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

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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, oncosuppressor 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 oncosuppressor 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); 1–14. ©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 miRNAs 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 miRNAs. 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 miRNAs 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).
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 themicroprocessor 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 stabilizes 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 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 translation inhibition (reviewed in ref. 28). Apart from this, it has also been shown that some miRNAs can actually increase the...
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 alterations of miRNA lead to tumor formation by inducing cell

![Figure 2. Role of oncomiRs and oncosuppressor 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 oncosuppressor miRNAs leads to their downregulation. Downregulation of oncosuppressor miRNAs results in upregulation of oncogenes and thus proliferation of cancer cells. OG, oncogene; TSG, tumor suppressor gene.](https://journals.aacr.org/content/mcr/11/4/OF3/F2.large.jpg)
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 oncogene (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 (TGFBR2), 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 include 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-29a 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 oncogenes 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 transacted by p53, and is an important component of the p53 transcripational 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 oncosuppressor 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 antagonimiRs, 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− 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 stem-like features of BCSCs. Many studies reported a set of miRNAs differentially expressed 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

<table>
<thead>
<tr>
<th>Breast cancer types</th>
<th>miRNAs upregulated</th>
<th>miRNAs downregulated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ductal carcinoma in situ</td>
<td>miR-21, miR-200c, miR-361-5p, miR-374a, miR-93, miR-182, miR-183</td>
<td>let-7d, miR-210, miR-221, miR-7b, miR-125b, miR-127-3p, miR-320, miR-10b, miR-126, miR-143, miR-143, miR-218, miR-335-5p</td>
<td>(82–83)</td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>let-7d, miR-210, miR-221</td>
<td></td>
<td>(82)</td>
</tr>
<tr>
<td>Lobular carcinoma in situ</td>
<td>miR-375, miR-182, miR-183, miR-96, miR-203, miR-425-5p, miR -565</td>
<td>—</td>
<td>(84)</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>miR-9, miR-375, miR-182, miR-183</td>
<td>—</td>
<td>(85)</td>
</tr>
</tbody>
</table>
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 miRNAs (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 coexpressed 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 coexpressed in serum and tissues. They also found a novel miRNA, miR-B51, 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 profound antimitastatic 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

<table>
<thead>
<tr>
<th>miRNA</th>
<th>OncomiRs/oncosuppressor</th>
<th>Targets</th>
<th>Cellular function/mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-9</td>
<td>OncomiRs</td>
<td>CDH1</td>
<td>Increased cell motility and invasion</td>
<td>(86)</td>
</tr>
<tr>
<td>miR-10b</td>
<td>OncomiRs</td>
<td>HOXD10</td>
<td>Favors cancer cell migration and invasion</td>
<td>(87)</td>
</tr>
<tr>
<td>miR-21</td>
<td>OncomiRs</td>
<td>PTEN, TPM1, PDCD4, Maspin, BCL2</td>
<td>Invasion, metastasis, and apoptosis</td>
<td>(51-52, 88-89)</td>
</tr>
<tr>
<td>miR-27a</td>
<td>OncomiRs</td>
<td>Zinc finger, ZBTB10, Myt-1</td>
<td>Cell-cycle progression (G2-M checkpoint regulation)</td>
<td>(90-91)</td>
</tr>
<tr>
<td>miR-92</td>
<td>OncomiRs</td>
<td>ERβ1</td>
<td>Downregulated in many breast cancers; inhibition of miR-92 in MCF-7 cells increased ERβ1 expression</td>
<td>(92)</td>
</tr>
<tr>
<td>miR-96</td>
<td>OncomiRs</td>
<td>FOXO3a</td>
<td>Cell proliferation</td>
<td>(93)</td>
</tr>
<tr>
<td>miR-103/107</td>
<td>OncomiRs</td>
<td>Dicer</td>
<td>High levels of miR-103/107 were associated with metastasis</td>
<td>(94)</td>
</tr>
<tr>
<td>miR-155</td>
<td>OncomiRs</td>
<td>RHOA, SOCS1</td>
<td>TGF-β signaling</td>
<td>(54, 95)</td>
</tr>
<tr>
<td>miR-204 and miR-510</td>
<td>OncomiRs</td>
<td>PDEF</td>
<td>Overexpression leads to metastasis progression</td>
<td>(96)</td>
</tr>
<tr>
<td>miR-221/222</td>
<td>OncomiRs</td>
<td>ERα</td>
<td>Reduce the expression of tumor suppressors</td>
<td>(97)</td>
</tr>
<tr>
<td>miR-373/520c</td>
<td>OncomiRs</td>
<td>CD44</td>
<td>Metastasis</td>
<td>(98)</td>
</tr>
<tr>
<td>miR-661</td>
<td>OncomiRs</td>
<td>Nectin-1, Star-D16</td>
<td>Required for efficient invasion of breast cancer cells</td>
<td>(99)</td>
</tr>
<tr>
<td>Let-7 family</td>
<td>OncomiRs</td>
<td>H-RAS, HMGA2, LIN28, PEBP1</td>
<td>Repress cell proliferation and growth; Let-7f promotes angiogenesis</td>
<td>(43, 100-101)</td>
</tr>
<tr>
<td>miR-17-5p</td>
<td>OncomiRs</td>
<td>AIB1, CyclinD, E2F</td>
<td>Estrogen and E2F-mediated growth, proliferation</td>
<td>(102-103)</td>
</tr>
<tr>
<td>miR-18a/b</td>
<td>OncomiRs</td>
<td>ERα</td>
<td>Inhibition of ER-mediated cell growth</td>
<td>(104-105)</td>
</tr>
<tr>
<td>miR-17/20</td>
<td>OncomiRs</td>
<td>AIB1, cyclin D1, E2F, Myc</td>
<td>Coordinates the timing of cell cycle by targeting multiple cell-cycle regulators</td>
<td>(106)</td>
</tr>
<tr>
<td>miR-30</td>
<td>OncomiRs</td>
<td>Ubc9, ITGj3</td>
<td>Constitutive expression of miR-30 in breast tumor-initiating cells inhibits their self-renewal capacity</td>
<td>(70)</td>
</tr>
<tr>
<td>miR-31</td>
<td>OncomiRs</td>
<td>FZD3, ITGAS, M-RIP, MMP16</td>
<td>Metastasis</td>
<td>(107)</td>
</tr>
<tr>
<td>miR-34a</td>
<td>OncomiRs</td>
<td>CCND1, CDK6, E2F3, MYC</td>
<td>DNA damage, proliferation</td>
<td>(108)</td>
</tr>
<tr>
<td>miR-125a,b</td>
<td>OncomiRs</td>
<td>HER2, HER3</td>
<td>Anchorage-dependent growth</td>
<td>(81, 91)</td>
</tr>
<tr>
<td>miR-145</td>
<td>OncomiRs</td>
<td>RTNK</td>
<td>Plays an important role in human stem cell growth and differentiation by targeting the pluripotency factors</td>
<td>(109)</td>
</tr>
<tr>
<td>miR-200</td>
<td>OncomiRs</td>
<td>BIM1, ZEB1, ZEB2</td>
<td>TGF-β signaling</td>
<td>(71, 110-111)</td>
</tr>
<tr>
<td>miR-205</td>
<td>Onco-suppressor</td>
<td>HER3 receptor</td>
<td>Oncosuppressor gene that is able to interfere with the proliferative pathway mediated by HER receptor family</td>
<td>(112)</td>
</tr>
<tr>
<td>miR-206</td>
<td>OncomiRs</td>
<td>ERα</td>
<td>ER signaling</td>
<td>(16, 77)</td>
</tr>
<tr>
<td>miR-497</td>
<td>OncomiRs</td>
<td>Bcl-w</td>
<td>Apoptosis</td>
<td>(113)</td>
</tr>
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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 Dicer mRNA, which reduce Dicer 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 lungs 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, DGC88, 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 Dicer 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 *XRC1* 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 cancers. 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

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**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 demethylases 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 DNMT3a, HDAC, and PRG (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 PC1 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 stage 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 not, 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.

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Molecular Cancer Research

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

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Mol Cancer Res  Published OnlineFirst January 29, 2013.

Updated version  Access the most recent version of this article at: doi:10.1158/1541-7786.MCR-12-0649

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