Chapter

Duplicitous Dispositions of Micro-RNAs (miRs) in Breast Cancer

Amal Qattan

Abstract

In 1993, a gene silencer known as lin-4 was first discovered in *Caenorhabditis elegans* and demonstrated to be critical for larval development. Lin-4 belongs to a family of signaling molecules known as non-protein coding microRNAs (miRNAs) which are not only highly conserved in humans, but also involved in the fundamental processes of oncogenesis. While miRNAs are not translated to proteins themselves, they are capable of regulating the expression and translation of other genes thus affecting a multitude of biological and pathological pathways as well as those essential to the malignant landscape. The aim of this chapter is to explore the diverse roles of miRNAs in the context of breast cancer. Following a brief overview of miRNA biogenesis, this chapter covers the production of miRNAs by tumor cells and stromal cells, onco-suppressor miRNAs, use as therapeutics, contribution to therapeutic resistance, and finally their emerging role as biomarkers.

Keywords: microRNAs (miRs), breast cancer epigenetic alteration, microRNA-based therapy, miRNA pharmacogenomics, miRSNPs, miR-polymorphisms, clinical trials

1. Introduction

A gene silencer known as *lin-4* was first discovered in *Caenorhabditis elegans* and demonstrated to be critical for larval development [1]. *Lin-4* belongs to a family of signaling molecules known as non-protein coding microRNA (miRNAs) which are not only highly conserved in humans, but also involved in the fundamental processes of oncogenesis [2]. Approximately 2000 miRNAs are present in the human genome [3]. While miRNAs are not translated into proteins themselves, they are implicated in the regulation of 30% of all genes and are thereby capable of regulating the expression and translation of other genes influencing a multitude of biological and pathological pathways [4]. This chapter explores the diverse roles of miRNAs in the most frequent cancer among women in the world: breast cancer (BC). BC impacts 2.1 million women yearly [5] and it also causes the greatest number of cancer-related deaths among women. Early detection and diagnosis are critical to survival. In the context of BC, miRNAs are dynamically regulated implicating their use in diagnosis, prognosis and tracking of drug efficacy during treatment. Following a brief overview of miRNA biogenesis, this chapter covers the production of miRNAs by tumor cells, onco-suppressor and tumor-suppressor miRNAs, their contribution to therapeutic resistance, therapeutic miRNAs (as well as therapeutics targeting of miRNAs), and finally their emerging role as biomarkers for BC prognosis, treatment responsiveness and efficacy.

2. MiRNA biogenesis and mechanisms of action

Since 1993, researchers have proceeded to learn that miRNAs were of ancient evolutionary origins. Single stranded, non-protein coding miRNAs with genetic suppression activities were found in algae, plants, invertebrates, vertebrates and even viruses [6]. Further characterization has revealed that miRNAs are not only critical for normal human development, but their aberrant expression is associated with diseases such as cancer [7, 8].

The miRNAs are encoded by genetic sequences which may be located within the introns of protein coding genes as well as in the exons and introns of long noncoding RNAs, and even intergenic regions [9]. According to the miRIAD database, 1157 (61.5%) miRNAs are intragenic (169 exonic and 988 intronic) and 724 (38.5%) are intergenic [10]. MiRNA's are single-stranded RNA transcripts that are transcribed from DNA sequences and are usually around 22 nucleotides in length. They often form distinct secondary folding conformational motifs. Most miRNAs are first transcribed into primary miRNAs (pri-miRNAs) and processed into precursor miRNAs (pre-miRNAs) and mature miRNAs. Usually they bind to the 3′-untranslated region (UTR) of target mRNAs to suppress their target's expression by inhibiting its translation. However, they can also interact with coding sequences, the 5′UTR and gene promoter regions. Though less common, some are involved in the translation activation and stabilization of target transcripts. Furthermore, the shuttling of miRNAs between different cellular compartments can also control rates of transcription and translation of their targets.

In the canonical pathway of miRNA biogenesis, RNA polymerase II transcribes miRNAs into primary miRNAs (pri-miRNAs) greater than 200 nucleotides long. Pri-mRNAs are then cleaved into pre-mRNAs by the RNAse III enzyme, Drosha with the help of double stranded RNA binding proteins Pasha and DiGeorge Syndrome Critical Region 8 (DGCR8). The 60–70 nucleotides long pre-mRNAs are then exported out of the nucleus and into the cell cytoplasm by exportin-5 and Ran GTPase. Once in the cytoplasm, pre-mRNAs are cleaved by the RNAse III enzyme Dicer which removes hairpin loops resulting in miRNA duplexes composed of a guide strand and a passenger strand. The passenger strand is discarded and the guide strand associates with Argonaute 2 (Ago2) to form the RNA-induced silencing complex (RISC) which brings the miRNA to its target mRNA. A 6–8 nucleotide sequence on the miRNA, referred to as the "seed sequence" locates the corresponding sequence of the target mRNA. A double stranded complex is formed which impedes the ribosome from translating the target [11]. Imperfect complementarity between the seed sequence and the target mRNA can also cause target degradation indirectly via deadenylation at the 3′-UTR. Non-canonical miRNA biogenesis is less common and can generally be grouped into Drosha/DGCR8-independent and Dicer-independent pathways which are outside the scope of this chapter. In addition to the inhibition of target miRNAs, there is evidence indicating that some miRNAs directly increase target translation via recruitment of protein synthesis complexes to the translation initiation region. Alternatively, target mRNA expression can also be increased due to inhibition of modulating repressors that block translation. Moreover, some miRNAs enhance ribosome biogenesis resulting in increased protein synthesis [12].

In summary, miRNA biogenesis is a multi-step process that requires various enzymes and shuttling proteins to reach a final product. Mature miRNAs are either stable molecules with half-lives of greater than 24 h or they display shorter half-lives of less than 12 h, depending on the functionality of the product [13]. More on the regulation of miRNA expression is discussed in the next section.

3. Regulation of miRNA expression

In general, just as protein-coding genes are regulated by transcription factors (TF), TFs are one of the central ways by which miRNA expression is regulated. Tissue and developmental stage specific TFs can control the transcription of miRNA genes. Many miRNAs and TFs form autoregulatory loops, in which they mutually regulate each other [14]. In addition, various physiological and pathological stimuli, such as steroid hormones, retinoids, hypoxia, interferons, stress, as well as estrogen, can affect miRNA expression [15]. Finally, while transcription regulates the magnitude of miRNA expression, decay rates influence miRNA dynamic regulation. Slow decay leads to a high level of accumulation while fast decay leads quick changes in miRNA expression levels implying that fast turnover may be involved in transient biological processes.

Epigenetic mechanisms are heritable changes in gene expression that occur without any modifications in the DNA sequence itself and include DNA methylation and histone modifications as well as miRNAs themselves [16]. The covalent binding of methyl groups to cytosine bases located among CpG dinucleotide sequences is the major modification of eukaryotic genomes which results in down regulation of gene expression. DNA methylation controls embryonic cell fate lineages and prevents reversion to an undifferentiated state [17]. Frequency of methylation is nearly one order of magnitude higher in human miRNA genes compared to the methylation of other protein-coding genes [18, 19]. This indicates strict epigenetic control of miRNA expression and also reveals how epigenetic changes in cancer cells can lead to dysregulated expression of miRNAs by cancer cells.

Genome variations include genetic mutations and polymorphisms; defined as a DNA variation in which a possible sequence is present in at least 1% of people. Single nucleotide polymorphisms (SNPs) constitute approximately 1% of the human genome. SNPs contribute to phenotypic diversity within a species as well as disease susceptibility. MiRSNPs/miR-polymorphisms are a new mechanism and novel class of functional SNPs. As miRNA molecular interactions with their targets are affected via base pairing as well as genetic variation, such as changes in genome sequence; which influences binding energy and annealing strength, SNPs can result in no change, off target or absence of miRNA binding to the predicted target [20]. Carcinogens such as those from cigarettes, dietary elements and other foreign chemical toxicities referred to as "xenobiotics," can also affect miRNA expression. Importantly, many more changes in miRNA expression were observed in cancer-target tissues than in the non-target tissues following acute or chronic exposure to carcinogens thus implicating their use as potential biomarkers for exposure to xenobiotics [21]. Finally, circadian rhythm control of miRNA expression has significant consequences for circadian timing as some miRNAs have promoter sequences inducible by circadian clock proteins. Moreover, some miRNAs can even be regulated by light and dark cycles which confer important rhythmic expressions in organs such as the liver and heart [22].

In summary, miRNA regulation is similar to other protein coding gene regulation as changes in expression can occur based on the presences or exposure to TFs, genetic polymorphisms, epigenetic factors, xenobiotics and carcinogens. How miRNA expression is regulated in the context of BC is discussed in the next section.

4. miRNAs (miRs) production by breast cancer cells

As summarized above, TF, SNPs, epigenetics, hormones and xenobiotics all affect the regulation of miRNAs; therefore, it is not surprising that breast cancer

(BC) leads to significant, dynamic changes in miRNA expression both by tumor cells and by surrounding stromal cells. This section describes BC tumor cell production of miRNAs as well as the surrounding non-cancerous stromal cells. In general, miRNAs either support or suppress tumorigenesis and are often dysregulated due to tumor-specific epigenetic changes. Likewise, tumor secreted factors such as exosomes and cytokines can also lead to aberrant signaling in the surrounding stromal cells. Furthermore, while all BCs begin in the breast, there are many subtypes which are named to reflect their particular molecular pathogenesis. Subtype diagnosis can help select appropriate therapies. Likewise, aberrant regulation of miRNAs can be subtype specific. Therefore, this section begins with a brief overview of cancer subtypes.

Breast carcinoma can begin either in the ducts or the lobules and as such, termed either ductal carcinoma in situ (DCIS), or lobular carcinoma in situ (LCIS). Both can either stay contained to the area or travel to surrounding tissue and lymph nodes in which case the clinical diagnosis is either invasive ductal carcinoma (IDC) or invasive lobular carcinoma (ILC). IDC is the most common type of BC (50–75%) followed by ILC (5–15%) [23]. Rare BC is characterized by tumor origination in the mucinous, papillary, medullary or cribriform compartments of the breast [24]. Metastasis of breast cancer to other organs is the main cause of mortality and up to 5% of patients will already have experienced metastasis at the time of diagnosis [25].

MiRNA microarray performed on 1542 breast tissue samples procured via the Molecular Taxonomy of Breast Cancer International Consortium and the Akershus University Hospital (AHUS) revealed that no miRNAs were differentially expressed in DCIS patients relative to IDC, supporting the idea that miRNA dysregulation occurs at an early stage of BC development [26]. Among the invasive subtypes, however, expression of seven miRNAs was consistently downregulated, including tumor suppressors let-7c-5p, miR-125b-5p, miR140-3p, miR-145-3p, miR-145-5p, miR-193a-5p, and miR378a-3p while expression of four oncogenic miRNAs was consistently upregulated including miR-106b-5p, miR-142, miR-342-3p, and miR425-5p. Taken together these miRNAs may significantly contribute to the transition to an invasive BC subtype [26].

While Bloom and Richardson's histologic grading system which was modified by Elston and Ellis in 1991 is the most commonly used system to gain prognostic insight, hormone receptors status, tumor size, nodal status and whether tumorous cells have invaded the lymph or blood vessels is also considered during initial diagnosis. Hormone receptor statuses including estrogen receptor (ER) and progesterone receptor (PR) as well as the tyrosine kinase receptor, human epidermal growth receptor type two (HER2) are always measured on newly diagnosed invasive BCs. Subtypes are identified via immunohistochemical staining for hormone receptors, HER2 expression status, and Ki-67 proliferation index as: luminal A (ER-positive and/or PR-positive, HER2-negative, low proliferation), luminal B (ER-positive and/or PR-positive, HER2-negative, high proliferation; or hormone receptor (HR)-positive and HER2-positive), HER2-positive (HR-negative and HER2-positive) and finally TNBC type (HR-negative and HER2-negative) [27]. ER+ breast cancer subtype is particularly prevalent in postmenopausal women taking hormone replacement therapy (HRT) which activates the transcription factor estrogen receptor alpha (ERα) which promotes the expression of numerous oncogenic genes. While ERα-signaling is targeted by miRNAs for degradation, aberrant activation of this receptor leads to aberrant expression of miRNAs controlled by ERα-signaling [28].

Several miRNAs are both tissue and cancer specific. As the primary role of miRNA is to decrease target mRNA expression, miRNAs that are upregulated by

cancerous cells are often those that support cancer growth and are referred to as oncomiRs. miR-10b, miR-21 and miR-155 are well characterized oncomiRs in BC [29]. Their main role is to downregulate tumor suppressor genes which results in the promotion of cancer cell proliferation, de-differentiation and invasion [30]. BC cells also produce less tumor-suppressor miRNAs (miR-31, miR-125b, miR-200 and miR-205) which downregulate oncogenic proteins. Cancer-initiating cells (CSCs) were first isolated from breast cancer tumors and are considered the seed-cells of tumor development [31]. While CSCs are similar to normal somatic stem cells in that they are capable of asymmetric cell division and the efflux of small molecules, they have more phenotypic plasticity. The family of miRNAs known as let-7 was demonstrated to be a master regulator of self-renewal and tumor-seeding ability [32]. Likewise, the process of epithelial to mesenchymal transition (EMT) which enables tumorigenicity and invasion, was facilitated via transforming growth factor β2 (TGF-β2) and Zeb1 transcription factor mediated repression of the miR-200 and miR-141; two miRNAs which are responsible for epithelial differentiation [33].

In summary, reflecting the cancer cells aim of aberrant, dysregulated gene expression needed for tumor cell survival and proliferation, a global downregulation of all miRNAs is observed in cancer. In tumor cells, the main mechanism by which global miRNA production is suppressed is via the upregulation of miRNAs that target the crucial miRNA biogenesis enzyme Dicer, miR-103 and miR-107 [34]. Likewise, chromatin remodeling that results in an increase in miRNAs that support EMT and self-renewal rather than continuation of a differentiated cell type is observed [35].

5. miRNAs affecting breast cancer chemotherapy efficacy and resistance

Chemoresistance is the primary cause of treatment failure in breast cancer. Dysregulation of some miRNAs can result in increases in drug efflux, alter drug targets and energy metabolism, stimulate DNA repair pathways and evasion of apoptosis and result in loss of cell cycle control. The first BC drug was a DNA-replication blocker called doxorubicin. Resistance to doxorubicin correlated with downregulation of miR-505, miR-128, and miR-145 tumor suppressors [36–41]. In contrast, miR-663, miR-181a, and miR-106b are oncogenic miRNAs whose downregulation resulted in enhancement of doxorubicin sensitivity in formerly resistant cells [41–43]. Like doxorubicin, cisplatin inhibits DNA replication and was also one of the first established therapies for BC. Upregulation of miR-345 and miR-7 contribute to cisplatin-resistance, while miR-302b can sensitize resistant cells to cisplatin therapy [44, 45]. A list of miRNA expression levels and targets of BC drug resistant is listed in **Table 1**.

In addition to doxorubicin and cisplatin, efficacy of the chemotherapeutic agents docetaxel and paclitaxel which inhibit microtubule formation during cell division, can also be compromised by miRNAs. Downregulation of miR-34a, miR-100, and miR-30c were observed in paclitaxel-resistant BC cell while the upregulation of miR-129-3p was found to contribute to resistance [57–61].

In ER+ breast cancer, *de novo* and acquired resistance to conventional endocrine therapies such as aromatase inhibitors, fulvestrant and tamoxifen, can occur in more than 30% of patients [63]. Evidence suggests that resistance to these drugs is in part mediated by miRNAs. As most BC patients have high estrogen receptor-α (ER-α) expression, targeting ER-α signaling is a critical therapy. Resistance to tamoxifen, an agent which blocks interaction between estrogen and estrogen receptor is associated with the downregulation of the following tumor suppressor miRNAs: miR-15a, miR-16, miR-214, miR-320, miR-342, miR-451, miR-873,

miRNA	BC therapy	Targets	Level	Mechanism/Refs.
miR-200	Carboplatin	Zeb	↓	Reverses EMT [46]
miR-345	Cisplatin	MRP1	↓	Not yet characterized [45]
miR-7				
miR-302b	Cisplatin	E2F1 (direct)	↓	Inhibit cell cycle progression [44]
		ATM (indirect)		
miR-24	Cisplatin	BimL F1H1	↑	Promotes EMT and cancer stem cells [47]
miR-106b~25 cluster	Doxorubicin	EP300	↑	Activates EMT [43]
miR-128	Doxorubicin	Bmi-1 ABCC5	↓	Increases apoptosis [48]
miR-145	Doxorubicin	MRP1	↓	Induces intracellular doxorubicin accumulation [36]
miR-181a	Doxorubicin	Bcl-2	↓	Increases apoptosis [41]
miR-181a	Doxorubicin	Bax	↑	Inhibits apoptosis [49]
miR-25	Doxorubicin	ULK1	↑	Inhibits autophagy [50]
miR-326	Doxorubicin	MDR-1	↓	Downregulates MRP-1 [51]
miR-505	Doxorubicin	Akt3 (indirect)	↓	Not yet investigated [37]
miR-644a	Doxorubicin	CTBP1	↓	Inhibits EMT [52]
miR-663	Doxorubicin	HSPG2	↑	Inhibits apoptosis [42]
miR-129-3p	Docetaxel	CP100	↑	Reduces cell cycle arrest and apoptosis [53]
miR-34a	Docetaxel	BCL-2 CCND1	↑	Inhibit apoptosis [54]
miR-484	Gemcitabine	CDA	↓	Promote proliferation and cell-cycle redistribution [55]
miR-218	MDR	Survivin	↓	Enhance apoptosis [56]
miR-100	Paclitaxel	mTOR	↓	Enhance cell cycle arrest and apoptosis [57]
miR-125b	Paclitaxel	Sema4C	↓	Reverses EMT [58]
miR-125b	Taxol	Bak1	↑	Inhibits apoptosis [59]
miR-30c	Doxorubicin	TWF1 (PTK9) VIM IL-11	↓	Reverses EMT [60]
	Paclitaxel			
miR-34a	Doxorubicin	HDAC1HDAC7	↑	Inhibits autophagic cell death [61]
	Cisplatin			

Abbreviations: Expression level of miRs: upregulation (↑) or downregulation (↓) of miRNAs in breast cancer therapy. The reference of each miR is included in the table. Table adapted from Hu et al. [62].

Table 1.
miRNAs involved in the regulation of common breast cancer drugs.

miRNA-375, miR-378a-3p, and miR-574-3p [64–71] .In contrast, oncogenic miRs: miR-101, miR-221/222, miR-301, and miRNAs-C19MC were highly expressed in tamoxifen resistant cells [72–75]. In addition, both the humanized monoclonal antibody targeting HER2 named trastuzumab, as well as lapatinib, which is a small-molecule tyrosine kinase inhibitor targeting both HER2 and epithelial growth factor receptor (EGFR), improve therapeutic outcome but result in resistance after 1 year.

Resistance to these two drugs is correlated with an upregulation of miR-21, miR-221 and miR-375 [76–80].

The role of miRNA in chemotherapeutic resistance is associated with the modification of drug transporters which has a net effect of drug efflux out of the cell via exosomes as well as modifications of autophagy and apoptosis pathways which lead to enhanced survival, the promotion of growth factors and activation EMT [81]. The tumor microenvironment which consists of the surrounding stromal cells serve as the normal foundation upon which the deviant tumor "house" is constructed supplying it with blood vessels, signaling molecules and ECM. Exosomes transport bioactive molecules and mediate cellular communication in the tumor microenvironment, facilitating a more cancerous and recalcitrant milieu [82]. For example, exosome-derived miRNAs such as miR-222 transfer doxorubicin-resistance by inhibiting PTEN in recipient cells, 22 miRNAs were concentrated in exosomes and correlated to chemotherapy resistance [83]. While the major function of exosomes in the context of BC and drug resistance is the shuttling of drugs out of the tumor, exosomes can also be bio-hacked for use as a prime chemotherapy delivery system [84–86].

In summary, in the context of breast cancer, tumor cells regulate miRNAs in a way that promotes tumor survival, growth and invasion. Aside from a global down-regulation of most miRNAs and especially tumor suppressor miRNAs, oncogenic miRNAs are increased and often exported via exosomes where they are taken up by non-cancerous cells, transforming the local environment to a pro-cancer milieu. Knowing how BC cells regulate miRNAs opens the door for potential therapies that target oncogenic miRNAs (antagomirs) or add back tumor suppresser miRNAs (mimic miRNAs). The targeting of miRs in breast cancer is discussed in the following section.

6. miRNAs as breast cancer therapy

As reviewed in this chapter, miRNAs are dynamically regulated in BC and can also contribute to drug resistance. Therefore, interventions that disrupt activities of dysregulated miRNAs offer promising targets for novel therapeutics in the form of mimics or antagomirs. In addition, mature miRNAs and their precursors can also be targeted by small molecules. In general, there are two strategies for targeting miRNA in BC. In the first strategy, tumor suppressor miRNAs which are down regulated by tumor cells can be added back to the tumor microenvironment using chemically synthesized miRNA mimics which imitate endogenous mature double-stranded miRNA [87]. MiRNA mimics could be delivered in viral vectors which would allow extended expression. The second strategy is to target oncogenic miRNAs which are highly expressed and exported by tumor cells. In this strategy, oligonucleotides, locked-nucleic-acids antisense oligonucleotides (LNAs), miRNA sponges, multiple-target anti-miRNA antisense oligo-deoxyribonucleotides (MTg-AMOs), miRNA-masking and nanoparticles are used to target for degradation or impede aberrantly expressed oncogenic miRNAs from reaching their targets [88–91].

As previously mentioned, the majority of highly expressed, dysregulated miRNAs in tumor cells are oncomirs, or those that support tumorigenesis, while tumor suppressor miRNAs are suppressed [92]. For example, miR-155 is an oncogenic miRNA upregulated in BC tumor tissue. Targeting of miR-155 with an antisense oligonucleotide (miR-155) in a BC cell line blocked proliferation and augmented apoptosis [93]. MiR-892b is an example of a tumor suppressor miRNA that is significantly downregulated in BC tissue specimens. By supplementing miR-892b

"mimics" in BC cells, a decrease in tumor growth, metastases rate, and angiogenesis was observed. MiR-892b mimic blocked impeded tumorigenesis by attenuating nuclear transcription factor kappa B (NF-kB) signaling [94]. Artificial miRNAs can also be constructed to inhibit targets that are not normally targeted by endogenous miRNAs. For example, a novel artificial miRNA (amiRNA) called miR-p-27-5p, which targets the 3′-UTR of cyclin-dependent kinase 4 (CDK4) mRNA, inhibited cell cycle progression via downregulation of CDK4 expression and suppression of retinoblastoma protein (RB1) phosphorylation [95]. Likewise, an a miRNA against a C-X-C motif chemokine receptor 4 (CXCR4) inserted into an expression vector reduced CXCR4 expression and suppressed migration and invasion of BC cells [96]. While in vitro experiments provide proof of concept for further development of miRNA targeting in oncogenic diseases, only clinical trial results can determine whether miRNA therapy is truly efficacious. Patents, clinical trials and biopharmaceutical companies invested in the development of miRNA therapies are summarized by Chakraborty *et al*, [97]. A seminal trial for miRNA replacement therapy took place employing the tumor suppressor miR-34 mimic (MRX34). MRX34 was formulated for intravenous injection using a liposome delivery system for patients with metastatic liver cancer. MRX34 along with dexamethasone was associated with safety and showed evidence of antitumor activity in a subset of patients with refractory advanced solid tumors [98]. However, there were adverse events in the trial which indicate the need for alternative approaches in formulation design and delivery.

In summary, there is much research to be done in the emerging field of miRNA therapeutics. Drug developers, pharmacists, physicians and molecular biologists must work together to develop novel strategies for miRNA delivery that is more targeted and controlled in order to mitigate off-target effects by affecting only cell signaling of targeted tumor cells.

7. miRNAs as breast cancer biomarkers

MiRNAs that maintain a stable presence in the serum are referred to as "circulating" miRNAs. Thus, in addition to therapeutic targeting, many studies have reported utility of miRNAs in the context of BC as biomarkers for diagnostic, prognostic, or predictive of drug efficacy. In this final section, miRNAs currently being used as biomarkers in the context of BC are discussed.

In the context of diagnostics, the current gold standard for BC is mammography. However, many women avoid mammograms for fear of pain or inconvenience in scheduling thus rendering assays performed on less invasive, routine blood draws amenable to early screening for BC. Global profiling of circulating miRNAs in early-stage ER+ BC (n = 48) and age-matched healthy controls (n = 24) revealed a panel of nine miRNAs (miR-15a, miR-18a, miR-107, miR-133a, miR-139-5p, miR-143, miR-145, miR-365 and miR-425) that discriminated between patients with early-stage ER+ BC and healthy controls [99]. A study in Japan performed on serum (n = 1280 BC, n = 2836 non-cancer controls) found a combination of five miRNAs: miR-1246, miR-1307-3p, miR-4634, miR-6861-5p and miR-6875-5p, could predict breast cancer with a sensitivity of 97.3% overall, 98% sensitivity for early stage BC and a specificity of 82.9% and accuracy of 89.7% [100]. A study based in Prague (n = 63 early stage BC, n = 21 non cancer controls) found that several oncogenic miRNAs were significantly elevated in early stage BC; including: miR-155, miR-19a, miR-181b, and miR-24 and unsurprisingly, their expression dropped following surgical resection of the tumor [101]. A study in Singapore performed global profiling

Source	miRNA	Expression/Refs	DX	PX	PR	VA
Blood	miR-195, let-7 and -155	↑ in BC [108]	Y	N	N	N
Serum	miR-214	Indicates malignant from benign and healthy [109]	Y	N	N	N
Plasma	miR-127-3p, -376a, -148b, -409-3p, -652 and -801	↑ in BC [110]	Y	N	N	Y
Plasma	miR-148b, -133a, and -409-3p	↑ in BC [111]	Y	N	N	Y
Serum	miR-15a	↑ in BC [99]	Y	N	N	Y
	miR-18a, -107, -425, -133a, -139-5p, -143, -145, and -365	↓ in BC [99]				
Serum	miR-484	↑ in BC [112]	Y	N	N	Y
Serum	miR-1246, -1307-3p, and -6861-5p	↑ in BC [100]	Y	N	N	Y
	miR-4634 and -6875-5p	↓ in BC [100]				
Serum	miR-155, -19a, -181b, and -24	↑ in BC [101]	Y	N	N	N
Serum	miR-1, -92a, -133a, and -133b	↑ in BC [102]	Y	N	N	Y
Plasma	miR-505-5p, -125b-5p, -21-5p, and -96-5p	↑ in BC [113]	Y	N	N	Y
Serum	let-7c	↓ in BC [103]	Y	N	N	N
Serum	miR-182	↑ in BC [114]	Y	N	N	N
Blood	miR-138	↑ in BC [115]	Y	N	N	N
Serum	miR-155	Correlates w/PR status [116]	Y	N	N	N
Serum	miR-21, -126, -155, -199a, and -335	Associated w/ histological tumor grade and sex hormone receptor expression [117]	Y	N	N	N
Serum; Plasma	miR-4270, -1225-5p, -188-5p, -1202, -4281, -1207-5p, -642b-3p, -1290, and -3141	↑ in BC and correlates w/stage and molecular subtype [118]	Y	N	N	Y
Serum	miR-202 and let-7b	↑ expression in BC and correlates w/ tumor aggressive and overall survival [119]	Y	Y	N	N
Serum	miR-148b-3p and -652-3p	↓ in the BC [120]	Y	Y	N	Y
	miR-10b-5p	↑ levels correlate w/ poor prognosis [120]				
Serum	miR-18b, -103, -107, and -652	Associated w/tumor relapse and overall survival in TNBC [105]	Y	Y	N	Y
Plasma	miR-10b and -373	↑ in breast cancer w/ LN metastasis [121]	Y	Y	N	Y
Serum	miR-10b, 34a, and -155	Correlates w/ tumor stage and/or metastasis [122]	Y	Y	N	N

Source	miRNA	Expression/Refs	DX	PX	PR	VA
Serum	miR-29b-2, miR-155, miR -197 and miR -205	Correlates w/tumor grade and metastasis [123]	Y	Y	N	N
Serum	miR-92a	↓ in BC, LN metastasis [124]	Y	Y	N	N
	miR-21	↑ in BC, LN metastasis [124]				
Serum	miR-21-5p, -375, -205-5p, and -194-5p	↑ in recurrent BC [125]	Y	Y	N	Y
	miR-382-5p, -376c-3p, and -411-5p	↓ in recurrent BC [125]				
Serum	miR-34a, -93, -373, -17, and -155	Expression correlated w/metastasis and HER2, PR, and ER status [126]	Y	N	N	N
Serum	miR-125b	↑ expression in non-responsive [127]	Y	N	Y	N
Serum	miR-122	↓ in NR and pCR [128]	N	N	Y	Y
	miR-375	↑ in NR and pCR [128]				
Serum	miR-155	↑ in BC; ↓ post chemo [107]	Y	N	Y	N

Abbreviations: DX, diagnostic; PX, prognostic; PR, predictive; VA, validated; BC, breast cancer; ddPCR, droplet digital PCR; DS, deep sequencing; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; miRNA (miR), microRNA; PR, progesterone receptor; qRT-PCR, quantitative reverse transcriptase real-time PCR; TNBC, triple-negative breast cancer; NR, non-relapse; pCR, Pathologic complete response.

Table 2.
Circulating miRNAs; diagnostic, prognostic, predictive and validated biomarkers in breast cancer.

of miRNA expression in BC tumor tissue, non-tumor tissue and serum samples obtained from BC patients (n = 132) and from healthy controls (n = 123) revealed miR-1, miR-92a, miR-133a and miR-133b as significantly upregulated diagnostic markers in BC sera [102]. In addition to upregulation of oncogenic miRNAs, tumor suppressor Let-7c was decreased in BC tissue and sera according to a study performed in China (n = 90 BC, n = 64 controls) [103]. Although some studies have suggested that let-7 and miR-195 restoration may be therapeutic, results of Qattan et al. in 2017 [104] supported literature indicating that tumor cells export hsa-miR-195 and let-7 miRNAs. While the data of this study did not generally support the use of these miRNAs as therapies, it suggested that these markers may be the most robust markers to use in a blood-based screen for the early detection of TNBC and luminal breast cancer [104].

The definition of a prognostic biomarker is one that indicates recurrence or progression; such as chance of survival, independent of the course of therapy. In a study based in Germany, pre-operative serum (n = 102) and post-operative serum (n = 34) of BC patients was compared to healthy women (n = 37) or those with benign breast disease (n = 26). The mean follow-up time of for BC patients was 6.2 years. In this study, high expression of miR-202 positively correlated with reduced overall survival (poor prognosis). In a European study, genome-wide miRNA expression profiling using serum from TNBC patients (n = 130) and healthy controls (n = 30), revealed a four-miRNA signature (miR-18b, miR-103, miR-107

and miR-652) that predicted tumor recurrence and overall survival [105]. While few studies have investigated the use of miRNA serum expression levels as a predictive metric for treatment response, clinically relevant outcomes were revealed in the studies performed indicating the need for incentivizing investigations into miRNA biomarkers. For example, elevated miR-125b expression predicts poor prognosis, is associated with tumor size and TNM stage in HER2+ BC as well as poor responsiveness to paclitaxel-based neoadjuvant chemotherapy [106]. Therefore, miR-125b may be a potential predictor of clinical outcome, particularly in HER2+ BC patients receiving paclitaxel-based neoadjuvant chemotherapy. In another example, miR-155 was significantly increased in BC patients (n = 103) compared with healthy normal (n = 55). Post-surgical resection and four cycles of chemotherapy, a subset of BC patient sera (n = 29) were collected to evaluate the effects of clinical treatment on serum levels of candidate miRNAs. Decreased levels of circulating miR-155 post-treatment was associated with response to therapy and stable disease [107].

In summary, the data from these studies and others suggest that BC patients with novel miRNA signatures correlating with poor prognosis are not receiving adequate treatment and should be selected for inclusion in novel randomized clinical trials for the chance to receive alternative life-saving therapies. **Table 2** summarizes studies revealing statistically significant regulation of circulating miRNAs with diagnostic (DX), prognostic (PX), predictive biomarkers (PR) potential for BC. Some studies were validated (VA) with alternative cohorts.

8. Conclusions

In conclusion, this chapter provided an overview of the most recent studies describing the dynamic roles of miRNAs in the context of BC. This overview demonstrates that just as miRNAs are integral to maintaining normal homeostasis, they are simultaneously sensitive to changes in overall physiology and local micro-environments thus studying them will likely lead to insight into the unique manifestation of BC in an individual. Given that they are actively released by tumor cells into the circulatory system, both monitoring and targeting miRNAs enables the diagnosis and monitoring of BC as well as the opportunity for the development of novel therapeutics. Future studies should employ well standardized methods for sample collection and multi-center global miRNA profiling to reveal novel nuances and robust results regarding miRNA signaling in the context of BC. Taken together, the emerging field of precision oncology may rely on understanding miRNA profiles.

Author details

Amal Qattan[1,2]

1 Department of Molecular Oncology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

2 Department of Biochemistry and Molecular Medicine, School of Medicine and Health Science (SMHS), GW University, Washington, DC, USA

*Address all correspondence to: aqattan5@gwu.edu; akattan@kfshrc.edu.sa

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