

Chapter 2

Molecular Pathogenesis of Breast Cancer and the Role of MicroRNAs

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Abstract Breast cancer is a leading cause of cancer-associated death in women worldwide. The therapy usually involves mastectomy or lumpectomy, followed by chemotherapy and/or radiation therapy in addition to hormonal therapy when indicated. While the area of research on the identification and potential use of microRNAs (miRNAs) as either a diagnostic, prognostic, or predictive biomarker is still in its early stages, there is increasing evidence that miRNAs are involved in tumor progression, chemoresistance, and survival. The miRNAs have enormous prospective in clinical research since they are detected in the serum, plasma, fresh tissues, and formalin-fixed paraffin-embedded tissue samples. Hence, it may be possible to develop novel therapeutic regimens of specific miRNAs as targets to prevent or treat breast cancer (BC). The miRNA expression profiling is now used extensively by many investigators to demonstrate specific miRNA signatures in both the body fluids and in the tumor tissue, indicating that miRNAs may likely be useful as diagnostic and prognostic tools in all cancers including BC. Numerous investigators, including our laboratory, have used strategies to deregulate miRNAs with either anti- and pre-miRNA molecular drugs or even natural compounds to prevent or control tumor progression, which will be discussed in this chapter. Moreover, the role of several natural and synthetic compounds as anticancer agents will also be discussed in this chapter. Finally, the role of several miRNAs as targets will be discussed especially because miRNA-based therapies are currently being exploited for cancer therapy.

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Introduction

Breast adenocarcinoma (breast cancer, BC) is the most commonly diagnosed cancer among women in the United States with an estimated 232,340 new cases in 2013. Sadly, one in eight is diagnosed with breast cancer in their lifetime, yet it is still a frequent cause of death in women [1]. Although research together with progress in targeted therapies led to increased patient survival, there is still an increased demand for the development of new diagnostic biomarkers and ground-breaking therapeutic strategies for improving the overall survival in all patients diagnosed with BC. Recent research suggests the involvement of deregulated genes at the levels of DNA, protein, RNA, and microRNAs (miRNAs), and as such miRNAs are becoming important players in the development, differentiation, and regulation of gene expression in cancer biology [2, 3]. The miRNAs are small noncoding endogenous single-stranded class of regulatory RNAs that posttranscriptionally inhibit gene expression through targeting specific messenger RNAs (mRNAs) [4]. Due to the smaller size, these miRNAs remain stable in body specimens including plasma, serum, and both fresh-frozen and formalin-fixed paraffin-embedded (FFPE) tissue samples and can serve as an excellent source of early detection of various cancers, including BC [5, 6]. Emerging evidence suggests that a single miRNA may target several mRNAs and vice versa and substantial basic-science research on these miRNAs has led to the development of new methodologies for cancer diagnosis, and this is now progressing to clinical research arenas. Comparison of cancerous against normal human plasma or tissue samples by miRNA microarray showed deregulation of several miRNAs in many cancers such as lung, pancreas, prostate, and BC [5–10]. Among the many deregulated miRNAs, several of them are common in most cancers and few are cancer specific [11]. Research on antisense oligonucleotides (ASO) is also on the rise; use of this technology in vivo has been successfully demonstrated by delivering ASO with chemokines to inactivate a subset of immune cells [12] and in many other conditions. In this book chapter, we review the current and developing methodologies of extracting miRNA from body fluids and fresh-frozen and FFPE tissue samples and will discuss few miRNAs and their use in the diagnosis and treatment of BC.

Detection of microRNAs in Clinical Specimens

The challenge that clinicians face is in the early detection of cancer to reduce mortality rates. Significant research in the fields of molecular biology has led to the development of newly identified miRNAs which has implications in the prevention

of disease progression and therapeutic targets for designing molecular therapies for the management of cancer patients. Many investigators including our own laboratory have shown miRNAs as suitable biomarkers for early detection of cancers since they are stable and not degraded in plasma, serum or fresh-frozen and fine-needle aspirates of FFPE tissue samples [5, 6]. The miRNA expression profiling has been helpful in differentiating normal patients from cancer patients due to differential expression of various miRNAs. Several miRNAs such as miR-34a, miR-155, and miR-10b have been shown to be deregulated in the serum of BC patients compared to healthy controls [13]. Another study showed differences in circulating miRNA level between Caucasian and African-American patients [14]. In addition, specific miRNAs may also predict drug response that is essential for developing precise molecular targeted therapy for each individual. Moreover, miRNAs can be up- or downregulated in cancers due to the genes' downstream signaling effect, hence controlling the expression of cancers [15]. The miRNAs that are elevated in cancer are oncogenic, and likewise, the miRNAs that have reduced expression function as tumor suppressor [16–19].

Methodology and Clinical Associations

Although new technologies have advanced cancer research from bench to bedside, it remains a common medical problem worldwide in all cancers leading to significant mortality, morbidity, and rising healthcare costs. This emphasizes the urgent need for novel molecular technologies both in the laboratory and in the clinic to identify high-risk patients. Small molecules, such as miRNAs, have enormous potential in the clinic since a single miRNA can target multiple genes, indicating that modulating one miRNA will have effects on multiple genes which is opening new doors for innovative therapies especially to target heterogeneous populations of cancer cells within a tumor mass. The discovery of noninvasive biomarkers for most cancer types has been a valuable tool to differentiate tumors from normal tissue with minimal discomfort and risk to the cancer patients. Their most important benefit is the ease of access and possibility of repeated testing in a noninvasive manner. Emerging evidence suggests that the expression level of miRNAs varies in various cancers and also changes as the disease progresses. Some miRNAs are tumor specific both in human and in mouse models [20], while others are common in many tumor types [21, 22].

Isolation of microRNAs, Reverse Transcription, and Polymerase Chain Reaction (RT-PCR) from Plasma Samples

A number of independent researchers studied the potential role of miRNAs in the plasma as early detection biomarkers of cancers including BC [5, 7, 11, 13]. The miRNAs have been found to be stable in many body fluids including plasma and serum which is believed to be important for their potential as disease biomarkers. The assay requires a very small amount of RNA as low as 10 ng which can be easily isolated from either plasma or serum for real-time PCR quantitative analysis. The detailed methodology of isolation of miRNAs from plasma has been described earlier [15]. Here we will highlight few important points and techniques. The total RNA including miRNAs is isolated from as low as 250 μ l of plasma sample using QIAGEN kit (QIAGEN, Valencia, CA) and eluted in 25 μ l of water. Although RNA obtained from plasma is low, and it cannot be quantified using single-drop NanoDrop technology, similar volume can be used together with housekeeping miRNAs as controls. Nonetheless, the reverse-transcription (RT) reaction can be carried out using the template mature miRNA by using Exiqon-Universal cDNA synthesis kit available from Exiqon. The RT reaction contains 4 μ l of 5X RT buffer, 2 μ l of enzyme, 4 μ l of either plasma miRNA or 250 nM of standard miRNA, and 10 μ l of water incubated for 1 h at 42 °C and 5 min at 95 °C.

The cDNA obtained from above is subjected to real-time polymerase chain reaction (PCR) using multiple housekeeping genes for data normalization. The analysis is performed by the standard Ct method for quantification using StepOnePlus Real-Time PCR (Applied Biosystems, Foster City, CA) which also serves as control for variability in sample loading. The miRNA standard cDNA and the plasma cDNA is diluted in water, and the reaction is set up using SYBR Green (Applied Biosystems) and PCR primer mix as described earlier [15].

MicroRNA Methodology Utilizing Archived Formalin-Fixed Paraffin-Embedded Tissues

The total RNA containing miRNA is isolated from FFPE tissue using RNeasy Kit (QIAGEN) following manufacturer's protocol using four 10- μ m thick and approximately 0.5–1 cm in diameter tissue curls as described previously [15]. The total RNA containing miRNA is then eluted with RNase-free water and measured and quantified using NanoDrop 2000 (Thermo Scientific, Pittsburgh, PA). The RT reaction is performed with SYBR Green miRNA-based assay using Exiqon-Universal cDNA synthesis kit (Exiqon, Woburn, MA) using 10 ng of total RNA. PCR reactions are performed in triplicate using StepOnePlus Real-Time PCR (Applied Biosystems), and expression levels of miRNAs are analyzed using Ct method.

Developments Made in Differentiating Normal and Disease State Using miRNA Profiling

We have described RNA extraction methods both from plasma and FFPE tissues previously [15], and few additional points are also discussed in this chapter. The ultimate challenge or concern to miRNA extraction from plasma is the low level of circulating miRNAs which are below the detection limit of spectrophotometry. Compared to microarray profiling, qRT-PCR have shown superiority in sensitivity [23], and hence these methodologies are often used to validate abnormal expression of miRNAs. To avoid unfair measurement of miRNAs, endogenous genes are used as internal controls for data normalization [6]. Initial research on human breast tumors identified the variation in gene expression using RNA from 42 patients with complimentary DNA microarrays that showed great variation in gene expression, yet it also showed specific gene expression relating to tumor types [24]. In addition, gene expression profiling of hereditary BC patients discovered exclusive expression patterns that were dependent on the *BRCA1* and *BRCA2* mutation status [25]. Although gene expression profiling was the standard for defining molecular subtypes, immunohistochemical analysis was also used for expression of hormone receptors and lack of *HER2/neu* overexpression and luminal cytokeratin [26]. Several developing technologies are looking outside of gene expression profiling such as toward the level of protein expression or gene methylation to understand the differences associated between normal tissue and cancer [27]. Finally, the probability of using miRNA expression profiling in clinical samples by microarray or by quantitative RT-PCR emerged in several studies as diagnostic and prognostic marker [27, 28]. The miRNA research has made a substantial impact on efficient profiling of deregulated miRNAs in plasma, serum, FFPE, and many other sample types because of their stability, serving as a potentially reliable biomarker [5–7, 11, 13, 14]. Expression analysis of miRNAs not only determines several miRNAs but also fully discriminates between normal healthy and diseased state. There are a number of miRNAs that are substantially upregulated in one type of cancer; for instance, miR-205 is overexpressed in the lung, pancreas, and bladder cancer [13, 29–31] and found to be decreased in breast, prostate, and esophageal cancer [32–34], suggesting that some miRNAs act as both oncogenic and tumor suppressor depending on the tumor type and expression pattern. Therefore, the field of miRNA research is highly complex and requires critical insights with respect to the function of a specific miRNA in a specific context.

Altered miRNA Expression in Breast Cancer

Despite substantial improvements in the field of cancer biology, the progress of validated biomarkers for BC has remained an overwhelming task. Numerous studies have been published identifying deregulated miRNAs in BC using

microarray profiling and subsequent validation of selected miRNAs by real-time PCR. The results of miRNA profiling suggest that many miRNAs are altered in all types of cancer and may provide a useful biomarker for detection of cancer. Overexpression of miRNAs in human BC is often a result of molecular genomic abnormalities, called OncomiRs. Inhibition of these OncomiRs can inhibit cell proliferation as well as tumor growth. Patients with triple negative BC (TNBC) have poor prognosis due to aggressive proliferation, migration, and invasion. One study with MDA-MB-231 parental cells and MDA-MB-231 cells stably expressing miR-221-ZIP or scramble-ZIP showed knockdown of miR-221 which inhibited tumor growth by altering the expression of E-cadherin, snail, and slug both in vivo and in TNBC cell lines in vitro [35]. In the subsequent section, we will discuss few miRNAs that are OncomiRs and tumor suppressors in BC.

miR-21

One well-described OncomiR globally found in many tumors including BC is miR-21. The important target of miR-21 is phosphatase and tensin homolog (PTEN) and PDCD4 [36]. Loss of PTEN has been found to be indirectly associated with miR-21 expression in the breast, pancreas, and colon cancer [36–39]. Iorio et al. reported aberrant expression of 29 miRNAs in breast cancer tissues using microarray analysis compared to normal tissues [7]. The miR-21 was also recognized as overexpressed miRNA in a large-scale miRNome analysis on 540 samples that included the lung, breast, stomach, prostate, colon, and pancreatic tumors [40]. By TaqMan real-time PCR methodology, miR-21 was proven to be upregulated in breast tumors compared to the normal breast tissue samples among 157 miRNAs analyzed [41]. A recent study demonstrated overexpression of miR-21 in FFPE tissue samples of atypical ductal hyperplasia, ductal carcinoma in situ, and invasive ductal carcinoma compared to normal tissue samples, suggesting its oncogenic role in all types of cancer including BC [42].

miR-155

The miR-155 has been shown to be upregulated in different tumor types, including BC [7, 43–45]. It directly inhibits RhoA expression, a gene that regulates cell adhesion, motility, and polarity [7]. It is also linked with cancer invasiveness in human BC [44]. Inhibition of miR-155 induced apoptosis and improved chemosensitivity in BC cell lines by targeting FOXO3a [43]. Inhibition of miR-155 with antisense oligonucleotide (ASO-miR-155) in MDA-MB-157 breast cancer cell line inhibited cell viability, induced apoptosis, and most importantly inhibited tumor growth in mouse model which was in part mediated via capase-3 upregulation [45].

miR-10b

The miR-10b is greatly expressed in hepatocellular, glioblastoma, pancreatic, and breast tumors [29, 46–48]. Chan et al. studied the expression of circulating miRNAs from Asian Chinese to compare miRNA expression from serum samples obtained from BC patients and healthy individuals using microarrays or locked nucleic acid real-time PCR panels. Among the significantly expressed miRNAs, miR-10b was significantly upregulated in serum of BC patients compared to serum obtained from healthy controls, suggesting the noninvasive diagnostic strategy could be a promising tool for clinical studies, although further validation in different subtypes of breast cancer is warranted [49]. Overexpression of miR-10b in metastatic BC cells regulated cell migration and invasion through the transcription factor Twist which, in turn, inhibited homeobox D10, resulting in increased expression of RHOC [47]. Higher expression of miR-10b in primary breast carcinomas was associated with clinical progression [47]. In addition, miR-10b overexpression was also observed in metastasis-positive patients compared to metastasis-free patients in hepatocellular carcinoma [48].

miR-34a

Overexpression of miR-34a decreases Akt signaling pathway and increases estrogen receptor-alpha (ER α)-phosphorylation status [50]. The expression of miR-34a is typically decreased in cancer causing activated signaling such as through Akt pathway. A recent article by Guo et al. stated upregulation of miR-34a with curcumin and its combination with another natural compound, emodin, led to the downregulation of Bcl-2 and Bmi-1 in breast cancer cells, indicating the involvement of both apoptosis regulator and self-renewal of adult stem cells [51]. Sensitization of MCF-7 cells to Adriamycin was also observed with ectopic overexpression of miR-34a, suggesting that deregulation of miR-34a plays a key role in acquired Adriamycin resistance of BC, to some extent by targeting Notch-1, another target of miR-34a [52]. Some miRNAs are differentially expressed in the blood of breast and colorectal cancer patients compared to controls. The analysis of the relative quantification of the miRNAs showed significantly reduced levels of expression of miR-34a both in breast and in colorectal cancer patients compared to controls, suggesting that miR-34a is not tissue specific and may be used in the future as a circulating biomarker for multiple cancers [53].

miR-125a,b

HDAC inhibitor entinostat inhibited erbB2/erbB3 protein translation through upregulation of miR-125a, miR-125b, and miR-205 via targeting erbB2 and/or erbB3 in BC cells [54]. Similarly, one investigator report using SKBR3 cells, a breast cancer cell line, as a model for ERBB2/ERBB3 dependence, showed through infection of cells with retroviral constructs expressing miR-125a or 125b which resulted in the inhibition of ERBB2/ERBB3, suggesting the possibility of using miRNAs as a beneficial strategy for therapeutic target [55]. Another report demonstrated that the expression of miR-125a was related with the expression of stress-induced RNA binding protein HuR, which is high in many cancers including BC. Restoration of miR-125a expression reduced HuR protein level and repressed cell growth in breast cancer cells, suggesting that miR-125a may play a role as a tumor suppressor in BC [56].

miR-200 Family

Several investigators have demonstrated the key role of miR-200 family in regulating epithelial-to-mesenchymal transition (EMT) via inhibition of the E-cadherin transcriptional repressors ZEB1/ZEB2 [57–61]. A recent study also demonstrated in a mouse model of BC metastasis that ectopic expression of the miR-200b/200c/429 limits tumor-cell invasion and metastasis [62]. Furthermore, moesin was found to be directly targeted by miR-200b, and thus restoration of miR-200b expression in cells alleviated metastatic suppression, suggesting the existence of a moesin-dependent pathway which is different from the ZEB1/ZEB2 pathway [62]. Micro-environmental signals including TGF β can direct tumor metastasis by varying miR-200 expression [58]. The role of miR-200 as tumor suppressor was also studied by Manavalan et al. in BC cell line model of advancing endocrine/tamoxifen resistance. The study showed that overexpression of miR-200b or 200c in endocrine therapy (tamoxifen)-resistant cells changed morphology to epithelial appearance, inhibited cell growth and migration, and were sensitized to tamoxifen [63]. Tamoxifen effect was also observed in endometrial cancer cells, which was in part through upregulation of snail and downregulation of E-cadherin and miR-200 expression, contributing to tamoxifen-induced EMT through c-Myc [64]. Lim et al. in a recent study suggested that cancer stemlike cells show loss of miR-200 expression, and restoration of its expression decreased stemlike characteristics and further stimulated epithelial phenotype in BC cells [65]. Studies in other cancers also showed upregulation of E-cadherin caused by ectopic expression of miR-200 family, suggesting miR-200 as a marker of the epithelial phenotype [61]. Many investigators reported anticancer properties of natural and synthetic compounds, such as reduction in proliferation [37, 60, 66] and cancer cell-specific induction of apoptosis in BC cells mediated through deregulation of several signaling pathways

[66, 67]. In this chapter we will discuss few natural and synthetic anticancer compounds and their attributes as modulators of miRNAs.

Natural and Synthetic Anticancer Compounds

Garcinol

Anticancer properties of edible fruit *Garcinia indica*-derived garcinol are opening new doors for chemotherapy or chemoprevention of many cancers, including BC [67–71]. It was demonstrated earlier by our group that garcinol-induced apoptosis in the breast, prostate, and pancreatic cancer cells is mediated through the downregulation of NF- κ B signaling pathway [67, 68]. Another recent report by our group showed the important role of garcinol in the reversal of EMT, as observed in aggressive triple negative MDA-MB-231 and BT-549 breast cancer cells which was mediated through the upregulation of epithelial marker E-cadherin and the expression of miR-200 and let-7 family miRNAs [69]. Another study investigated the effect of garcinol on a human hepatocellular cancer cell line Hep3B that lacks functional p53. Garcinol activated the mitochondrial apoptotic pathways along with the ER stress modulator GADD153, indicating a potential therapeutic role of garcinol in p53-independent apoptosis in cancer [71]. In addition, garcinol inhibited cell proliferation in nicotine-induced human BC, MDA-MB-231 cells, through the downregulation of α 9-nAChR and cyclin D3 expression, suggesting that cyclin D3 would be a suitable molecular target for assessing the activity of chemotherapeutics and/or garcinol could be a powerful chemopreventive agent in BC patients in the clinical setting [70].

Plumbagin

Many investigators reported potent anticancer activity of a plant metabolite plumbagin, a naturally occurring naphthaquinone (5-hydroxy-2-methyl-1, 4-naphthoquinone) [66, 72–74]. Plumbagin has been shown to inhibit cancer cell migration and invasion and suppressed the expression of osteoclast-activating factors [66]. It also inhibited breast tumor-bone metastasis and osteolysis by controlling the tumor-bone microenvironment in a mouse model, suggesting that plumbagin may serve as an innovative agent for the treatment of tumor-bone metastasis [66]. Plumbagin can induce estrogen-dependent cell signaling and apoptosis in BRCA1-blocked ovarian cancer cells. It was observed to be the most effective anticancer agent when compared to other structurally related compounds and indicated to enhance numerous pathways of apoptosis and cell cycle arrest in BRCA1-blocked cells compared to unblocked cells [72]. Another investigator

observed the potential role of plumbagin toward the expression of CXCR4 and its function in various tumor cells. It was observed that plumbagin downregulated CXCR4 expression in BC cells regardless of their HER2 status and was not cell type specific. For example, inhibition with plumbagin also occurred in the gastric, lung, renal, oral, and hepatocellular cancer cell lines [74]; however, no specific miRNA has been found to be associated with the biological activity of plumbagin.

Curcumin

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a polyphenolic compound found in the spice turmeric and is considered as a pleiotropic molecule which interacts with a variety of molecular targets and has antitumor, anti-inflammatory, and various other biological activities [75]. Curcumin inhibited the growth of BC cell lines, and increased the percentage of cells in sub-G0 phase, representing the apoptotic cell population, which was further confirmed by PARP-1 cleavage [75]. In vivo antitumor activity of both early and in an advanced stage of mammary carcinogenesis induced tumor-free survival and a reduction in tumor multiplicity with the administration of safe curcumin [75]. Curcumin was observed to stabilize p27 levels in BC with concomitant decrease in Skp2, Her2, Cyclin E, and CDK kinases expression in MDA-MB-231/Her2 cells, suggesting the potential role of curcumin as a chemopreventive agent in BC [76]. Curcumin is not only considered as chemopreventive/chemotherapeutic drug but is also associated with obesity-related cancers. It modifies several molecular targets by reversing insulin resistance to prevent obesity-related cancers [77]. Demethoxycurcumin, an active compound of curcuminoids originated in turmeric powder, showed reduced levels of ECM degradation-associated proteins such as matrix metalloproteinase-9 (MMP-9), membrane type-1 matrix metalloproteinase (MT1-MMP), urokinase plasminogen activator (uPA), and uPA receptor (uPAR) curcumin-treated in MDA-MB-231 cells [78].

Although in vitro and some limited preclinical model study showed promising results with curcumin, human clinical trial has been disappointing which was partly attributed to the target tissue bioavailability of curcumin. The low bioavailability of curcumin prompted the synthesis of many analogs of curcumin. One such example is CDF, a difluorinated synthetic analog of curcumin with greater bioavailability [38]. Although the effect of CDF has not been demonstrated in BC yet, it has been proven to be more effective than curcumin in both pancreatic and colon cancer [37, 38, 79]. In order to ensure that the efficiency of CDF was similar to that of curcumin, tests were conducted in pancreatic cancer cell lines comparing the two in terms of their ability to inhibit cell growth both in vitro and in vivo [37]. CDF also reduced the presence of cancer stem cell markers in chemoresistant colon cancer cells compared to curcumin [79]. The above two reports confirmed that CDF was more effective than curcumin, and the biological activity was in part mediated through deregulation of miRNAs [38].

Table 2.1 The list of up- and downregulated microRNAs and their Targets

miRNAs	Up- or downregulated	Target genes	References
miR-221	Upregulated	E-cadherin, snail, slug	[35]
miR-21	Upregulated	PTEN, PDCD4	[36–40]
miR-155	Upregulated	RhoA, FOXO3a, Caspase-3	[7, 43, 45]
miR-10b	Upregulated	Twist, RHOC	[47]
miR-34a	Downregulated	Akt, Bcl2, Bmi-1, Notch1	[50–52]
miR-125a,b	Downregulated	erbB2, erbB3, HUR	[54–56]
miR-200 family	Downregulated	E-cadherin, ZEB1, ZEB2, c-Myc	[57–61, 64]

Conclusion

For the past decade or more, numerous studies have shown an intricate relationship between the expression of miRNA and human malignancies including BC. The miRNAs are important regulators of numerous biological processes and are implicated in the pathogenesis of not only cancers but also other human diseases. Recent studies have shown the stability of miRNAs in serum, plasma, and in both fresh and FFPE tissue samples. Microarray expression profiling of miRNAs provides a high-throughput molecular resource to classify countless number of diseases including BC associated with deregulated expression of miRNAs, which is also concurrently cost-effective. Therefore, the expression of miRNAs plays a significant role in differentiating cancer from normal, suggesting its role as a future diagnostic, prognostic, and predictive biomarker for cancer therapy. Since a single miRNA can regulate the expression of multiple target genes, it has a substantial potential for therapeutic use. Forced overexpression of pre-miRNA or inhibition of miRNA expression by antisense miRNA as demonstrated in mouse models could reduce tumor growth [80, 81]. These antisense miRNAs are now being tested in the clinical setting [82]. However, the use of natural agents or their synthetic analogs appears to have a great promise toward cancer prevention and therapy which indeed could be attributed to its function as the deregulators of miRNAs. Thus the use of natural agents or their derivatives as a single agent or in combination with conventional therapeutics will become a better strategy for prevention and/or treatment of human malignancies including BC. Although the understanding of miRNA function and regulation has increased in recent years, we are still in need of new ideas and techniques involving miRNA-based research especially for miRNA-targeted cancer therapy. Nevertheless, the future looks brighter for developing miRNA-targeted therapy for all human cancers including BC (Table 2.1).

References

- Desantis C, Ma J, Bryan L, Jemal A (2013) Breast cancer statistics, 2013. *CA Cancer J Clin* 64 (1):52–62, PM:24114568
- Ali AS, Ahmad A, Ali S, Bao B, Philip PA, Sarkar FH (2013) The role of cancer stem cells and miRNAs in defining the complexities of brain metastasis. *J Cell Physiol* 228:36–42, PM:22689345 PMC3443527
- Sethi S, Ali S, Kong D, Philip PA, Sarkar FH (2013) Clinical implication of microRNAs in molecular pathology. *Clin Lab Med* 33:773–86, PM:24267185
- Melo SA, Esteller M (2011) Dysregulation of microRNAs in cancer: playing with fire. *FEBS Lett* 585:2087–99, PM:20708002
- Ali S, Almhanna K, Chen W, Philip PA, Sarkar FH (2010) Differentially expressed miRNAs in the plasma may provide a molecular signature for aggressive pancreatic cancer. *Am J Transl Res* 3:28–47, PM:21139804 PMC2981424
- Ali S, Saleh H, Sethi S, Sarkar FH, Philip PA (2012) MicroRNA profiling of diagnostic needle aspirates from patients with pancreatic cancer. *Br J Cancer* 107:1354–1360, PM:22929886 PMC3494446
- Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S et al (2005) MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 65:7065–7070, PM:16103053
- Liu J, Mao Q, Liu Y, Hao X, Zhang S, Zhang J (2013) Analysis of miR-205 and miR-155 expression in the blood of breast cancer patients. *Chin J Cancer Res* 25:46–54, PM:23372341 PMC3555294
- Peng X, Guo W, Liu T, Wang X, Tu X, Xiong D et al (2011) Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. *PLoS One* 6:e20341, PM:21647377 PMC3103579
- Wang Y, Gu J, Roth JA, Hildebrandt MA, Lippman SM, Ye Y et al (2013) Pathway-based serum microRNA profiling and survival in patients with advanced stage non-small cell lung cancer. *Cancer Res* 73:4801–4809, PM:23774211 PMC3760306
- Ferracin M, Querzoli P, Calin GA, Negrini M (2011) MicroRNAs: toward the clinic for breast cancer patients. *Semin Oncol* 38:764–775, PM:22082762
- Biragyn A, Bodogai M, Olkhanud PB, Denny-Brown SR, Puri N, Ayukawa K et al (2013) Inhibition of lung metastasis by chemokine CCL17-mediated in vivo silencing of genes in CCR4+ Tregs. *J Immunother* 36:258–267, PM:23603860 PMC3707614
- Zhu W, Qin W, Atasoy U, Sauter ER (2009) Circulating microRNAs in breast cancer and healthy subjects. *BMC Res Notes* 2:89, PM:19454029 PMC2694820
- Zhao H, Shen J, Medico L, Wang D, Ambrosone CB, Liu S (2010) A pilot study of circulating miRNAs as potential biomarkers of early stage breast cancer. *PLoS One* 5:e13735, PM:21060830 PMC2966402
- Sethi S, Ali S, Kong D, Philip PA, Sarkar FH (2013) Clinical implication of microRNAs in molecular pathology. *Clin Lab Med* 33:773–786, PM:24267185
- Czyzyk-Krzeska MF, Zhang X (2014) MiR-155 at the heart of oncogenic pathways. *Oncogene* 33(6):677–678, PM:23416982
- Orso F, Balzac F, Marino M, Lembo A, Retta SF, Taverna D (2013) miR-21 coordinates tumor growth and modulates KRIT1 levels. *Biochem Biophys Res Commun* 438:90–96, PM:23872064 PMC3750217
- Piva R, Spandidos DA, Gambari R (2013) From microRNA functions to microRNA therapeutics: novel targets and novel drugs in breast cancer research and treatment (Review). *Int J Oncol* 43:985–994, PM:23939688 PMC3829774
- Sun X, Qin S, Fan C, Xu C, Du N, Ren H (2013) Let-7: a regulator of the ERalpha signaling pathway in human breast tumors and breast cancer stem cells. *Oncol Rep* 29:2079–2087, PM:23467929
- Liang Y, Ridzon D, Wong L, Chen C (2007) Characterization of microRNA expression profiles in normal human tissues. *BMC Genomics* 8:166, PM:17565689 PMC1904203

21. Gall TM, Frampton AE, Krell J, Castellano L, Stebbing J, Jiao LR (2013) Blood-based miRNAs as noninvasive diagnostic and surrogate biomarkers in colorectal cancer. *Expert Rev Mol Diagn* 13:141–145, PM:23477554
22. Si H, Sun X, Chen Y, Cao Y, Chen S, Wang H et al (2013) Circulating microRNA-92a and microRNA-21 as novel minimally invasive biomarkers for primary breast cancer. *J Cancer Res Clin Oncol* 139:223–229, PM:23052693 PMC3549412
23. Chen Y, Gelfond JA, McManus LM, Shireman PK (2009) Reproducibility of quantitative RT-PCR array in miRNA expression profiling and comparison with microarray analysis. *BMC Genomics* 10:407, PM:19715577 PMC2753550
24. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA et al (2000) Molecular portraits of human breast tumours. *Nature* 406:747–752, PM:10963602
25. Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R et al (2001) Gene-expression profiles in hereditary breast cancer. *N Engl J Med* 344:539–548, PM:11207349
26. Tang P, Skinner KA, Hicks DG (2009) Molecular classification of breast carcinomas by immunohistochemical analysis: are we ready? *Diagn Mol Pathol* 18:125–132, PM:19704256
27. Gruver AM, Portier BP, Tubbs RR (2011) Molecular pathology of breast cancer: the journey from traditional practice toward embracing the complexity of a molecular classification. *Arch Pathol Lab Med* 135:544–557, PM:21526953
28. Kulshreshtha R, Ferracin M, Wojcik SE, Garzon R, Alder H, Agosto-Perez FJ et al (2007) A microRNA signature of hypoxia. *Mol Cell Biol* 27:1859–1867, PM:17194750 PMC1820461
29. Bloomston M, Frankel WL, Petrocca F, Volinia S, Alder H, Hagan JP et al (2007) MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 297:1901–1908, PM:17473300
30. Gottardo F, Liu CG, Ferracin M, Calin GA, Fassan M, Bassi P et al (2007) Micro-RNA profiling in kidney and bladder cancers. *Urol Oncol* 25:387–392, PM:17826655
31. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M et al (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 9:189–198, PM:16530703
32. Feber A, Xi L, Luketich JD, Pennathur A, Landreneau RJ, Wu M et al (2008) MicroRNA expression profiles of esophageal cancer. *J Thorac Cardiovasc Surg* 135:255–260, PM:18242245 PMC2265073
33. Ichimi T, Enokida H, Okuno Y, Kunitomo R, Chiyomaru T, Kawamoto K et al (2009) Identification of novel microRNA targets based on microRNA signatures in bladder cancer. *Int J Cancer* 125:345–352, PM:19378336
34. Sempere LF, Christensen M, Silahtaroglu A, Bak M, Heath CV, Schwartz G et al (2007) Altered MicroRNA expression confined to specific epithelial cell subpopulations in breast cancer. *Cancer Res* 67:11612–11620, PM:18089790
35. Nassirpour R, Mehta PP, Baxi SM, Yin MJ (2013) miR-221 promotes tumorigenesis in human triple negative breast cancer cells. *PLoS One* 8:e62170, PM:23637992 PMC3634767
36. Qi L, Bart J, Tan LP, Platteel I, Sluis T, Huitema S et al (2009) 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* 9:163, PM:19473551 PMC2695476
37. Ali S, Ahmad A, Banerjee S, Padhye S, Dominiak K, Schaffert JM et al (2010) Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF. *Cancer Res* 70:3606–3617, PM:20388782 PMC2978024
38. Bao B, Ali S, Kong D, Sarkar SH, Wang Z, Banerjee S et al (2011) Anti-tumor activity of a novel compound-CDF is mediated by regulating miR-21, miR-200, and PTEN in pancreatic cancer. *PLoS One* 6:e17850, PM:21408027 PMC3052388
39. Roy S, Yu Y, Padhye SB, Sarkar FH, Majumdar AP (2013) Difluorinated-curcumin (CDF) restores PTEN expression in colon cancer cells by down-regulating miR-21. *PLoS One* 8:e68543, PM:23894315 PMC3722247

40. Chen J, Wang X (2014) MicroRNA-21 in breast cancer: diagnostic and prognostic potential. *Clin Transl Oncol* 16(3):225–233, PM:24248894
41. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY (2007) miR-21-mediated tumor growth. *Oncogene* 26:2799–2803, PM:17072344
42. Chen L, Li Y, Fu Y, Peng J, Mo MH, Stamatakis M et al (2013) Role of deregulated microRNAs in breast cancer progression using FFPE tissue. *PLoS One* 8:e54213, PM:23372687 PMC3553092
43. Kong W, He L, Coppola M, Guo J, Esposito NN, Coppola D et al (2010) MicroRNA-155 regulates cell survival, growth, and chemosensitivity by targeting FOXO3a in breast cancer. *J Biol Chem* 285:17869–17879, PM:20371610 PMC2878550
44. O'Day E, Lal A (2010) MicroRNAs and their target gene networks in breast cancer. *Breast Cancer Res* 12:201, PM:20346098 PMC2879559
45. Zheng SR, Guo GL, Zhai Q, Zou ZY, Zhang W (2013) Effects of miR-155 antisense oligonucleotide on breast carcinoma cell line MDA-MB-157 and implanted tumors. *Asian Pac J Cancer Prev* 14:2361–2366, PM:23725141
46. Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G et al (2005) Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem Biophys Res Commun* 334:1351–1358, PM:16039986
47. Ma L, Teruya-Feldstein J, Weinberg RA (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449:682–688, PM:17898713
48. Tan HX, Wang Q, Chen LZ, Huang XH, Chen JS, Fu XH et al (2010) MicroRNA-9 reduces cell invasion and E-cadherin secretion in SK-Hep-1 cell. *Med Oncol* 27:654–660, PM:19572217
49. Chan M, Liaw CS, Ji SM, Tan HH, Wong CY, Thike AA et al (2013) Identification of circulating microRNA signatures for breast cancer detection. *Clin Cancer Res* 19:4477–4487, PM:23797906
50. Zhao G, Guo J, Li D, Jia C, Yin W, Sun R et al (2013) MicroRNA-34a suppresses cell proliferation by targeting LMTK3 in human breast cancer MCF-7 cell line. *DNA Cell Biol* 32:699–707, PM:24050776 PMC3864372
51. Guo J, Li W, Shi H, Xie X, Li L, Tang H et al (2013) Synergistic effects of curcumin with emodin against the proliferation and invasion of breast cancer cells through upregulation of miR-34a. *Mol Cell Biochem* 382:103–111, PM:23771315
52. Li XJ, Ji MH, Zhong SL, Zha QB, Xu JJ, Zhao JH et al (2012) MicroRNA-34a modulates chemosensitivity of breast cancer cells to adriamycin by targeting Notch1. *Arch Med Res* 43:514–521, PM:23085450
53. Nugent M, Miller N, Kerin MJ (2012) Circulating miR-34a levels are reduced in colorectal cancer. *J Surg Oncol* 106:947–952, PM:22648208
54. Wang S, Huang J, Lyu H, Lee CK, Tan J, Wang J et al (2013) Functional cooperation of miR-125a, miR-125b, and miR-205 in entinostat-induced downregulation of erbB2/erbB3 and apoptosis in breast cancer cells. *Cell Death Dis* 4:e556, PM:23519125 PMC3615747
55. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC (2007) Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J Biol Chem* 282:1479–1486, PM:17110380
56. Guo X, Wu Y, Hartley RS (2009) MicroRNA-125a represses cell growth by targeting HuR in breast cancer. *RNA Biol* 6:575–583, PM:19875930 PMC3645467
57. Ahmad A, Aboukameel A, Kong D, Wang Z, Sethi S, Chen W et al (2011) Phosphoglucose isomerase/autocrine motility factor mediates epithelial-mesenchymal transition regulated by miR-200 in breast cancer cells. *Cancer Res* 71:3400–3409, PM:21389093 PMC3085607
58. Creighton CJ, Gibbons DL, Kurie JM (2013) The role of epithelial-mesenchymal transition programming in invasion and metastasis: a clinical perspective. *Cancer Manag Res* 5:187–195, PM:23986650 PMC3754282

59. Kong D, Banerjee S, Ahmad A, Li Y, Wang Z, Sethi S et al (2010) Epithelial to mesenchymal transition is mechanistically linked with stem cell signatures in prostate cancer cells. *PLoS One* 5:e12445, PM:20805998 PMC2929211
60. Li Y, VandenBoom TG, Kong D, Wang Z, Ali S, Philip PA et al (2009) Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. *Cancer Res* 69:6704–6712, PM:19654291 PMC2727571
61. Park SM, Gaur AB, Lengyel E, Peter ME (2008) The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. *Genes Dev* 22:894–907, PM:18381893 PMC2279201
62. Li X, Roslan S, Johnstone CN, Wright JA, Bracken CP, Anderson M et al (2013) MiR-200 can repress breast cancer metastasis through ZEB1-independent but moesin-dependent pathways. *Oncogene*. doi:10.1038/onc.2013.370, PM:24037528
63. Manavalan TT, Teng Y, Litchfield LM, Muluhngwi P, Al-Rayyan N, Klinge CM (2013) Reduced expression of miR-200 family members contributes to antiestrogen resistance in LY2 human breast cancer cells. *PLoS One* 8:e62334, PM:23626803 PMC3633860
64. Bai JX, Yan B, Zhao ZN, Xiao X, Qin WW, Zhang R et al (2013) Tamoxifen represses miR-200 microRNAs and promotes epithelial-to-mesenchymal transition by up-regulating c-Myc in endometrial carcinoma cell lines. *Endocrinology* 154:635–645, PM:23295740
65. Lim YY, Wright JA, Attema JL, Gregory PA, Bert AG, Smith E et al (2013) Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem-cell-like state. *J Cell Sci* 126:2256–2266, PM:23525011
66. Li Z, Xiao J, Wu X, Li W, Yang Z, Xie J et al (2012) Plumbagin inhibits breast tumor bone metastasis and osteolysis by modulating the tumor-bone microenvironment. *Curr Mol Med* 12:967–981, PM:22574935
67. Ahmad A, Wang Z, Ali R, Maitah MY, Kong D, Banerjee S et al (2010) Apoptosis-inducing effect of garcinol is mediated by NF-kappaB signaling in breast cancer cells. *J Cell Biochem* 109:1134–1141, PM:20108249
68. Ahmad A, Wang Z, Wojewoda C, Ali R, Kong D, Maitah MY et al (2011) Garcinol-induced apoptosis in prostate and pancreatic cancer cells is mediated by NF- kappaB signaling. *Front Biosci (Elite Ed)* 3:1483–1492, PM:21622152
69. Ahmad A, Sarkar SH, Bitar B, Ali S, Aboukameel A, Sethi S et al (2012) Garcinol regulates EMT and Wnt signaling pathways in vitro and in vivo, leading to anticancer activity against breast cancer cells. *Mol Cancer Ther* 11:2193–2201, PM:22821148 PMC3836047
70. Chen CS, Lee CH, Hsieh CD, Ho CT, Pan MH, Huang CS et al (2011) Nicotine-induced human breast cancer cell proliferation attenuated by garcinol through down-regulation of the nicotinic receptor and cyclin D3 proteins. *Breast Cancer Res Treat* 125:73–87, PM:20229177
71. Cheng AC, Tsai ML, Liu CM, Lee MF, Nagabhushanam K, Ho CT et al (2010) Garcinol inhibits cell growth in hepatocellular carcinoma Hep3B cells through induction of ROS-dependent apoptosis. *Food Funct* 1:301–307, PM:21776480
72. K A T, T R, G R, K C S, Nair RS, G S et al (2013) Structure activity relationship of plumbagin in BRCA1 related cancer cells. *Mol Carcinog* 52:392–403, PM:22290577
73. Lee JH, Yeon JH, Kim H, Roh W, Chae J, Park HO et al (2012) The natural anticancer agent plumbagin induces potent cytotoxicity in MCF-7 human breast cancer cells by inhibiting a PI-5 kinase for ROS generation. *PLoS One* 7:e45023, PM:23028742 PMC3441601
74. Manu KA, Shanmugam MK, Rajendran P, Li F, Ramachandran L, Hay HS et al (2011) Plumbagin inhibits invasion and migration of breast and gastric cancer cells by downregulating the expression of chemokine receptor CXCR4. *Mol Cancer* 10:107, PM:21880153 PMC3175200
75. Masuelli L, Benvenuto M, Fantini M, Marzocchella L, Sacchetti P, Di SE et al (2013) Curcumin induces apoptosis in breast cancer cell lines and delays the growth of mammary tumors in neu transgenic mice. *J Biol Regul Homeost Agents* 27:105–119, PM:23489691

76. Sun SH, Huang HC, Huang C, Lin JK (2012) Cycle arrest and apoptosis in MDA-MB-231/Her2 cells induced by curcumin. *Eur J Pharmacol* 690:22–30, PM:22705896
77. Shehzad A, Khan S, Sup LY (2012) Curcumin molecular targets in obesity and obesity-related cancers. *Future Oncol* 8:179–190, PM:22335582
78. Yodkeeree S, Ampasavate C, Sung B, Aggarwal BB, Limtrakul P (2010) Demethoxycurcumin suppresses migration and invasion of MDA-MB-231 human breast cancer cell line. *Eur J Pharmacol* 627:8–15, PM:19818349
79. Kanwar SS, Yu Y, Nautiyal J, Patel BB, Padhye S, Sarkar FH et al (2011) Difluorinated-curcumin (CDF): a novel curcumin analog is a potent inhibitor of colon cancer stem-like cells. *Pharm Res* 28:827–838, PM:21161336 PMC3792588
80. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M et al (2006) miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 3:87–98, PM:16459310
81. Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW et al (2009) Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 137:1005–1017, PM:19524505 PMC2722880
82. Nana-Sinkam SP, Croce CM (2013) Clinical applications for microRNAs in cancer. *Clin Pharmacol Ther* 93:98–104, PM:23212103

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