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Role of Circulating microRNA as a Potential Biomarker in Metastatic Breast Cancer

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ABSTRACT

Background: Breast cancer is the highest incidence among cancers in women, which diagnosed by tissue biopsy. These disadvantages, such as the invasive technique itself and the suffer from surgical procedure, make liquid or biofluid biopsies are further studied. The extracellualar or circulating microRNAs (miRNAs), has opened up new opportunities for early cancer diagnosis, because the method is non-invasive and simpler than tissue biopsies. Their presence in the circulation can be either cell-free or wrapped in vesicle membranes such as exosomes, microparticles, and apoptotic bodies, making miRNAs a potential biomarker in breast cancer. In addition, miRNA is currently being developed as a targeted therapy in breast cancer.
Methods: This literature was collected from significant journal databases, included published original article from 2005 until 2021. We only included freely accessible journals, using "miRNA breast cancer and circulating microRNA" as keywords.
Results: This review introduces a perspective on the function of miRNAs by illustrating their potential biomarker in diagnosis breast cancer.
Conclusion: miRNAs play an important role in regulating breast cancer development and their clinical role in diagnosis, prognosis and therapeutic targets in breast cancer patients.

Keywords: microRNA, breast cancer, metastatic, biofluid biopsy

INTRODUCTION

There are various methods of diagnosing breast cancer, one of which is a tissue biopsy. Tissue biopsy has the disadvantage of being an invasive technique and some patients suffer enough to require a surgical procedure. Liquid biopsies or biofluid biopsies are currently being widely studied because they are non-invasive and simpler than tissue biopsies. Liquid biopsies also have many advantages such as enabling early diagnosis in potential patients who have not yet developed cancer. In addition, liquid biopsies can also be used to observe the progress of treatment in patients who already have cancer. Therefore, liquid biopsies are expected to be able to screen patients with high risk groups for breast cancer and as an early diagnosis method that is simpler and more affordable compared to tissue biopsies that require invasive procedures.

METHODS

This literature was collected from significant journal databases, included published original article from 2005 until 2021. We only included freely accessible

journals, using "miRNA breast cancer and circulating microRNA" as keywords.

RESULTS

miRNAs are expressed in cells and their expression can also be found in various human tissues, including in breast cancer. Study from Kodahl et al. have shown that there were nine circulating miRNA which are capable to differentiate patients with early stage of breast cancer and healthy controls. Those miRNA are miR-15a, miR-18a, miR-107, miR-133a, miR-139-5p, miR-143, miR-145, miR-365, miR-425.[1] A meta-analysis carried by Wang et al. shown that circulating miR-155 have 79% sensitivity and 85% specificity in diagnosing breast cancer.[2] Another study by Chan et al. have identified four miRNAs as significant biomarker for diagnosis breast cancer. Those are miR-1, miR-92a, miR-133a, dan miR-133b which increase in serum patients with breast cancer. microRNA is considered as a potential and promising diagnostic biomarker because it can be detected at early stage of breast cancer. Study from Cuk et al. have shown miR-148b, miR-376c, miR-409-3p dan miR-801 significantly upregulate in plasma patients with stage I or stage II breast cancer. [3] These studies introduce a new perspective on the function of miRNAs by illustrating their potential biomarker in diagnosis breast cancer.

DISCUSSION

The emergence of microRNAs (miRNAs), which are small non-protein coding RNAs that play an important role in tumor initiation and development, has opened up new opportunities for early cancer diagnosis. miRNAs are non-coding RNA molecules that regulate the expression levels of various genes by base pairs in specific sequences on the untranslated regions of the target mRNA, resulting in mRNA degradation or translation inhibition. One miRNA can target more than one mRNA and one mRNA can be affected by several miRNAs. Because of this ability, miRNAs may have more than 100 targets, making miRNAs one of the largest classes of regulators or the so-called master of regulators, and allowing them to act in a coordinated manner to regulate cellular processes. Through various processes make miRNAs as powerful oncogenes or tumor suppressors because of their ability to modulate proteins. miRNAs are highly specific for certain tissues and stages of development. miRNA can be involved in various important cellular processes, such as cell cycle, angiogenesis and apoptosis which are very important in the process of cancer development. Therefore, it is not surprising that miRNA dysregulation is frequently associated with many diseases, including breast cancer. Another important role of miRNAs is as signaling between cells. Extracellular miRNAs or so-called circulating miRNAs can be found in a variety of body fluids, including serum and plasma. Their presence in the circulation can be either cell-free or wrapped in membrane vesicles such as exosomes, microparticles, and apoptotic bodies, making miRNAs a potential biomarker in breast cancer. In addition, miRNA is currently being developed as a therapeutic target in breast cancer.

Micro-RNA and Central Dogma

The "central dogma" of modern molecular biology has provided the principle of transfer genomic information into the structure and function of organisms. According to the central dogma, RNA (ribonucleic acid) is a mediator between the genetic material, namely DNA (deoxyribonucleic acid) and the final product, namely protein. Deoxyribonucleic acid is transcribed into messenger ribonucleic acid (messenger RNA/mRNA) and then undergoes mRNA translation into protein. DNA allows for long-term, stable and orderly storage of the genetic code. mRNA allows short-term storage and is unstable. Meanwhile, the protein, which is the program of the cell, is the physical manifestation of the information recorded in

the genome. Reverse transcriptase, the enzyme that synthesizes DNA from RNA, is an exception to the central dogma.

The discovery of microRNAs (miRNAs) with mRNAs as their targets has uncovered new mechanisms that regulate gene expression beyond this central dogma. MiRNA research has been linked to the faulty "junk DNA" hypothesis, a theory that suggests that only a small percentage of the human genome encodes proteins. For decades it was believed that the majority of human DNA, i.e. 80-90%, was considered to have no biological purpose. Thus, this condition is not sufficient to explain the functionality of 80-90% of transcribed RNA. In a 2001 human genome project, it was said that only 2% of human DNA encodes proteins (coding proteins), about 50% are unique sequences and the remaining 50% are repetitive sequences, mainly transposable element.[1] RNA cannot code for proteins, known as non-coding RNA (ncRNA). From various new discoveries, the theory of non-coding RNA is currently developing as a new central dogma in biology.[1,2]

RNA was originally known as a messenger, conveying information from DNA genes to the cytoplasm for protein synthesis. An RNA molecule has a messenger or coding part for a protein known as messenger RNA (mRNA). In addition, RNA also has noncoding and nonmessenger sections which have been extensively studied.[3] Non-coding RNA (ncRNA) is any RNA molecule that functions without being translated into protein. An ncRNA is also known as small RNA (sRNA). Less commonly, ncRNAs are referred to as non-messenger RNA (nmRNA), small non-messenger RNA (snmRNA), tiny ncRNA (tncRNA), small modulator RNA (smRNA), or small regulatory RNA.[4] MicroRNA (miRNA) is part of the molecule highly conserved small non-coding RNA (sncRNA) involved in the regulation of gene expression.

microRNAs are noncoding regulatory RNAs ~18–25 nt long and are responsible for mediating post-transcriptional regulation of gene expression, usually by inhibition of translation, or initiation of specific mRNA degradation. miRNAs are endogenous single-stranded RNA encoded by eukaryotic nuclear DNA.[2] The genes encoding miRNAs are transcribed from DNA to produce primary transcripts (pri-miRNA) which are processed into shorter miRNA precursors (pre-miRNA), which are then processed into mature single-stranded miRNAs that are 18 to 24 nucleotides long. A mature miRNA binds to a target mRNA at a complementary sequence to downregulate gene expression by inhibiting translation of the mRNA into protein or by inducing mRNA degradation.[4]

According to the release of miRbase 12.0, a total of 695 human miRNA genes have been described, although the discovery of new miRNAs is an ongoing process. miRNAs were categorized using standard nomenclature. The first three letters indicate the species (hsa for human, mmu for mouse, rno for rat, cel for C. elegans, etc.), and the numbers indicate the order. For example, hsa-miR-1 is identical to cel-miR-1 and nearly identical to rno-miR-1. When a new miRNA is identified, it is first compared with other known miRNAs before a number is assigned. Identical or closely related miRNAs within the same species are assigned sub-names, usually letters, but sometimes numbers (i.e., hsa-miR-199a/hsa-miR-199b and hsa-miR-138-1/hsa-miR-138-2).[1]

Biogenesis of miRNA

miRNA was first observed in C. elegans as an 18–23 nt RNA molecule that complements the 3' untranslated target transcript region (UTR), including the lin-4 and let-7 genes. miRNAs are thought to be transcribed from DNA which are not translated but regulate the expression of other genes. miRNA biogenesis begins in the nucleus. The primary transcript of a miRNA gene, namely pri-miRNA, is a long RNA transcript that is a miRNA precursor. Pri-miRNA is affected by RNA polymerase

II and III. The resulting molecule is a hairpin-like structure, which contains a loop at one end (hair-pin like structure).[5] These primordial miRNA precursors, which usually consist of hundreds of nucleotides, are then processed sequentially by two RNase III enzymes. First, pri-miRNAs are processed in the nucleus by the Drosha ribonuclease or DCGR8 into pre-miRNAs, which are new hairpin-like structures with about 70 nucleotides. With the help of Exportin-5, the pre-miRNA is transported from the nucleus to the cytoplasm. Next, the pre-miRNA in the cytoplasm is cleaved again by the Ago2/Dicer complex leading to a short and mature double-stranded miRNA. Next, one of the strands, usually known as the guide strand, will be integrated into the RNA-induced silencing complex (RISC), while the other, known as the passenger strand, will degraded, although on several occasions it was also found to be functional. In most cases, the strand containing the less stable 5′ end or the uracil early is more likely to be selected as the guide strand. In those situations, where the passenger strand is not degraded and both are incorporated into the miRISC complex, the mature miRNA in the guide strand will be the dominant one.

miRNA is also referred to as the master of regulatory genes. Interactions between miRNAs and target mRNAs can lead to mRNA degradation through cleavage, mediated by endonucleases in RISC, where miRNAs bind. The small size of miRNAs and tolerability of partial complementarity with their targets allow miRNAs to interact with many mRNAs. Therefore experimental verification is required because the cellular effects of miRNAs depend on the function of the target mRNA. One miRNA may have opposite biological effects in different cells. Therefore, to understand the roles of different miRNAs, the transcript profile of mRNA in each cell type must be taken into account. Given the complexity of miRNA-mRNA interaction networks, bioinformatics modeling can be used for deeper understanding of these relationships and recognition of additive or contradictory effects of different miRNAs. Each mRNA can be the target of more than one miRNA. To understand the desired miRNA effect, interactions between the target mRNA and other miRNAs must be considered, because the results of such interactions may reflect the end result of multiple miRNAs being expressed in the same cell. Therefore, it can be argued that a miRNA can regulate opposite processes in different cell types, such as increased cell proliferation and apoptosis rate. Nonetheless, similar levels of specific miRNAs may have different biological effects, as these depend on targets and interactions with other molecules in a given cell type.[6] Due to this function, miRNAs can serve as master gene regulators, influencing a variety of cellular pathways important for normal cellular function as well as cancer development.[3]

Circulating miRNAs

Another important role played by miRNAs is signaling between cells. Although most miRNAs are found inside cells, a large proportion migrates outside and can be found in body fluids. These are referred to as circulating miRNAs and are excreted in blood, urine, saliva, seminal fluid, breast milk and other fluids by tissue damage, apoptosis, and necrosis, or by active pathways, in microvesicles, exosomes, or by binding to proteins.[5] It is known that miRNAs are expressed in cells, their expression can also be found in various human tissues, one of which in this discussion is breast cancer. Because of the presence of RNase in blood, RNA is thought to be absent in ribonuclease-rich body fluids such as serum, or considered to be a result of cell death and lysis. While most miRNAs are detected in the cellular microenvironment, studies have shown that miRNAs are also present in extracellular or also called miRNAs circulating in the blood. The presence of miRNAs in blood indicates their potential as biomarkers. In addition, the expression profiles of extracellular miRNAs from different types of biofluids in

relation to different pathophysiological conditions showed specific patterns indicating that extracellular miRNAs may not be released passively from necrotic or injured cells, but rather be released selectively from cells. This raises the question of the stability of miRNAs in blood. In contrast to cellular miRNAs and other RNA species, which degrade in the extracellular environment within seconds, extracellular miRNAs are highly stable and can persist under unfavorable conditions for a long time. This shows that miRNA has resistance to endogenous RNase activity. Due to its stability and resistance to endogenous RNase activity, this miRNA has been proposed as a diagnostic and prognostic biomarker for diseases, such as cancer.[7,8]

The stability of circulating microRNAs is then related to their carriers, i.e. different types of microvesicles (microparticles, exosomes and other vesicular structures) provide protection of trapped miRNAs against digestion by RNase. Exosomes are small homologous membrane-bound vesicles (50–90 nm) that originate from endosomes and are present in almost all biological fluids. The main components of the exosome membrane are lipids and proteins. These vesicles can function as transmitters between cells to convey their contents, particularly miRNAs, from one cell to another. Formation and release of exosomes by cells is a complex and coordinated process that requires enzymatic activation and energy (ATP) and the miRNA profile of exosomes derived from bio-fluids and culture media is largely different from that of their parent cells. Exosomes can carry a wide variety of molecules including lipids, proteins, DNA, mRNAs and miRNAs, of which miRNAs receive research priority because of their diverse functions and implications.[7]

Pathogenesis of miRNAs in Cancer Development

Cancer-associated miRNAs can be classified according to their function to mRNAs target as tumor suppressor miRNAs (tsmiRs) and oncogenic miRNAs or oncomiRs. This classification is based on the ability of miRNA to interfere with processes related to carcinogenesis, including mechanisms related to cell migration and invasion, apoptosis, and proliferation. Given that most miRNAs inhibit expression of target mRNAs, miRNAs and target RNAs may have opposite classifications. Tumor suppressor miRNAs regulate the expression of mRNAs necessary for cell division or survival, whereas oncomiR is more strongly expressed in cancer cells and downregulates tumor suppressor genes, leading to increased cancer cell division. Changes in miRNA expression patterns detected in cancer cells emphasize the significant role of miRNAs in cancer development.[9]

microRNA has a role in the development of cancer cell regulation, one of which is through its interaction with EMT. The epithelial-mesenchymal transition (EMT) is a complex molecular and cellular process, in which polarized epithelial cells lose their epithelial features and acquire mesenchymal characteristics (increased motility, invasion, and resistance to apoptosis). The main feature of EMT is the loss of E-cadherin. Snail1, Slug, ZEB1, and SIP1 (ZEB2) are major transcription factors that suppress E-cadherin either directly or indirectly to promote EMT. Twist1 indirectly suppresses E-cadherin and exerts important downstream effects on cellular function. Loss of E-cadherin increases Twist1 and ZEB1 expression. Signals from the tumor microenvironment can also significantly influence EMT. For example, hypoxic conditions will result in Snail translocation through promotion of the Wnt/β-catenin pathway, which can lead to delayed migration and sustained invasion. The reverse process of EMT is the mesenchymal-epithelial transition (MET), which reestablishes apical-basal polarity, tight junctions, and expression of cell-cell adhesion molecules such as E-cadherin. MET is thought to be essential for tumor

growth in distant organs (distant metastases), supplying tumor cells with epithelial characteristics similar to cells in the primary tumor.[2]

Studies have identified a number of miRNAs that may participate in the TGF-β-induced EMT pathway. For example, miR-21 and miR-31 expression significantly increased in response to TGF-β stimulation. miR-21 and miR-31, together with TGF-β, synergistically increase EMT by targeting T-cell lymphoma invasion and metastasis-inducing protein 1 (TIAM1). Expression of the miR-200 family cluster (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) was markedly decreased in EMT cells and invasive breast cancer cell lines.[2]

Role of miRNAs in Metastatic Breast Cancer

Breast cancer-associated miRNAs can be further divided into oncogenic miRNAs (oncomiRs) and tumor suppressor miRNAs (tsmiRs). In various cancer cases, oncomiRs were found to be overexpressed or upregulated, whereas tsmiRs were downregulated.[10] Both oncomiRs and tsmiRs (Table 1 and Table 2) critically regulate the development and progression of breast tumors by participating in complex tissue regulation.

Abnormal expression of miRNAs is also considered a potential biomarker in breast cancer because they are stably detected in tumor tissues and in patient body fluids, including blood, serum, plasma, and saliva.[11,12] miRNAs in body fluids, also called circulating miRNAs, are highly stable, packaged into extracellular microparticles or bound to lipoproteins, which protect them from RNase digestion. MiRNA profiling has been effectively used to classify breast cancer patients as treatment-responsive or non-responsive groups.[13] Therefore, miRNAs have been shown to unequivocally potentially regulate breast cancer progression and be used as novel diagnostic, prognostic, and predictive biomarkers of breast cancer.[14]

Table 1. List of several oncomiRs and their oncogenic pathways.[15]

oncomiRs	Target	Oncogenic pathways	Oncogenic behavior
miR-20b	PTEN	PI3K/AKT/mTOR	Metastases
miR-21	PTEN Cdc25,MSH2 Mespin	PI3K/AKT/mTOR Repair DNA	Proliferation
miR-93	LATS2	Formasi mikrotubul	Metastases
miR-103/107	Dicer DAPK, KLF4	Proses miR	Proliferation
miR-142	APC	Wnt/ β-catenin	Metastases
miR-146	BRCA1	Repair DNA NFκB	Angiogenesis
miR-182	BRCA1	Repair DNA	Metastases
miR-373	CD44	Wnt/ β-catenin	Self renewal
miR-489	E-Cadherin Smad3	Wnt/ β-catenin	Metastases
mir-520c	CD44	Wnt/ β-catenin	Proliferation

Table 2. List of several tsmiRNAs [15,16]

tsmiRs	Target	Influenced pathways	Tumor suppression behavior
miR-22	CDK6, SIRT1, SP1	Cell aging	Anti-metastatic
miR-29b	Integrin β1 MMP2, TIAM1	Focal adhesions Cell cycle Epigenetics	Anti-proliferation Anti-metastatic Anti-angiogenesis
miR-107	CDK8	Cell cycle	Anti-proliferation Anti-metastatic
miR-128	BMI1, ABCC5	Cell cycle	Anti-self-renewing
miR-143	HER3	PI3K/AKT	Anti-proliferation Anti-metastatic
miR-145	EGF receptor, c-Myc, VEGF, N-cadherin, HIF-2α, Mucin 1, HER3	Cell cycle MAP kinase PI3K/AKT	Anti-angiogenesis Anti-proliferation Anti-metastatic
miR-205	HER3, E2F, P53	MAP kinase	Anti-metastatic Anti-proliferation Anti-self-renewing

The molecular profiling of breast cancer has provided interesting observations of miRNA dysregulation in this disease. In one study, miRNA profiling was used to differentiate between luminal and basal epithelial subsets of breast tumors. In another study, miRNAs have been successfully applied to differentiate breast tumor subtypes based on their hormone status, namely estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor (ErBB2/HER2). This leads to the possible use of miRNAs as potential diagnostic and prognostic markers in therapeutic interventions. Genomic mRNA profiling using microarrays has further classified breast cancer into four intrinsic subtypes: luminal A (ER+ and low grade), luminal B (ER+ and high grade), HER2, and basal like (mainly triple negative). MiRNA profiling has been used to identify the multiple roles of miRNAs in tumorigenesis and breast cancer development. For example, expression of Dicer, one of the most important components of miRNA processing, is attenuated by upregulation of miR-103 and miR-107 particularly in metastatic breast cancer. The miR-200 family has been investigated for its function of maintaining the integrity of the epithelial phenotype in the breast epithelium and its expression is often downregulated or lost in invasive and metastatic breast cancer.[16]

Proliferation and cell cycle

Cell proliferation is the most important feature of breast cancer and dysregulation is the main cause of breast tumorigenesis. The development of the cell cycle is essential for maintaining the balance of proliferation and suppression of cell growth. Several studies have suggested that miRNAs have a role in the initiation and development of breast cancer through their interactions in various signaling pathways. Some of them are miRNA interactions with TGF-β, Wnt/ β-catenin, PI3K, MMP, NF-κB, and MAPK.[17,18] Cell cycle abnormalities that are frequently observed in breast cancer, including loss of function of retinoblastoma (Rb), reduced inhibition of overexpression of cyclin-dependent kinases (CDK) p21 (Waf1/Cip1) and p27 (Kip1), as well as increased amounts of cyclin types D and E. Cyclin D1 encodes a key regulator of the cell cycle transition from G1 to the DNA synthetic phase and is overexpressed in more than 50% of breast cancers, serves as a rate-limiting factor for human breast cancer cell proliferation in vivo and in vitro. Cyclin E, another important cell cycle regulator, is overexpressed in more than 10% of breast cancers and is a strong prognostic predictor of early-stage breast cancer as well as a significant determinant of tumor aggressiveness. miRNAs interact with inhibitors E2F, Rb, cyclins, CDKs, and CDK, conferring the potential to regulate cellular division and cell cycle progression. The mechanisms governing cell cycle control by miRNAs are increasingly well understood. Five groups of miRs, including the miR-15a/16 cluster, miR-17/20 cluster, miR-221/222 cluster, and the let-7 and miR-34 families, can regulate cell cycle progression by directly targeting cell cycle regulators. miR-34 is involved in cell cycle regulation by suppressing the expression of E2F, cyclin D1, and cyclin E. miR-34 itself is a direct transcription target of p53. The miR-17/20 cluster is transcriptionally regulated by myc, E2Fs, and cyclin D1, and in turn regulates expression of E2F, pRb, and cyclin D1 at the translational level. miR-15/16 regulates cell cycle control by inhibiting cyclin D1, cyclin E, and CDK4/6.[19]

Cell senescence is a state of irreversible cell cycle arrest, which limits the proliferative capacity of cells exposed to stress signals, but the cells do not actually die. Oncogene activation can induce this cellular senescence, which is a natural barrier against tumorigenesis. However, if there is inactivation of tumor suppressors such as P53 and RB, the cells will regain their proliferative ability.[20] While oncogenic stress leads to P53 activation and P21 induction, resulting in cell cycle arrest and senescence, introduction of miR-372 or

miR-373 prevents CDK inhibition by targeting the tumor suppressor LATS2 directly, thereby enabling the proliferation and tumorigenesis of transformed cells.[21]

Invasion and metastasis

Invasion is a series of processes that begins with damage to the basement membrane, which is mostly composed of type IV collagen. As a result of damage to the basement membrane, it will allow cancer cells to enter the stroma and connective tissue. To be able to invade locally, tumor cells must acquire an additional set of gene activity or characteristics, in addition to being able to grow without restriction in the primary tumor. The epithelial-mesenchymal transition (EMT) regulates the steps of local invasion of cancer at the epithelial stage. EMTs were controlled by a transcription program consisting of Twist, Slug, Snail, ZEB1, ZEB2, and miR-200s.[23] These factors respond to external stimuli, such as TGFβ, WNT, or hypoxia, and act together to induce changes in the surface molecular profile, including loss of E-cadherin and gain of N-cadherin or vimentin. There are also studies showing that reduced levels of E-cadherin in cancer cells might facilitate the Wnt/β-catenin signaling pathway. Vimentin overexpression and the switch of E-cadherin to N-cadherin expression are also EMT promoters that contribute to the metastatic phenotype of cancer cells.[18]

To be able to spread from the breast to other organs, breast cancer cells or breast cancer cells (BCC) need to go through a series of processes, which are commonly referred to as metastatic cascades.[24] This cascade process consists of: (1) local infiltration of malignant cells into surrounding tissues ; (2) intravasation, namely transendothelial migration (TEM) of BCC into the blood vessels to reach circulation; (3) circulation and survival in the bloodstream; (4) capture and extravasation to target organs; and (5) proliferation and colonization in competent organs. It is known that miRNAs can influence and play a role in the metastatic process through this cascade process.[25] Many miRNAs have been described to regulate EMT and are involved in BCC detachment and local invasion. Specifically in breast cancer, members of the miR-200 family (miR-141, miR-429, miR-200a, miR-200b and miR-200c) have been shown to be potent regulators of EMT, by being highly expressed in epithelial cells and downregulated in cells with a mesenchymal phenotype. This family promotes epithelial state by downregulating the transcriptional repressor of the ZEB1/ZEB2 epithelial genes. ZEB1/ZEB2 are known to induce EMT by strongly suppressing E-cadherin expression, while ZEB2 directly activates vimentin.[25,26]

MiR-373 and miR-520c promote BCC detachment by mediating loss of cell-ECM interaction by downregulating CD44, a cell surface receptor for hyaluronan, a major component of the ECM. Another miRNA that is upregulated in BCC is miR-9, which promotes loss of cell-cell interactions by targeting CDH1, the gene that encodes the epithelial cell adhesion molecule E-cadherin. Thus, by downregulating E-cadherin, miR-9 may also be involved in EMT regulation. Collectively, by influencing EMT and BCC invasion, miRNAs may become a determinant of patient prognosis and may become valuable therapeutic targets.[27]

Apoptosis

Deregulation of apoptosis is an important step in cancer development as it allows genetically unstable cells to survive and accumulate further mutations that eventually lead to tumorigenesis. One of the mechanisms by which miRNAs affect tumor development is by regulation of proteins involved in the process of apoptosis. miRNAs that function to promote or inhibit apoptosis are called pro- and anti-apoptotic miRNAs. As many as 22 miRNAs have been reported so far to be involved in induction of apoptosis indicating them as tumor suppressors. Tumor suppressor miRNAs prevent tumor development by negatively regulating the expression of

genes that promote cell proliferation, differentiation, migration, or apoptosis. A total of 11 miRNAs have been shown to inhibit apoptosis indicating that they are oncomiRs in breast cancer. Among them are miR-21, miR-155, miR-96, miR-182, miR-196a, and miR-210 which were found to be highly regulated in breast cancer patients.[28]

Hypoxia and Angiogenesis

Angiogenesis is a complex process of forming new blood vessels from pre-existing ones. It begins by stimulating, migrating, proliferating, and differentiating endothelial cells in response to signals from surrounding tissues, such as hypoxia (low oxygen levels). Each step is determined by various factors called pro- and anti-angiogenesis.[29] HIF (hypoxia inducable factor) is a family of hypoxia-induced transcription factors that regulate various critical breast cancer pathological processes, including stem cell homeostasis, cell proliferation, metastasis, and therapeutic resistance. In addition, VEGF is another important pro-angiogenic factor that stimulates engorgement of blood vessels by endothelial cells. Therefore, knowledge of the regulatory mechanisms of these hypoxia/angiogenesis-related genes by miRNAs may inform the development of promising anti-angiogenic agents for breast cancer. miR-210 was the most consistently and significantly induced miRNA during hypoxia.[18]

miRNA angioregulators can suppress or promote angiogenesis through hyperactivation or hypoactivation of tumor cell signaling mediators. Aggressive breast cancer exhibits angioregulatory miRNAs and increased pro-angiogenic factors that can mediate communication between tumor cells and their microenvironment. Phosphoinositide 3-kinase (PI3K)/AKT/mTOR/ vascular endothelial growth factor (VEGF), mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription 3 (STAT3), and Notch are the main signaling pathways that can be affected by miRNAs angioregulators in breast cancer. The PI3K/AKT and MAPK signaling pathways are normally hyperactive in a variety of human malignancies. This pathway regulates various cellular processes including cell growth, apoptosis, proliferation, migration, and survival.[30] VEGF, a pro-angiogenic factor and prognostic marker with various types of cancer including breast cancer, plays an important role in tumor development and metastasis. In breast cancer, VEGF plays a key role in disease development through its influence on tumor angiogenesis and through its autocrine function in breast cancer cell migration and invasion.[31] VEGF and cyclin D1, and all are related to tumorigenesis. Therefore, detection of miRNA-373 in circulating blood could help better early diagnosis of breast cancer, and the prognosis of the disease.[32]

microRNA as a Potential Biomarker in Breast Cancer

Some miRNAs, which are present in the circulating blood, can be measured in peripheral blood and serum easily.[33] Due to their high tissue specificity, high stability, and aberrant expression in different tumor types, miRNAs are considered as specific biomarkers with potential diagnostic, predictive and prognostic. As a diagnostic biomarker, microRNA can be used to differentiate tumors from healthy tissue, detect early stages of cancer, and can classify different subgroups of breast cancer. Early detection of breast cancer is important to reduce the risk of disease. Current diagnostic methods for early detection of breast cancer are still limited to several procedures such as tissue biopsy and histological examination. However, a sensitive and specific marker is still needed. Because miRNAs have small size, high specificity, and greater stability, they can be used as diagnostic and predictive biomarkers. Diagnostic biomarkers based on their ability to discriminate between normal patients and breast cancer, predictive biomarkers based on response to

conventional therapy (sensitivity/ resistance).[28] Several studies have demonstrated the role of miRNAs in the diagnosis and prognosis of breast cancer. Research by Iori et al. in 2005 identified 13 miRNAs that can differentiate breast cancer from normal breast tissue with 100% accuracy.[34]

In a recent study, there were nine circulating miRNAs, namely miR-15a, miR-18a, miR-107, miR-133a, miR-139-5p, miR-143, miR-145, miR-365, miR-425 was able to discriminate between early-stage breast cancer and healthy controls.[35] A meta-analysis of miR-155 demonstrated a highly sensitive and specific diagnostic accuracy. The meta-analysis stated that circulating miR-155 had a sensitivity of 79% and a specificity of 85% in the diagnosis of breast cancer. Its expression was found to be increased in the sera of patients with breast cancer.[36] By comparing the miRNA profiles between sera samples from breast cancer patients and healthy individuals, Chan et al. identified four miRNAs as diagnostically significant markers, namely miR-1, miR-92a, miR-133a, and miR-133b whose expression was found to be increased in serum.[37] Cuk et al. found four other miRNAs that were upregulated in the plasma of patients with breast cancer, capable of detecting stage I or II breast cancer, making them attractive candidates for early detection of breast cancer.[38] The four miRNAs, miR-148b, miR-376c, miR-409-3p and miR-801, significantly experienced upregulation in the plasma of breast cancer patients.[38] In another study, it was suggested that the combination of miR-145 and miR-451 was the best biomarker ($p<0.0001$) in differentiating breast cancer from healthy controls and all other types of cancer. The positive predictive value is 88% and the negative predictive value is 92%. Changes in plasma levels of this miRNA have been detected not only in advanced but also early stages of tumors. The positive predictive value for ductal carcinoma in situ (DCIS) is 96%.[39] A prospective study identified three serum miRNAs (miR-18a, miR-181a, miR-222) that were significantly expressed in women who eventually developed breast cancer compared with breast cancer-free controls. This study introduces a new perspective on the function of miRNAs by describing their potential to predict an increased risk of developing breast cancer.[40] The following is a summary table of several miRNAs that have been studied as potential biomarkers for breast cancer diagnosis.[41]

Table 3. Several miRNAs as markers for early diagnosis of breast cancer. [41]

miRNA	Expression (in breast cancer vs normal)	Sample
miR-15a	*Upregulation*	Serum
miR-18a	*Upregulation*	Serum
miR-107	*Upregulation*	Serum
miR-425	*Upregulation*	Serum
miR-133a	*Downregulation*	Serum
miR-139-5p	*Downregulation*	Serum
miR-143	*Downregulation*	Serum
miR-145	*Downregulation*	Serum
miR-365	*Downregulation*	Serum
miR-155	*Upregulation*	Serum
miR-1	*Upregulation*	Serum
miR-133b	*Upregulation*	Serum
miR-92a	*Upregulation*	Serum
miR-148b	*Upregulation*	Plasma
miR-376c	*Upregulation*	Plasma
miR-409-3p	*Upregulation*	Plasma
miR-801	*Upregulation*	Plasma
miR-16	*Upregulation*	Plasma and tissue
miR-21	*Upregulation*	Plasma and tissue
miR-451	*Upregulation*	Plasma and tissue

miRNAs are also known as predictors for disease development and progression. Using miRNAs as predictors can be beneficial because this method has the advantage of

being non-invasive. miRNAs can be biomarkers in cancer for the following reasons. First, the expression of this molecule changes in cancer, the expression of these molecules is tissue specific, these molecules are stable in the FFPE network. miRNAs have also recently been studied to differentiate subgroups in breast cancer.[33] Based on the presence or absence of generally evaluated hormone receptors, namely estrogen (ER), progesterone (PR) and human epidermal growth factor 2 (HER2), breast cancer is divided into four main subtypes, namely luminal A (ER+ and/or PR+, HER2−) , luminal B (ER+ and/or PR+/HER2+), HER2 positive (ER−/PR−/HER2+) and triple negative/basal cell like/BCL type (ER−/PR−/HER2−). Luminal A tumors proved to have a good prognosis and less aggressive behavior when compared to the BCL or HER2 positive group. The BCL subtype has been associated with aggressive behavior, less response to hormonal therapy, and shorter survival.[42]

There are several miRNAs used for breast cancer subgroups, as shown in Table 4. miRNAs can be used even as a diagnostic tool to check whether a tumor has metastasized. miR-21 and miR-155 are increased in metastatic tumors, whereas the miR-200 family is decreased in metastatic tumors. miR-21 is also seen, especially in invasive breast cancer. The profiling capabilities of miRNAs in breast tumor subgroups can be used today. Therefore, this ability may help select cancer patients to receive adjuvant therapy. MiRNA profiling can also be useful in discovering the molecular basis of breast cancer subgroups, and therefore can be effective in defining new therapeutic targets.[33]

Table 4. miRNAs used to differentiate breast cancer subgroups.[33]

Breast Cancer Subgroups	Change in miRNA
HER-2 positive	Upregulation miR-150, miR-142-3p, miR-142-5p, miR-148a, miR-106b, miR-93, miR-155, miR-25, miR-187
Luminal A	Overexpression of miR-126, miR-136, miR-100, miR-99a, miR-145, miR-10a, miR-199a/b, miR-130a, miR-30a-3p, miR-30a-5p, miR- 224, miR-214, let-7a/b/c/f, miR-342
Luminal B	Overexpression of miR-106a/b, miR-93, miR-25, miR-10a, miR-30a-3p, miR-30a-5p, miR-224, let-7b/c/f, miR-342c

In another study, 453 miRNAs in 29 early-stage breast cancer tumors were profiled, identifying which miRNAs accurately predicted ER, progesterone receptor (PR) and HER2 status. miR-342 showed highest expression in ER positive and HER2/neu-positive Luminal B tumors, which was verified in a recent study and showed reduced expression in TNBC. MiR-520 is downregulated in ER and PR positive tumors.[41]

As a predictive biomarker, miRNA is thought to provide information based on response to therapy, hormones and chemotherapy. Predictors help to individualize breast cancer therapy and diagnosis, correlate with response to treatment given, and determine treatment benefit. Recently, several miRNAs have been described as therapeutic targets. Chemotherapy is the current method of therapy used for breast cancer, especially for the triple-negative form, but patients usually do not receive the desired results. miRNAs can suggest new alternative therapy methods to produce better results of breast cancer chemotherapy. Studies have shown that miRNA expression levels may be related to a patient's response to chemotherapy. For example, upregulation of miR-663 occurs in breast cancer and is associated with chemoresistance. miRNAs may have a role in drug resistance. miR-326 causes reduced drug resistance and is downregulated in advanced breast cancer. miR-21 has an oncogenic role in breast cancer, so suppression of this miRNA can delay tumor growth. On the other hand, induction of miRNAs with a tumor suppressor role can lead to tumor shrinkage or prevent tumor development. Similarly, in a study using anti-miR-21, the amount of this miRNA was reduced, and through inhibition of cell proliferation and

promotion of apoptosis, led to decreased cell growth. Therefore, the combination of anti-miR-21 with chemotherapy can reduce tumor growth. Given that this miRNA (miR-21) has an oncogenic role, suppression of this molecule could sensitize tumor cells to anticancer therapies.[33]

As prognostic biomarkers, miRNAs are known to provide information about disease course and outcomes such as survival and recurrence. Several gene expression studies have identified new or improved miRNA prognostic markers, providing information about disease course and outcome in different patient subgroups. Broadly speaking, miRNAs can be divided into miRNAs associated with a positive prognosis and miRNAs associated with a poor prognosis.

CONCLUSION

In this review article, it has been reviewed that miRNAs play an important role in regulating breast cancer development and their clinical role in diagnosis, prognosis and therapeutic targets in breast cancer patients. An increasing number of studies have reported the role of circulating miRNAs in providing prognostic and diagnostic value in breast cancer. miRNAs affect breast cancer development through multiple pathways, either as oncomiR or tsmiR. Thus, miRNAs have clinical potential in terms of breast cancer diagnosis and prognosis. miRNAs can also be potential biomarkers to help predict tumor response to certain chemotherapeutic agents. The potential of miRNAs is not limited to their use as biomarkers for breast cancer. miRNA also plays a role in controlling specific cellular processes in breast cancer, such as invasion, migration, proliferation, and apoptosis. miRNAs also act as tumor suppressor or oncogenic miRNAs that facilitate the occurrence, progression, and metastasis in breast cancer. Further research on miRNA molecular mechanisms in the regulation of tumorigenesis or breast cancer development may provide new therapies for breast cancer.

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REFERENCES

1. Robinson VL. Rethinking the central dogma: Noncoding RNAs are biologically relevant. *Urol Oncol Semin Orig Investig* 2009; 27: 304–306.
2. Saw PE, Xu X, Chen J, Song EW. Non-coding RNAs: the new central dogma of cancer biology. *Sci China Life Sci* 2021; 64: 22–50.
3. MacFarlane L-A, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. *Curr Genomics* 2010; 11: 537.
4. Sun W, Julie Li YS, Huang H Da, et al. MicroRNA: A master regulator of cellular processes for bioengineering systems. *Annu Rev Biomed Eng* 2010; 12: 1–27.
5. Condrat CE, Thompson DC, Barbu MG, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells* 2020; 9: 1–32.
6. Jorge AL, Pereira ER, Oliveira CS de, et al. MicroRNAs: understanding their role in gene expression and cancer. *Einstein (Sao Paulo)* 2021; 19: eRB5996.
7. Hamam R, Hamam D, Alsaleh KA, et al. Circulating microRNAs in breast cancer: novel diagnostic and prognostic biomarkers. *Cell Death Dis* 2017; 8: e3045.
8. Sohel MH. Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges. *Achiev Life Sci* 2016; 10: 175–186.
9. Garzon R, Fabbri M, Cimmino A, et al. MicroRNA expression and function in cancer. *Trends Mol Med* 2006; 12: 580–587.
10. Joshi M, Singh Sodhi K, Pandey R, et al. MicroRNA: Biomarker for cancer diagnosis and prognosis. *J Pharm Biomed Sci* 2014; 4: 600–610.
11. Ashby J, Flack K, Jimenez LA, et al. Distribution profiling of circulating MicroRNAs in serum. *Anal Chem* 2014; 86: 9343–9349.

12. Shen J, Stass SA, Jiang F. MicroRNAs as potential biomarkers in human solid tumors. *Cancer Lett* 2013; 329: 125–136.
13. Cava C, Bertoli G, Ripamonti M, et al. Integration of mRNA expression profile, copy number alterations, and microRNA expression levels in breast cancer to improve grade definition. *PLoS One* 2014; 9. doi:10.1371/JOURNAL.PONE.0097681.
14. Yang Z, Liu Z. The Emerging Role of MicroRNAs in Breast Cancer. *J Oncol* 2020; 2020. doi:10.1155/2020/9160905.
15. Kaboli PJ, Rahmat A, Ismail P, Ling KH. MicroRNA-based therapy and breast cancer: A comprehensive review of novel therapeutic strategies from diagnosis to treatment. *Pharmacol Res* 2015; 97: 104–121.
16. Goh JN, Loo SY, Datta A, et al. microRNAs in breast cancer: Regulatory roles governing the hallmarks of cancer. *Biol Rev* 2016; 91: 409–428.
17. Abolghasemi M, Tehrani SS, Yousefi T, et al. MicroRNAs in breast cancer: Roles, functions, and mechanism of actions. *J Cell Physiol* 2020; 235: 5008–5029.
18. Loh HY, Norman BP, Lai KS, et al. The regulatory role of microRNAs in breast cancer. *Int J Mol Sci* 2019; 20. doi:10.3390/IJMS20194940.
19. Yu Z, Baserga R, Chen L, et al. MicroRNA, cell cycle, and human breast cancer. *Am J Pathol* 2010; 176: 1058–1064.
20. Pérez-Mancera PA, Young ARJ, Narita M. Inside and out: the activities of senescence in cancer. *Nat Rev Cancer 2014 148* 2014; 14: 547–558.
21. Voorhoeve PM, Sage C, Schrier M, et al. A Genetic Screen Implicates miRNA-372 and miRNA-373 As Oncogenes in Testicular Germ Cell Tumors. 2006; : 1169–1181.
22. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. *Cell* 2011; 147: 275–292.
23. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; 133: 704–715.
24. Custódio-Santos T, Videira M, Brito MA. Brain metastasization of breast cancer. *Biochim Biophys Acta - Rev Cancer* 2017; 1868: 132–147.
25. Sereno M, Videira M, Wilhelm I, et al. miRNAs in Health and Disease: A Focus on the Breast Cancer Metastatic Cascade towards the Brain. *Cells* 2020; 9. doi:10.3390/CELLS9081790.
26. Bindels S, Mestdagt M, Vandewalle C, et al. Regulation of vimentin by SIP1 in human epithelial breast tumor cells. *Oncogene 2006 2536* 2006; 25: 4975–4985.
27. Zhao M, Ang L, Huang J, Wang J. MicroRNAs regulate the epithelial–mesenchymal transition and influence breast cancer invasion and metastasis. *Tumor Biol* 2017; 39: 1–8.
28. Sharma S, Patnaik PK, Aronov S, Kulshreshtha R. ApoptomiRs of breast cancer: Basics to clinics. *Front Genet* 2016; 7: 1–14.
29. Hussen BM, Abdullah ST, Rasul MF, et al. MicroRNAs: Important Players in Breast Cancer Angiogenesis and Therapeutic Targets. *Front Mol Biosci* 2021; 8: 1043.
30. Soheilifar MH, Masoudi-Khoram N, Madadi S, et al. Angioregulatory microRNAs in breast cancer: Molecular mechanistic basis and implications for therapeutic strategies. *J Adv Res* 2022; 37: 235–253.
31. Perrot-Applanat M, Di Benedetto M. Autocrine functions of VEGF in breast tumor cells. *http://dx.doi.org/104161/cam23332* 2012; 6: 547–553.
32. Bakr NM, Mahmoud MS, Nabil R, et al. Impact of circulating miRNA-373 on breast cancer diagnosis through targeting VEGF and cyclin D1 genes. *J Genet Eng Biotechnol* 2021; 19: 1–10.
33. Mehrgou A, Akouchekian M. Therapeutic impacts of microRNAs in breast cancer by their roles in regulating processes involved in this disease. *J Res Med Sci | Publ by Wolters Kluwer* 2017. doi:10.4103/jrms.JRMS_967_16.
34. Iorio M V, Ferracin M, Liu CG, et al. MicroRNA Gene Expression Deregulation in Human Breast Cancer. *Cancer Res* 2005; 65: 7065–7070.
35. Kodahl AR, Lyng MB, Binder H, et al. Novel circulating microRNA signature as a potential non-invasive multi-marker test in ER-positive early-stage breast cancer: A case control study. *Mol Oncol* 2014; 8: 874–883.
36. Wang F, Hou J, Jin W, et al. Increased circulating microRNA-155 as a potential biomarker for breast cancer screening: A

meta-Analysis. *Molecules* 2014; 19: 6282–6293.
37. Chan M, Liaw CS, Ji SM, Tan HH, Wong CY, Thike AA *et al.* Identification of circulating microRNA signatures for breast cancer detection. *Clin Cancer Res* 2013; 19: 4477–4487.
38. Cuk K, Zucknick M, Heil J, et al. Circulating microRNAs in plasma as early detection markers for breast cancer. *Int J Cancer* 2013; 132: 1602–1612.
39. Ng EKO, Li R, Shin VY, et al. Circulating microRNAs as Specific Biomarkers for Breast Cancer Detection. *PLoS One* 2013; 8: 4–13.
40. Godfrey AC, Xu Z, Weinberg CR, et al. Serum microRNA expression as an early marker for breast cancer risk in prospectively collected samples from the Sister Study cohort. *Breast Cancer Res* 2013; 15. doi:10.1186/bcr3428.
41. van Schooneveld E, Wildiers H, Vergote I, et al. Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management. *Breast Cancer Res* 2015; 17: 1–15.
42. Shomaf M, Masad J, Najjar S, Faydi D. Distribution of breast cancer subtypes among Jordanian women and correlation with histopathological grade: molecular subclassification study. *JRSM Short Rep* 2013; 4: 2042533313490516.

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