MicroRNAs: Important Players in Breast Cancer Angiogenesis and Therapeutic Targets

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The high incidence of breast cancer (BC) is linked to metastasis, facilitated by tumor angiogenesis. MicroRNAs (miRNAs or miRs) are small non-coding RNA molecules that have an essential role in gene expression and are significantly linked to the tumor development and angiogenesis process in different types of cancer, including BC. There’s increasing evidence showed that various miRNAs play a significant role in disease processes; specifically, they are observed and over-expressed in a wide range of diseases linked to the angiogenesis process. However, more studies are required to reach the best findings and identify the link among miRNA expression, angiogenic pathways, and immune response-related genes to find new therapeutic targets. Here, we summarized the recent updates on miRNA signatures and their cellular targets in the development of breast tumor angiogenetic and discussed the strategies associated with miRNA-based therapeutic targets as anti-angiogenic response.

Keywords: breast cancer, microRNA, vascular endothelial growth factor, angiogenesis, therapeutic target

INTRODUCTION

Breast cancer (BC) is one of the more prevalent occurring forms of cancer in females and the second most frequently occurring type of cancer worldwide (Siegel et al., 2021), and 90 percent of breast cancer deaths are due to the formation of distant organ metastases (Chaffer and Weinberg, 2011). In breast cancer, angiogenesis, or the development of new blood vessels, is essential for both local tumor growth and distant metastasis (Folkman, 1971). Angiogenesis is a multi-step complicated process characterized by 1) MMP damages the basement membranes of tissues on a local basis, causing degradation and hypoxia immediately, 2) endothelial cells migrate in response to angiogenic factors, 3) proliferation and stabilization of endothelial cells, and 4) angiogenic factors still influence the angiogenic mechanism. The development of new vessels is regulated by a concerted action of multiple cytokines and growth factors such as anti-angiogenic and proangiogenic factors (Figure 1). miRNAs regulate tumor angiogenesis in two ways: They can inhibit or promote it (Leone et al., 2019) (Figure 2). Victor Ambros’ lab was the first to publish an article regarding miRNAs (Lee et al., 1993), which have a length of 21–25 nt, are known as short non-coding RNAs (short-RNAs) and regulate the expression of a variety of cellular proteins by modulating their messenger RNA levels (Lewis et al.,...
They regulate gene expression in health and disease cells by binding to the 3′-untranslated region (UTR) or other regions such as the 5′ UTR, gene promoters, and the coding sequence (Broughton et al., 2016). Also, it has been established that crosstalk exists between long non-coding RNAs and miRNAs, where these two networks interact and form complex networks in gene regulation pathways (Ghafouri-Fard et al., 2021a; Ghafouri-Fard et al., 2021b; Dastmalchi et al., 2021).

According to TargetScan studies, miRNAs regulate one-third of all human genes (Lewis et al., 2005) and are essential regulators of a variety of processes, including cancer-related developments like angiogenesis (Goradel et al., 2019), metastasis (Martello et al., 2010), and drug resistance (Taheri et al., 2021). For example, global miRNA depletion inhibits the angiogenic process because miRs control the angiogenesis process (Chen et al., 2014). In addition, several investigations have described miRNA signatures in clinical breast specimens and cell lines. In particular, miRNAs can influence angiogenesis directly by affecting endothelial cell function or indirectly by changing the production of proteins that prevent or promote angiogenesis (Wang et al., 2018a). Consequently, miRNAs have attracted attention as potential targets for new anti-angiogenic therapies.

In this review, we summarized the recent updates on miRNA signatures and their cellular targets in the development of breast tumor angiogenic and discussed the strategies associated with miRNA-based therapeutic potential as anti-angiogenic response.

In the current review, the key questions attempted to be answered were “What are the miRNA’s contribution to breast cancer angiogenesis?”, “How can miRNAs contribute to BC angiogenesis? and “What are miRNA-based therapies for angiogenesis in BC?”

The terms “microRNA” or “miRNA,” “breast cancer,” “therapeutic target,” and “angiogenesis” were searched in PubMed and Google Scholar. To determine whether the retrieved papers were relevant to the subject, we evaluated the abstracts of all articles. Then, all related papers (in vitro, in vivo, and human-based research) to the subject were selected to be included in the study.

**BIOGENESIS OF MICRORNA**

MiRNAs are small, single-stranded non-coding RNAs that come from pri-miRNA, which is an early-stage transcript produced by...
RNA polymerase II (Zeng and Cullen, 2006). The pri-miRNAs are defined by the presence of one or many incomplete hairpin structures with a stem of about 33 base pairs (Bartel, 2004). Drosha and Dicer, two RNase III family ribonucleases, process the pri-miRNA precursor in two steps (Kuehbacher et al., 2008). First, Drosha cleaves the pri-miRNA in the nucleus to create a pre-miRNA of roughly 70 nucleotides in length transported to the cytoplasm through an exportin-5 (XPO5) process (Bohnsack et al., 2004; Wu et al., 2018). Then, Dicer converts the pre-miRNA into a mature, functional, double-stranded (ds) miRNA (Carthew and Sontheimer, 2009). The mature miRNA is then covalently coupled to RISC, a multiprotein complex that includes the AGO protein, necessary for RNA silencing. Through Watson-Crick base pairing, RISC utilizes the leading strand to target the mRNA complementary, and the other strand is eliminated (Gregory et al., 2005). The miRNA binding to a 3′-UTR causes mRNA destruction or translational inhibition. The level of mRNA degradation or translational repression is determined by the degree of miRNA complementarity to the 3′-UTR. In addition, RISC can target and trigger 5′-UTR mRNA translation (Vasudevan et al., 2007). Figure 3 shows the biogenesis of miRNA.

**TUMOR ANGIOGENESIS**

Angiogenesis is a multi-step and complex process to form new blood vessels from pre-existing ones. It begins with the stimulating, migrating, proliferating, and differentiating endothelial cells in response to signals from the surrounding tissue, such as hypoxia (low oxygen levels) (Bentley and Chakravartula, 2017). Each step is progressed and controlled by several pro- and anti-angiogenic factors.

VEGF, PDGF, and FGF promote new blood vessel development. VEGF-A, VEGF-B, VEGF-C, and VEGF-D are the primary forms of VEGF, and they bind to VEGF tyrosine kinase receptors (VEGFR-1-2-3) to regulate angiogenesis (Holmes and Zachary, 2005). Additionally, angiopoietins have a role in controlling endothelial cell signaling pathways. They associate with other angiogenic factors to bind with Tie-2 tyrosine kinase receptors and help the formation of endothelial tubes.

Despite the angiogenic factors, the body also produces endogenous anti-angiogenic substances such as TSP1 (Good et al., 1990), proteolytic fragments of basement membrane or extracellular matrix that comprises an inhibitor of blood vessel formation, an anti-angiogenic factor. Another anti-angiogenic factor is Endostatin, a proteolytic fragment of collagen XVIII (O’Reilly et al., 1997). The last two anti-angiogenic factors are canstatin (Magnon et al., 2005) and tumstatin (Maeshima et al., 2002), cleavage fragments of collagen IV. Furthermore, the body also produces endogenous anti-angiogenic soluble substances like IFN-α and IFN-β and angiostatin, a proteolytic fragment of plasmin (Baeriswyl and Christofori, 2009).

The balance between pro- and anti-angiogenic factors together with differential expression, release, or activation of the numerous factors control new blood vessel formation under pathological or physiological situations (Figure 4). Under physiological conditions, stromal and endothelial cells and released chemicals constitute a dynamic system that constantly alters and produces anti-angiogenic substances that keep the vasculature quiet. First, the “angiogenic switch” is turned on because proangiogenic factors are overabundant within tumor...
cells, and angiogenesis occurs as a response. Then, tumor cells and invading inflammatory/immune cells can release proangiogenic factors (Baeriswyl and Christofori, 2009) (Figure 1).

A key feature of tumor development is the ability of the tumor to generate its blood supply. It is an essential step in tumor initiation, which can vary based on the characteristics of the tumor and its microenvironment (Carmeliet and Jain, 2000). In addition, this tumor vasculature facilitates the tumor’s ability to obtain appropriate oxygen, nutrition, and waste removal. Moreover, neovascularization has the potential to create an “escape route” for tumor cells to enter the circulation, allowing them to disseminate and metastasis (Aguilar-Cazares et al., 2019).

Physiological angiogenesis and tumor angiogenesis are significantly different. First, tumor angiogenesis (the formation of new blood vessels to nourish the tumor) is a long-term process. Secondly, tumor blood vessels are disorganized, chaotic vascular networks, irregular in shape, dilated, hyper permeable, and not made up of distinctive arterioles, venules, and capillaries like normal vascular systems (Siemann, 2011). Additional aspects of tumor angiogenesis, tumor cells can undergo a process known as vasculogenic mimicry, during which they alter their gene expression to look like endothelial cells (Luo et al., 2020). Vasculogenic mimicry may be induced by hypoxia due to mass tumor growth (Wei et al., 2021), which occurs when tumor cells are far away from blood vessels and consequently do not receive enough oxygen and nutrients. Individuals with malignancies that display the characteristics of vasculogenic mimicry have a poor prognosis (Yang et al., 2016). The microenvironment in anoxic conditions collaborates with oncogenic pathways to trigger new blood vessel formation and leads to cancer development (Dayan et al., 2008). Hypoxia-inducible factor-1 (HIF-1) is a major transcriptional factor influencing the development of hypoxia-induced conditions. HIF-1 is composed of α and β polypeptides. HIF-1α induces...
angiogenesis by upregulating the production of proangiogenic factors, including VEGF-A (Hashimoto and Shibasaki, 2015). von Hippel-Lindau (VHL) regulates the proteasome via ubiquitin degradation in normoxic conditions to keep HIF-1 levels low (Tanimoto et al., 2000). While hypoxia causes HIF-1α breakdown, the effect is that HIF-1α levels increase, and angiogenesis is promoted (Jin et al., 2020).

**KEY MICRORNAS IN BREAST CANCER ANGIOGENESIS**

In the human genome, about 2,300 mature miRNAs have been identified (Alles et al., 2019), 10% of these have been shown to target EC activity and angiogenesis (Sun et al., 2018), like let7a, let-7b, let-7d, mir-16, mir-9, mir-21, mir-20, mir-23a, mir-34a, mir-29, mir-99a, mir-93, mir-100, mir-124, mir-125-5p, mir-126, mir-146, mir-155a, mir-195, mir-276, mir-181a, mir-181b, mir-199b, mir-204, mir-200b, mir-201b and mir-202b, mir-296, mir-320, mir-361-5p, mir-874. Moreover, they are upregulated in ECs and linked to angiogenesis control (Poliseno et al., 2006; Kuebhaber et al., 2007; Suárez et al., 2007; Caporali and Emanuelli, 2011). Thus, miRNAs are divided into two categories based on miRNA expression and angiogenesis function: 1) miRNAs that control the angiogenesis process primarily through well-characterized target genes, and 2) miRNAs controlled by pro- or anti-angiogenic factors or hypoxia (Caporali and Emanuelli, 2011).

**Anti-angiogenic microRNAs in Breast Cancer**

The expression of miRNAs, which regulate oncogenes and tumor suppressor genes, is changed in different types of human cancers (Hussen et al., 2021a). Therefore, these molecules have been termed “oncomiRs.” MiRNAs that are directing the process of angiogenesis are called angiomiRs, as they govern angiogenic processes in pathological and physiological circumstances (Table 1) (Suárez and Sessa, 2009). Overexpression of miRNAs is usually associated with a poor prognosis, chemotherapy resistance, and low survival (Babashah and Soleimani, 2011; Bitaraf et al., 2020). Various research groups have discovered many different miRNA expression patterns and individual miRNAs in breast cancer patients, and they have been linked to angiogenesis, invasion, and prognosis.

**microRNA-126**

miRNA-126 is expressed mainly in ECs, and it is significantly linked with angiogenesis throughout normal development and wound healing (Harris et al., 2008). The two mature strands of the pre-miR-126 are mir-126-3p and mir-126-5p, which have distinct cell-type and strand-specific functions in angiogenesis (Zhou et al., 2016). MiRNA-26, which targets both VEGFA and PIK3R2, plays a significant role in angiogenesis in human breast cancer, and its expression was decreased (Zhu et al., 2011). In addition, Overexpression of miR-26a in MCF7 cells has been found to reduce cancer development, tumor angiogenesis and induce apoptosis by inhibiting VEGF/P3K/AKT signaling pathway (Fish et al., 2008; Zhu et al., 2011). Furthermore, ectopic expression of miR-126 in BC cells reduced CD97, a G-coupled receptor that promotes cell invasion and angiogenesis via integrin signaling. (Lu et al., 2014). Another study found that miR-126 controls metastasis and angiogenesis through targeting the pro-angiogenic genes (IGFBP2, PITPNC1, and c-Mer kinase) (Png et al., 2011). These findings suggest that a single miRNA (miR-126) regulates cell survival and angiogenesis, with the possibility to help control vascular function and development.

**microRNA-497**

According to the miRNA growing evidence, miR-497 has been found that regulate the proliferation, migration, and survival of BC cells (Liu et al., 2016). In a mouse xenograft model, miR-497 inhibits tumor development and endothelial cell tube formation.
## TABLE 1 | miRNAs that inhibit angiogenesis in Breast cancer (ANTS: adjacent non-cancerous tissues).

| miRNA | Numbers of clinical samples | Assessed cell line | Animal model | Targets/Regulators | Signaling Pathways | Association with patients’ outcome | Function | References |
|-------|----------------------------|--------------------|--------------|--------------------|-------------------|------------------------------------|----------|------------|
| miR-126-3p | 50 formalin-fixed and paraffin-embedded BC tissue | MDA-MB-231, SKBR3, MDA-MB-1739, HCC70, MDA-MB-361, MCF-7 | Rat | VEGF-A and PK3P2 | VEGF/PI3K/AKT | — | — | miR-206 downregulated in BC through targeting VEGF, MAPK3, and SOX9 | Salgado et al. (2016) |
| miR-206 | — | MDA-MB-231, MCF-7 | — | VEGF, MAPK3, and SOX9 | — | — | — | In BC cells, miR-206 controls VEGF and MAPK3 expression | Salgado et al. (2018) |
| miR-4500 | — | MCF10A, MCF7, BT474, MDA-MB-231, MDA-MB-468, MCF-7 | — | RRM2 | MAPK | — | — | miRNA-4500 suppresses MAPK signaling via regulating RRM2 | Li et al. (2020a) |
| miR-497 | 45 pairs of clinical samples and ANTs | MCF-7, T-74D, MDA-MB-468, MDA-MB-453, MDA-MB-43L | — | HIF-1α | — | — | — | miRNA-497 suppresses BC angiogenesis by direct targeting HIF-1α | Wu et al. (2016) |
| miR-100 | Six healthy persons | MCF-7, MDA-MB-231, and T47D | — | mTOR | mTOR/HIF1α/VEGF | — | — | miR-100 regulates neovascularization via mTOR | Pakravan et al. (2017) |
| miR-153 | — | HBE23T, MCF10A, MDA-MB-231, HCC1937 | — | HIF1α | IRE1α-XBP1 | — | — | Hypoxia promotes miRNA-153 to fine-tune HIF-1α/VEGFA-stimulated angiogenesis in BC. | Liang et al. (2018a) |
| miR-190 | 12 pairs of tumors and ANTs | MCF-7, MCF-10A, T47D, MDA-MB-231, Bcap37, MCF-7 | 36 Nude mice | STC2 | AKT-ERK | Overexpression of miRNA-190 leads to inhibition of tumor growth in BC. | — | — | Angiogenesis in BC is inhibited by miR190, which targets STC2 | Sun et al. (2019) |
| miR-200 family | — | MDA-MB-231, T47D, HS7ST, BT-549, MCF-7 | Nude mice | IL-8 and CXCL1 | — | — | Through targeting interleukin-8 and CXCL1, miRNA-200 inhibits angiogenesis | Pecot et al. (2013) |
| miR-145 | — | MDA-MB-231 | Nude mice | IRS1 | PI3K/Akt and Ras/Raf/MAPK | — | — | miR-145 inhibits angiogenesis by inhibiting vascular endothelial cell tube formation | Pan et al. (2021) |
| miR-148a-3p | — | MDA-MB-231 | Nude mice | IGF-1R and VEGF | — | — | Angiogenic factors, such as VEGF and IGF-1R, are targeted by miR-148a-3p, which decreases the expression of these factors | Lacerda et al. (2019) |
| miR-148a | — | HS57ST, MDA-MB-231 | — | Wnt-3' UTRs | Wnt/β-catenin signaling pathway | — | — | miR-148a inhibits angiogenesis by suppressing GLA via the Wnt/β-catenin pathway | Mu et al. (2017) |
| miR-148a | 68 breast tissues and 22 pairs of tumors and ANTs | MCF-7, T47D, MDA-MB-231 | — | IGF-IR and IRS1 | AKT and MAPK/ERK signaling pathways | — | — | miR-148a inhibits tumor growth by binding to IGF-IR and IRS1 | Xu et al. (2012) |

(Continued on following page)
| miRNA     | Numbers of clinical samples | Assessed cell line | Animal model | Targets/Regulators | Signaling Pathways | Association with patients’ outcome | Function | References |
|-----------|-----------------------------|--------------------|--------------|-------------------|-------------------|------------------------------------|----------|------------|
| miR-152  | —                          | MDA-MB-468         | Nude mice    | IGF-IR            | —                 | —                                 | miRNA-152 suppresses HIF-1 and VEGF via modulating IGF-IR and IRS1 | Marques et al. (2018) |
| miR-153  | Seven pairs of tumors and ANTs | MCF7, MDA-MB-231, and HCC1937 | —            | ANG1              | —                 | —                                 | miR-153 reduced endothelial cell migration and tube formation by targeting ANG1 in breast cancer | Liang et al. (2018b) |
| miR-139  | —                          | HCC180, MCF-10A    | Six and B549 | SOX8              | —                 | —                                 | miR-139 reduced TNBC cell growth by targeting SOX8 | Dong et al. (2020) |
| miR-10a  | —                          | MCF-7, MDA-MB-231, and SK-BR3 T47D, and ZR-75-30 | —            | p-Akt, p-mTOR, p-p70S6K, PI3K/Akt, Cyt-C IRS1 | —                 | —                                 | miRNA-10a inhibited phosphoinositide/Akt/mTOR signaling in breast cancer cells | Ke and Lou, (2017) |
| miR-195  | —                          | MDA-MB-231, MCF-7, MDA-MB-435, MDA-MB-453, SK-BR3 T47D, SK-BR3 T47D | —            | IRS1              | —                 | —                                 | miR-195 reduces tumor angiogenesis by inhibiting the IRS1-VEGF axis | Wang et al. (2016) |
| miR-19b-1 | —                         | MDA-MB-231 MCF-7   | Nude mice    | VEZF1              | —                 | —                                 | miR-19b-1 can activate VEGFR to decrease angiogenesis in BC. | Yin et al. (2018) |
| miR-199b-5p | —                      | T47D, MCF-7, and BT474 | —            | ALK1              | ALK1/Smad/Id1     | —                                 | Breast cancer miR-199b-5p targets Angiopoietin-2 to decrease tumor angiogenesis | Lin et al. (2019) |
| miR-542-3p | 72 breast cancer patients | HMECs, SVEC4-10, 4T1 and HEB293T | —            | Ang2              | —                 | With the late stages of BC, miRNA-542-3p could be useful for tracking disease progression. Lower miR-542-3p expression was linked to increased ANG expression. | Breast cancer miR-542-3p targets Angiopoietin-2 to decrease tumor angiogenesis | He et al. (2014) |
| miR-542-3p | 52 breast cancer patients | MDA-MB-231 and HEB293T | Nude mice    | Angpt2             | —                 | —                                 | Breast cancer miR-542-3p targets Angiopoietin-2 to decrease tumor angiogenesis | He et al. (2015) |
| miR-497  | 45 pairs of tumors and ANTs | MCF-7 T-47D, MDA-MB-435, MDA-MB-453, MDA-MB-468 | BALB/c nu/nu mice | HIF-1a and VEGF | —                 | —                                 | miR-497 overexpression reduced the expression of HIF-1 and VEGF. | Wu et al. (2016) |
| miR-519c | 34 breast cancer tissues | MCF-7, SK-BR3, MDA-MB431, MDA-MB231 T47D T-47D SK-BR3 T47D | BALB/c Nude mice | HIF-1a            | HGFR/c-Met signaling pathway | — | miR-519c controls angiogenesis by inhibiting HIF-1 in vitro and in vivo | Cha et al. (2010) |
| miR-573 and miR-578 | 43 FFPE and | HEB293, MCF-7 and SUM149PT | —            | VEGFA and ANGII | —                 | —                                 | miR-573 and miRNA-578 can control angiogenic markers like HIF-1, VEGF, and focal adhesion kinase. | Danza et al. (2015) |
| miR-29b | —                         | MDA-MB-231         | Nude Akt3    | —                 | —                 | —                                 | miR-29b slows tumor development by targeting Akt3 and decreasing angiogenesis. | Li et al. (2017) |
| miR-199b-5p | —                      | MDA-MB-231         | ALK1         | ALK1/Smad/Id1     | —                 | —                                 | miR-199b-5p overexpression decreased tumor development and angiogenesis. | Lin et al. (2019) |
miR-204 — inhibiting proangiogenic molecules (HIF-1α that miR-497 may serve as a novel treatment approach for BC via VEGFR2-luc. Further, it has been recommended (Wu et al., 2016) and VEGFR2 expression when injected in transgenic mice with 497 in 4T1 cells suppressed the growth of BC cells, angiogenesis, group (Tu et al., 2015) described that increasing levels of miR-542-3p has been shown to serve as a tumor suppressor gene and has been linked to the control of angiogenesis indicating that miRNAs are key modulators of angiogenesis. The control of Angpt2 by miRNAs has been established, microR-542-3p being well-known for promoting metastasis in several malignancies, such as BC, Hotair has the potential to alter the expression of gene patterns and inhibit the production of miR-568, a critical tumor suppressor gene (Li et al., 2014). Furthermore, it appears that miR-568 is causing low levels of NFAT5 expression, which in turn sustains VEGFC and S100A4 expression, both of which are angiogenic and metastatic transcriptional activators of NFAT5 (Li et al., 2014). Despite these findings, additional study is required to identify or better understand the connection between lncRNAs and miRNAs for more efficient treatment approaches.

microR-542-3p

The control of Angpt2 by miRNAs has been established, indicating that miRNAs are key modulators of angiogenesis (He et al., 2014). MiR-542-3p has been shown to serve as a tumor suppressor gene and has been linked to the control of various cancers (Long et al., 2016; Wang et al., 2018b), including breast cancer (Lyu et al., 2018). The level of miR-542-3p is inversely correlated to the clinical progression of patients with advanced-stage of BC. The use of an in-vitro BC mouse model revealed that the overexpression of miR-542-3p could suppress tumor growth, formation of tubules, and metastasis (He et al., 2015). He et al. miR-542-3p downregulates the expression of angiogenin, allowing it to be overexpressed in tumor cells and promote angiogenic activation in both in vitro and in vivo models (He et al., 2015). CEBPB and POU2F1, which are transcription factors for miR-542-3p, were shown to be suppressed by angiogenin, which might function as a new tumor-endothelial cell signal pathway (He et al., 2015). As a result, miR-542-3p presents novel targets for BC prevention and therapy.

TABLE 1

| miRNA | Numbers of clinical samples | Assessed cell line | Animal model | Targets/ Regulators | Signaling Pathways | Association with patients' outcome | Function | References |
|-------|-----------------------------|-------------------|--------------|---------------------|-------------------|---------------------------------|---------|-----------|
| miR-204 | 58 breast cancer samples | MCF-7, MDA-MB-231, ZR-75, MDA-MB-453 | Nude mice | FOXC1, RAB29A, SMAD4 | — | Deregulated expression of miRNAs has been associated with angiogenesis | miR-204 reduces angiogenesis in BC cells by targeting FOXC1, RAB29A, and SMAD4 | Flores-Pérez et al. (2016) |
| miR-204 | — | MCF-7, MDA-MB-231, T457-D, ZR-75, MDA-MB-453 | Athymic nude mice | ANGPT1 and TGFβ2 genes | angiopoietin signaling | — | miR-204 targets proangiogenic genes (ANGPT1 and TGFβ2) to decrease cell proliferation, invasion, and angiogenesis | Flores-Pérez et al. (2016) |

(Wu et al., 2016). Furthermore, it was shown that the levels of VEGF and HIF-1α protein were decreased due to the overexpression of miR-497 (Wu et al., 2016). Tu and his group (Tu et al., 2015) described that increasing levels of miR-497 in 4T1 cells suppressed the growth of BC cells, angiogenesis, and VEGFR2 expression when injected in transgenic mice with VEGFR2-luc. Further, it has been recommended (Wu et al., 2016) that miR-497 may serve as a novel treatment approach for BC via inhibiting proangiogenic molecules (HIF-1α and VEGF).

microR-155

MiR-155 expression is considerably increased in BC and is highly associated with high tumor grade, progressing stage, and lymph node, but is negatively associated with cancer survival (Chen et al., 2012). Tumors respond to hypoxia by activating a genomic pathway, including miRNAs dependent on HIF1α and the hypoxia-induced pathway. Recent data showed that miR-155 has a crucial role in HIF1α-induced angiogenesis, and its expression is differentially regulated in BC (Chang et al., 2011). In BC tissues, the comparative expression of miR-155 was substantially greater than in normal tissues. Increased levels of miR-155 were all associated with tumor grade, tumor stage, and lymph node metastasis (Chen et al., 2012). Furthermore, the levels of miR-155 are negatively related to VHL protein; an E3 ubiquitin ligase inhibits members of the HIF1α family (Kong et al., 2014). Additionally, in patients with breast cancer, an increase in the expression of miR-155 may offer a prognostic marker and therapeutic target.

microR-568

The miR-568 has been found to be present in the circulation of women with breast cancer (Leidner et al., 2013). lncRNA Despite being well-known for promoting metastasis in several malignancies, such as BC, Hotair has the potential to alter the expression of gene patterns and inhibit the production of miR-568, a critical tumor suppressor gene (Li et al., 2014). Additionally study is required to identify or better understand the connection between lncRNAs and miRNAs for more efficient treatment approaches.

microR-204

miR-204 is a new multi-target anti-angiogenic miRNA that fights BC. In MCF7 BC cells, miR-204 mediates tumor-suppressing effect, and expression of miR-204 induces the inhibition of proliferation, invasion, and metastasis through targeting p-AKT and p-PI3K significantly (Fan et al., 2019). In vivo vascularization and angiogenesis were similarly reduced in nu/nu mice by miR-204, which is consistent with previous findings (Salinas-Vera et al., 2019). The levels of proangiogenic ANGPT1 and TGFβ2 proteins were decreased in MDA-MB-231 BC cells after treating with miR-204 (Salinas-Vera et al., 2019). Furthermore, ectopic expression of miR-204 exhibits decreasing vascular endothelial growth factor (VEGF) levels and a reduced number of branches of capillary tubes (Salinas-Vera et al., 2018). Conclusively, multiple proteins associated with the PI3K/AKT, RAF1/MAPK, FAK/SRC, and VEGF pathways were downregulated and phosphorylated due to increased levels of miR-204 (Salinas-Vera et al., 2018; Hong et al., 2019a). This
new finding reveals miR-204 has a new, yet-unproven role in regulating the crucial synergy of the PI3K/AKT/FAK mediators that are important in VM development.

**microR-29**
The family of miR-29a, miR-29b, and miR-29c genes have similar structures, have a high degree of sequence similarity, and serve as a target-identifying foundation for molecules (Sun et al., 2018). miR-29b acts as a tumor suppressor, which inhibits angiogenesis and tumor development. However, in several malignancies, including endometrial carcinoma (Chen et al., 2017), HCC (Fang et al., 2011), ovarian cancer (Sugio et al., 2014), and BC (Li et al., 2017), miR-29b levels are downregulated. miRNA-29b expression in breast cancer impairs the development of capillary-like tubular structures in HUVECs, as well as their ability to proliferate, migrate, and stop tumor progression (Li et al., 2017). Moreover, miR-29b acts to target Akt3 and inhibit angiogenesis and tumor growth by acting as an anti-angiogenesis and anti-tumorigenesis agent through the VEGF and C-myc arrest in BC cells (Li et al., 2017). Importantly, miR-29b might be an efficient anti-cancer treatment by way of therapeutic administration.

**microR-4500**
MiR-4500 was expressed poorly in BC cell lines, and RRMI2 was a target gene (Li et al., 2020a). Additionally, a high expression of miR-4500 is seen in BC cells. Its expression in BC cells impairs the MAPK signaling through control of RRMI2, which decreases proliferation, invasion, and angiogenesis while causing apoptosis. (Li et al., 2020a). Based on the findings that therapeutic methods should target the elevation of miR-4500, which might be a therapeutically feasible target in breast cancer treatment, this implies that therapies should focus on raising miR-4500 levels. However, to completely understand the precise processes and mechanisms of miR-4500 in BC, further studies are required on tissues from patients and additional BC cell lines.

**microR-200**
It has been determined that miR-200 can inhibit angiogenesis in breast cancer’s environment and make it a therapeutic potential substance. Pecot et al. showed that miR-200 reduces angiogenesis via targeting interleukin-8 and CXCL1, which are produced by the tumor endothelium and cancer cells, and indirectly by targeting interleukin-8 (Pecot et al., 2013).

Furthermore, miR-200 family members have been shown to regulate the formation of blood vessels and angiogenesis by suppressing VEGF signaling (Choi et al., 2011). In vitro, angiogenesis was inhibited by miR-200b, which caused activation of the Notch system, which then triggered wound healing (Qu et al., 2021). Thus, by modulating the expression of VEGF, the miR-200 family may offer a possible anti-angiogenesis treatment for treating cancer and other illnesses dependent on angiogenesis.

**microR-190**
In BC cells, miRNA-190 has the potential to target STC2 negatively, and through suppressing the AKT-ERK pathway, it has the potential to impede migration, invasion, EMT, and angiogenesis (Sun et al., 2019). Angiogenesis is influenced by the tumor microenvironment, which alters the cellular mechanisms required for vascular growth. MiRNA-190 suppresses the tumor microenvironment by targeting a set of angiogenic genes including RAS2, TCF4, HGF, Smad2, Smad4, IGF1, Jak2, and VEGF in vivo and in vitro (Hao et al., 2014). Furthermore, these genes, targeted by miRNA-190, have been shown to control VEGF expression (Hao et al., 2014). Moreover, it has been demonstrated that miR-190 substantially inhibits tumor metastasis, particularly angiogenesis (Hao et al., 2014). These findings collectively suggest that miR-190 is a promising anti-cancer target in therapeutic applications.

**microR-148a**
In various kinds of cancer, including BC (Yu et al., 2011), HCC (Pan et al., 2014), and ovarian cancer (Wang et al., 2018c), miRNA-148a-3p works as a tumor suppressor that is substantially downregulated. miRNA-148b-3p has also been implicated in the control of carcinogenesis, according to recent research. Interestingly, both miRNA-148a and miRNA-148b are essential regulators of EC migration in responses to VEGF, and they are also important regulators of EC proliferation. Because miRNA-148a/b-3p targets NRP1, upregulating its expression in ECs from its normally low endogenous levels has a significant inhibitory effect on VEGF-induced activation of VEGFR2 and downstream signaling (Kim et al., 2019). HIF-1α expression, which is required to form a functional HIF-1 transcription factor, was likewise reduced by MiR-148a. HIF-1 is a transcription factor that regulates the production of VEGF and other angiogenesis regulators (Semenza, 2003). As a result, we believe that miR-148a suppresses angiogenesis in breast tumors.

Furthermore, it has been established that miR-148a overexpression inhibited the angiogenesis produced by MCF7 cells in BC by directly targets ERBB3 (Yu et al., 2011). Additionally, overexpression of miR-148a targets IGF-IR and IRS1 suppresses BC cells proliferation and tumor angiogenesis by suppressing their downstream AKT and MAPK/ERK pathways (Xu et al., 2013). Following these findings, it appears that miRNA-148a might potentially be a promising therapeutic target in cancer treatment in the upcoming years.

**MicroR-199b-5p**
According to previous studies, miRNA-199b-5p is a tumor suppressor. It was revealed to be downregulated in various BC cell lines and reduced in VEGF-induced human umbilical vein epithelial cells (HUVECs) (Lai et al., 2018; Lin et al., 2019). Interestingly, the migration and angiogenesis of HUVECs were decreased by ectopic expression of miRNA-199b-5p, whereas inhibition of miRNA-199b-5p induced the reverse effect. Similarly, HUVECs treated with high levels of miR-199b-5p exhibited suppressed ALK1 mRNA and protein production due to direct binding to the 3’UTR of ALK1 (Lin et al., 2020). Aside from that, high levels of miR-199b-5p in HUVECs reduced the activation of the ALK1/Smad1/Id1 signaling by BMP9 in BC. As a result of these findings, miR-199b-5p, which primarily affects ALK1, has been identified as an anti-angiogenic factor, suggesting that miR-199b-5p might be used as an anti-angiogenic strategy in treating cancer.
| miRNA       | Numbers of clinical samples | Assessed cell line | Animal model | Targets/Regulators | Signaling Pathways | Association with patients’ outcome | Function                                                                 | References                  |
|-------------|-----------------------------|--------------------|--------------|--------------------|--------------------|-----------------------------------|---------------------------------------------------------------------------|-----------------------------|
| miR-20a     | breast cancer patients \(n=108\) | MCF7, MDA-MB-231   | —            | VEGFA              | —                  | Promoting metastasis              | miR-20a promotes aberrant vascular mesh size and excessive VEGFA expression in BC patients, VEGF-A and HIF-1alpha target proteins correlated negatively with miR-20a/b | Luengo-Gil et al. (2018) |
| miR-20a/b   | 32 breast cancer patients 16 controls | —                  | —            | VEGF-A, HIF-1alpha | —                  | metastatic heterogeneity          | miR-20b acted as a tumor promoter by targeting PTEN expression             | Li et al. (2012)            |
| miR-20b     | 23 paired clinical breast cancer tissues and ANTs | MCF7, SK-BR-3 T-47D, ZR-75-30, MVT1 and E071, FVB/N nude mice | PTEN          | —                  | PTEN-P3K-Akt pathway            | —                                | miR-21 promotes tumor metastasis and angiogenesis by suppressing the CSF1-ETS2 pathway | Zhou et al. (2014)          |
| miR-21      | 79 breast cancer samples and 60 pairs of tumors | MCF-10A, MCF-7, MDA-453, MDA-231, BALB/c nude mice | —            | VEGFA, VEGF-D, VEGF-C, COX-2 | PI3K/Akt and ERK pathway | —                                | miR-29a stimulates BC cell proliferation and EMT through TET1          | Pei et al. (2016)           |
| miR-93      | —                           | MT-1               | —            | LATS2              | —                  | —                                | miR-93 targets LATS2 to promote angiogenesis and metastasis               | Fang et al. (2012)          |
| miR-10b     | —                           | HMEC-1, MDA-MB-231 | —            | HoxD10             | —                  | —                                | miR-10b targets HoxD10 mRNA to induce angiogenesis                        | Shen et al. (2011)          |
| miR-655/526b | 105 tumor tissue samples MCF7 and MDA-MB231 | —                  | —            | VEGFA, VEGF-D, VEGF-C, COX-2, P7E | PI3K/Akt and ERK pathway | —                                | miR-526b-655 induce both angiogenesis and lymphangiogenesis in BC.       | Hunter et al. (2019)        |
| miR-10b     | 20 non-cancerous tissues    | —                  | —            | HOXD10             | —                  | —                                | miR-10b expression associated with metastases and angiogenesis in node-negative breast cancer | Liu et al. (2017)           |
| miR-210     | 299 paraffin-embedded breast cancer tissue | —                  | —            | VEGF and cyclin D1 | —                  | —                                | miR-210 induced angiogenesis by targeting HIF-1α-VEGF signaling          | Foekens et al. (2008)       |

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microRNA-195

miRNA-195 is one of the genetic markers found on chromosome 17p13.1, known as the origin of intron 7. miRNA-195 has been characterized as a tumor suppressor molecule that is often dysregulated in many malignancies, such as BC (Yu et al., 2018). miR-195’s anti-cancer activity is attributed to its target molecules, FASN, ACACA, HMGCR, and IRS1, which help slow down BC cell growth, migration, angiogenesis, and metastasis. (Singh et al., 2015; Wang et al., 2016). In both BC cell lines and BC tissues, miR-195 is negatively linked to Insulin receptor substrate 1 (IRS1) (de Sales et al., 2021). IRS1 expression is downregulated by induction miR-195 using a miR-195 oligo transfection method or infection with a lentivirus containing the miR-195 gene (Wang et al., 2016; Lai et al., 2018). These findings imply that miR-195 replicas are promising BC treatment agents.

Pro-angiogenic microRNAs in Breast Cancer

miRNAs are involved in the initiation and progression of several tumor characteristics, such as tumor invasion, angiogenesis, and metastasis. Recent investigations have suggested that several miRNAs suppress angiogenesis in the BC. These studies present novel therapy options for treating angiogenesis in BC (Table 2).

microR-20b

miRNA-20 is a member of the miRNA-17–92 cluster, and it has been characterized as an oncogenic miRNA molecule that is often dysregulated in many malignancies, such as BC (Li et al., 2012). miRNA-20b is a potential oncogene that affects the control of VEGF expression in MCF-7 breast cancer cells by targeting HIF-1α and STAT3 (Cascio et al., 2010). The other study found that miR-20b was elevated in human BC tissues and predicted that the anti-oncogenic PTEN gene might target miRNA-20b (Zhou et al., 2014). These data suggest that miR-20b may be used as a potential biomarker and a viable target for diagnosing and treating breast cancer.

microR-29a

In several types of malignancies, including esophageal (Liu et al., 2015a), colon cancer (Leng et al., 2021) and BC (Rostas et al., 2014) miR-29a was shown to be upregulated. The essential process that leads to metastasis is the EMT. miR-29a, which is upregulated in many types of cancer, has been shown to promote EMT in BC (Rostas et al., 2014). Furthermore, in vivo and in vitro, miRNA-29a upregulation led to TET1 reduction, which increased cell proliferation and EMT in BC (Pei et al., 2016). Thus, it appears that miR-29a is a novel biomarker for BC detection and a possible treatment target.

microR-9

The miRNA-9 expression is abnormal in many cancers; however, the Role of miR-9 in cancers is still debated, in some studies indicating that it is a proangiogenic oncomiR as in BC (Bertoli et al., 2015) or as a tumor suppressor such as in melanoma (Bu et al., 2017). Kim et al. recently discovered that miRNA-9, an angiogenic mediator, selectively targets mRNA from genes involved in stimulating VEGF expression (Kim et al., 2017). Their study observed that miR-9 could inhibit the production of VEGFA by binding to the products of the ITGAV6 gene, which encodes α6β4 integrin complex subunit in BC cell lines (Kim et al., 2017). In vitro, it has already been established that the integrin (α6β4) subunit enhances the VEGF expression by activating the mTOR pathway (Soung et al., 2013). Similarly, miR-9-mediated E-cadherin increases VEGFA expression in breast cancer via activating beta-catenin signaling in animal models and cell lines (Ma et al., 2010). Furthermore, the same study found that miRNA-9 targets the CDH1 expression, which is translated into E-cadherin protein and increases the nuclear localization and activity of β-catenin (Ma et al., 2010), which are both essential in tumorigenesis (Yu et al., 2019). Lastly, they observed that upregulation or elevated levels of miRNA-9 in breast cancers promotes angiogenesis (Ma et al., 2010). These findings provide evidence that miRNA-9 has a proangiogenic function for the development of cancer.

microR-10b

The miR-10b genomic location is in front of the HOXD4 gene and has more attention because of its highly conservative (Tehler et al., 2011). In vivo and in vitro studies have revealed the importance of miR-10b in angiogenesis (Lin et al., 2012). It was shown that axillary lymph node-negative breast cancer patients had an increased microvessel density (MVD), which was correlated with raised miRNA10b (Liu et al., 2017). Plummer and his colleagues found that miR-10b shows increased expression and promotes stimulation of VEGF in high-grade human breast cancer (Plummer et al., 2013). The increased expression of miR-10b is considered to have a role in increasing the capacity of endothelial cells to create blood vessels by reducing the anti-angiogenic pathway gene expression (Shen et al., 2011). By binding to the 3’ UTR of HoxD10 mRNA, miRNA-10b is capable of targeting HoxD10 mRNA and inhibits the production of HoxD10 protein (Shen et al., 2011). Interestingly, FLT1 is believed to inhibit VEGF and VEGFR2 interaction from stopping the development of new blood vessels (Fong et al., 1999). It has been observed that inhibiting the expression of miR-10 in HUVECs exposed to low concentrations of VEGF decreases the VEGFR2 phosphorylation, which inhibits the VEGF-dependent angiogenesis (Hassel et al., 2012).

microR-21

miR-21, a hypoxia-inducing miRNA, has participated in developing cancer, angiogenesis, and stimulation of VEGF signaling in patients with BC (Foekens et al., 2008). Oncogenic mi R-21, which is related to the advanced tumor stage, lymph node, and poor patient mortality, was found as a potential molecular prognosis mark for BC development (Yan et al., 2008). In a VEGFR2-Lucent mice model of BC, a miR-21 antagonist substantially decreased cancer growth and tube formation by directly inhibiting the VEGF/VEGFR2/HIF1 pathway (Zhao et al., 2013). Interestingly, this work demonstrated that miR-21 inhibition causes apoptosis in BC.
| Compound          | miRNA | Assessed cell line | Animal model | Target genes                                      | Signaling Pathways                     | References                  |
|-------------------|-------|--------------------|--------------|--------------------------------------------------|----------------------------------------|-----------------------------|
| Melatonin         | †miR-152-3p | MDA-MB-468, MDA-MB-231 | Nude mice    | ↓ angiogenesis by ↓ IGF-1R, ↑ HIF-1α, ↓ VEGF, ↑ angiogenesis by ↓ IGF-1, ↓ VEGF, migration, ↓ invasion of BC cells | miR-152/IGF-1R, HIF-1α, VEGF pathway | Marques et al. (2018)       |
|                   | †miR-148a-3 | MDA-MB-231 | Nude mice    | ↓ migration, ↓ proliferation, ↓ angiogenesis, ↓ TGF-β, ↓ PTEN, ↓ SMA, ↑ [Silibilin + EGCG] | IGF-1R/VEGF pathway | Lacerda et al. (2019)       |
| Metformin         | †miR-21  | HUVEC              | —            | ↓ cell viability, ↓ Bcl-2, ↓ PTEN, ↑ Ezh2          | PTEN/AKT/PKB pathway                  | Cabello et al. (2016)       |
|                   | †miR-26a | MDA-MB-468, MDA-MB-231 | MCF-7        | ↓ miR-17, ↑ miR-16, ↓ miR-27a                       | TGFβ-signal pathway                   | Olveras-Ferraro et al. (2011) |
|                   | †miR-181a | MDA-MB-231 | MCF-7        | ↓ miR-27a/ZBTB10/Sp-1 axis                        | MCF-10F/Sp1, Sp2, Sp3, Sp4, ↑ Bcl-2 | Wang et al. (2017)          |
|                   | †miR-96  | MCF-7              | —            | ↓ cell viability, ↓ Bcl-2, ↓ PTEN, ↑ Ezh2          | PTEN/AKT/PKB pathway                  | Cabello et al. (2016)       |
|                   | †miR-21-5p | BT-549, BT-474, SUM159PT | —            | ↓ AMPK, ↓ CAB39L, ↑ Sestrin-1 → ↓ mTOR synergistically with everolimus → ↓ cell invasion and growth | AMPK/mTOR pathway                  | Puito et al. (2017)         |
|                   | †miR-155 | MDA-MB-23 | MCF-7        | ↓ ROS → ↑ SOD, ↓ MMP-2, ↓ MMP-9, ↓ Bcl-2 | ROS-independent pathway | Sharma and Kumar (2018)     |
|                   | †miR-200c | MDA-MB-231 | SCID mice    | ↓ apoptosis and ↓ proliferation                    | metformin/L-Myc/miR-200c/AKT2/Bcl-2/Bcl-2 | Zhang et al. (2017)         |
| Cardamonin        | †miR-21  | HUVEC              | —            | ↓ VEGF mediated angiogenesis, ↓ proliferation and migration of endothelial cells | miR-21/VEGF signaling pathway        | Jiang et al. (2015)         |
| (Silibilin + EGCG) | † miR-17 | HUVEC A549         | —            | Synergistically ↓ VEGF and ↓ VEGFR2                | VEGF/VEGFR2 pathway                  | Mirzaghahai et al. (2019)   |
|                   | † miR-18a | MCF-7              | —            | ↓ Bcl-2                                             | miR-15a/16-Bcl-2 apoptotic pathway   | Yang et al. (2010)          |
|                   | † miR-15a | MCF-7              | —            | ↓ Bcl-2                                             | miR-15a/16-Bcl-2 apoptotic pathway   | Yang et al. (2010)          |
|                   | † miR-16 | MDA-MB-231 | MCF-7        | ↓ proliferation, survival, invasion and apoptosis  | Bcl-2 mediated apoptotic pathway     | Guo et al. (2013)           |
|                   | † miR-34a | MDA-MB-231 | MCF-7        | ↓ Bcl-2, ↓ Bmi-1, ↓ proliferation, ↓ apoptosis by ↓ Bcl-2, ↓ survivin, ↓ MMP-1, ↓ MMP-3 | Bcl-2 mediated apoptotic pathway     | Kronski et al. (2014)       |
|                   | † miR-181b | MDA-MB-231 | MCF-7        | Anti-metastatic effect by ↓ CXCL1, ↓ CXCL 2        | anti-metastatic pathway | Gallardo et al. (2020)      |
|                   | † miR-34a | MDA-MB-231 | MCF-10F      | ↓ cell viability, ↓ cell migration and invasive ness | Rho -signaling pathway | Gallardo et al. (2020)      |
|                   | † miR-21 | MCF-7              | —            | ↓ caspase 3/9 → ↓ apoptosis                        | miR-21/PTEN/Akt pathway              | Wang et al. (2017)          |
|                   | † miR-16 | MCF-7              | —            | ↓ cell viability, ↓ apoptosis, ↓ Bcl-2            | Bcl2/Bax apoptotic pathway           | Magura et al. (2020)       |
| Hesperidin and luteolin | † miR-21 | MCF-7              | —            | ↓ cell viability, ↓ apoptosis, ↓ Bcl-2            | Bcl2/Bax apoptotic pathway           | Magura et al. (2020)       |
|                   | † miR-16 | MCF-7              | —            | ↓ cell viability, ↓ apoptosis, ↓ Bcl-2, ↓ Bax     | Bcl2/Bax apoptotic pathway           | Magura et al. (2020)       |
| Quercetin         | † miR-21  | MCF-7              | —            | ↓ Maspin, ↓ PTEN, ↓ cell viability                | PTEN/maspin pathway                  | Panahi, (2018)              |
| betulinic acid    | † miR-27a | MCF-7              | —            | ↓ Bcl-2, ↓ Bcl-2                                 | PTEN/AKT2/Bcl-2 pathway             | Talcott et al. (2008)      |
|                   | † miR-27a | MCF-7              | —            | ↓ cell viability, ↓ apoptosis, ↓ Bcl-2, ↓ Bax     | Bcl2/Bax apoptotic pathway           | Talcott et al. (2008)      |
|                   | † miR-34a | MCF-7              | —            | ↓ cell viability, ↓ apoptosis, ↓ Bcl-2, ↓ Bax     | Bcl2/Bax apoptotic pathway           | Talcott et al. (2008)      |

(Continued on following page)
cells and HUVECs via upregulating the PTEN gene. This miRNA has also been shown to be effective in inhibiting angiogenesis.

Evolving angiogenesis in mice carrying BCs with a luciferase gene that activates the VEGF/VEGFR2 pathway may be stopped if a molecule called miR-21 is inhibited by blocking HIF-1α/VEGF/VEGFR2 signaling (Zhao et al., 2013). Furthermore, this work demonstrated that blocking miR-21 leads to PTEN overexpression, contributing to BC cell and HUVEC death. The presence of miR-21-1 has also been shown to inhibit the growth of new blood vessels. Liu et al. exposed that a BC oncogene, metadherin (MDT), can promote angiogenesis by stimulating the miR-21/ERK-VEGF-MMP2 pathway (Liu et al., 2015b). According to recent research, exercise and hormone treatment decreased tumor growth and angiogenesis in a mouse model of invasive breast cancer by reducing levels of miR-21, ER, HIF-1, VEGF, and raising levels of miR-20 (Chen et al., 2015). According to recent research, exercise and hormone treatment decreased tumor growth and angiogenesis in a mouse model of invasive breast cancer by reducing levels of miR-21, ER, HIF-1, VEGF, and raising levels of miR-20 (Chen et al., 2015).

miR-93

It is located on the 7th chromosome and is part of the miRNA-106b-25 cluster. miR-93 is one of the miRNAs frequently found to be overexpressed in tumors (Sun et al., 2018). Previous research has discovered that miR-93 is increased in BC and that it functions as an oncomiR, promoting angiogenesis (Fang et al., 2012; Liang et al., 2017; Sun et al., 2018). Fang and his team found that miR-93 is required to promote angiogenesis, enhanced EC proliferation and migration, and tube formation (Fang et al., 2012). Also, miRNA-93 is upregulated in breast cancer, stimulating new blood vessel growth by blocking the homology 2 gene (LATS2) (Fang et al., 2012). MiR-93, on the other hand, appears to have a function in the inhibition of angiogenesis in some pathological conditions (Fabbi et al., 2016). According to the study by Liang et al., TNBC specimens with greater levels of miR-93-5p had increased blood vessel density. They also revealed that overexpressing miRNA-93-5p in HUVECs enhanced proliferation, migration, and cell sprouting in vitro, but inhibiting miRNA-93-5p reduced migration and angiogenic ability (Liang et al., 2017). miR-93 is involved in tumor angiogenesis by inhibiting several targets, particularly VEGF, EPLIN, integrin-β3, IL-8, and LATS2 in TNBC tissues (Liang et al., 2017). Fang et al. have demonstrated that tumor xenografts formed from breast cancer cell lines transfected with miR-93 showed increased vascular density and metastatic ability and a greater capacity for lung metastasis than tumors transfected with a vector without miR-93 (Fang et al., 2012).

Additionally, they found that miR-93 might promote invasion and tumor angiogenesis by silencing LATS2 expression. Furthermore, miR-93, an oxygen-responsive microRNA, might disrupt NCOA3, an epigenetic factor that mediated tumor suppression and inhibits cGAS-mediated antitumor immunity in breast cancer (Wu et al., 2017). Due to this, tumor angiogenesis may be promoted by the hypoxia-regulated miRNAs like miR-93, which also participates in immunosuppression. Altogether, miRNA-93 has a dual impact on angiogenesis in various human tissues and cells, and these effects are mediated through a variety of molecular pathways.

miR-182

The miRNA-182-183-96 cluster contains miR-182, which is located on chromosome 7q32. Overexpression of miR-182 has been found in BC cells, and this miRNA inhibits FOXO1, MTSS1, MIM, and BRCA1 and, therefore, negatively impacts cell proliferation angiogenesis and DNA damage response (Guttilla and White, 2009; Lei et al., 2014). Furthermore, increasing miR-182 expression leads to an increase in HIF-1α and VEGFA activation by direct targeting FBXW7 induces angiogenesis in BC tissues (Chiang et al., 2016). In addition to its involvement in regulating ubiquitin ligase (SCF) activity, tumor suppressor FBXW7 is essential for SCF complex activity, which controls the degradation of a variety of oncogenic proteins, such as HIF-1, Notch, cyclin E, and c-myc (Flügel et al., 2012). Thus, we conclude that miR-182 contributes to breast cancer invasion, angiogenesis, and metastasis based on the above studies.
**microR-210**

miR-210, a hypoxia-inducing miRNA, has been primarily described as an oncomiR. Overexpression of miRNA-210 is a critical component of EC survival, angiogenesis, and differentiation in response to hypoxia (Fasanaro et al., 2008). In research conducted by Jung et al. (Jung et al., 2017), HIF-1 and miR-210 were overexpressed in exosomes produced from mouse BC cells during a hypoxic microenvironment. Exosomes carrying miR-210 were transfected into HUVEC cells, efficiently decreasing PTP1B and Ephrin-A3 expression and promoting angiogenesis by targeting VEGF signaling (Jung et al., 2017). MiR-210 expression was highly correlated with VEGF expression, hypoxia, and angiogenesis in breast cancer patients, suggesting that miR-210 may play a role in tumor angiogenesis. Although this association is significant, it is not adequate to evaluate whether miR-210 is a functional regulator of BC angiogenesis due to its substantial increase under hypoxic circumstances. An additional miR-210 target, the protein tyrosine phosphatase Ptp1b, has been discovered as a factor that promotes angiogenesis and suppresses cellular death in the setting of a mouse myocardial infarction (Hfu et al., 2010). Earlier studies demonstrated that PTP1B might bind to and inhibit the activation of a VEGF receptor, VEGFR2, and inhibit the tyrosine phosphorylation of VEGFR2 in endothelial cells stabilizing cell-cell adhesions (Nakamura et al., 2008). Taken together, because of its ability to suppress Ptp1b and Efna3, miR-210 might facilitate angiogenesis.

**microR-467**

The miR-467 was found to be a specific inhibitor of TSP-1, which was reported to be elevated in the BC cells after glucose stimulation (Soheilifar et al., 2021). It was demonstrated that the miR-467 mimic increased the number of BC cells in the matrigel plugs in mice, indicating the proangiogenic activity of miR-467 in vivo. MiR-467, on the other hand, was unable to stimulate angiogenesis in the absence of TSP1. Additionally, it was demonstrated that the amount of miR-467 in BC tumors increased, and a link between the expression miR-467 and tumor mass was shown in STZ-treated hyperglycemic mice. Similar results were found in the hyperglycemic Leprdb/db mice, with miRNA-467 hyperactivity leading to increased tumor growth and angiogenesis (Krukovets et al., 2015). Also, in the animal models, it was discovered that miRNA-467 blockers reduced tumor development and angiogenesis indicators (Krukovets et al., 2015). These findings show that hyperglycemia causes angiogenesis by increasing the expression of miR-467.

**microR26b and microR562**

Both miR26b and miR562, tumor suppressor microRNAs, are found on human chromosomes 2q37.1 and 2q35, respectively. The expression of both NF-kB1 (p105) and NF-kB subunit RELA (p65) are directly repressed by miR26b and miR562, and this is linked with angiogenesis in breast cancer patients (Anbalagan et al., 2014). Many pathways, including the PI3K/AKT pathway, are associated with NF-kB signaling (Ghalouri-Fard et al., 2021a; Hussen et al., 2021b). In addition, the phosphatidyl inositol 3-/kinase/Akt signaling is involved in the production of HIF-1 and VEGF, and it is essential for the development of blood vessels in the BC (Karar and Maity, 2011; Li et al., 2015). Because of this, miR-26b and miR-562 lead to BC angiogenesis through the activation of NF-kB, PI3K/AKT, HIF-1α, and VEGF pathways.

**microR-655/526b**

The expression of miRNA-655/526b is considerably greater in human BC, and the higher expression of miR-655/526b is linked with a poorer prognosis (Gervin et al., 2020). In vitro research showed that COX2, an inflammatory enzyme elevated in BC, increases the expression of miR-655/526b (Majumder et al., 2015; Majumder et al., 2018). Furthermore, the researchers discovered that miR-655/526b transfection led to increases in the levels of angiogenic molecules like VEGF, VEGF-D, COX2, and LYVE1 (Hunter et al., 2019). It was also shown that the expression of VEGFRI, which controls the growth of blood vessels, was increased in cell lines treated with both miRNAs. Additionally, HUVEC cells treated with medium containing miR-655/526b generated tubular structures (Hunter et al., 2019).

**ANTI-ANGIOGENIC MICRORNA-BASED THERAPY**

One of the most essential proposed strategies to combat and prevent cancer metastasis is to target angiogenesis pathways (Lee et al., 2015). Tumors start to produce a myriad of proangiogenic factors early during tumorigenesis to form their vasculature (Carmeliet and Jain, 2011), the anti-angiogenesis strategy, which was first suggested by Judah Folkman in 1971 now a day is considered an effective and promising antitumor strategy (Folkman, 1971), (Ebos and Kerbel, 2011). Therefore, targeting angiogenic miRNAs can be gained by either 1) miRNA-based therapeutics or 2) drugs and phytochemicals that already affect angiogenic miRNA. This category has additional advantages of being available and almost safe, and their toxicity and side effects are well studied (Varghese et al., 2020).

**microRNA-Based Therapeutics in Breast Cancer**

miRNA-based therapeutics with antitumor and/or anti-angiogenic effects are achieved by either substituting or restoring tumor suppressor miRNAs activity or silencing overexpressed endogenous oncogenic miRNAs (Boca et al., 2020). In which mimic sequencing is used for restoring tumor suppressor miRNA (miRNA mimics) and exogenous delivery of antagonists (oligonucleotides that are chemically modified) for silencing endogenous oncogenic miRNAs (antagonim) (Caporal and Emanueli, 2011). Achieving efficient and targeted delivery of miRNAs mimics or antagonims to targeted cancer tissues is of paramount importance. Some successful modalities have been researched extensively, yet a major obstacle still in progress is to be translated more successfully into clinical practice (Ganju et al., 2017). miRNA therapeutics can be delivered by either viral or non-viral vectors. Nano-technology delivery of miRNA
Drugs and Phytochemicals Harboring Anti-angiogenic Activity in Breast Cancer Melatonin

Melatonin is a flexible anti-cancer agent that has been studied extensively in various malignancies, including breast cancer. According to a recent study, melatonin has anticancer effects ranging from antiproliferative to increased apoptosis of breast cancer cells at physiological and pharmacological doses. Melatonin also has anti-metastatic properties and can reduce antitumor resistance and toxicity (Kong et al., 2020). Cheng et al. showed that melatonin reduces cellular viability and has an anti-angiogenic effect on HUVECs via the downregulation of the HIF1/ROS/VEGF axis, in which melatonin exerts these effects by directly inhibiting hypoxia-induced HIF1 and indirectly acting as a free radical scavenger, resulting in a reduction of ROS and VEGF crosstalk (Cheng et al., 2019).

Additionally, melatonin can also disrupt the development of vasculogenic mimicry (VM) via breast cancer cell lines in both normoxic and hypoxic conditions. VM mediates resistance toward anti-angiogenic drugs and has a significant role in breast cancer metastasis (Maroufi et al., 2020). Another study found that melatonin not only downregulates VEGF mRNA but also simultaneously downregulates ANG1 and ANG2 in breast cancer cells (González-González et al., 2018). miR-148a/152 overexpression is associated with marked inhibition of breast cancer cell proliferation and angiogenesis by targeting IGF-1R and IRS1 and consequently their downstream signaling pathway (Xu et al., 2013). Melatonin can also regulate the aforementioned angiogenic miRNAs, and it can upregulate expression of miR-152-3p, “a tumor suppressor found to be downregulated in breast cancer,” which in turn reduces the protein expression of IGF-insulin-like-like growth factor-1 receptor), HI, F-1,α, and VEGF (Marques et al., 2018). In another study, melatonin upregulated miR-148a-3 and reduced gene expression of IGF-1R and VEGF (Lacerda et al., 2019).

Metformin

Metformin, a widely used anti-diabetic, has been very extensively investigated in recent years for having several anti-cancer effects in breast cancer (DeCensi et al., 2014), (Goodwin et al., 2008), (Alimoradi et al., 2021). The most extensive ongoing clinical trial (NCT01101438) for using metformin in breast cancer will reveal metformin’s Role in breast cancer in detail. Metformin has several mechanisms for its beneficiary effect in breast cancer; among them, it affects miRNAs. Metformin exerted an anti-angiogenic effect in breast cancer models by inhibiting HER2-mediated VEGF upregulation and HIF-1α-mediated VEGF up-regulation, suggesting a novel mechanism of metformin targeting the HER2/HIF-1α/VEGF signaling axis (Wang et al., 2015).

Additionally, it was found out that metformin inhibited proliferation, tube formation, and migration of HUVECs by downregulation of miR-21 and TGF-β protein expression, consequently increasing PTEN and SMAD7 protein expression (Luo et al., 2017). Furthermore, Metformin was found to reduce breast cancer cell viability, upregulated miR-26a, and reduced expression of miR-26a targets PTEN and EH2Z in several breast cancer cell lines (Cabello et al., 2016). Although, metformin might have an impact on metastasis, potentially via altering the levels of miR-21 in various cancer cell lines and breast cancer patients (Pulito et al., 2017). Metformin was also found to suppress miR-21 and miR-155 and up-regulate miR-200c in breast cancer cells, accordingly suppressing proliferation and metastasis of breast cancer (Alimoradi et al., 2021), (Sharma and Kumar, 2018), (Zhang et al., 2017).

These metformin effects showed synergism with everolimus, providing a potential role for metformin to be used in conjunction with breast cancer treatments.

Phytochemicals

Cardamonin suppressed miR-21 in HUVECs and accordingly suppressed VEGF-induced angiogenesis and cell migration (Jiang et al., 2015). Epigallocatechin-3-gallate (EGCG) from green tea and silibinin from milk thistle is widely consumed shown to have a powerful anti-angiogenic effect. Both synergistically, they were found to downregulate VEGF and miR-17–92 cluster while upregulated anti-angiogenic miR-19b in HUVECs (Mizraghaei et al., 2019). Interestingly EGCC was also found to inhibit tumor cell growth and angiogenesis via suppressing HIF-1α, NFκB α, and VEGF (Gu et al., 2013). Meanwhile, silibinin downregulated miR-21 and miR-155 in T47D breast cancer cells (Zadeh et al., 2015).

Furthermore, curcumin was found to suppress the proliferation and induce apoptosis of cancer cells (Ghaderi et al., 2021), (Sobhkhizi et al., 2020) as well as impinge MCF-7 cells by upregulating miR-15a and miR-16, which caused downregulation of Bcl-2 (Fix et al., 2010). Curcumin also upregulated miR-34a and miR-181b in breast cancer cell lines and inhibited invasion and metastasis of them (Guo et al., 2013), (Kronsiki et al., 2014), (Norouzi et al., 2018). In addition, curcumin efficiently targeted and elevated protein expression of miR-34a in MCF-10F and MDA-MB-231 cell lines and consequently affected regulatory genes of EMT and Rho-A and attenuated tumor cell migration and invasiveness (Gallardo et al., 2020). Beside of the above, several additional phytochemicals, such as resveratrol, luteolin, and betulinic acid, have been shown to influence angiogenic miRNAs (Varghese et al., 2020) (Table 3).

CONCLUSIONS AND FUTURE PERSPECTIVES

MicroRNAs regulate tumor angiogenesis, a key component in cancer growth and metastasis. Identification of novel molecular features of angiogenesis regulation, and a greater understanding of cancer progression strategies, will allow the development of new therapeutic options. Numerous genes which involved in angiogenesis are regulated by miRNAs, therefore identifying...
miRNA-target interaction networks might be useful in describing anti-angiogenic therapy and novel diagnostic biomarkers in BC. Angio-regulatory miRNAs may be used to produce a new generation of medicines such as nano-based therapeutics. Additionally, phytochemical medicines might modulate the expression of angio-regulatory miRs, which in turn could enhance survival in BC patients. Furthermore, we revealed the inhibiting and stimulating pathways of angio-regulatory miRNAs in cancer-related angiogenesis process, which may be useful in the developing anti-angiogenic methods in cancer therapy.

**AUTHOR CONTRIBUTIONS**

MT, SF and HJ wrote the draft and revised it. BH, SA, MR and AS collected the data, designed the figures and tables. All the authors read and approved the submitted version.
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