Published in final edited form as:
J Mammary Gland Biol Neoplasia. 2012 March ; 17(1): 33–42. doi:10.1007/s10911-012-9245-5.

Non-Coding RNAs as Regulators of Mammary Development and Breast Cancer

Hai-long Piao and **Li Ma**
Department of Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

Li Ma: lma4@mdanderson.org

Abstract

Over the past decade, non-coding RNAs (ncRNAs) have become a new paradigm of gene regulation. ncRNAs are classified into two major groups based on their size: long non-coding RNAs (lncRNAs) and small non-coding RNAs (including microRNAs, piRNAs, snoRNAs, and endogenous siRNAs). Here we review the recently emerging role of ncRNAs in mammary development, tumorigenesis, and metastasis, with the focus being on microRNAs (miRNAs) and lncRNAs. These findings shed new light on normal development and malignant progression, and suggest the potential for using ncRNAs as new biomarkers of breast cancer and targets for treatment.

Keywords

MicroRNA; Long non-coding RNA; Epithelial-mesenchymal transition; Mammary development; Breast cancer; Metastasis

Introduction

The human genome contains approximately 20,000–25,000 protein-coding genes, far fewer than had been anticipated before its sequencing. In fact, only 1.5% of the human genome encodes proteins, whereas the rest consists of introns, regulatory sequences, and non-coding RNA genes [1–3]. To date, more than 1,500 miRNAs (www.mirbase.org) and over 8,000 long non-coding RNAs (lncRNAs) [4] are known to be encoded by the human genome.

The first miRNA, lin-4, was reported in 1993 by Victor Ambros and Gary Ruvkun, who discovered the first example of this type of molecule by genetic screening in *C. elegans*, and demonstrated that lin-4 turns off the expression of a specific gene, lin-14, whose protein product is crucial for development [5, 6]. The second miRNA, let-7, was found 7 years later, also through forward genetics approaches in worms [7, 8]. By now, miRNAs have been recognized as widely acting regulatory molecules. These endogenously expressed small non-coding RNAs are 18–25 nucleotides long, and they regulate gene expression through base pairing with target mRNAs, leading to degradation of target mRNAs and/or inhibition of their translation [9–11]. MiRNAs have been shown to orchestrate a variety of cellular processes, and the oncogenic or tumor-suppressing functions of a number of miRNAs have been characterized [12–17]. Moreover, we and others have demonstrated the existence of pro-metastatic and anti-metastatic miR-NAs [18, 19]; the molecular mechanisms by which these individual miRNAs function in malignant progression require further investigation.

In addition to the well-annotated protein-coding and miRNA genes, lncRNAs, transcripts that are more than 200 nt in length and lack significant open reading frames (ORFs), have also emerged as important regulatory molecules of cellular processes [20, 21]. A lncRNA

could act as a scaffold that keeps proteins together, or as a guide that helps recruit proteins to specific genomic DNA sequences [20]. For instance, several well-characterized lncRNAs, including AIR, HOTAIR, and XIST, interact with chromatin-remodeling complexes and target them to specific genes, thereby affecting the ability of these complexes to regulate gene transcription [22–24].

In this article, we review recent progress in the regulation of mammary development and breast cancer by ncRNAs. These findings confirm and extend the importance of cellular regulatory RNAs in physiological and pathological processes, and illustrate the value of analyzing normal and neoplastic cells for new leads that may improve breast cancer outcomes.

Non-coding RNAs in Mammary Development

The basic components of a mature female mammary gland are the alveoli that are lined with milk-secreting cuboidal epithelial cells and surrounded by myoepithelial cells. The morphogenesis of mammary glands begins during embryonic development and proceeds through puberty, pregnancy, lactation, and involution [25]. Most of the mammary gland development and differentiation steps occur after birth. During these steps, the mammary gland undergoes profound morphological and functional changes [26], which involve cell proliferation, differentiation, and apoptosis in conjunction with extensive changes in gene expression [27–33], and are regarded as a succession of cell fate determinations [34]. Endocrine and paracrine signals, as well as cell-matrix and cell-cell interactions, regulate these processes.

The mammary morphogenesis has been extensively studied at the physiological, molecular, and genetic levels. In the past few years, ncRNAs, particularly miRNAs, have begun to be implicated in regulating mammary gland development (Table 1). Avril-Sassen et al. reported the first comprehensive miRNA profiling in the postnatal mammary gland throughout juvenile, puberty, mature virgin, gestation, lactation, and involution stages. MiRNAs were found to be represented in seven temporally co-expressed clusters, suggesting co-regulation of miRNA groups during mammary gland development [35]. This study has provided a valuable source for the future functional characterization of individual miRNAs. Consistent with another report that the let-7 miRNA family is underexpressed in mammary progenitor cells and that enforced let-7 expression induced loss of self-renewing cells [36], expression of many let-7 family members showed a peak in puberty and the mature virgin phase, followed by a marked reduction during lactation [35]. On the other hand, miR-22 and miR-205, miRNAs previously reported to promote expansion of mammary progenitor cells [36, 37], displayed enrichment during gestation [35]. Thus, these miRNAs may control the balance between mammary progenitors and differentiated cells. How such ncRNAs themselves are regulated during the course of differentiation remains unknown.

Increased miR-101a levels were observed throughout differentiation and involution of mammary tissues [38]. Ectopic expression of miR-101a inhibited mammary epithelial cell proliferation and reduced expression of β-casein, a milk protein and a marker of cell differentiation; such changes correlated with the ability of miR-101a to directly downregulate cyclooxygenase-2 (COX-2) [38]. These findings suggest that miR-101a may regulate cell proliferation by targeting COX-2 expression, which may be important for the differentiation and involution of mammary glands. Future studies are needed to elucidate the relationship between miR-101a, COX-2, mammary cell proliferation and differentiation.

Mammary gland development is controlled by locally acting breast mitogens such as EGF, as well as endocrine hormones such as estrogen, progesterone, and prolactin [39]. It has been demonstrated that the mRNAs encoding receptors of these endocrine hormones are

direct targets of specific miRNAs. For instance, the miR-126-3p miRNA has been shown to directly downregulate expression levels of progesterone receptor (PR), leading to a reduction in both mammary epithelial cell proliferation and in β-casein expression [40]. Interestingly, miR-126-3p is highly expressed in the virgin mammary gland and during involution, but is markedly downregulated during pregnancy and lactation, indicating that this miRNA could play an important role in postnatal mammary gland development [40]. Similarly, another two miRNAs, miR-138 and miR-221, can suppress the viability and proliferation of mammary epithelial cells, probably through their ability to downregulate prolactin receptor (PRL-R) and growth hormone receptor (GHR), respectively [41, 42].

Molecules whose expression is restricted to the mammary stroma can also be critical regulators of mammary development through epithelial-stromal interaction. Mice lacking the miR-212/132 family (which comprises miR-212 and miR-132) had normal rudimental ductal trees in the pre-pubertal phase, but displayed profound defect in pubertal ductal outgrowth [43]. Transplantation experiments in which wild-type mammary epithelial cells were implanted into the cleared fat pads of miR-212/132 knockout mice recapitulated the ductal outgrowth defect, whereas pre-pubertal miR-212/132-null mammary epithelia transplanted into the cleared wild-type fat pads formed normal mammary glands, which suggested that these two miRNAs function in the stroma but not in the epithelium [43]. Indeed, both miR-212 and miR-132 are expressed exclusively in the mammary stroma and directly target the mRNA encoding the matrix metalloproteinase MMP-9; mammary glands that lack these two miRNAs displayed accumulation of MMP-9 around the ducts and hyperactivation of the TGF-β pathway, which might underlie the impaired ductal outgrowth [43]. These findings represent the first example of a miRNA that functions in a developmental process through the tissue microenvironment.

Although lncRNAs are much less annotated and characterized functionally compared with proteins and miRNAs, some of them have also been recognized as regulators of developmental processes. Mattick and colleagues found that a non-coding transcript antisense to the protein-coding transcript Znfx1, named Zfas1, is among the highest and most differentially expressed lncRNAs during mouse mammary development [44]. Zfas1 hosts three small nucleolar RNAs (snoRNAs), but intriguingly, knockdown of Zfas1 in a mammary epithelial cell line resulted in an increase in cell proliferation and differentiation without altering levels of the three snoRNAs. In support of this result, the Zfas1 transcript is remarkably stable, with a half-life of over 16 h [44]. Thus, in contrast with the assumption that the host transcripts of snoRNAs act only as originators of snoRNAs, a lncRNA transcript which generates snoRNAs can actually have a function independent of the snoRNAs it gives rise to; the molecular mechanisms underlying this snoRNA-independent function remain to be determined.

Non-coding RNAs in Mammary Tumorigenesis

Deregulation of oncogenic and tumor-suppressing pathways results in neoplastic transformation and tumorigenesis, and these pathways have been shown to be controlled by small and large ncRNAs at the transcriptional, post-transcriptional, and epigenetic levels [17, 20, 21]. Iorio et al. performed miRNA microarray analysis of clinical breast cancers. They found that 29 miRNAs were differentially expressed in human mammary tumors compared with normal breast tissues, and that miR-21 and miR-155 were among the most consistently upregulated miRNAs [45]. In fact, both of these two miRNAs have been recognized as oncogenic miRNAs and are overexpressed in a variety of human cancers. Transfection of MCF-7 human breast cancer cells with anti-miR-21 oligonucleotides resulted in a reduction of cell proliferation in vitro as well as tumor growth in xenograft experiments, which was accompanied by increased apoptosis and decreased expression of

the BCL-2 anti-apoptotic protein [46]. Subsequently, the mRNAs produced by several tumor suppressor genes have been identified as direct miR-21 targets in breast cancer and other cancer cells, including *PDCD4* (programmed cell death 4) [47], *TPM1* (tropomyosin 1) [48] and *PTEN* (phosphatase and tensin homolog on chromosome 10) [49]. In a synthetic miRNA library screen to identify modulators of the TRAIL (tumor necrosis factor–related apoptosis-inducing ligand)-induced apoptosis pathway in the MDA-MB-453 human breast cancer cell line, miR-155 stood out as a potent suppressor of apoptosis [50]. Ectopic expression of miR-155 in breast cancer cells promoted proliferation and colony formation in soft agar in vitro and development of tumors in nude mice; these effects were found to depend on the negative regulation of *SOCS1* (suppressor of cytokine signaling 1) by miR-155 [51].

The oncogenic roles of miR-21 and miR-155 have been further validated in genetically engineered mouse models (GEMMs). Croce and colleagues generated Eμ-miR-155 transgenic mice in which the expression of miR-155 is under the control of a V_H promoter-Ig heavy chain Eμ enhancer, which becomes active at the late pro-B cell stage of B cell development. These mice initially exhibited a pre-leukemic pre-B cell proliferation in spleen and bone marrow, which was followed by lymphoblastic leukemia and high-grade lymphoma, providing strong evidence that miR-155 is directly implicated in the initiation and progression of these diseases in which this miRNA is overexpressed [52]. Slack and colleagues developed a miR-21 transgenic line in which Cre and Tet-Off technologies were employed to achieve tissue-specific and doxycycline-controlled expression of miR-21; this model exhibited 16-fold overexpression of miR-21 in hematopoietic tissues and development of pre-B-cell lymphoma, both of which could be completely reversed within 1 week of doxycycline treatment, demonstrating that miR-21 is responsible for both initiation and maintenance of lymphoma [53]. Taken together, miR-21 and miR-155 are *bona fide* oncogenic ncRNAs in lymphoma; whether these two miRNAs are sufficient and/or required for breast cancer initiation and progression remains to be determined in GEMMs.

Interestingly, the second miRNA discovered in *C. elegans* and the first miRNA discovered in human, the let-7 miRNA family, has been shown to be downregulated in many human cancers as well as in stem cells [54, 55]. In particular, a recent study found that the expression of let-7 is markedly reduced in breast tumor-initiating cells (BT-ICs) and increases with differentiation. Knockdown of let-7 induced self-renewal of non-BT-ICs; conversely, restoring let-7 expression in BT-ICs decreased their proliferative potential, their ability to form mammospheres, as well as tumor formation and metastasis in vivo. Known oncogenic targets of let-7, *H-RAS* and *HMGA2*, mediate the effect of let-7 on suppressing tumorigenicity [56]. In breast cancer cells, *CCND1* (encoding cyclin D1), an oncogene overexpressed in many human tumor types, has been recently identified as a direct target of the miR-17-5p/miR-20a miRNA cluster. miR-17/20 functions to suppress proliferation and colony formation of breast cancer cells by inhibiting cyclin D1 protein synthesis; on the other hand, cyclin D1 induces miR-17-5p and miR-20a expression [57]. Therefore, this miRNA cluster serves as a negative feedback mechanism to limit cyclin D1 expression, which may impede tumor formation or progression. Additional miRNAs, such as miR-145 [58], miR-34a [59], miR-214 [60], and miR-205 [61, 62], have also been shown to function as putative tumor-suppressing miR-NAs in breast cancer cells (Table 2). In vivo confirmation of the role of these miRNAs in mammary tumorigenesis still awaits new transgenic and knockout mouse models.

Aided by the rapid development and widespread availability of new tiling microarray and high-throughput RNA sequencing technologies, investigators are beginning to probe lncRNAs that are differentially expressed in breast cancer and other cancers compared with normal tissues (Table 2). GAS5 (growth arrest-specific 5) has been observed to be

downregulated in clinical breast cancer tissues, and overexpression of this lncRNA in the MCF-7 breast cancer cell line promoted growth arrest and apoptosis [63]. Interestingly, GAS5 is induced in cells that are growth-arrested due to lack of nutrients or growth factors, and it competes with glucocorticoid response elements (GREs) for binding to glucocorticoid receptor (GR), thereby blocking transcriptional activation by GR, which, in turn, leads to reduced cell metabolism [64]. Hence, downregulation of GAS5 in cancer may help to keep tumor cells viable and metabolically active even under starvation conditions. The highly conserved 8 kb lncRNA MALAT-1 (metastasis associated lung adenocarcinoma transcript 1) is overexpressed in many different cancer types, including lung, breast, colon, prostate, pancreatic, and hepatocellular carcinomas [65–67]. Overexpression of MALAT-1 in the NIH3T3 cell line and a human melanoma cell line promoted colony formation in soft agar and tumor formation in nude mice, while silencing MALAT-1 in tumor cells by RNAi inhibited tumorigenicity [68], which suggested that MALAT-1 is an oncogenic lncRNA. BC200, a neuron-specific lncRNA, is upregulated in invasive breast carcinomas and correlates with tumor grade [69]. It is tempting to speculate that this lncRNA might contribute to the development of aggressive mammary tumors.

Non-coding RNAs in Breast Cancer Metastasis

Ninety percent of solid tumor-related deaths are caused by metastasis, a multi-step process which begins as primary tumor cells invade adjacent tissues and enter the blood and lymphatic vessels. These cells travel through the vasculature, extravasate into the parenchyma of distant organs, and finally proliferate from minute growths (micrometastases) into macroscopic secondary tumors [70]. A growing list of miRNAs has been implicated in metastasis of various tumor types and has been recently reviewed [19, 71]; here we focus on miRNAs which are functionally important in breast cancer metastasis.

In an initial screen for miRNAs differentially expressed in human breast cancer cells, the three most significantly upregulated miRNAs were identified: miR-10b, miR-9, and miR-155 [72]. Among them, miR-10b is highly expressed in cultured metastatic cancer cells as well as in metastatic breast tumors from patients. Overexpression of miR-10b triggered tumor invasion and distant metastasis from primary mammary tumors in xenograft models. miR-10b is induced by the epithelial-mesenchymal transition (EMT)-inducing transcription factor Twist, and this miRNA inhibits synthesis of the *HOXD10* protein, permitting expression of the metastasis-promoting protein RhoC [72]. This was the first report of a miRNA that regulates metastasis. Recently, several independent groups showed that miR-10b induces metastatic behaviors of various types of tumor cells in vitro and in vivo, and that in advanced-stage tumors, miR-10b correlates with high-grade malignancy in clinical cancers, including breast cancer, pancreatic cancer, and glioblastoma [73–76]. A second miRNA that stood out in the initial screen, miR-9, has been shown to directly target *CDH1,* the E-cadherin-encoding mRNA, leading to increased cell migration/invasion and to a context-dependent EMT-like conversion [77]. miR-9-mediated downregulation of E-cadherin leads to the activation of β-catenin signaling, which in turn contributes to increased expression of VEGF; this then results in enhanced tumor angiogenesis. Overexpression of miR-9 in otherwise non-metastatic breast cancer cells induced metastasis in mice, whereas silencing miR-9 in highly malignant cells suppressed metastasis formation [77]. Whether miR-9 promotes metastasis in an E-cadherin-independent manner (i.e., in E-cadherin-negative tumor cells) remains to be investigated.

Huang, Agami, and colleagues exploited a functional genomics approach to screen for miRNAs that promote cell motility. They infected the non-metastatic breast cancer cell line MCF-7 with a miRNA expression library and then used Transwell migration assays as the functional readout. Both miR-373 and miR-520c increased cell migration and were further

found to induce metastasis in vivo. In clinical breast cancers, miR-373 is upregulated in lymph node metastases compared with paired primary tumors [78]. The key enzyme of miRNA biogenesis, Dicer, has been identified as a suppressor of metastasis [79] and epithelial-mesenchymal transition (EMT), an embryonic program that has been proposed to be resurrected by carcinoma cells in order to acquire motility and invasiveness [80]. Interestingly, a miRNA family that attenuates miRNA biosynthesis by targeting Dicer, miR-103/107, induces EMT and metastatic dissemination of otherwise non-metastatic breast cancer cells [81]. miR-221/ 222, a miRNA cluster that targets *ESR1* (encoding estrogen receptor) [82], has also been shown to directly downregulate Dicer [83] and TRPS1 (trichorhinophalangeal syndrome type 1) [84], leading to induction of EMT in breast cancer cells [84]. These findings provided an explanation for the observed high expression of miR-221/222 and low expression of Dicer in ER-negative, basal-like breast cancer, and suggested a possible metastasis-promoting role of miR-221/ 222 in this tumor type which is highly aggressive and non-responsive to hormonal therapy.

Several miRNAs have been established as anti-metastatic molecules. miR-126, miR-206, and miR-335 were found to be downregulated in the highly metastatic variants of the MDA-MB-231 breast cancer cell line [85]. Overexpression of these miRNAs in the metastatic variants reduced both lung and bone metastases, but their mechanisms of action are different: miR-126 suppressed proliferation and tumorigenesis but had little effect on migration and invasion; in contrast, miR-335 or miR-206 inhibited motility and invasiveness, but did not affect the proliferation or apoptosis of primary tumor cells [85]. By using gain-of-function and loss-of-function approaches, Valastyan et al. demonstrated that miR-31 is a suppressor of breast cancer metastasis. This suppressing effect was not seen on primary tumor growth, but instead was observed at multiple steps of the invasion-metastasis cascade, including local invasion, anoikis, and metastatic colonization [86, 87]. Nuclear factor κB (NFκB) is a transcription factor associated with enhanced survival and metastasis of cancer cells [88]. Two miRNAs, miR-146a and miR-146b, suppress NFκB activity through their ability to target *IRAK1* and *TRAF6* in the MDA-MB-231 breast cancer cell line, leading to reduced migration and invasion in vitro as well as suppressed experimental lung metastases in vivo [89, 90]. Collectively, these miRNAs can impede metastasis through inhibition of either a single step or multiple steps of metastasis.

miR-205 and the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429), miRNAs that target the EMT-inducing transcription factors ZEB1 and ZEB2, are downregulated in cells that have undergone EMT [91, 92]. Overexpression of miR-200 inhibited transforming growth factor (TGF)-β-induced EMT; conversely, silencing miR-200 in epithelial cell lines resulted in EMT [91, 92]. Interestingly, ZEB1 directly represses the transcription of miR-200 genes [93]. Consistent with its role in suppressing EMT and promoting mesenchymal-epithelial transition (MET), miR-200 has been found to inhibit cell migration [94]. However, when overexpressed in the 4TO7 mesenchymal-like breast cancer cells, miR-200 promoted metastatic colonization, i.e., formation of macroscopic lung metastases [95, 96]. These results suggest that miR-200 plays opposing roles at early and late stages of metastatic process, and underscore the importance of both EMT and MET in metastasis.

Certain lncRNAs exhibit distinct expression patterns between primary tumors and metastases. A 2.2 kb lncRNA, HOTAIR, is significantly overexpressed in clinical breast cancers. Moreover, HOTAIR levels in primary mammary tumors correlate with metastasis and poor survival rate [97]. HOTAIR serves as a scaffold molecule that bridges the Polycomb Repressive Complex 2 (PRC2) and the LSD1 H3K4 demethylase complex together and recruits these two complexes to target genes, such as the *HOXD* locus, leading to repressed transcription of specific genes and induction of metastasis [23, 97, 98]. These

findings illustrate how a specific lncRNA can remodel the epigenetic machinery to repress metastasis suppressor genes (e.g., *HOXD10*). A future challenge will be to identify the full complement of functional lncRNAs in breast cancer formation, progression, and metastasis, and to determine their roles in normal development as well as their mechanisms of action.

Non-coding RNAs as Breast Cancer Biomarkers and Therapeutic Targets

mRNA profiles have been used to define molecular subclasses of breast cancer, i.e., luminal A, luminal B, basal-like, *HER2*-amplified, and normal-like, which are predictive of prognosis [99]. A further interrogation by miRNA expression profiling might enable more precise classification. Using a bead-based flow cytometric method, Lu et al. systematically profiled 217 mammalian miRNAs in 334 samples, including multiple human cancers. A general downregulation of miRNAs in tumor tissues was observed compared with normal tissues, and remarkably, the expression pattern of this relatively small set of miRNAs classified tumor types better than the expression data of 16,000 mRNAs [100]. These results underscore the potential of miRNA profiling in cancer diagnosis.

Iorio et al. performed a miRNA microarray analysis of 76 breast tumors and 34 normal tissues, and found that miRNA expression was associated with clinical parameters such as estrogen receptor (ER)/progesterone receptor (PR) status (e.g., miR-30) and tumor stage (e.g., miR-213 and miR-203) [45]. Several let-7 miRNA family members correlated with PR status (let-7c), high proliferative index (let-7c and let-7d), or lymph node metastasis (let-7f-1, let-7a-3, and let-7a-2) in breast tumors [45]. Mattie et al. identified distinct miRNA subsets in human breast tumors that correlated with their ER/PR status or HER2 status [101], and notably, there is a significant overlap between the miRNAs in these profiles and Iorio's miRNA profiles [45].

In pursuit of non-invasive/minimally invasive biomarkers for cancer diagnosis, investigators have found stable circulating miRNAs in serum from cancer patients [102, 103]. The increased levels of tumor-associated circulating miR-NAs were first identified in serum of diffuse large B-cell lymphoma patients [104]. Subsequently, higher levels of circulating miRNAs, e.g., miR-155 and miR-195, were found in serum from breast cancer patients than in serum from healthy individuals [105–107]. These studies have provided new promise for improved cancer detection.

Development of miRNA-based therapeutic strategies is under intensive investigation. The feasibility of restoring cancer-suppressing miRNAs and silencing cancer-promoting miRNAs has been addressed in preclinical models of various tumor types. Several tumor-suppressing miR-NAs have been actively studied as targets for restoration. Intratumoral injection of lipid-based let-7 or miR-34 miRNA mimics reduced tumor growth in a subcutaneous H460 NSCLC model [108, 109]. In a K-RAS model of NSCLC, systemically delivered let-7 or miR-34 was well tolerated and effectively inhibited lung tumors [110]. In an orthotopic model of pancreatic cancer, systemic delivery of miR-34 or miR-143/145 decreased tumor growth with no signs of toxicity [111]. In addition, systemic miR-34 delivery suppressed prostate cancer metastasis and improved survival in mice [112]. On the other hand, miRNAs that promote tumor formation and/or metastasis can be silenced therapeutically using engineered antisense RNA oligonucleotides. In collaboration with Regulus Therapeutics (Carlsbad, CA), we developed a miR-10b antagomir—a new class of chemically modified, cholesterol-conjugated anti-miRNA oligonucleotides, and tested it in a 4T1 mouse mammary tumor model (4T1 cells are highly metastatic and express high miR-10b levels). Systemically delivered miR-10b antagomirs had a potent and sequence-specific metastasis-suppressing effect on 4T1 cells without affecting their ability to grow as primary tumors. This work is the first report of systemic delivery of antagomirs for cancer

therapy in mice [113]. Taken together, these studies suggest the potential for manipulating miRNAs as therapeutic strategies. Whether the benefits observed in preclinical models can be recapitulated in human patients remains to be seen. Doses and schedules of treatment with such agents need to be carefully assessed.

Concluding Remarks

Here we have reviewed ncRNAs which regulate mammary development and breast cancer. Every single year, more than 1.3 million women will be diagnosed with breast cancer in the world, and around 465,000 will die from this disease (Susan G. Komen For The Cure; ww5.komen.org). There is an urgent need to diagnose and treat breast cancer appropriately at its early stage. The emerging roles of regulatory RNAs in breast cancer formation and progression have stimulated extensive efforts toward using ncRNAs as cancer biomarkers and therapeutic targets. Circulating miRNAs could be valuable as diagnostic and/or prognostic factors. In the next decade, we anticipate big steps forward in developing ncRNA-based breast cancer therapy.

Acknowledgments

The ncRNA research in the Ma Lab is supported by an NIH Pathway to Independence (K99/R00) Award CA138572, a CPRIT First-Time, Tenure-Track Faculty Award R1004, a University of Texas STARS Award, and a Faculty Development Award from MD Anderson's Cancer Center Support Grant CA016672 from NIH.

Abbreviations

AIR	Antisense to IGF2R
BC200	Brain cytoplasmic RNA 200 nt
COX-2	Cyclooxygenase-2
EMT	Epithelial-mesenchymal transition
ER	Estrogen receptor
GAS5	Growth arrest-specific 5
GEMM	Genetically engineered mouse model
GHR	Growth hormone receptor
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
HER2	Human epidermal growth factor receptor 2
HMGA2	High-mobility group AT-hook 2
HOTAIR	Hox antisense intergenic RNA
IRAK1	Interleukin-1 receptor-associated kinase 1
lncRNA	Long non-coding RNA
LSD1	Lysergic acid diethylamide 1
MALAT1	Metastasis associated lung adenocarcinoma transcript 1
MET	Mesenchymal-epithelial transition
miRNA	microRNA
MMP-9	Matrix metalloproteinase-9

ncRNA	Non-coding RNA
NFκB	Nuclear factor κB
NSCLC	Non-small cell lung cancer
PDCD4	Programmed cell death 4
piRNA	Piwi-interacting RNA
PLR-R	Prolactin receptor
PR	Progesterone receptor
PRC2	Polycomb repressive complex 2
PTEN	Phosphatase and tensin homolog on chromosome 10
siRNA	Small-interfering RNA
snoRNA	Small nucleolar RNA
SOCS1	Suppressor of cytokine signaling 1
TGF-β	Transforming growth factor β
TPM1	Tropomyosin 1
TRAF6	TNF receptor associated factor 6
TRAIL	Tumor necrosis factor–related apoptosis-inducing ligand
TRPS1	Trichorhinophalangeal syndrome type 1
VEGF	Vascular endothelial growth factor
XIST	X-inactive-specific transcript
ZEB1	Zinc finger E-box binding homeobox 1
ZEB2	Zinc finger E-box binding homeobox 2
Zfas1	Antisense to the 5′ end of the protein-coding gene Znfx1

References

1. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature. 2001; 409:860–921. [PubMed: 11237011]
2. Finishing the euchromatic sequence of the human genome. Nature. 2004; 431:931–45. [PubMed: 15496913]
3. Stein LD. Human genome: end of the beginning. Nature. 2004; 431:915–6. [PubMed: 15496902]
4. Cabili MN, Trapnell C, Goff L, et al. Integrative annotation of human large intergenic noncoding RNAs reveals global properties and specific subclasses. Genes Dev. 2011; 25:1915–27. [PubMed: 21890647]
5. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75:843–54. [PubMed: 8252621]
6. Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell. 1993; 75:855–62. [PubMed: 8252622]
7. Reinhart BJ, Slack FJ, Basson M, et al. The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature. 2000; 403:901–6. [PubMed: 10706289]
8. Slack FJ, Basson M, Liu Z, et al. The lin-41 RBCC gene acts in the C. elegans heterochronic pathway between the let-7 regulatory RNA and the LIN-29 transcription factor. Mol Cell. 2000; 5:659–69. [PubMed: 10882102]

9. Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science. 2001; 294:862–4. [PubMed: 11679672]
10. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–97. [PubMed: 14744438]
11. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009; 136:215–33. [PubMed: 19167326]
12. Jovanovic M, Hengartner MO. miRNAs and apoptosis: RNAs to die for. Oncogene. 2006; 25:6176–87. [PubMed: 17028597]
13. Hayashita Y, Osada H, Tatematsu Y, et al. A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. Cancer Res. 2005; 65:9628–32. [PubMed: 16266980]
14. Zhang B, Pan X, Anderson TA. MicroRNA: a new player in stem cells. J Cell Physiol. 2006; 209:266–9. [PubMed: 16791837]
15. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human oncogene. Nature. 2005; 435:828–33. [PubMed: 15944707]
16. O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs modulate E2F1 expression. Nature. 2005; 435:839–43. [PubMed: 15944709]
17. Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer. 2006; 6:857–66. [PubMed: 17060945]
18. Ma L, Weinberg RA. Micromanagers of malignancy: role of microRNAs in regulating metastasis. Trends Genet. 2008; 24:448–56. [PubMed: 18674843]
19. Nicoloso MS, Spizzo R, Shimizu M, et al. MicroRNAs–the micro steering wheel of tumour metastases. Nat Rev Cancer. 2009; 9:293–302. [PubMed: 19262572]
20. Huarte M, Rinn JL. Large non-coding RNAs: missing links in cancer? Hum Mol Genet. 2010; 19:R152–61. [PubMed: 20729297]
21. Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol. 2011; 21:354–61. [PubMed: 21550244]
22. Nagano T, Mitchell JA, Sanz LA, et al. The air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science. 2008; 322:1717–20. [PubMed: 18988810]
23. Rinn JL, Kertesz M, Wang JK, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007; 129:1311–23. [PubMed: 17604720]
24. Zhao J, Sun BK, Erwin JA, et al. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science. 2008; 322:750–6. [PubMed: 18974356]
25. Rijnkels M, Kabotyanski E, Montazer-Torbati MB, et al. The epigenetic landscape of mammary gland development and functional differentiation. J Mammary Gland Biol Neoplasia. 2010; 15:85–100. [PubMed: 20157770]
26. Topper YJ, Freeman CS. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol Rev. 1980; 60:1049–106. [PubMed: 7001510]
27. Stein T, Morris JS, Davies CR, et al. Involution of the mouse mammary gland is associated with an immune cascade and an acute-phase response, involving LBP, CD14 and STAT3. Breast Canc Res. 2004; 6:R75–91.
28. Master SR, Stoddard AJ, Bailey LC, et al. Genomic analysis of early murine mammary gland development using novel probe-level algorithms. Genome Biol. 2005; 6:R20. [PubMed: 15693949]
29. McBryan J, Howlin J, Kenny PA, et al. ERalpha-CITED1 co-regulated genes expressed during pubertal mammary gland development: implications for breast cancer prognosis. Oncogene. 2007; 26:6406–19. [PubMed: 17486082]
30. Rudolph MC, McManaman JL, Phang T, et al. Metabolic regulation in the lactating mammary gland: a lipid synthesizing machine. Physiol Genom. 2007; 28:323–36.
31. Clarkson RW, Wayland MT, Lee J, et al. Gene expression profiling of mammary gland development reveals putative roles for death receptors and immune mediators in post-lactational regression. Breast Canc Res. 2004; 6:R92–R109.

32. Kendrick H, Regan JL, Magnay FA, et al. Transcriptome analysis of mammary epithelial subpopulations identifies novel determinants of lineage commitment and cell fate. BMC Genom. 2008; 9:591.
33. Raouf A, Zhao Y, To K, et al. Transcriptome analysis of the normal human mammary cell commitment and differentiation process. Cell Stem Cell. 2008; 3:109–18. [PubMed: 18593563]
34. Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. Genes Dev. 2009; 23:2563–77. [PubMed: 19933147]
35. Avril-Sassen S, Goldstein LD, Stingl J, et al. Characterisation of microRNA expression in post-natal mouse mammary gland development. BMC Genom. 2009; 10:548.
36. Ibarra I, Erlich Y, Muthuswamy SK, et al. A role for microRNAs in maintenance of mouse mammary epithelial progenitor cells. Genes Dev. 2007; 21:3238–43. [PubMed: 18079172]
37. Greene SB, Gunaratne PH, Hammond SM, et al. A putative role for microRNA-205 in mammary epithelial cell progenitors. J Cell Sci. 2010; 123:606–18. [PubMed: 20103531]
38. Tanaka T, Haneda S, Imakawa K, et al. A microRNA, miR-101a, controls mammary gland development by regulating cyclooxygenase-2 expression. Differentiation. 2009; 77:181–7. [PubMed: 19281778]
39. Anderson E. The role of oestrogen and progesterone receptors in human mammary development and tumorigenesis. Breast Canc Res. 2002; 4:197–201.
40. Cui W, Li Q, Feng L, et al. MiR-126-3p regulates progesterone receptors and involves development and lactation of mouse mammary gland. Mol Cell Biochem. 2011; 355:17–25. [PubMed: 21526342]
41. Wang CM, Li QZ, Li Y. miR-138 function and its targets on mouse mammary epithelial cells. Progr Biochem Biophys. 2008; 35:834–8.
42. Lu LM, Li QZ, Wang CM, Li Y, Gao XJ. Impact of miR-221 on mouse mammary epithelial cells and lactation. Chin J Biochem Mol Biol. 2009; 25:454–8.
43. Ucar A, Vafaizadeh V, Jarry H, et al. miR-212 and miR-132 are required for epithelial stromal interactions necessary for mouse mammary gland development. Nat Genet. 2010; 42:1101–8. [PubMed: 21057503]
44. Askarian-Amiri ME, Crawford J, French JD, et al. SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. RNA. 2011; 17:878–91. [PubMed: 21460236]
45. Iorio MV, Ferracin M, Liu CG, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005; 65:7065–70. [PubMed: 16103053]
46. Si ML, Zhu S, Wu H, et al. miR-21-mediated tumor growth. Oncogene. 2007; 26:2799–803. [PubMed: 17072344]
47. Frankel LB, Christoffersen NR, Jacobsen A, et al. Programmed cell death 4 (PDCD4) is an important functional target of the microRNA miR-21 in breast cancer cells. J Biol Chem. 2008; 283:1026–33. [PubMed: 17991735]
48. Zhu S, Si ML, Wu H, et al. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). J Biol Chem. 2007; 282:14328–36. [PubMed: 17363372]
49. Meng F, Henson R, Wehbe-Janek H, et al. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007; 133:647–58. [PubMed: 17681183]
50. Ovcharenko D, Kelnar K, Johnson C, et al. Genome-scale microRNA and small interfering RNA screens identify small RNA modulators of TRAIL-induced apoptosis pathway. Cancer Res. 2007; 67:10782–8. [PubMed: 18006822]
51. Jiang S, Zhang HW, Lu MH, et al. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. Cancer Res. 2010; 70:3119–27. [PubMed: 20354188]
52. Costinean S, Zanesi N, Pekarsky Y, et al. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. Proc Natl Acad Sci U S A. 2006; 103:7024–9. [PubMed: 16641092]
53. Medina PP, Nolde M, Slack FJ. OncomiR addiction in an in vivo model of microRNA-21-induced pre-B-cell lymphoma. Nature. 2010; 467:86–90. [PubMed: 20693987]

54. Bussing I, Slack FJ, Grosshans H. let-7 microRNAs in development, stem cells and cancer. Trends Mol Med. 2008; 14:400–9. [PubMed: 18674967]
55. Boyerinas B, Park SM, Hau A, et al. The role of let-7 in cell differentiation and cancer. Endocr Relat Canc. 2010; 17:F19–36.
56. Yu F, Yao H, Zhu P, et al. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell. 2007; 131:1109–23. [PubMed: 18083101]
57. Yu Z, Wang C, Wang M, et al. A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. J Cell Biol. 2008; 182:509–17. [PubMed: 18695042]
58. Spizzo R, Nicoloso MS, Lupini L, et al. miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-alpha in human breast cancer cells. Cell Death Differ. 2010; 17:246–54. [PubMed: 19730444]
59. Mackiewicz M, Huppi K, Pitt JJ, et al. Identification of the receptor tyrosine kinase AXL in breast cancer as a target for the human miR-34a microRNA. Breast Canc Res Treat. 2011; 130:663–79.
60. Derfoul A, Juan AH, Difilippantonio MJ, et al. Decreased microRNA-214 levels in breast cancer cells coincides with increased cell proliferation, invasion and accumulation of the Polycomb Ezh2 methyltransferase. Carcinogenesis. 2011; 32:1607–14. [PubMed: 21828058]
61. Wu H, Zhu S, Mo YY. Suppression of cell growth and invasion by miR-205 in breast cancer. Cell Res. 2009; 19:439–48. [PubMed: 19238171]
62. Iorio MV, Casalini P, Piovan C, et al. microRNA-205 regulates HER3 in human breast cancer. Cancer Res. 2009; 69:2195–200. [PubMed: 19276373]
63. Mourtada-Maarabouni M, Pickard MR, Hedge VL, et al. GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. Oncogene. 2009; 28:195–208. [PubMed: 18836484]
64. Kino T, Hurt DE, Ichijo T, et al. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci Signal. 2010; 3:ra8. [PubMed: 20124551]
65. Perez DS, Hoage TR, Pritchett JR, et al. Long, abundantly expressed non-coding transcripts are altered in cancer. Hum Mol Genet. 2008; 17:642–55. [PubMed: 18006640]
66. Ji P, Diederichs S, Wang W, et al. MALAT-1, a novel non-coding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene. 2003; 22:8031–41. [PubMed: 12970751]
67. Lin R, Maeda S, Liu C, et al. A large noncoding RNA is a marker for murine hepatocellular carcinomas and a spectrum of human carcinomas. Oncogene. 2007; 26:851–8. [PubMed: 16878148]
68. Li L, Feng T, Lian Y, et al. Role of human noncoding RNAs in the control of tumorigenesis. Proc Natl Acad Sci U S A. 2009; 106:12956–61. [PubMed: 19625619]
69. Iacoangeli A, Lin Y, Morley EJ, et al. BC200 RNA in invasive and preinvasive breast cancer. Carcinogenesis. 2004; 25:2125–33. [PubMed: 15240511]
70. Fidler IJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer. 2003; 3:453–8. [PubMed: 12778135]
71. Le XF, Merchant O, Bast RC, et al. The roles of MicroRNAs in the cancer invasion-metastasis cascade. Cancer Microenviron. 2010; 3:137–47. [PubMed: 21209780]
72. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature. 2007; 449:682–8. [PubMed: 17898713]
73. Ma L. Role of miR-10b in breast cancer metastasis. Breast Canc Res. 2010; 12:210.
74. Preis M, Gardner TB, Gordon SR, et al. MicroRNA-10b expression correlates with response to neoadjuvant therapy and survival in pancreatic ductal adenocarcinoma. Clin Cancer Res. 2011; 17:5812–21. [PubMed: 21652542]
75. Sasayama T, Nishihara M, Kondoh T, et al. MicroRNA-10b is overexpressed in malignant glioma and associated with tumor invasive factors, uPAR and RhoC. Int J Cancer. 2009; 125:1407–13. [PubMed: 19536818]
76. Sun L, Yan W, Wang Y, et al. MicroRNA-10b induces glioma cell invasion by modulating MMP-14 and uPAR expression via HOXD10. Brain Res. 2011; 1389:9–18. [PubMed: 21419107]

77. Ma L, Young J, Prabhala H, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol. 2010; 12:247–56. [PubMed: 20173740]
78. Huang Q, Gumireddy K, Schrier M, et al. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol. 2008; 10:202–10. [PubMed: 18193036]
79. Su X, Chakravarti D, Cho MS, et al. TAp63 suppresses metastasis through coordinate regulation of Dicer and miRNAs. Nature. 2010; 467:986–90. [PubMed: 20962848]
80. Thiery JP, Acloque H, Huang RY, et al. Epithelial-mesenchymal transitions in development and disease. Cell. 2009; 139:871–90. [PubMed: 19945376]
81. Martello G, Rosato A, Ferrari F, et al. A MicroRNA targeting dicer for metastasis control. Cell. 2010; 141:1195–207. [PubMed: 20603000]
82. Di Leva G, Gasparini P, Piovan C, et al. MicroRNA cluster 221–222 and estrogen receptor alpha interactions in breast cancer. J Natl Canc Inst. 2010; 102:706–21.
83. Cochrane DR, Cittelly DM, Howe EN, et al. MicroRNAs link estrogen receptor alpha status and dicer levels in breast cancer. Horm Cancer. 2010; 1:306–19. [PubMed: 21761362]
84. Stinson S, Lackner MR, Adai AT, et al. miR-221/222 targeting of trichorhinophalangeal 1 (TRPS1) promotes epithelial-to-mesenchymal transition in breast cancer. Sci Signal. 2011; 4:pt5. [PubMed: 21868360]
85. Tavazoie SF, Alarcon C, Oskarsson T, et al. Endogenous human microRNAs that suppress breast cancer metastasis. Nature. 2008; 451:147–52. [PubMed: 18185580]
86. Valastyan S, Reinhardt F, Benaich N, et al. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell. 2009; 137:1032–46. [PubMed: 19524507]
87. Valastyan S, Benaich N, Chang A, et al. Concomitant suppression of three target genes can explain the impact of a microRNA on metastasis. Genes Dev. 2009; 23:2592–7. [PubMed: 19875476]
88. Karin M. Nuclear factor-kappaB in cancer development and progression. Nature. 2006; 441:431–6. [PubMed: 16724054]
89. Bhaumik D, Scott GK, Schokrpur S, et al. Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. Oncogene. 2008; 27:5643–7. [PubMed: 18504431]
90. Hurst DR, Edmonds MD, Scott GK, et al. Breast cancer metastasis suppressor 1 up-regulates miR-146, which suppresses breast cancer metastasis. Cancer Res. 2009; 69:1279–83. [PubMed: 19190326]
91. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol. 2008; 10:593–601. [PubMed: 18376396]
92. Park SM, Gaur AB, Lengyel E, et al. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. 2008; 22:894–907. [PubMed: 18381893]
93. Burk U, Schubert J, Wellner U, et al. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep. 2008; 9:582–9. [PubMed: 18483486]
94. Korpal M, Lee ES, Hu G, et al. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem. 2008; 283:14910–4. [PubMed: 18411277]
95. Dykxhoorn DM, Wu Y, Xie H, et al. miR-200 enhances mouse breast cancer cell colonization to form distant metastases. PLoS One. 2009; 4:e7181. [PubMed: 19787069]
96. Korpal M, Ell BJ, Buffa FM, et al. Direct targeting of Sec23a by miR-200 s influences cancer cell secretome and promotes metastatic colonization. Nat Med. 2011; 17:1101–8. [PubMed: 21822286]
97. Gupta RA, Shah N, Wang KC, et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature. 2010; 464:1071–6. [PubMed: 20393566]
98. Tsai MC, Manor O, Wan Y, et al. Long noncoding RNA as modular scaffold of histone modification complexes. Science. 2010; 329:689–93. [PubMed: 20616235]

99. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001; 98:10869–74. [PubMed: 11553815]
100. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435:834–8. [PubMed: 15944708]
101. Mattie MD, Benz CC, Bowers J, et al. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. Mol Canc. 2006; 5:24.
102. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008; 105:10513–8. [PubMed: 18663219]
103. Chen X, Ba Y, Ma L, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008; 18:997–1006. [PubMed: 18766170]
104. Lawrie CH, Gal S, Dunlop HM, et al. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol. 2008; 141:672–5. [PubMed: 18318758]
105. Corcoran C, Friel AM, Duffy MJ, et al. Intracellular and extracellular microRNAs in breast cancer. Clin Chem. 2011; 57:18–32. [PubMed: 21059829]
106. Zhu W, Qin W, Atasoy U, et al. Circulating microRNAs in breast cancer and healthy subjects. BMC Res Notes. 2009; 2:89. [PubMed: 19454029]
107. Heneghan HM, Miller N, Lowery AJ, et al. Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg. 2010; 251:499–505. [PubMed: 20134314]
108. Trang P, Medina PP, Wiggins JF, et al. Regression of murine lung tumors by the let-7 microRNA. Oncogene. 2010; 29:1580–7. [PubMed: 19966857]
109. Wiggins JF, Ruffino L, Kelnar K, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Res. 2010; 70:5923–30. [PubMed: 20570894]
110. Trang P, Wiggins JF, Daige CL, et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. Mol Ther. 2011; 19:1116–22. [PubMed: 21427705]
111. Pramanik D, Campbell NR, Karikari C, et al. Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. Mol Cancer Ther. 2011; 10:1470–80. [PubMed: 21622730]
112. Liu C, Kelnar K, Liu B, et al. The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Nat Med. 2011; 17:211–5. [PubMed: 21240262]
113. Ma L, Reinhardt F, Pan E, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol. 2010; 28:341–7. [PubMed: 20351690]
114. Adams BD, Furneaux H, White BA. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. Mol Endocrinol. 2007; 21:1132–47. [PubMed: 17312270]

Table 1

Non-coding RNAs in mammary development

Non-coding RNA	Function	Target gene or effector	Reference
miRNA			
let-7	Inhibit self-renewal and promote differentiation; deplete mammary stem and progenitor cell population	RAS, HMGA2	[36]
miR-205	Expand mammary progenitor cell population	PTEN, ZEB1, ZEB2	[37]
miR-101a	Inhibit mammary epithelial cell proliferation and reduce β-casein expression	COX-2	[38]
miR-126-3p	Inhibit mammary epithelial cell proliferation and reduce β-casein expression	PR	[40]
miR-138	Suppress mammary epithelial cell viability and proliferation	PRL-R	[41]
miR-221	Suppress mammary epithelial cell viability and proliferation	GHR	[42]
miR-132/212 family	Regulate epithelial-stromal interaction	MMP-9	[43]
lncRNA			
Zfas1	Suppress mammary epithelial cell proliferation and differentiation		[44]

Table 2

Non-coding RNA in mammary tumorigenesis and metastasis

Non-coding RNA	Function	Target gene or effector	Reference
miRNA			
miR-21	Increase tumor growth and decrease apoptosis	TPM1, PDCD4, PTEN, MASPIN	[45–49, 53]
miR-155	Promote proliferation and oncogenic transformation	SOCS1	[50–52]
let-7	Inhibit tumorigenicity and stemness	H-RAS, HMGA2	[56]
miR-17/20	Suppress proliferation and oncogenic transformation	CCND1	[57]
miR-145	Promote apoptosis	ESR1	[58]
miR-34a	Induce growth arrest and apoptosis	AXL	[59]
miR-214	Inhibit cell proliferation and invasion	EZH2	[60]
miR-205	Suppress cell growth and invasion; suppress EMT and induce MET	HER3, VEGFA, ZEB1, ZEB2	[61, 62, 91]
miR-10b	Induce tumor invasion and metastasis	HOXD10	[72–76]
miR-9	Promote migration, invasion, and metastasis	CDH1	[72, 77]
miR-373	Promote migration, invasion, and metastasis	CD44	[78]
miR-103/107	Promote migration, invasion, and metastasis	Dicer	[81]
miR-221/222	Promote EMT, migration, and invasion	ESR1, Dicer, TRPS1	[82–84]
miR-126	Suppress proliferation and tumorigenesis		[85]
miR-206	Inhibit cell growth, motility, and invasiveness	ESR1	[85, 114]
miR-335	Inhibit motility and invasiveness	SOX4, TNC	[85]
miR-31	Suppress multiple steps of breast cancer metastasis	ITGA5, RDX, RHOA	[86, 87]
miR-146a/b	Reduce migration, invasion, and metastasis	IRAK1, TRAF6	[89, 90]
miR-200 family	Suppress EMT and induce MET; inhibit migration but promote metastatic colonization	ZEB1, ZEB2, sec23a	[91–96]
lncRNA			
GAS5	Promote growth arrest and apoptosis	GREs	[63, 64]
MALAT1	Promote tumor formation		[65–68]
BC200	Is associated with invasive breast cancer		[69]
HOTAIR	Induce metastasis	HOXD genes	[23, 97, 98]