pathways of metastasis. A study by Baffa and colleagues identified the cancer metastatic miRNA signatures as miR-10b, miR-21, miR-30a, miR-30e, miR-125b, miR-141, miR-200b, miR-200c, and miR-205 (126). Certain miRNAs also target *Dicer* for controlling metastasis (94). In breast cancer patients with increased risk of developing metastasis, it was found that miR-103/107 family targets 3′UTR of *Dicer1* mRNA, which reduce *Dicer1* expression, and as a consequence, mature miRNAs were downregulated.

Metastasis-promoting miRNAs

The metastamirs, which promote metastasis in breast cancer, include the well-characterized oncomiR, miR-21, which has a critical role in cell transformation and tumorigenesis. It actively affects tumor invasion and metastasis by targeting TSGs *TPM1, PDCD4*, and *maspin*. Another oncomiR highly expressed in breast cancer metastasis is miR-10b (87). The overexpression of miR-10b in nonmetastatic breast tumors is able to initiate invasion and metastasis in breast cancer xenograft models (127). Findings by Huang and colleagues indicated that human miR-373 and miR-520c stimulated MCF-7 cancer cell migration and invasion *in vitro* and *in vivo*, which implicates miR-373 and miR-520c as metastasis-promoting miRNAs (98).

Metastasis-suppressing miRNAs

Tavazoie and colleagues (128) showed that restoring the expression of miRNAs in malignant cells whose expression is specifically lost as human breast cancer cells develop metastatic potential and suppresses lung and bone metastasis in metastatic breast cancer. MiR-126 restoration reduced overall tumor growth and proliferation, whereas miR-335 inhibited metastatic cell invasion. They had identified the first suppressing metastamir. Another miRNA, miR-31, which is expressed in normal mammary cells, is specifically lost in metastatic breast cancer cell lines. MiR-31 inhibits multiple steps of metastasis including invasion, anoikis, and colonization leading to a 95% reduction in lung metastasis in an orthotopic model of breast cancer (107). MiR-146a and b have been seen to inhibit invasion and migration of breast cancer cells by downregulating *NF-κB* by targeting *IRAK1* and *TRAF6* (129). In an expression study of another miRNA, miR-497, it was seen that its expression was decreased in breast cancer specimens and negatively correlated with tumor–node–metastasis stage, lymphatic metastasis, tumor size, and HER2. It induced apoptosis of BCSCs by targeting *Bcl-w* (113).

Factors for miRNA Dysregulation in Breast Cancer

Defects in miRNA biogenesis pathway

Drosha, DGCR8, and Dicer, the proteins involved in the biogenesis pathway are the 3 well-established regulators of miRNA processing. Defects in the miRNA biogenesis machinery can be closely correlated to oncogenesis. Khoshnaw and colleagues reported the association of loss of Dicer expression with breast cancer progression and recurrence. They reported a gradual loss of Dicer protein expression in malignant tissues compared with normal breast tissues (130). In invasive breast cancer, the loss of Dicer expression was associated with features of aggressive behavior including higher histologic grade, loss of hormone receptor and *BRCA1* protein expression, and shorter disease-free survival (DFS). *BRCA1* is a target of miR-182 (131) and miR-146 (132), which are both upregulated in breast cancer. Passon and colleagues have reported the deregulation of Dicer and Drosha in triple-negative breast cancer (133). A triple-negative breast cancer refers to any breast cancer that does not express the genes for ER, PR, or Her2 receptors. They found that Drosha mRNA levels in triple-negative breast cancer were significantly higher than those in normal breast tissues. Martello and colleagues in 2010 reported from an analysis of chromosomal arrays from breast cancers that tumors with a reduced copy number of the *Dicer1* locus had an increased probability of developing metastasis (94). There are also recent reports that downregulation of Drosha and Dicer is associated with specific subgroups of breast cancer (134).

Genomic alternations of miRNAs contributing to breast cancer

Of all the known human miRNA genes, many are frequently located at fragile sites of the genome, which are usually either amplified or deleted in human cancer (40). The aberrant miRNA expression in breast cancer and in other cancers results, in part, due to these genomic alternations. The miR-125b, downregulated in breast cancer, is located at *11q23-24*, one of the most frequently deleted regions (135). In 2006, Zhang and colleagues studied 283 known human miRNA genes in breast cancer and showed that 72.8% of miRNA genes are located in regions that exhibit DNA copy number abnormalities (gain or loss; ref. 136). In another study, mir-320 is found to be located in regions with DNA copy number loss in breast cancer. The predicted target of miR-320 is methyl CpG-binding protein 2 (*MECP2*), which is overexpressed in breast cancer and serves as an oncogene promoting cell proliferation (137). Multiple genetic changes like germline mutation in *BRCA1* or *BRCA2* predisposes to breast and ovarian cancer as well as other cancers (138). Certain non-*BRCA* mutations, which also lead to breast cancer are *TP53, PTEN, STK11, CHEK2, ATM, BRIP1*, and *PALB2* (139).

Genome-wide association studies (GWAS) on breast cancer have also uncovered a number of single-nucleotide polymorphisms (SNP) associated with susceptibility to the disease. Many of these polymorphisms may be found in regulatory regions and regions containing ncRNAs. A SNP in a miRNA can have a significant effect on its hybridization to the target sites or can affect the processing of the mRNA. For example, a SNP within the 3′UTR of *SET8*, a methyltransferase that represses *p53* activity, generates a new miR-502 binding site and is associated with early breast cancer onset in premenopausal women (140). In a study on the

impact of 11 miRNA target site SNPs located in the 3′UTR of cancer associated genes, a significant association with familial breast cancer risk was observed (141). Nicoloso and colleagues analyzed SNPs associated with breast cancer risk and identified SNPs in *TGFβ1* and *XRCC1* that could modulate their expression by differential interaction with miR-187 and miR-138, respectively (142). In another study, Y-P Fu and colleagues reported that breast cancer-associated rs11249433 located in the *1p11.2* region is associated with mRNA expression of NOTCH2 and its expression was dependent on the mutational status of the *p53* and ER status of the tumors (143). A recent GWAS of breast cancer defined by hormone receptor status has revealed loci at *6q14* and *20q11* contributing to susceptibility of ER^- subtypes (144). SNP rs17530068 at *6q14* was associated with both ER^+ and ER^- breast cancer. SNP rs2284378 at *20q11* was associated with ER^- breast cancer but showed no association with ER^+ disease.

Epigenetics–miRNA regulatory circuit in breast cancer

It is known that epigenetic factors play a prominent role in regulation of gene expression. The epigenetic factors encompass changes in DNA methylation, histone modifications, miRNA expression, nucleosome positioning, and higher order chromatin. miRNAs are regulated by epigenetic mechanisms, similar to conventional PCGs. Conversely, miRNAs also target the important epigenetic regulators (termed as epi-miRNAs) and modulate their expression. The former attributes to decipher the mechanisms that underlie the abnormal miRNA expression in different biological systems or conditions. This complex network of feedback between miRNAs and epigenetic pathways seems to form a epigenetics-miRNA regulatory circuit that controls the normal physiologic processes of an organism. Figure 4 depicts the crosslinking network between the miRNAs and epigenetic effectors. Below, we describe both the mechanisms of disruption encompassing miRNAs as targets of epigenetic changes and miRNAs targeting epigenetic factors that contribute to breast cancer.

miRNAs as targets of epigenetic changes

Abnormal epigenetic regulation of miRNA genes is responsible for aberrant miRNA expression in cancer. MiRNAs are regulated by the effectors of the epigenetic machinery such as *DNMTs*, *HDACs*, and polycomb repressive complex (*PRC*) genes. Genome-wide analysis of different cancer types has shown the influence of DNA methylation and histone modifications on the global regulation of miRNAs (145–146). The first evidence of the altered expression of miRNAs and epigenetic modifications in tumor cells was reported by Scott and colleagues in SKBr3 breast cancer cells (91). They showed that the treatment with *HDAC* inhibitor resulted in upregulation of 5 miRNAs and downregulation of 22 miRNAs, indicating that some miRNAs are mainly silenced by histone modifications. Using next generation sequencing and microarrays, Morita and colleagues showed epigenetic similarities and differences between miRNAs and PCGs, as well as highlighting the relationship between DNA methylation and transcription of miRNAs (147). They found that several genes were hypermethylated in the MCF-7 breast cancer cell line and also showed that DNA methylation in the proximal promoter of miRNAs is tightly

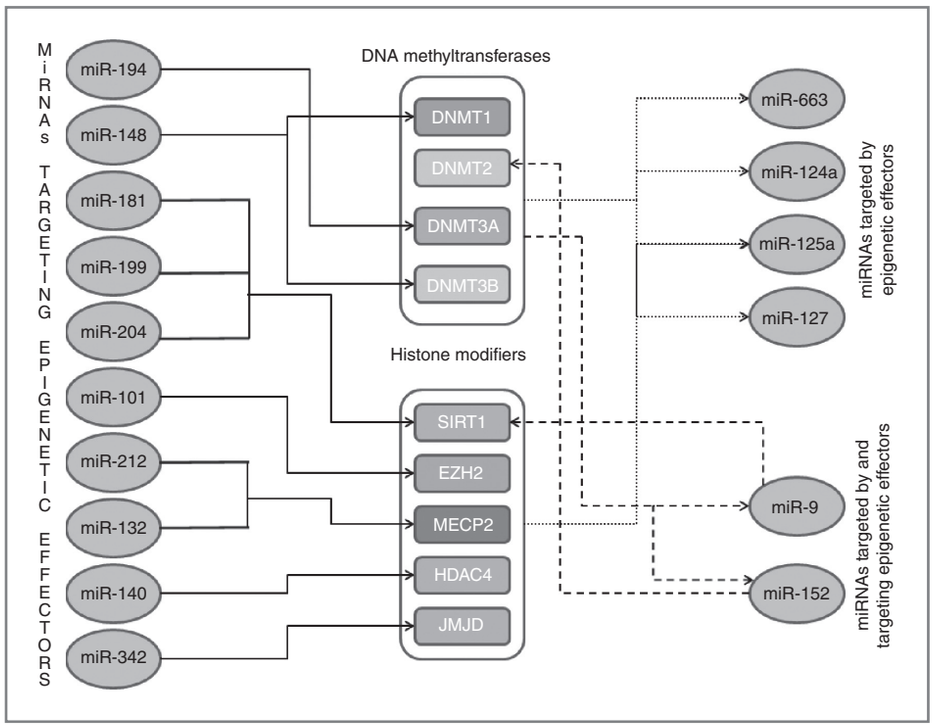

Figure 4. Crosslinking network between miRNAs and epigenetic effectors. ⟶, epi-miRNAs targeting different epigenetic effectors; ⇢, miRNAs being targeted by the epigenetic effectors; and ⇢, miRNAs targeting and being targeted by epigenetic effectors. *DNMT*, DNA methyltransferase; *SIRT1*, sirtuin (silent mating type information regulation 2 homolog) 1; *EZH2*, Histone-lysine N-methyltransferase; *MECP2*, methyl CpG-binding protein 2 (Rett syndrome); *HDAC4*, histone deacetylase 4; *JMJD*, histone demythylases Jumonji domain-containing proteins.

linked to transcriptional silencing, as is the case with PCGs. Even the role of nonpromoter methylation, such as in enhancers/far-upstream elements and within the body of the gene of miRNAs was examined (147). Lehmann and colleagues found that in breast cancer cell lines, 5-aza-2'-deoxycytidine reactivates miR-9-1 (hypermethylated in up to 86% of primary tumors) and miR-663 (also hypermethylated; ref. 148). They also showed epigenetic silencing of miR-9 and miR-148a (together with miR-152, -124a, and -663). Recently, the role of CCCTC-binding factor (CTCF) in the regulation of miR-125b1 and miR-375 in breast cancer cells has been studied (149). CTCF is known to bind insulators and exhibits an enhancer-blocking and barrier function along with maintenance of three-dimensional organization of the genome. In breast cancer cells, there is loss of CTCF binding at the miR-125b1 CpG island, which is associated with CpG island methylation and the gain of repressive histone modifications. Similarly, CTCF binds to unmethylated DNA in the CpG islands of ERα^- breast cancer cells and induces silencing of miR-375 expression. MiR-375 plays an important role in cell proliferation and is overexpressed in ERα^+ breast cancer cell lines. Recently, an oncomiR, miR-155 has been seen to be epigenetically controlled by a TSG, *BRCA1*, which is implicated in the development of a subset of breast and ovarian cancers (150). Thus, it is seen that miRNAs undergo epigenetic regulation similar to PCGs and epigenetics can direct its control indirectly on oncogenes and TSGs through miRNAs.

Epi-miRNAs—regulation of epigenetic machinery by miRNAs

The components of the epigenetic machinery, such as DNMTs and histone modifiers, can themselves be subject to regulation by miRNAs. Such miRNAs are called epi-miRNAs. Epi-miRNAs target *DNMTs, HDACs*, and *PRCs* (151). They indirectly affect the expression of TSGs, whose expression is controlled by epigenetic factors. These miRNAs can also indirectly regulate gene expression by affecting their epigenetic regulation. The first evidence of the existence of epi-miRNAs was obtained in lung cancer cell lines, where a family of miRNAs (miR-29a, -29b, and -29c) directly targets *DNMT3a* and *DNMT3b* (152). Two independent groups have shown in mouse embryonic stem cells that members of the miR-290 cluster directly target *RBL2*, an inhibitor of *DNMT3* genes (153–154). *BMI1*, a known regulator of stem cell self-renewal, is a component of PCR1 complex whose expression is repressed by miR-200c (71). In a study on the miRNA-mediated drug resistance in breast cancer, epigenetics has been hypothesized to play a role. Different mechanisms have recently been shown to be targeted by miRNAs in drug-resistant breast cancer of which perturbations in DNA methylation and histone modifications is one (155). miRNAs target many effectors of the epigenetic machinery like *HDAC4, DNMT1, DNMT3b*, and so on. Though there are different studies of regulation of the epigenetic machinery by epi-miRNAs involved in different types of cancer, a clear concept of the epi-miRNAs involved in breast cancer has to be drawn. Thus, decoding the connection between miRNA and epigenetics will lead to a better understanding of gene regulation and human carcinogenesis, therefore allowing the rendition of this knowledge into new treatments for patients with cancer.

Future Perspectives

With the remarkable advances in research in the past decade on ncRNAs and their functionality, these small RNA molecules play a crucial role in regulation of gene expression of a wide range of biological and pathological processes. As miRNAs are involved in breast cancer from the onset of the malignant state through the progression to metastasis, they may be ideal targets for the development of new drugs for treating patients with breast cancer. For the development of miRNA-targeted therapies, it is necessary to understand the interplay between miRNAs and mRNAs leading to breast cancer. MiRNA and their targets seem to form complex regulatory networks and unwinding and understanding it will make individualized and tailored therapies conceivable. miRNAs have emerged as suitable diagnostic and prognostic biomarkers with the competence for guiding treatment decisions. The upregulation and downregulation of particular miRNAs in breast cancer can serve as the hallmark for the disease. The most exciting and emerging area to be explored is where miRNAs serve as novel, minimally invasive blood-based biomarkers for early detection and diagnosis of breast cancer. Though there are advances in research on the use of miRNAs in targeted therapies for breast cancer, success has been limited. Research into identification, validation, and determination of the unwanted side effects of erroneous targeting of miRNAs is of prime importance. Epigenetics having a major function in gene expression regulation, aberrancies in the epigenome are implicated in carcinogenesis. With a significant role of miRNAs in genetic and epigenetic regulations in breast cancer and the chance of them being interconnected, exploring this field remains a challenge. The patterns of epigenetic modifications associated with development and progression of cancer are potentially useful. Studying these patterns like DNA methylation of whole genome or CpG methylation of promoters of miRNA genes using next-generation sequencing methods like MeDIP-seq (methylated DNA immunoprecipitation sequencing) and bisulfite sequencing will be useful. Analysis of MeDIP-seq data of miRNA genes associated with breast cancer can provided insights into the development of DNA methylation markers for early detection, diagnosis, and prognosis of the disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Samantarrai, S. Dash, B. Mallick
Development of methodology: B. Mallick
Writing, review, and/or revision of the manuscript: D. Samantarrai, B. Chhetri, B. Mallick
Study supervision: B. Mallick

Acknowledgments

D. Samantarrai thanks the National Institute of Technology Rourkela for providing fellowship assistance under the institute research scheme. The authors also thank

Subhrata Jena, one of their lab members, for her technical assistance. The authors apologize to those whose works were not cited due to space limitation.

Received November 19, 2012; revised December 20, 2012; accepted January 2, 2013; published OnlineFirst January 29, 2013.

References

1. Negrini M, Calin GA. Breast cancer metastasis: a microRNA story. Breast Cancer Res 2008;10:203.
2. Ghosh Z, Mallick B, Chakrabarti J. Cellular versus viral microRNAs in host-virus interaction. Nucleic Acids Res 2009;37:1035–48.
3. Ivey KN, Srivastava D. MicroRNAs as regulators of differentiation and cell fate decisions. Cell Stem Cell 2010;7:36–41.
4. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003;425:415–9.
5. Jovanovic M, Hengartner MO. miRNAs and apoptosis: RNAs to die for. Oncogene 2006;25:6176–87.
6. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215–33.
7. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433:769–73.
8. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004;5:522–31.
9. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 2000;403:901–6.
10. Aigner A. MicroRNAs (miRNAs) in cancer invasion and metastasis: therapeutic approaches based on metastasis-related miRNAs. J Mol Med (Berl) 2011;89:445–57.
11. Lee YS, Dutta A. MicroRNAs in cancer. Annu Rev Pathol 2009;4:199–227.
12. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, et al. MicroRNA expression profiling of human breast cancer identifies new markers of tumor subtype. Genome Biol 2007;8:R214.
13. van Schooneveld E, Wouters MC, Van der Auwera I, Peeters DJ, Wildiers H, Van Dam PA, et al. Expression profiling of cancerous and normal breast tissues identifies microRNAs that are differentially expressed in serum from patients with (metastatic) breast cancer and healthy volunteers. Breast Cancer Res 2012;14:R34.
14. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 2012;4:143–59.
15. Sato F, Tsuchiya S, Meltzer SJ, Shimizu K. MicroRNAs and epigenetics. FEBS J 2011;278:1598–609.
16. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005;65:7065–70.
17. Fu SW, Chen L, Man YG. miRNA biomarkers in breast cancer detection and management. J Cancer 2011;2:116–22.
18. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, et al. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2005;102:19075–80.
19. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005;435:839–43.
20. Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: rationale, strategies and challenges. Nat Rev Drug Discov 2010;9:775–89.
21. Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 2006;13:1097–101.
22. Landthaler M, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and Its D. melanogaster homolog are required for miRNA biogenesis. Curr Biol 2004;14:2162–7.
23. Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, et al. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 2006;125:887–901.
24. Yi R, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. Genes Dev 2003;17:3011–6.
25. Zeng Y, Cullen BR. Structural requirements for pre-microRNA binding and nuclear export by Exportin 5. Nucleic Acids Res 2004;32:4776–85.
26. MacRae IJ, Ma E, Zhou M, Robinson CV, Doudna JA. *In vitro* reconstitution of the human RISC-loading complex. Proc Natl Acad Sci U S A 2008;105:512–7.
27. Doench JG, Petersen CP, Sharp PA. siRNAs can function as miRNAs. Genes Dev 2003;17:438–42.
28. Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. Nat Struct Mol Biol 2012;19:586–93.
29. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science 2007;318:1931–4.
30. Berezikov E, Chung WJ, Willis J, Cuppen E, Lai EC. Mammalian mirtron genes. Mol Cell 2007;28:328–36.
31. Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in *Drosophila*. Cell 2007;130:89–100.
32. Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. Nature 2007;448:83–6.
33. Flynt AS, Greimann JC, Chung WJ, Lima CD, Lai EC. MicroRNA biogenesis via splicing and exosome-mediated trimming in *Drosophila*. Mol Cell 2010;38:900–7.
34. Ender C, Krek A, Friedlander MR, Beitzinger M, Weinmann L, Chen W, et al. A human snoRNA with microRNA-like functions. Mol Cell 2008;32:519–28.
35. Bogerd HP, Karnowski HW, Cai X, Shin J, Pohlers M, Cullen BR. A mammalian herpesvirus uses noncanonical expression and processing mechanisms to generate viral MicroRNAs. Mol Cell 2010;37:135–42.
36. Cazalla D, Xie M, Steitz JA. A primate herpesvirus uses the integrator complex to generate viral microRNAs. Mol Cell 2011;43:982–92.
37. Cifuentes D, Xue H, Taylor DW, Patnode H, Mishima Y, Cheloufi S, et al. A novel miRNA processing pathway independent of Dicer requires Argonaute2 catalytic activity. Science 2010;328:1694–8.
38. Kayani M, Kayani MA, Malik FA, Faryal R. Role of miRNAs in breast cancer. Asian Pac J Cancer Prev 2011;12:3175–80.
39. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002;99:15524–9.
40. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999–3004.
41. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103:2257–61.
42. Hammond SM. MicroRNAs as oncogenes. Curr Opin Genet Dev 2006;16:4–9.
43. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, et al. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635–47.
44. Tagawa H, Seto M. A microRNA cluster as a target of genomic amplification in malignant lymphoma. Leukemia 2005;19:2013–6.
45. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res 2005;65:9628–32.
46. Inomata M, Tagawa H, Guo YM, Kameoka Y, Takahashi N, Sawada K. MicroRNA-17-92 down-regulates expression of distinct targets in different B-cell lymphoma subtypes. Blood 2009;113:396–402.

47. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, et al. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008;14:2348–60.
48. Papagiannakopoulos T, Shapiro A, Kosik KS. MicroRNA-21 targets a network of key tumor-suppressive pathways in glioblastoma cells. Cancer Res 2008;68:8164–72.
49. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology 2007;133: 647–58.
50. Schramedei K, Morbt N, Pfeifer G, Lauter J, Rosolowski M, Tomm JM, et al. MicroRNA-21 targets tumor suppressor genes ANP32A and SMARCA4. Oncogene 2011;30:2975–85.
51. Zhu S, Wu H, Wu F, Nie D, Sheng S, Mo YY. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. Cell Res 2008; 18:350–9.
52. Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). J Biol Chem 2007;282: 14328–36.
53. Mattiske S, Suetani RJ, Neilsen PM, Callen DF. The oncogenic role of miR-155 in Breast Cancer. Cancer Epidemiol Biomarkers Prev 2012;21:1236–43.
54. Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, et al. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res 2010;70:3119–27.
55. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005;102:13944–9.
56. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 2007;26:745–52.
57. Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih Ie M, Zhang Y, et al. MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 2008;3:e2436.
58. O'Hara AJ, Wang L, Dezube BJ, Harrington WJ Jr, Damania B, Dittmer DP. Tumor suppressor microRNAs are underrepresented in primary effusion lymphoma and Kaposi sarcoma. Blood 2009;113:5938–41.
59. Dobos GJ, Voiss P, Schwidde I, Choi KE, Paul A, Kirschbaum B, et al. Integrative oncology for breast cancer patients: introduction of an expert-based model. BMC Cancer 2012;12:539.
60. Broderick JA, Zamore PD. MicroRNA therapeutics. Gene Ther 2011;18:1104–10.
61. Bader AG, Brown D, Stoudemire J, Lammers P. Developing therapeutic microRNAs for cancer. Gene Ther 2011;18:1121–6.
62. Mallick B, Chakrabarti J, Ghosh Z. MicroRNA reins in embryonic and cancer stem cells. RNA Biol 2011;8:415–26.
63. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 2008;100:672–9.
64. Andl T, Murchison EP, Liu F, Zhang Y, Yunta-Gonzalez M, Tobias JW, et al. The miRNA-processing enzyme dicer is essential for the morphogenesis and maintenance of hair follicles. Curr Biol 2006;16: 1041–9.
65. Greene SB, Herschkowitz JI, Rosen JM. Small players with big roles: microRNAs as targets to inhibit breast cancer progression. Curr Drug Targets 2010;11:1059–73.
66. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100:3983–8.
67. Pece S, Tosoni D, Confalonieri S, Mazzarol G, Vecchi M, Ronzoni S, et al. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. Cell 2010;140:62–73.
68. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 2009;11: 1487–95.
69. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 2007;131: 1109–23.
70. Yu F, Deng H, Yao H, Liu Q, Su F, Song E. Mir-30 reduction maintains self-renewal and inhibits apoptosis in breast tumor-initiating cells. Oncogene 2010;29:4194–204.
71. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, et al. Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 2009;138:592–603.
72. Sun X, Fan C, Hu LJ, Du N, Xu CW, Ren H. [Role of let-7 in maintaining characteristics of breast cancer stem cells]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2012;28:789–92.
73. Buechner J, Tomte E, Haug BH, Henriksen JR, Lokke C, Flaegstad T, et al. Tumour-suppressor microRNAs let-7 and mir-101 target the proto-oncogene MYCN and inhibit cell proliferation in MYCN-amplified neuroblastoma. Br J Cancer 2011;105:296–303.
74. Dangi-Garimella S, Yun J, Eves EM, Newman M, Erkeland SJ, Hammond SM, et al. Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7. EMBO J 2009;28:347–58.
75. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003;1:882–91.
76. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74.
77. Adams BD, Furneaux H, White BA. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. Mol Endocrinol 2007;21:1132–47.
78. Zhao JJ, Lin J, Yang H, Kong W, He L, Ma X, et al. MicroRNA-221/222 negatively regulates estrogen receptor alpha and is associated with tamoxifen resistance in breast cancer. J Biol Chem 2008;283: 31079–86.
79. Zhou X, Marian C, Makambi KH, Kosti O, Kallakury BV, Loffredo CA, et al. MicroRNA-9 as potential biomarker for breast cancer local recurrence and tumor estrogen receptor status. PLoS ONE 2012;7: e39011.
80. Rothe F, Ignatiadis M, Chaboteaux C, Haibe-Kains B, Kheddoumi N, Majjaj S, et al. Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. PLoS ONE 2011;6:e20980.
81. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, et al. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. Mol Cancer 2006;5:24.
82. Volinia S, Galasso M, Sana ME, Wise TF, Palatini J, Huebner K, et al. Breast cancer signatures for invasiveness and prognosis defined by deep sequencing of microRNA. Proc Natl Acad Sci U S A 2012; 109:3024–9.
83. Hannafon BN, Sebastiani P, de las Morenas A, Lu J, Rosenberg CL. Expression of microRNA and their gene targets are dysregulated in preinvasive breast cancer. Breast Cancer Res 2011;13:R24.
84. Giricz O, Reynolds PA, Ramnauth A, Liu C, Wang T, Stead L, et al. Hsa-miR-375 is differentially expressed during breast lobular neoplasia and promotes loss of mammary acinar polarity. J Pathol 2012;226:108–19.
85. Krell J, Frampton AE, Jacob J, Pellegrino L, Roca-Alonso L, Zeloof D, et al. The clinico-pathologic role of microRNAs miR-9 and miR-151-5p in breast cancer metastasis. Mol Diagn Ther 2012;16:167–72.
86. Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 2010;12:247–56.
87. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 2007;449: 682–8.
88. Frankel LB, Christoffersen NR, Jacobsen A, Lindow M, Krogh A, Lund AH. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem 2008;283:1026–33.
89. Qi L, Bart J, Tan LP, Platteel I, Sluis T, Huitema S, et al. Expression of miR-21 and its targets (PTEN, PDCD4, TM1) in flat epithelial atypia of

the breast in relation to ductal carcinoma *in situ* and invasive carcinoma. BMC Cancer 2009;9:163.
90. Mertens-Talcott SU, Chintharlapalli S, Li X, Safe S. The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. Cancer Res 2007;67:11001–11.
91. Scott GK, Mattie MD, Berger CE, Benz SC, Benz CC. Rapid alteration of microRNA levels by histone deacetylase inhibition. Cancer Res 2006;66:1277–81.
92. Al-Nakhle H, Burns PA, Cummings M, Hanby AM, Hughes TA, Satheesha S, et al. Estrogen receptor {beta}1 expression is regulated by miR-92 in breast cancer. Cancer Res 2010;70:4778–84.
93. Lin H, Dai T, Xiong H, Zhao X, Chen X, Yu C, et al. Unregulated miR-96 induces cell proliferation in human breast cancer by downregulating transcriptional factor FOXO3a. PLoS One 2010;5:e15797.
94. Martello G, Rosato A, Ferrari F, Manfrin A, Cordenonsi M, Dupont S, et al. A MicroRNA targeting dicer for metastasis control. Cell 2010;141:1195–207.
95. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, et al. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol 2008;28:6773–84.
96. Findlay VJ, Turner DP, Moussa O, Watson DK. MicroRNA-mediated inhibition of prostate-derived Ets factor messenger RNA translation affects prostate-derived Ets factor regulatory networks in human breast cancer. Cancer Res 2008;68:8499–506.
97. Di Leva G, Gasparini P, Piovan C, Ngankeu A, Garofalo M, Taccioli C, et al. MicroRNA cluster 221-222 and estrogen receptor alpha interactions in breast cancer. J Natl Cancer Inst 2010;102:706–21.
98. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, et al. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol 2008;10:202–10.
99. Vetter G, Saumet A, Moes M, Vallar L, Le Bechec A, Laurini C, et al. miR-661 expression in SNAI1-induced epithelial to mesenchymal transition contributes to breast cancer cell invasion by targeting Nectin-1 and StarD10 messengers. Oncogene 2010;29:4436–48.
100. Wu L, Fan J, Belasco JG. MicroRNAs direct rapid deadenylation of mRNA. Proc Natl Acad Sci U S A 2006;103:4034–9.
101. Orom UA, Nielsen FC, Lund AH. MicroRNA-10a binds the 5′UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 2008;30:460–71.
102. Hossain A, Kuo MT, Saunders GF. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. Mol Cell Biol 2006;26:8191–201.
103. Yu Z, Wang C, Wang M, Li Z, Casimiro MC, Liu M, et al. A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. J Cell Biol 2008;182:509–17.
104. Hurst DR, Edmonds MD, Scott GK, Benz CC, Vaidya KS, Welch DR. Breast cancer metastasis suppressor 1 up-regulates miR-146, which suppresses breast cancer metastasis. Cancer Res 2009;69:1279–83.
105. Li XF, Yan PJ, Shao ZM. Downregulation of miR-193b contributes to enhance urokinase-type plasminogen activator (uPA) expression and tumor progression and invasion in human breast cancer. Oncogene 2009;28:3937–48.
106. Yu Z, Baserga R, Chen L, Wang C, Lisanti MP, Pestell RG. microRNA, cell cycle, and human breast cancer. Am J Pathol 2010;176:1058–64.
107. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, et al. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 2009;137:1032–46.
108. Sun F, Fu H, Liu Q, Tie Y, Zhu J, Xing R, et al. Downregulation of CCND1 and CDK6 by miR-34a induces cell cycle arrest. FEBS Lett 2008;582:1564–8.
109. Wang S, Bian C, Yang Z, Bo Y, Li J, Zeng L, et al. miR-145 inhibits breast cancer cell growth through RTKN. Int J Oncol 2009;34:1461–6.
110. Dykxhoorn DM, Wu Y, Xie H, Yu F, Lal A, Petrocca F, et al. miR-200 enhances mouse breast cancer cell colonization to form distant metastases. PLoS ONE 2009;4:e7181.
111. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008;10:593–601.
112. Iorio MV, Casalini P, Piovan C, Di Leva G, Merlo A, Triulzi T, et al. microRNA-205 regulates HER3 in human breast cancer. Cancer Res 2009;69:2195–200.
113. Shen L, Li J, Xu L, Ma J, Li H, Xiao X, et al. miR-497 induces apoptosis of breast cancer cells by targeting Bcl-w. Exp Ther Med 2012;3:475–80.
114. Zen K, Zhang CY. Circulating microRNAs: a novel class of biomarkers to diagnose and monitor human cancers. Med Res Rev 2012;32:326–48.
115. Gibbings DJ, Ciaudo C, Erhardt M, Voinnet O. Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity. Nat Cell Biol 2009;11:1143–9.
116. Zhao H, Shen J, Medico L, Wang D, Ambrosone CB, Liu S. A pilot study of circulating miRNAs as potential biomarkers of early stage breast cancer. PLoS ONE 2010;5:e13735.
117. Roth C, Rack B, Muller V, Janni W, Pantel K, Schwarzenbach H. Circulating microRNAs as blood-based markers for patients with primary and metastatic breast cancer. Breast Cancer Res 2010;12:R90.
118. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, Kerin MJ. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 2010;251:499–505.
119. Guo LJ, Zhang QY. Decreased serum miR-181a is a potential new tool for breast cancer screening. Int J Mol Med 2012;30:680–6.
120. Schrauder MG, Strick R, Schulz-Wendtland R, Strissel PL, Kahmann L, Loehberg CR, et al. Circulating micro-RNAs as potential blood-based markers for early stage breast cancer detection. PLoS ONE 2012;7:e29770.
121. Backes C, Meese E, Lenhof HP, Keller A. A dictionary on microRNAs and their putative target pathways. Nucleic Acids Res 2010;38:4476–86.
122. Wu Q, Wang C, Lu Z, Guo L, Ge Q. Analysis of serum genome-wide microRNAs for breast cancer detection. Clin Chim Acta 2012;413:1058–65.
123. Chalmin F, Ladoire S, Mignot G, Vincent J, Bruchard M, Remy-Martin JP, et al. Membrane-associated Hsp72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. J Clin Invest 2010;120:457–71.
124. Madhavan D, Zucknick M, Wallwiener M, Cuk K, Modugno C, Scharpff M, et al. Circulating miRNAs as surrogate markers for circulating tumor cells and prognostic markers in metastatic breast cancer. Clin Cancer Res 2012;18:5972–82.
125. Hurst DR, Edmonds MD, Welch DR. Metastamir: the field of metastasis-regulatory microRNA is spreading. Cancer Res 2009;69:7495–8.
126. Baffa R, Fassan M, Volinia S, O'Hara B, Liu CG, Palazzo JP, et al. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. J Pathol 2009;219:214–21.
127. Ma L. Role of miR-10b in breast cancer metastasis. Breast Cancer Res 2010;12:210.
128. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, et al. Endogenous human microRNAs that suppress breast cancer metastasis. Nature 2008;451:147–52.
129. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Campisi J, Benz CC. Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. Oncogene 2008;27:5643–7.
130. Khoshnaw SM, Rakha EA, Abdel-Fatah TM, Nolan CC, Hodi Z, Macmillan DR, et al. Loss of Dicer expression is associated with breast cancer progression and recurrence. Breast Cancer Res Treat 2012;135:403–13.
131. Moskwa P, Buffa FM, Pan Y, Panchakshari R, Gottipati P, Muschel RJ, et al. miR-182-mediated downregulation of BRCA1 impacts DNA repair and sensitivity to PARP inhibitors. Mol Cell 2011;41:210–20.
132. Garcia AI, Buisson M, Bertrand P, Rimokh R, Rouleau E, Lopez BS, et al. Down-regulation of BRCA1 expression by miR-146a and miR-

146b-5p in triple negative sporadic breast cancers. EMBO Mol Med 2011;3:279–90.
133. Passon N, Gerometta A, Puppin C, Lavarone E, Puglisi F, Tell G, et al. Expression of Dicer and Drosha in triple-negative breast cancer. J Clin Pathol 2012;65:320–6.
134. Dedes KJ, Natrajan R, Lambros MB, Geyer FC, Lopez-Garcia MA, Savage K, et al. Down-regulation of the miRNA master regulators Drosha and Dicer is associated with specific subgroups of breast cancer. Eur J Cancer 2011;47:138–50.
135. Negrini M, Rasio D, Hampton GM, Sabbioni S, Rattan S, Carter SL, et al. Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: identification of a new region at 11q23.3. Cancer Res 1995;55:3003–7.
136. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, et al. microRNAs exhibit high frequency genomic alterations in human cancer. Proc Natl Acad Sci U S A 2006;103:9136–41.
137. Muller HM, Fiegl H, Goebel G, Hubalek MM, Widschwendter A, Muller-Holzner E, et al. MeCP2 and MBD2 expression in human neoplastic and non-neoplastic breast tissue and its association with oestrogen receptor status. Br J Cancer 2003;89:1934–9.
138. Pasche B. Cancer genetics. Introduction. Cancer Treat Res 2010; 155:xi–xii.
139. Gage M, Wattendorf D, Henry LR. Translational advances regarding hereditary breast cancer syndromes. J Surg Oncol 2012;105:444–51.
140. Song F, Zheng H, Liu B, Wei S, Dai H, Zhang L, et al. An miR-502-binding site single-nucleotide polymorphism in the 3′-untranslated region of the SET8 gene is associated with early age of breast cancer onset. Clin Cancer Res 2009;15:6292–300.
141. Tchatchou S, Jung A, Hemminki K, Sutter C, Wappenschmidt B, Bugert P, et al. A variant affecting a putative miRNA target site in estrogen receptor (ESR) 1 is associated with breast cancer risk in premenopausal women. Carcinogenesis 2009;30:59–64.
142. Nicoloso MS, Sun H, Spizzo R, Kim H, Wickramasinghe P, Shimizu M, et al. Single-nucleotide polymorphisms inside microRNA target sites influence tumor susceptibility. Cancer Res 2010;70:2789–98.
143. Fu YP, Edvardsen H, Kaushiva A, Arhancet JP, Howe TM, Kohaar I, et al. NOTCH2 in breast cancer: association of SNP rs11249433 with gene expression in ER-positive breast tumors without TP53 mutations. Mol Cancer 2010;9:113.
144. Siddiq A, Couch FJ, Chen GK, Lindstrom S, Eccles D, Millikan RC, et al. A meta-analysis of genome-wide association studies of breast cancer identifies two novel susceptibility loci at 6q14 and 20q11. Hum Mol Genet 2012;21:5373–84.
145. Choudhry H, Catto JW. Epigenetic regulation of microRNA expression in cancer. Methods Mol Biol 2011;676:165–84.
146. Pavicic W, Perkio E, Kaur S, Peltomaki P. Altered methylation at microRNA-associated CpG islands in hereditary and sporadic carcinomas: a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA)-based approach. Mol Med 2011;17: 726–35.
147. Morita S, Takahashi RU, Yamashita R, Toyoda A, Horii T, Kimura M, et al. Genome-wide analysis of DNA methylation and expression of microRNAs in breast cancer cells. Int J Mol Sci 2012;13:8259–72.
148. Lehmann U, Hasemeier B, Christgen M, Muller M, Romermann D, Langer F, et al. Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. J Pathol 2008;214:17–24.
149. Saito Y, Saito H. Role of CTCF in the regulation of microRNA expression. Front Genet 2012;3:186.
150. Chang S, Sharan SK. Epigenetic control of an oncogenic microRNA, miR-155, by BRCA1. Oncotarget 2012;3:5–6.
151. Valeri N, Vannini I, Fanini F, Calore F, Adair B, Fabbri M. Epigenetics, miRNAs, and human cancer: a new chapter in human gene regulation. Mamm Genome 2009;20:573–80.
152. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, et al. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A 2007;104:15805–10.
153. Benetti R, Gonzalo S, Jaco I, Munoz P, Gonzalez S, Schoeftner S, et al. A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. Nat Struct Mol Biol 2008;15:268–79.
154. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, et al. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. Nat Struct Mol Biol 2008;15:259–67.
155. Kutanzi KR, Yurchenko OV, Beland FA, Checkhun VF, Pogribny IP. MicroRNA-mediated drug resistance in breast cancer. Clin Epigenetics 2011;2:171–85.