Review Article

MicroRNA: A New Player in Breast Cancer Development

Vivien Wang and Wei Wu[1]

Department of Pathology, Evanston Northwestern Hospital, Evanston, IL 60201, USA [V. Wang]; Division of Cancer Biology, Department of Medicine, Northwestern University, Evanston, IL 60201, USA [W. Wu]

Breast cancer is the leading cause of death in women in North America. The etiology of breast cancer is complex, and genetic background and environmental factors are believed to contribute to the complexities. Over the past decades, a large body of literature has demonstrated that gene expression profile may be a useful tool to define the signature of cancer and predict the prognosis or response to treatment. Recently, microRNA (miRNA) expression profile calls a great attention to define various types of cancers. miRNAs are small non-coding RNAs that bind to the 3' untranslated region of target mRNAs and down-regulate their translation to protein or degrade the mRNAs. miRNAs play critical roles in many different cellular processes including metabolism, apoptosis, differentiation, and development. They are also linked to human diseases, including cancer. In this paper, we discuss the recent miRNA studies in breast cancer and provide a summary of the literatures focusing on miRNA signalling pathways and their potential involvement in breast cancer development.

Journal of Cancer Molecules 3(5): 133-138, 2007.

Keywords:

microRNA

breast cancer

miRNA pathways

microRNA microarray

MicroRNAs (miRNAs[2]) are endogenous ~21 nucleotide small non-coding RNAs encoded in genomes, which regulate protein-coding gene expression in a sequence-specific manner for cleavage or translational repression [1-4]. miRNAs are processed sequentially from primary miRNA transcripts to pre-miRNAs to mature miRNAs. The primary miRNA are transcribed by RNA polymerase II in nucleus, and are usually several kilobases in length. The primary miRNAs processed by Drosa/DGCR8/Pasha "microprocessor" are cleaved into ~70 to 100 nucleotide hairpin pre-miRNAs, which are rapidly exported into cytoplasma. Subsequently, pre-miRNAs are cleaved by cytoplasmic RNase III Dicer enzyme into ~21 nucleotide miRNA duplex. One of the strands serves as mature miRNA, which is guided by miRNA-associated multiprotein RNA-Induced Silencing Complex (RISC) to negatively regulate the mRNA [5]. Following the first miRNA lin-4 was discovered in the worm *C. elegans* and was shown to control the development and differentiation of cells [4], many other members of miRNAs were identified in different species with conventional experimental methods or computational prediction. Based on miRBase release 10.0, 533 human miRNAs were registered (http://microrna.sanger.ac.uk/cgi-bin/sequences/browse.pl) and expected to increase up to 1000 [6]. miRNA expression is highly specific for tissues and developmental stages, and the functions of miRNAs have been appreciated in various fundamental biological processes such as cell death [7], cell proliferation [8], and stem cell division [9]. miRNAs can act as oncogenes [10] and tumor suppressor genes [11]. Expression profiling in various cancer tissues have been intensively studied [12,13]. Here we only discuss the association of miRNA research with breast cancer and the potential targets for breast cancer treatment.

Received 4/17/07; Revised 10/16/07; Accepted 10/18/07.

[1]**Correspondence:** Dr. Wei Wu, Division of Cancer Biology, Department of Medicine, Northwestern University, ENH Research Institute, No. 1001 University Place, Evanston, IL 60201, USA. Phone: 1-224-3647514. E-mail: wei-wu-0@northwestern.edu

[2]**Abbreviations:** miRNA, microRNA; ER, estrogen receptor; PR, progesterone receptor; Ecd, ecdysone; TPM1, tropomyosin 1; AIB1, Amplified in Breast Cancer-1 protein.

Aberrant expression of miRNAs in breast cancer

The association of miRNA with breast cancer pathogenesis is supported by the studies examining expression of miRNAs in breast cancer cell lines and clinical samples (Table 1). The earliest study documenting abnormalities of miRNA expression in MCF-7 and T47-D breast cancer cell lines has reported that miR-143, miR-145, miR-16, and let-7a-1 are down regulated in these cell lines [14], suggesting that change of specific miRNA expression is associated with malignant transformation. In 2005, three studies published in *Nature* further establish close correlations between altered expression of specific miRNA and tumorigenesis [10,11,15]. For example, Lu *et al.* [15] utilized a new bead-based flow cytometric technique to obtain miRNA expression profiles. Using this technique, the expressions of 217 miRNA in 332 tissue samples including breast cancer tissues were analyzed. The authors found that miRNA profiles obtained are informative, reflecting the lineage and differentiation status of the tumor, and overall miRNA expression tended to be down regulated. Similar to other tumors, most miRNAs are expressed at lower levels in breast tumors than normal tissues. Moreover, miRNA level is lower in poorly differentiated breast tumors with respect to well-differentiated breast tumors. Interestingly, the miRNA profile is more accurately correlated with cell differentiation and development status compared mRNA expression profile. Subsequently, Iorio *et al.* [16] demonstrated the existence of a breast cancer-specific miRNA signature with a genome-wide miRNA expression profiling in a large set of normal and tumor breast tissues, in which 29 miRNAs were differentially expressed in breast cancer *versus* normal tissues. Among them, confirmed by Northern blot, miR-10b, miR-125b and miR-145 were down-regulated; while miR-21 and miR-155 were up-regulated, suggesting that these miRNAs may potentially act as tumor suppressor genes or oncogenes,

Table 1: Aberrant expression of miRNAs in breast cancer

Authors (year)[Ref.]	Samples used*	Method(s) used	Key miRNA changes	
			Up-regulated	Down-regulated
Michael *et al.* (2003)[14]	MCF-7, T47D breast cancer cell line	Northern blot		miR-143 miR-145 miR-16 let-7a-1
Lu *et al.* (2005)[15]	Breast cancer tumor tissues (6) *vs.* normal tissues (5) MCF-7 cell line	Bead-based flow cytometry Northern blot	miR-21	let-7
Iorio *et al.* (2005)[16]	Breast cancer tumor tissues (76) *vs.* normal tissues (30) Breast cancer cell lines	miRNA microarray Northern blot	miR-21 miR-155 miR-206 miR-122a miR-210	let-7 miR-10b miR-125a miR-125b miR-145
Volinia *et al.* (2006)[17]	Breast cancer tumor tissues (79) *vs.* normal tissues (6)	miRNA microarray	miR-21 miR-155 miR-206 miR-122a miR-210	let-7 miR-10b miR-125a miR-125b miR-145
Si *et al.* (2006)[29]	Breast cancer tumor tissues (5) *vs.* normal tissues (5) MCF-7 cell line	TaqMan RT-PCR	miR-21	

*The number in parentheses at the 2nd column represents how many samples were used in the study.

respectively. The same group further examined the miRNA expression signature of human solid tumors including breast cancer. The authors identified that 27 miRNAs were differentially expressed in breast cancer *versus* adjacent normal tissues, i.e., 15 miRNAs were up-regulated and 12 miRNAs were down-regulated in breast cancer *versus* normal tissues [17]. However, when comparing these two dataset, only one third of identified miRNAs are reproducible. The common changed miRNAs are list in Figure 1. During the preparation of this paper, Blenkiron *et al.* [18] reported that miRNA expression in 22 breast cancer cell lines were largely deregulated over breast tissues, but unlike previous reports, the authors did not observe the perfect separation of miRNA expression between the normal breast and tumor samples. In contrast, a subset of miRNAs was differentially expressed among five molecular breast tumor subtypes (luminal A, luminal B, basal-like, HER2+, and normal-like) with clinical implications [18]. The discrepancy of these studies may reflect the application of the different miRNA platforms, the various techniques, the targets of detection such as pre-miRNAs *versus* mature miRNAs, and the analytical tools. Nevertheless, these findings from diverse aspects provide new insight into the molecular mechanisms of breast cancer initiation, progression and metastasis.

Aberrant miRNA expression as biomarkers for breast cancer

ErbB2, estrogen receptor (ER) and progesterone receptor (PR) represent the clinically important breast cancer subtypes and guide the choice of treatment. ErbB2-positive and ER-positive breast cancers have been shown to exhibit significantly different gene expression profiles [19]. As we mentioned above, the miRNA profile is more accurately correlated with cell differentiation and development status when compared with mRNA expression profile, it is therefore of great interest for researchers to explore how miRNA expression is linked to known breast cancer markers. Interestingly, a subset of miRNA expression was correlated with specific breast cancer biopathologic features, such as ER and PR expression, tumor stage, vascular invasion and proliferation index [16]. Moreover, miRNA profiles were able to clearly discriminate ErbB2-positive/ER positive and ErbB2-positive/ER-negative breast cancers [20]. In contrast, a more recent study reported the miRNA expression profiling in 193 primary breast cancer tissues, and did not find specific miRNAs were strongly associated with tumor stage, vascular invasion, and ErbB2 status [18]. In terms of the miRNAs and ErbB2 status, these three studies do not support each other due to multiple reasons as we mentioned before. Of note, all three studies have identified a subset of miRNAs is associated with ER status regardless the experimental conditions [16,18,20]. The link of hormones and miRNA has been reported previously in *Drosophila* [21] and plants [22]. In *Drosophila*, the up-regulation of three miRNAs (miR-100, miR-125, and let-7) and the down-regulation of miR-34 require the hormone ecdysone (Ecd) and the activity of Ecd-inducible *Broad-Complex* gene [21]. The plant hormone auxin is regulated by miRNAs in plant *Arabidopsis thaliana* [22]. More recently, Adams *et al.* [23] have provided the first evidence that estrogen can enhance miR-206 expression and in turn miR-206 binds to 3'UTR of ERα mRNA and represses translation of ERα protein. This reveals a very important function of miRNAs in nuclear hormone biochemical circuits. Taken together, specific miRNA expression associated with biopathologic features such as ER and PR status may be identified which, upon further evaluation, can serve as important diagnostic or prognostic biomarkers for breast cancer.

Genomic change of miRNA in breast cancer

It has been reported that miRNA genes are frequently located in the chromosomal regions characterized by nonrandom aberrations in human cancer, suggesting that resident miRNA expression might be affected by these genetic abnormalities [24]. The mechanism for aberrant expression of miRNAs in breast cancer tissues is, in part, resulted from genomic changes. miR-125b, which is down regulated in breast cancer, is located at chromosome 11q23-24, one of the regions most frequently deleted in breast tumors [25]. miR-33 and miR-320 expression was found to

Print ISSN 1816-0735; Online ISSN 1817-4256

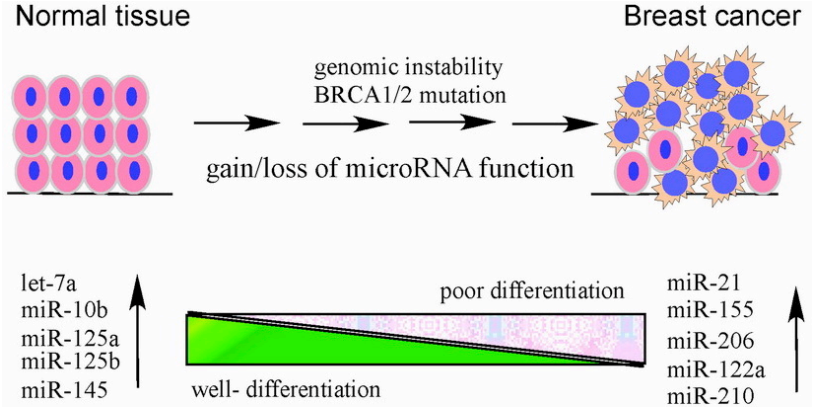

Figure 1: Loss- or gain-of-function of specific miRNAs contributes to breast epithelial cellular transformation and tumorigenesis. Simplified model of breast cancer development from normal breast epithelial cells to cancerous cells through multiple genetic changes such as BRCA1/2 mutations, gain/loss of miRNA functions. A subset of miRNAs differentially expressed in breast tumors compared to normal breast tissues has been identified, such as the expression levels of let-7a, miR-10b, miR-125a, miR-125b and miR-145 are higher in normal epithelial cells relative to breast tumors; whereas, miR-21, miR-155, miR-206, miR-122a, and miR-210 are higher in breast tumors against normal tissues.

be strongly associated with the genomic alteration [18]. The alteration of DNA copy number may be a critical factor affecting expression of miRNA in breast cancer. A high-resolution CGH data shows that 72.8% of miRNA genes are located in regions that exhibit DNA copy number abnormalities in breast cancer [26]. Moreover, if comparing the breast cancer CGH data with independent miRNA expression by miRNA microarrays, 81.8% miRNAs demonstrated increased expression level and high DNA copy number, but 60% miRNAs exhibited decreased expression level with loss of DNA copy number [16]. Therefore, changes of DNA copy display good correlation with miRNA transcript expression in breast cancer. In addition, chromatin remodeling may also account for the frequent miRNA dysregulation in breast cancer. When breast cancer cell line SK-Br-3 was treated with histone deacetylase inhibitor LAQ824, within 5 h of exposure to a proapoptotic dose of LAQ824, significant changes were measured in 40% of the > 60 different miRNA species expressed in SK-Br-3 cells with 22 miRNA species down-regulated and 5 miRNAs up-regulated [27]. It is speculated that methylation and dymethylation of miRNA promoter regions during cancer development may be responsible for the changes of miRNA expression level as well. There is no report that whether mutation of miRNA exists in breast cancer, which may also affect the detection of expression. In sum, genomic instability, epigenetic change and mutation of miRNA all cause miRNA dysregulation in breast cancer. We expect to understand more about miRnome in breast cancer in the nearest future.

miRNA signaling pathways in breast cancer development

The available evidence clearly demonstrates that miRNAs are intertwined with cellular pathways regulated by classical oncoproteins and tumor suppressors such as ErbB2, Akt, NF-κB, Myc, Ras, pTEN, p53, and Rb. Incorporation of miRNA regulation into current models of molecular cancer pathogenesis will be essential to achieve a complete understanding of breast cancer development. miRNAs with pro-proliferative and anti-apoptotic activity would likely promote oncogenesis and thus may be over-expressed in cancer cells. Likewise, miRNAs with anti-proliferative and pro-apoptotic activity are likely to function as tumor suppressor genes and thus may be under-expressed in cancer cells [28]. Based on current literature, breast cancer-related miRNAs and their targets/pathways are listed in Figure 2.

miR-21 is consistently overexpressed in breast cancer [16,17,29], and has been shown to promote cell survival and inhibit the apoptotic caspase activities [30]. Importantly, anti-miR-21 oligonucleotides suppress both breast cancer cell growth *in vitro* and tumor growth *in vivo*, which is associated with increased apoptosis and down-regulation of the anti-apoptotic protein Bcl-2 [29]. Recently, tropomyosin 1 (TPM1) has been identified as a potential miR-21 target in tumors and experimentally confirmed in breast cancer cell line [31]. TPM1 exhibits an anti-oncogenic function through binding microfilament and regulating cytoskeleton [32,33]. Similar to tumor suppressor pTEN, down-regulation of TPM1 is a consistent biochemical change observed in transformed breast epithelial cells. The interaction with miRNAs such as miR-19a::pTEN and miR21::TPM1 was found to be an crucial silencing effect. Loss of pTEN function contributes to uncontrolled cell growth [34]. Inhibition of TPM1 function under miR-21 overexpression results in progressive cell migration and invasion [31]. Akin to miR-21 effect, the level of miR-10b expression in primary breast carcinoma correlates with clinical progression, and miR-10b induced by transcription factor Twist proceeds to inhibit translation of the mRNA encoding homeobox D10, resulting in increased expression of pro-metastatic gene, RHOC, leading to tumor cell invasion and metastasis [35]. The TGF-β gene has been predicted as a potential target of miR-21 by multiple methods [16]. TGF-β signaling is a well-studied inhibitory signaling pathway [36], which induces cell-cycle arrest in part through direct suppression of c-Myc-mediated gene expression, and ectopic expression of c-Myc abrogates TGF-β-mediated growth inhibition [37]. We then hypothesize that overexpression of miR-21 inhibits TGF-β gene, diminishes the inhibitory effect of TGF-β signaling, enhances cell growth, and promotes breast cancer development. The experiments are underway to prove this signaling pathway.

The expression of various let-7 miRNAs is down-regulated in breast cancer samples with either lymph node metastasis or higher proliferation index, suggesting that a reduced let-7 expression could be associated with a poor prognosis [16]. An association between let-7 down-regulation and poor prognosis was previously reported in human lung cancer [38,39]. The finding that the let-7 family of miRNAs regulates expression of the *ras* oncogene family provides a potential explanation for the role of let-7 miRNAs in human cancer [40].

Transcriptional co-activators play a critical role in breast cancer development. AIB1 (Amplified in Breast Cancer-1 protein) is a member of p160/SRC family of co-activators and acts as an oncogene product [41]. A recent study has shown that miR-17-5p does not only negatively regulate E2F1, but also bind to 3'UTR of AIB1 mRNA, inhibiting its trans-activation function. Furthermore, miR-17-5p expression level decreases while AIB1 increases in a series of breast cancer cell lines, and the discrepancy regarding miR-17-5p expression changes in various conditions has been

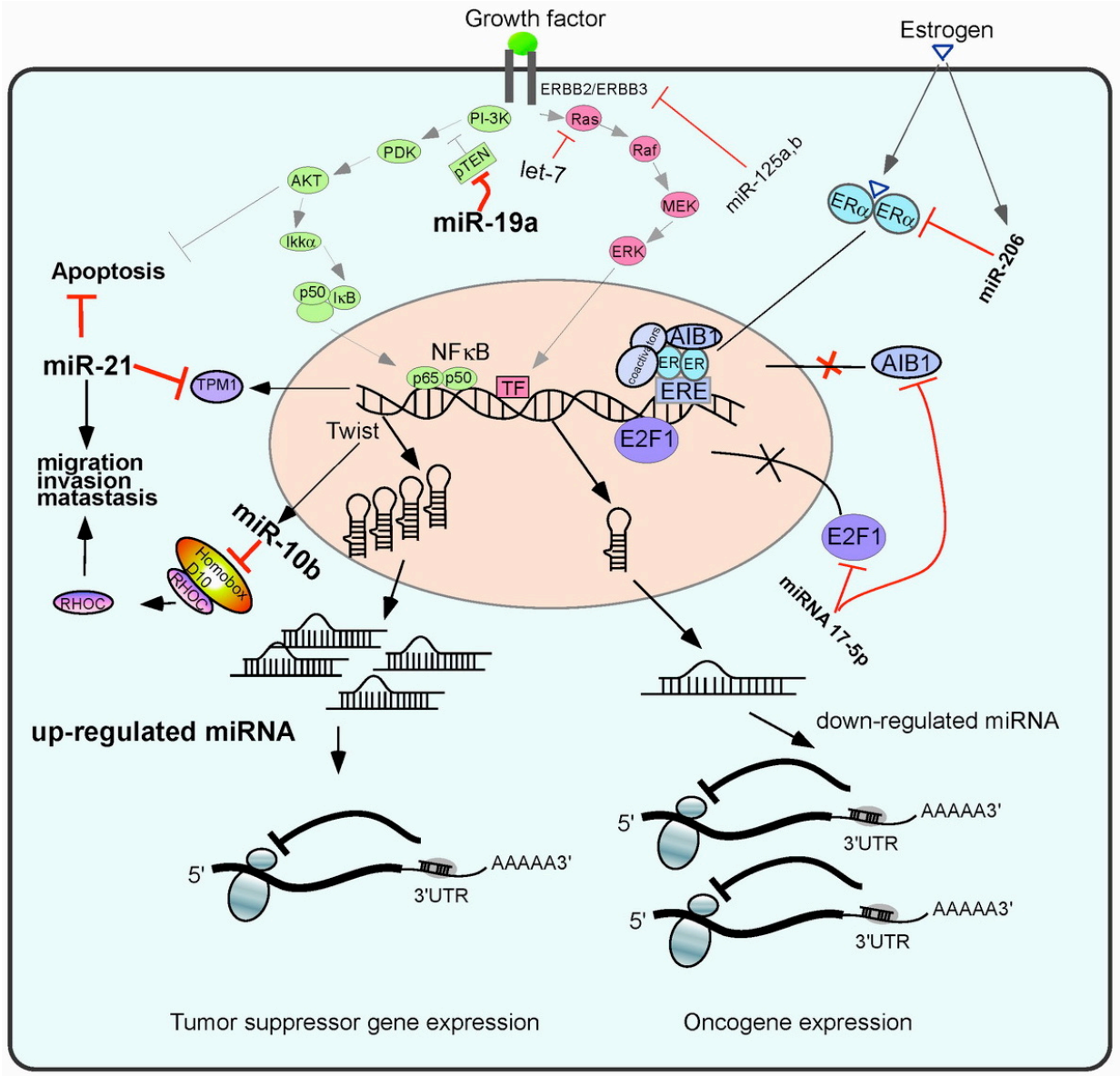

Figure 2: A model of miRNA involvement in breast cancer by modulating the expression of tumor suppressor genes and oncogenes. Overexpression of miRNAs by amplification of the miRNA-encoding locus leads to decreased expression of targets. Under expression of miRNAs by deletion or methylation of the miRNA locus results in increased expression of targets. miR-21 and miR-10b overexpressed in breast cancer interact with TPM1 and homeobox D10, respectively, resulting in progressive cell invasion and metastasis; miR-19a may inhibit tumor suppressor pTEN and thus activate PI-3K/Akt signaling pathway; deficiency of let-7 and miR-125a,b, which inhibit Ras and ErbB2/3, correspondingly, triggers Ras/Raf/ERK signaling pathway. Down-regulation of miR-206 and miR-17-5p, which block ER and AIB1, respectively, simultaneously magnify the ER signaling pathway.

explained by Hossain *et al.* [42]. Interestingly, miR-17-5p suppresses estrogen-dependent and independent breast cancer cell proliferation by targeting AIB1 [42]. The levels of miR-125a,b are down-regulated in breast cancer [16,17]. ErbB2 and ErbB3 have been recently identified as the targets of miR-125a,b, and enforced expression of miR-125a,b suppresses ErbB2 and ErbB3 at transcription and protein levels in SKBR3 breast cancer cell line, with the downstream signaling molecule ERK1/2 and Akt activities diminished. This results in impairment of breast cancer cell growth and invasion [43]. The miR-206 interacting with estrogen and ERα has been discussed above. It is of great interest to us that the global downstream gene changes of miRNA-mediated ER signaling would be further explored with ChIP on chip assay. Taken together, the available evidence has demonstrated that miRNAs are involved in modulating multiple signaling pathways during breast cancer development and may serve as potential targets for breast cancer therapy.

Concluding remarks

Over past decades, scientists have put many efforts to study the protein-coding genes to elucidate the molecular mechanisms of breast cancer. DNA microarrays have been widely used to identify potential signature genes as biomarkers for breast cancer diagnosis or prognosis [44]. The successful example is that HER2/Neu/ErbB2 is used as a biomarker, the specific antibody targeting ErbB2 (trastuzu-

Print ISSN 1816-0735; Online ISSN 1817-4256

mab; Herceptin®) has been developed for treatment. Now researchers take advantage of miRNA to target HER2/Neu in ovarian cancer cells overexpressing HER2/Neu protein [45]. The emerging view from studies discussed above is that miRNAs are involved in breast cancer development, and these findings highlight the potential utility of miRNA profiling for diagnostic and prognostic application. Due to the various miRNA microarray methods (from preparation of miRNA, hybridization to data mining) utilized by different investigators, the reliability of miRNAs identified in breast cancer tissues is still at unsatisfactory level. The golden standard of miRNA microarray technology remains to be developed. However, the miRNAs such as miR-21 as the promising molecular targets for breast cancer treatment have been arisen, at least in mouse model [29]. We are still at an early stage in our understanding for the roles of miRNAs in breast cancer development. There are many biological questions which need to be addressed such as how miRNA expression is involved in the normal mammary gland development, and how miRNAs participate in fine tuning of breast cancer stem cell maintenance. Indeed, the differential miRNA expression profiling on mouse mammary gland at different stages (virgin, pregnancy, lactation, and involution) has been reported [46], this provide valuable information on mammary gland biology. Undoubtedly, continued efforts to delineate miRNA function in mammary physiological and pathophysiological conditions will reveal novel insights into normal cellular and breast cancer biology, and eventually provide a new molecular target for alternative therapy.

Acknowledgments

We thank Yousaf Mian and Alo Nag for critical reading of the manuscript. We apologize to those whose work was not cited due to space limitation and highly selective topic.

References

1. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science* 294: 853-858, 2001.
2. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294: 858-862, 2001.
3. Lee RC, Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans*. *Science* 294: 862-864, 2001.
4. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 75: 843-854, 1993.
5. Kim VN, Nam JW. Genomics of microRNA. *Trends Genet* 22: 165-173, 2006.
6. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z. Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet* 37: 766-770, 2005.
7. Jovanovic M, Hengartner MO. miRNAs and apoptosis: RNAs to die for. *Oncogene* 25: 6176-6187, 2006.
8. Hayashita Y, Osada H, Tatematsu Y, Yamada H, Yanagisawa K, Tomida S, Yatabe Y, Kawahara K, Sekido Y, Takahashi T. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res* 65: 9628-9632, 2005.
9. Zhang B, Pan X, Anderson TA. MicroRNA: a new player in stem cells. *J Cell Physiol* 209: 266-269, 2006.
10. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM. A microRNA polycistron as a potential human oncogene. *Nature* 435: 828-833, 2005.
11. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature* 435: 839-843, 2005.
12. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. *Nat Rev Cancer* 6: 259-269, 2006.
13. Wu W, Sun M, Zou GM, Chen J. MicroRNA and cancer: current status and prospective. *Int J Cancer* 120: 953-960, 2007.
14. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. *Mol Cancer Res* 1: 882-891, 2003.
15. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 435: 834-838, 2005.
16. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Ménard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. *Cancer Res* 65: 7065-7070, 2005.
17. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci USA* 103: 2257-2261, 2006.
18. Blenkiron C, Goldstein LD, Thorne NP, Spiteri I, Chin SF, Dunning MJ, Barbosa-Morais NL, Teschendorff AE, Green AR, Ellis IO, Tavare S, Caldas C, Miska EA. MicroRNA expression profiling of human breast cancer identifies new markers of tumour subtype. *Genome Biol* 8: R214, 2007.
19. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale A-L. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 98: 10869-10874, 2001.
20. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Getts R, Haqq C. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. *Mol Cancer* 5: 24, 2006.
21. Sempere LF, Sokol NS, Dubrovsky EB, Berger EM, Ambros V. Temporal regulation of microRNA expression in *Drosophila melanogaster* mediated by hormonal signals and *Broad-Complex* gene activity. *Dev Biol* 259: 9-18, 2003.
22. Wang JW, Wang LJ, Mao YB, Cai WJ, Xue HW, Chen XY. Control of root cap formation by microRNA-targeted auxin response factors in *Arabidopsis*. *Plant Cell* 17: 2204-2216, 2005.
23. Adams BD, Furneaux Dagger H, White B. The microRNA miR-206 targets the human estrogen receptor α, and represses ERα mRNA and protein expression in breast cancer cell lines. *Mol Endocrinol* 21: 1132-1147, 2007.
24. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 101: 2999-3004, 2004.
25. Negrini M, Rasio D, Hampton GM, Sabbioni S, Rattan S, Carter SL, Rosenberg AL, Schwartz GF, Shiloh Y, Cavenee WK, Croce CM. Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: identification of a new region at 11q23.3. *Cancer Res* 55: 3003-3007, 1995.
26. Zhang L, Huang J, Yang N, Greshock J, Megraw MS, Giannakakis A, Liang S, Naylor TL, Barchetti A, Ward MR, Yao G, Medina A, O'Brien-Jenkins A, Katsaros D, Hatzigeorgiou A, Gimotty PA, Weber BL, Coukos G. MicroRNAs exhibit high frequency genomic alterations in human cancer. *Proc Natl Acad Sci USA* 103: 9136-9141, 2006.
27. Scott GK, Mattie MD, Berger CE, Benz SC, Benz CC. Rapid alteration of microRNA levels by histone deacetylase inhibition. *Cancer Res* 66: 1277-1281, 2006.
28. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. *Oncogene* 25: 6188-6196, 2006.
29. Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY. miR-21-mediated tumor growth. *Oncogene* 26: 2799-2803, 2006.
30. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 65: 6029-6033, 2005.
31. Zhu S, Si ML, Wu H, Mo YY. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J Biol Chem* 282: 14328-14336, 2007.
32. Mahadev K, Raval G, Bharadwaj S, Willingham MC, Lange EM, Vonderhaar B, Salomon D, Prasad GL. Suppression of the transformed phenotype of breast cancer by tropomyosin-1. *Exp Cell Res* 279: 40-51, 2002.
33. Shah V, Braverman R, Prasad GL. Suppression of neoplastic transformation and regulation of cytoskeleton by tropomyosins. *Somat Cell Mol Genet* 24: 273-280, 1998.
34. Maehama T. PTEN: its deregulation and tumorigenesis. *Biol Pharm Bull* 30: 1624-1627, 2007.
35. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449: 682-688, 2007.

36. Ten Dijke P, Goumans MJ, Itoh F, Itoh S. Regulation of cell proliferation by Smad proteins. *J Cell Physiol* 191: 1-16, 2002.
37. Alexandrow MG, Moses HL. Kips off to Myc: implications for TGF-β signaling. *J Cell Biochem* 66: 427-432, 1997.
38. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res* 64: 3753-3756, 2004.
39. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu C-G, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 9: 189-198, 2006.
40. Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. *Cell* 120: 635-647, 2005.
41. Torres-Arzayus MI, Font de Mora J, Yuan J, Vazquez F, Bronson R, Rue M, Sellers WR, Brown M. High tumor incidence and activation of the PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. *Cancer Cell* 6: 263-274, 2004.
42. Hossain A, Kuo MT, Saunders GF. Mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol Cell Biol* 26: 8191-8201, 2006.
43. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC. Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J Biol Chem* 282: 1479-1486, 2007.
44. Sims AH, Ong KR, Clarke RB, Howell A. High-throughput genomic technology in research and clinical management of breast cancer. Exploiting the potential of gene expression profiling: is it ready for the clinic? *Breast Cancer Res* 8: 214, 2006.
45. Tsuda N, Kawano K, Efferson CL, Ioannides CG. Synthetic microRNA and double-stranded RNA targeting the 3'-untranslated region of HER-2/neu mRNA inhibit HER-2 protein expression in ovarian cancer cells. *Int J Oncol* 27: 1299-1306, 2005.
46. Silveri L, Tilly G, Vilotte JL, Le Provost F. MicroRNA involvement in mammary gland development and breast cancer. *Reprod Nutr Dev* 46: 549-556, 2006.

Print ISSN 1816-0735; Online ISSN 1817-4256