Review Article

The Role of microRNAs Regulated Breast Cancer Stem Cells in the Pathogenesis, Prognosis and Aggressiveness of Breast Cancer

Abstract

Breast cancer is a leading cause of cancer related death among women worldwide and it is a complex disease characterized by its heterogeneity. The heterogenous characteristic of the breast cancer is responsible for its aggressiveness and treatment resistance. This review is to provide an overview of the pathogenic mechanism of microRNAs expressions on the breast cancer stem cells in controlling the prognosis and aggressiveness of breast cancer disease. Breast cancer stem cells (BCSCs) are population of heterogenous cells with increased propensity for proliferation, differentiation, migration, invasion and mammosphere formation with increased resistance to treatment. They have ability to initiate and propagate cancer cells population and share similarity with the normal stem cells in relation to their expressions of cell surface markers; Cluster of Differentiation 44 positive ($CD44^{+}$), Cluster of Differentiation 24 negative/low ($CD24^{-/low}$) and Alkaline dehydrogenase 1 (ALDH1).

MicroRNAs (miRNAs) are responsible for the RNA silencing and post-transcriptional regulation of gene expression. They control gene expression by acting as oncogenes or tumor suppressor genes. The epigenetic mechanisms are used for the regulation of miRNAs expression in the breast cancer and this can be targeted to reverse the cancer progression. Dysregulation of microRNAs expression were identified in breast cancer and breast cancer stem cells (BCSCs), including miR-10b, miR-21, miR-155, miR-181, miR-183, miR-210 and miR-221/222, were upregulated in BCSCs and the downregulated microRNAs in BCSCs are: Let-7, miR-22, miR-30e, miR-31, miR-103/107, miR-200, miR-205, miR-335, miR-449a, miR-519c, miR-600, miR-708, miR-760.

Studies showed association between the miRNA expression and breast cancer metastasis and aggressiveness. MicroRNAs has regulatory effect on neovascularization, drug resistance, and metastasis. They can be used as the predictive indicators in determining the prognosis of breast cancer following treatment. The miRNAs expression can be used to determine the metastatic BCSCs for an efficient targeting mechanism and identification of these metastasis.

Key words: microRNAs, Breast cancer stem cells, epigenetic mechanism, dysregulation, breast cancer aggressiveness, cell surface markers

1. Introduction

Breast cancer is a leading cause of cancer related death among women worldwide particularly in the developed worlds. It is a complex disease characterized by its heterogeneity with variation in its molecular and genetic composition [1]. The heterogeneous characteristic of the breast cancer is responsible for its aggressiveness and treatment resistance. Breast cancer disease has different molecular subtypes based on their gene expression including human epidermal growth factor 2^+ ($HER2^+$) type, luminal and basal-like types. $HER2^+$ subtype of breast cancer disease is associated with amplification or overexpression of epidermal growth factor (EGF) oncogene, the luminal subtype expresses the hormone receptors (estrogen and progesterone receptors), and basal-like subtype lacks the HER2 and hormone receptors (triple negative). The inheritance germ line gene mutations had also been implicated in the etiopathogenesis of breast cancer disease including Breast cancer type 1 (BRCA1) and breast cancer type 2 (BRCA2), Ataxia-Telangiectasia Mutated (ATM) gene and Phosphatase and tensin homolog (PTEN) genes. [1]

Breast cancer stem cells (BCSCs) are a population of heterogeneous cells with increased propensity for proliferation, differentiation, migration, invasion and mammosphere formation with increased resistance to treatment. Breast cancer stem cells are self-proliferative in nature with ability to initiate and propagate cancer cells population. They share similarity with the normal stem cells in relation to their expressions of cell surface markers; Cluster of Differentiation 44 positive ($CD44^+$), Cluster of Differentiation 24 negative/low ($CD24^{-/low}$) and Alkaline dehydrogenase 1 (ALDH1). BCSCs are characterized based on their tumor initiating and the differentiation capability. [1]

MicroRNAs (miRNAs) are non-coding single stranded fragments of RNA molecules, which consist of 20-22 nucleotides responsible for RNA silencing and post-transcriptional regulation of gene expression, through base pairing with the complementary sequences within mRNA molecules. MicroRNAs are involved in stem cell replication, differentiation, and apoptosis by controlling the expression of oncogenes and tumor suppressor genes. Dysregulation of microRNAs expression had been identified in breast cancer and breast cancer stem cells

(BCSCs), including miR-10b, miR-21, miR-155, miR-181, miR-183, miR-210 and miR-221/222, were upregulated in BCSCs while Let-7, miR-22, miR-30e, miR-31, miR-103/107, miR-200, miR-205, miR-335, miR-449a, miR-519c, miR-600, miR-708, miR-760 were found to be downregulated in breast cancer and BCSCs [2][3] Thus, these miRNAs regulate gene expression in cancer stem cells by acting either as an oncogene or tumor suppressor genes .[3]

A better understanding of the mechanism of regulation of BCSCs by the microRNAs in breast cancer can be extremely useful in determining the pathogenesis, prognosis, and the aggressiveness of breast cancer for the formulation of an effective treatment regimen for breast cancer. Numerous studies have been performed on the regulatory effects of microRNA expression patterns in human diseases including cancer, but only a few of these studies highlighted the regulatory roles of the miRNAs on breast cancer stem cells in relation to breast cancer prognosis and aggressiveness. The focus of our study is to highlight the roles of miRNAs in regulating breast cancer stem cells in the pathogenesis, prognosis, and the aggressiveness of breast cancer.

2. Aims and Objectives

This review is to provide an overview of the pathogenic mechanism of microRNAs regulatory effect on the breast cancer stem cells in controlling the prognosis and aggressiveness of breast cancer disease. This will be done by providing highlight of the biogenesis of miRNAs and functions. We will briefly highlight the effects of BCSCs on the pathogenesis of breast cancer and summarize the regulatory mechanism of miRNA expressions on the BCSCs. We would highlight the regulatory role of the dysregulated oncogenic and tumor suppressor microRNAs on BCSCs for the pathogenesis, prognosis, and aggressiveness of breast cancer. We will then summarize the regulatory and signaling mechanism of miRNAs expressions on BCSCs for the breast cancer prognosis, aggressiveness, and metastasis.

3. METHODS

3.1. Data sources

The data for this study review were obtained from the Medline on OvidSP, includes PubMed of the US National Library of Medicine and the search was done through the University of Bristol Library services.

3.2. Search strategy

The search was done by signing into Ovid, Wolters, and Kluwer portal and all the resources were selected. Three separate keywords were used for the search and the first search was done using the keyword “MicroRNAs” and this yielded a total number of 68,168 publications. The second search was done using the keyword “breast cancer” and this gave a total number of 338,066 publications while the third search was done with the keyword “stem cells” and this gave a total number of 155,033 publications. A combination of the search for “MicroRNAs” using the Boolean operator “AND” with “breast cancer” and “stem cells” yielded a total number of 132 publications. We next hand screened these 132 publications to see those that fit into the inclusion criteria for our study, and we arrived at a total 69 publications. Other data were included in this review, and these were obtained from the University of Bristol Library services using the search phrase “microRNAs,” “breast cancer” and “stem cells.” The publications generated were hand screened to fit into the inclusion criteria, and 21 publications were selected. Also included were relevant references from previously selected publications as well as many other recommended publications. A total of 104 articles were reviewed.

3.3. Inclusion Criteria

The publications selected were thoroughly analyzed to ensure they focused on our study objectives which are on the pathogenic mechanism of how miRNAs regulate breast cancer stem cells to affect the aggressiveness and prognosis of breast cancer. We included the studies that focused on the miRNAs and breast cancer stem cells and those that emphasized on various novel techniques in the understanding of the aggressiveness and pathogenesis of breast cancer. We considered publications on the roles and limitations of miRNAs and cancer stem cells (CSCs) in the diagnosis, and treatment of breast cancer and the progress that had been made to address these limitations.

4. MicroRNAs Biogenesis

MicroRNAs were first discovered in Caenorhabditis elegans and described by Lee et al. in 1993. [4] The genes are transcribed in the nucleus into primary miRNAs (Pri-miRNA) by RNA polymerase II. Drosha enzyme cleaves the Pri-miRNAs to precursor miRNAs (Pre-miRNAs) which are hairpin like structures. The Pre-miRNA are cleaved in the cytoplasm by dicer to form a mature miRNA. One strand of the miRNA is associated with the RNA-induced silencing complex (RISC). The miRNAs regulate gene expression through cleavage of mRNA by binding to the 3'-untranslated regions (3'-UTR) of the target messenger RNAs (mRNAs) through base pairing and this results in cleavage of the target mRNA. The complete complementarity between the miRNA and 3' UTR recognition site will result in the degradation of mRNA and incomplete complementarity will lead to repression of translation. Thus, miRNAs modulate expression of the target genes by degrading their target mRNA or inhibition of the translation through pairing of their sequence with the complementary bases of the target mRNA. [4]

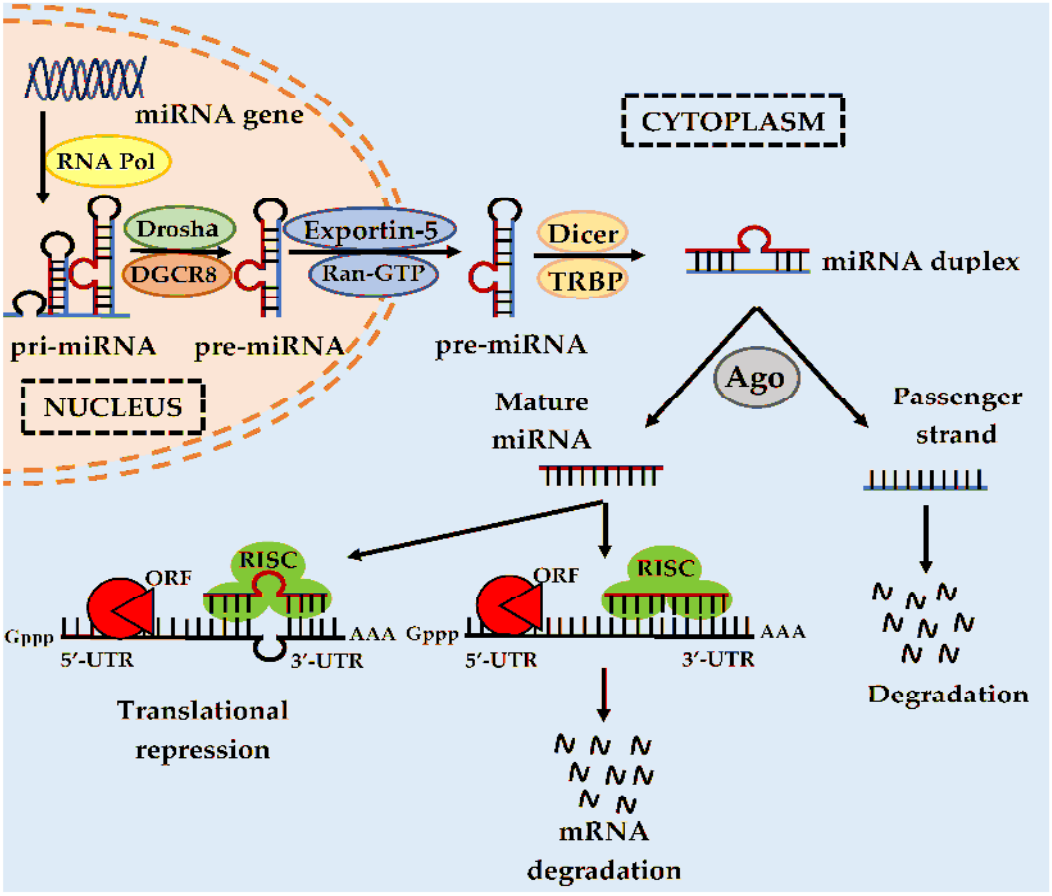

FIGURE 1: MicroRNAs BIOGENESIS

Source: The Regulatory Role of MicroRNAs in Breast Cancer - PubMed (nih.gov)

5. BREAST CANCER STEM CELLS

BCSCs are a small fraction of normal stem cells with self-renewable properties such as high proliferative, and differentiative capability [5]. The epigenetic mechanisms are vital for the generation of cancer stem cells by transforming normal stem cells to cancer stem cells, leading to loss of their differentiation capability by upregulating genes for stem cell replication and downregulating genes for differentiation. [6]

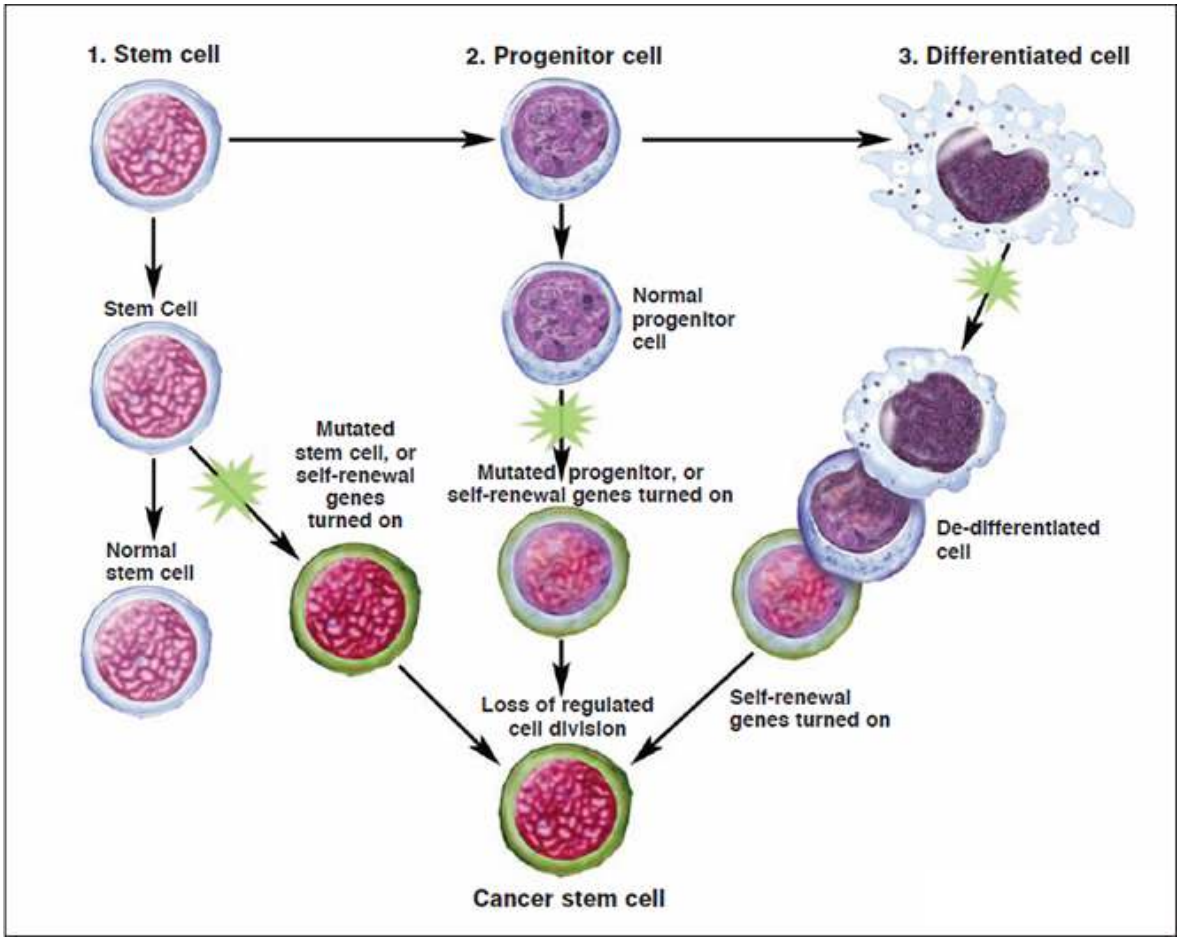

Figure 2- DEVELOPMENT OF BREAST CANCER STEM CELLS (HOW DO THEY ARISE?)

Source: Semantic Scholar [ARE STEM CELLS INVOLVED IN CANCER |

Cancer stem cells (CSCs) have the capacity to generate colonies of cancer cell progeny and share similar characteristics and properties with the normal stem cells such as marker expressions, proliferation, and self-renewable capability. Cancer stem cells are found to be resistant to treatment and can be associated with the initiation, progression, maintenance, and cancer recurrence. Thus, CSCs initiation capacity and resistance to treatments are major contributor to the cancer aggressiveness. Localized breast cancer is considered a manageable disease and majority of patients die from local disease that has spread to other sites in the body .[7] Thus, as miRNAs play a role in breast cancer cell metastasis, they have been implicated as one of the major contributors of death from breast cancer.[7]

Breast cancer stem cells (BCSCs) were first described in a publication in 2004. [8] They were identified and isolated based on their cell surface marker expressions $CD44^{+}$, $CD24^{-/low}$ and epithelial surface antigen (ESA). The expression of high levels of aldehyde dehydrogenase 1 (ALDH1) on BCSCs and the analysis of CD44 and ALDH1 on the cells in the serum showed high level of CD44 expressions were associated with reduced cancer survival when compared to the low serum expression of CD44. [9] Thus, CD44 is a cell surface antigen that is important for cell to cell interaction, migration, and adhesion.

The activation of epithelial to mesenchymal cell transformation are responsible for the tumor initiating capability of the cancer stem cells with the loss of E- cadherin and cell to cell interactions. Mutations are implicated as the cause of the transformation of normal stem cells into the cancer stem cells (CSCs) and research theory identified (misplacement somatic stem cell theory) problem with the somatic cells positioning as the mechanisms for the formation of .[10][11]

6. MicroRNAs Regulatory roles in BCSCs

The regulatory role of microRNAs in breast cancer was first described in 2007 by Yu et. al. (2007) where the expression of miRNAs in breast cancer stem cells were compared with the miRNAs from the self-proliferating and differentiating cells of the breast cancer cells . [12] MiRNAs are involved in the regulation of the expression of stem cell markers with the activation of the signaling pathways and the transcription of the breast cancer stem cells controlling cell proliferation, migration, survival, apoptosis, tumor growth, cell cycle progression, neovascularization, epithelial to mesenchymal transformation (EMT) and the stem cells maintenance.

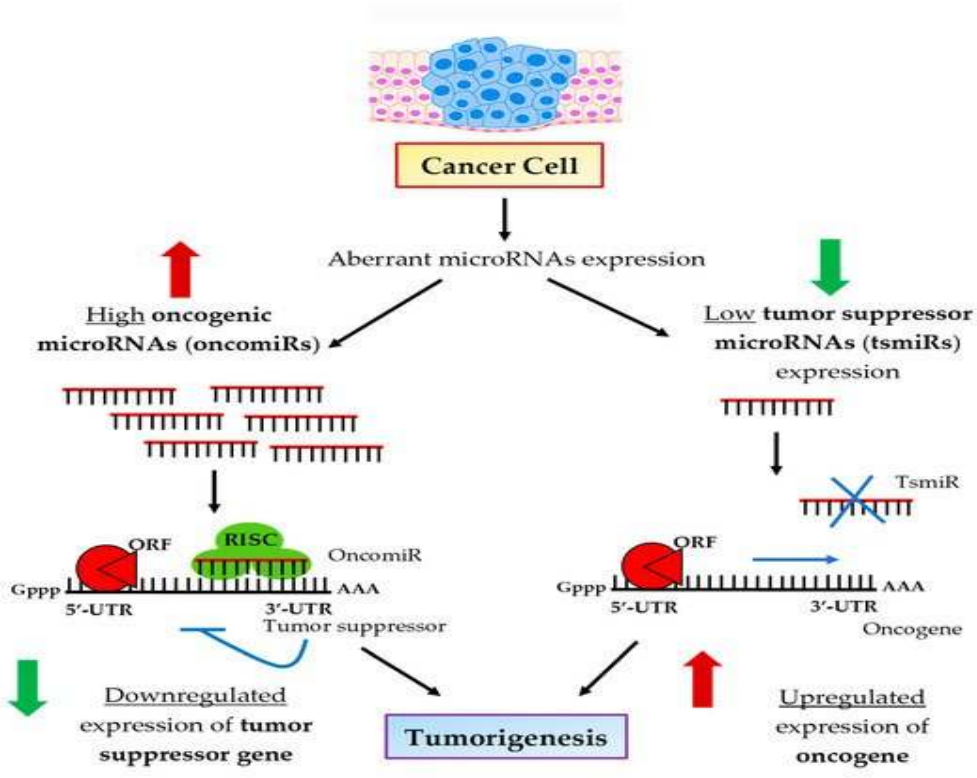

Figure 3: MicroRNAs Regulatory roles in BCSCs

Source: The Regulatory Role of MicroRNAs in Breast Cancer - PubMed (nih.gov)

MicroRNAs are found to play vital role in cancer initiation, progression, and prognosis. The regulatory role of miRNAs on the cancer stem cells by targeting the signaling pathways, including Notch gene, WNT/ β-Catenin, phosphatidylinositol 3-kinase B (PI3)/AKT) and nuclear factor kappa-light-chain-enhancer of activated B (NF-Kb) have been implicated in the cancer stem cell proliferation, migration, cell survival, differentiation, invasion, metastasis, and cancer recurrence. [12][13]

Shimono et. al. in 2009 identified miRNA expression in BCSCs and non-cancerous stem cells, including miR-200, let-7, miR-1 and miR-27. The miR-200 family are important for preserving the stem cells, let-7 for inhibition of self-renewal and differentiation by targeting the H-RAS (Harvey rat sarcoma oncogene) and High mobility Group AT-Hook 2 (HMGA2) genes while miR-1 suppresses migration and proliferation of BCSCs by targeting the Wnt pathway. MiR-27 were noticed to upregulate VEGF (vascular endothelial growth factor) in BCSCs to promote neovascularization and metastasis [6][13]. A study revealed that miR-27

targets the phosphodiesterase family 1 to regulate the drug resistance effects of the BCSCs [13] [14). Thus, miRNAs can function as an effective therapeutic target for the management of breast cancer through the re-expression of the downregulated miRNAs in the breast cancer stem cells.

MiR-204, miR-200c, miR-34a, and miR-10b target the self-renewal and epithelial to mesenchymal transformation (EMT) pathways by increasing the survival of the breast cancer stem cells via upregulation of the octamer- binding transcription 4 (OCT4), Sex determine region Y box 2 (SRY/SOX2), Kruppel like factor 4 (KLF4), Cellular myelocytomatosis oncogen (C-MYC), Notch homolog 1 translocation associated (NOTCH 1), Zinc finger protein (SNAIL), Zinc finger E-box binding homeobox 1 (ZERB1) and Cadherin 2 (CDH2). These genes are targeted in the regulation of pluripotency of the Mitogen activated protein kinase (MAPK), Wingless related integration site (WNT), Hedgehog, Tumor protein 53 (p53) and the Transforming growth factor β (TGF-β) pathways providing major insights to the potential role of targeting the core regulatory miRNAs in the eradication of the BCSCs.[5][8] [13][14]

7. DYSREGULATION OF MicroRNAs in the BCSCs

Epigenetic alteration of the genetic composition in the protein coding genes were implicated in cancer initiation and aggressiveness. The epigenetic mechanism involved in the miRNA dysregulation include the DNA methylation and histone acetylation that affect the gene transcription and expression . DNA methylation is involved in the loss of pluripotency in breast cancer stem cells and epigenetic reprograming can be used to generate cancer stem cells by downregulating the gene for differentiation and upregulating the gene for the expression and proliferation. DNA methylation is associated with wingless related integration site (Wnt)/β-catenin activation by the methylation of the promoter and silencing of the Wnt inhibitors in the breast cancers. Wnt/ β- catenin is involved in the differentiation and proliferation of the cancer stem cells. DNA methylation of the E-cadherin promoter recruits histone deacetylases (HDACs) and cause histone acetylation with silencing of the gene transcription. [14][15]

7.1. Downregulated (Tumor suppressor) MicroRNAs in the BCSCs

7.1.1. Let-7 Family

Let-7 family are tumor suppressor genes that are downregulated in breast cancer stem cells and can regulate the expression of BCSCs. The downregulation of Let 7 associated with the formation of breast cancer. Let 7 is a tumor suppressor gene, but also found to have anti-apoptotic properties for the regulation of cell differentiation and apoptosis. The Let 7 role in the BCSCs include the formation of cancer and mesenchymal cells differentiation. Lentiviral mediated re-expression of Let 7 was associated with a reduction in mammosphere formation, reduced rates of proliferation, and decreased numbers of undifferentiated stem cells, while reduction in the level of Let-7 expression inhibited the differentiation of BCSCs and maintained their proliferation [12].

Let 7 is a potential target for anti- cancer therapy in the treatment of breast cancer and Lin 28 is an RNA binding protein that was found to control the Let 7 family. The expression of Lin 28 blocks the formation of Let 7 by promoting cell proliferations and increase the formation of cancer cells. Reduction in the level of Let 7 expression would inhibit BCSCs differentiation, maintain their proliferation, and promote epithelial to mesenchymal transformation. Thus, suppression of Let 7 through Lin 28 promotes the formation of breast cancer cells. [12][13]

Let 7 also targets the RAS proto oncogene and high mobility group AT-Hook 2 (HMGA2) genes to inhibit the proliferation and metastasis of the cancer cells and engages the HMGA2 gene in the differentiation of the epithelial to mesenchymal cell transformation resulting in an increased expression of mesenchymal cell markers. Inflammatory cytokines activate the signaling transduction and stimulation of STAT transcription factor 3 (STAT 3) to promote Lin 28 transcription and cause the repression of Let 7 expression while the upregulation of Let 7 would target the HMGA2. [12]

7.1.2. miR-200 Family

The miR-200 family are downregulated in breast cancer stem cells and target Bmi-1 and Suz12 genes. They were found to be involved in the regulation of epithelial to mesenchymal transformation (EMT), self-renewal, clonal expansion, and differentiation of the BCSCs. They comprised of five members in the family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and are grouped into 2 clusters; MIR-200a, miR-200b/miR-429 genes are clustered on chromosome 1 while miR-200c/miR-141 genes clustered on chromosome 12.

The miR-200 family expression in the BCSCs and $CD44^{+}$, $CD24^{-/low}$ lineage expressions were compared in the BCSCs and the findings revealed downregulation of the expressions in the clusters of miRNAs. MiR-200c inhibits the proliferation of the BCSCs and inhibits the cell growth and promote cell differentiation .[2]

Expressions of some of the miR-200 family were found to be inhibitory to EMT that was induced by transforming growth factor- β (TGF-β) targeting the repression of E-cadherin through transcriptional repressor gene Zinc finger E-box binding homeobox 1(ZERB1) and ZERB2 .[15] The transcription of miR-200C and miR-141 were found to be strong inducers for the epithelial differentiation and the suppression by the ZERB1 resulted in epithelial to mesenchymal transformation. The histone modification of DNA promoter methylation epigenetically regulated the expression of miR-200 and transactivated the p53 in the miR-200c promoter. [17][18][19] Thus, the loss of p53 would result in a reduction in the levels of miR-200c. The increase in EMT and levels of stem cell marker expression were found to be associated with the risk of high-grade tumor formation. [17][18][19][20]

MiRNAs	Targets	Function	Reference
Let-7	RAS, HMGA2	Inhibits proliferation and metastasis	[11][12]
miR-708	Rhodopsin	Decreases migration and metastasis	[16]
miR-103/107	CDK5R1	Decreases migration	[20][21]
miR-200	ZEB1, ZEB2	Inhibits invasion and survival	[13][15]
miR-519c	HIF-1α	Decreases angiogenesis	[12][26]

MiRNAs	Targets	Function	Reference
miR-31	Radixin, RhoA	Inhibits metastasis and invasion	[13][25]
miR-335	SOX4, TNC	Inhibits metastasis and invasion	[13][23][24]
miR-30e	ITGB3, UBC9, p53	Prevents tumorigenesis	[12]
miR-205	HER3	Inhibits proliferation	[22]
miR-22	Erα, Sp1, C-MYC	Inhibits proliferation, invasion and metastasis	[17][18]
miR-449a	PLAGL2	Inhibits proliferation and metastasis	[27]
miR-600	SCD1	Reduces BCSC self-renewal and tumorigenicity	[28]
miR-760	NANOG	Inhibits proliferation and migration	[5][29][42]

TABLE 1: TUMOR SUPPRESSOR MicroRNAs

Source: MicroRNAs and breast cancer stem cells: Potential role in breast cancer therapy - PubMed (nih.gov)

7.1.3. miR-30 Family

MicroRNA 30 family are downregulated in BCSCs, most commonly miR-30e. They were found to modulate the self-renewal and anti-apoptotic properties of BCSCs. The expression of miR-30e in the breast cancer stem cells would result in the inhibition of cell proliferation by

decreasing the levels of Ubiquitin conjugating enzyme 9 (Ubc9) and induced apoptosis through repression of the integrin b3gene (ITGB3). The blocking of miR-30e expression in the differentiated breast cancer stem cells would result in the proliferation of the cancer cells. AVEN anti-apoptotic protein are downregulated after the overexpression of miR-30 confirming the involvement of miR-30 family in the regulation of genes for the non-attachment proliferation of mammospheres formation and the apoptosis of BCSCs. [30] [31] [32]

7.1.4. miR-708

The expressions of miR-708 are downregulated by the polycomb suppressor complex 2 induced H3K27 trimethylation in the breast cancer by targeting neuronatin to reduce the activation of focal adhesion kinase (FAK) that results in a decrease cell migration and cancer metastasis. MiR-708 expressions are suppressed when targeting rhodopin. Thus, the study showed overexpression of Neurontin result in resistance to miR-708 suppression with a decrease cell migration and metastasis. MiR-708 has a repressive metastatic effect in the breast cancer and could have a potential role in the treatment of metastatic cancer.[16]

7.1.5. miR-22

MicroRNA-22 overexpression decrease the (estrogen receptor α (Erα) gene by promoting the cleavage of mRNA targeted by the 3'UTR and results in the inhibition of proliferation, invasion and metastasis [17]. The overexpression of miR-22 would reduce the metastasis, invasion, and proliferation of BCSCs . [18] A study revealed that miR-22 would decrease the expression of cluster of differentiation 147 (CD147), which is an extracellular matrix metalloproteinase inducer that is associated with cancer growth, invasiveness, and metastasis. CD147 downregulates the specific protein 1 (Sp1) gene expression and the miR-22/Sp1/C-myc network have a regulatory effect on the transcription of CD147 gene to reduce the invasive and metastatic ability of the BCSCs. Frankel et al in 2008 revealed that tumor suppressor protein programmed cell death 4 (PDCD4) are regulated by miR-21 in the BCSCs while another study by Wickramasinghe et. al in 2009 revealed reduction in the level of expression of miR-21 in the BCSCs by estradiol E2 .[19][21]

7.1.6. miR-103/107

Overexpression of miR-103/miR-107 and the silencing of cyclin dependent kinase 5 activator 1 (CDK5R1) gene would reduce the migration of cancer cells .[20] They are found to modulate expression of CDK5R1 and affecting the effect of Cyclin dependent kinase 5 (CDK5) and the pathway.[20] [21]

7.1.7. miR-205

MiR-205 are downregulated in breast cancers and they interfere with the HER receptor by suppressing the proliferation of cells. MiR-205 targets the HER3 receptor to inhibit the activation of Akt mediators. They are downregulated in the stem cells that have undergone EMT in response to TGF-β .[15] Ectopic expression of miR-205 in the BCSCs would result in the suppression of cancer cell proliferation, invasion, and tumor growth by targeting Her3 and VEGF-A genes. A study showed that miR-205 expression would enhance cancer responsiveness to therapy by targeting Her3 gene and interfere with the PI3K/Akt survival pathway to improve the responsiveness to tyrosine kinase inhibitors.[22] MiR-205 can act as a tumor suppressor gene through p53 and re-expression would result in decreased cell proliferation and cell cycle arrest.

7.1.8. miR-335

The expression of miR-335 are downregulated in the BCSCs were found to be associated with reduced cancer cell invasion, migration, and metastatic ability .[23] It was noted that Tenascin-C (TNC) which is an extracellular matrix protein, and transcription factor SOX4 oncogene targeted the miR-335 .[24]

Study revealed that miR-31 targets radicin, RhoA, and integin-5 genes to promote metastasis and spread of breast cancer. The overexpression of miR-31 resulted in the decrease expression of targeting genes and reduced breast cancer metastasis and the invasiveness.[25]

7.1.10. miR-519c

miR-519c regulate neovascularization by targeting the hypoxia inducible factor- 1α (HIF-1α) to regulate the neovascularization in the breast cancer cells by the stimulation of VEGF

(vascular endothelial growth factor), basic fibroblast growth factor, and interleukin-8. A study revealed decreased angiogenesis due to the overexpression of miR-519c .[26]

7.1.11. miR-449a

A research study revealed overexpression of miR-449a suppressed the proliferation and metastasis of breast cancer by targeting the Pleomorphic adenoma gene like 2 (PLAGL2) gene. The miR-449a levels were found to be decreased in the breast cancer cells.[27]

7.1.12. miR-600

Overexpression of miR-600 inhibit the production of WNT proteins and promote breast cancer stem cells differentiation .[28] Study showed that expression of miR-600 would regulates the BCSCs colony. The level of expression of miR-600 is associated with the survival of the patient with the breast cancer. The upregulation of miR-600 regulates the WNT signaling pathway by suppressing the stearoyl-CoA desaturase-1 expression. The WNT signaling pathway is active and promotes proliferation of cells in the absence of miR-600. [28][35]

7.1.13. miR-760

MiR-760 targets the NANOG gene to inhibit the proliferation and migration of breast cancer cells. [29]

7.2. Upregulated (Oncogenic) miRNAs in the BCSCs

7.2.1. miR 181 Family

MiR-181 are upregulated in the breast cancer stem cells. The increase level of expression of miR-181 in the BCSCs initiated the mammosphere formation .[30] Study revealed the effect of transforming growth factor β mediated by miR-181 on the BCSCs would increase the proliferation of cells.[11] The up-regulation of the miR-181 were identified to be an inducer for the mammosphere formation at the post-transcriptional level by the TGF-β and identified BRCA1 as the targeted gene .[31] Ataxia telangietasia (ATM) can also act as a tumor suppressor gene to reduce the mammosphere formation.

7.2.2. miR-183

niR-183 expression was associated with the estrogen receptor and HER2/neu receptor to dysregulate the invasion and migration of the cancer cells. Study showed miR-183 targeted VIL2-coding protein Ezrin and have a target for the early growth response protein 1 (EGR1) mRNA .[32]

7.2.3. miR-210

The expression of miR-210 in the breast cancer was associated with the level of hypoxia and its overexpression was stimulated by hypoxia (HIF-1α and Von Hippel Lindau dependent factor). The level of miR-210 was found to be inversely related to the survival of the patient with breast cancer. Thus, they can be used as a prognostic indicator and predictive index for breast cancer .[33]

7.2.4. miR-10b

miR-10b were noticed to be highly upregulated in the breast cancer and study revealed the miR-10b expressions were associated with the metastasis, invasion, and migration of the breast cancer cells .[34] Thus, the level of miR-10b expression associated with the metastatic ability of the cancer .[35] E-cadherin are found to have a potential in targeting the miR-10b with the associated increase in the tumor size, tumor grading, stage, proliferation, metastasis, and level of expression of Her2 expressions .[36] Transcription factor twist can induce the miR-10b and promote the cancer invasion and metastasis by targeting the homeobox D10 (HOXD10) gene.

Oncogenic miRNAs	**Targets**	**Function**	**Reference**
miR-10b	ZEB1, PIK3CA	Promotes EMT, metastasis, and proliferation	[36]
miR-21	PTEN, PCDC4, TPM1, TIMP3	Promotes metastasis and tumor growth	[2][12][37]

Oncogenic miRNAs	Targets	Function	Reference
miR-155	TP53INP1	Promotes proliferation, Prevents apoptosis	[9]
miR-181	BRCA1	Promotes tumor growth	[30]
miR-183	VIL2, EGR1	Promotes migration and metastasis	[32]
miR-210	Efna3, Ptp1b	Decreases survival	[33]
miR-221/222	P27Kip1, p57, TRPS1, PTEN	Enhances proliferation and invasion	[38][41][42]

TABLE 2: Oncogenic MicroRNAs

NOTE: Partly Adapted from Fatemeh Vahidian, Hamed Mohammadi et. al (2018)

7.2.5. miR-21

MiR-21 expressions were associated with the migration and invasion of breast cancer stem cells. This was implicated in the progression and metastasis of breast cancer. The expression of miR-21 found to be associated with the bad prognosis .[37] They are located on chromosome 17q23.1 and primary miRNAs in the humans .[38] Study showed HIF-1α and miR-21 were up-regulated in the BCSCs and decrease level of their expression would result in the reversal of the epithelial to mesenchymal transformation. Thus, reduce expression of HIF-1α will reduce the cancer cells migration and invasiveness.[29]

Decreased expression of miR-21 would inhibits cancer growth by targeting the Programmed cell death 4 (PDCD4), metalloproteinase inhibitor 3 and alpha-tropomyosin 1 . [2] PDCD4 and p53 are the targets for the miR-21. Mammary serine protease inhibitor (Maspin) suppress the invasion and metastasis of breast cancer cells by reducing their ability to invade, induce apoptosis and neovascularization. Thus, PDCD4 and Maspin were targeted by miR-21 for cancer invasion and metastasis. Study identified a tumor suppressor tropomyosin 1 (TPM1) as

a target for miR-21 and showed negative correlation with the expression of miR-21 and PTEN in the BCSCs related to the cancer advanced stage, metastasis, and poor survival.[39] Transforming growth factor- β upregulate the expression of miR-21 with associated increase risk of high-grade tumor and negative hormone receptor status.

7.2.6. miR-221 and miR-222

Upregulation of miR-221 and miR-222 increase the ability of the cancer cells invasion and migration .[40] They are found to suppress the target protein Tricho-rhino-phalangeal syndrome type 1 protein which is a ZERB 2 GATA family. [41]

miR-221 and miR-222 are expressed more in the basal type of breast cancer and their suppression in the p57 and p27/Kip1 proteins result in the decreased cell cycle and increase proliferation .[13] They increase tumor growth by targeting the PTEN .[42] MiR-222 were noticed to be overexpressed in the BCSCs and they can be used as a marker in the differentiation of BCSCs from the normal progenitor stem cells.

7.2.7. miR-155

miR-155 are overexpressed in the breast cancer and associated with the metastasis, diagnosis, and prognosis of the breast cancer .[43][44] Study showed inflammation and hypoxia increase the level of miR-155 expression .[45] Tumor protein p53 inducible nuclear protein 1 (TP53INP1) in Michigan Cancer Foundation-7 (MCF-7) cells identified as the target for the miR-155 and overexpression of the miR-155 increase the cancer cell migration and elicits anti-apoptotic effect by inhibiting the caspases-3,8,9 and p21 through suppression of TP53INP1 in MCF-7 cells.

8. Mechanism of MicroRNA regulated BCSCs in Breast Cancer Aggressiveness, Metastasis and Prognosis

MiRNAs can control gene expression by acting as an oncogenes or tumor suppressor genes .[3] The epigenetic mechanisms can be used for the regulation of miRNAs expression in the breast cancer and can be potentially targeted to reverse cancer progression. MiRNAs are involved in post-translational gene silencing of the target mRNAs and this is important for stem cell proliferation, differentiation, and apoptosis. They can be used to determine prognosis and predict breast cancer survival. MiRNAs can be used as a diagnostic tool due to their biostability that enable their detection in the blood.

The overexpression of the miR-125 target the pro-apoptotic gene Bcl-2 antagonist killer 1 in the breast cancer resulting in the tumor advancement and metastasis. Problem with the apoptosis are associated with the malignant advancement of the breast cancer.[46][47] miR-195, miR-24-2 and miR-365 target and suppress the Bcl-2 gene resulting in the activation of apoptosis in hormone dependent BCSCs.[48] miR-21 inhibits tumor suppressor gene PTEN and formed the death inducing signaling complex (DISC complex) in the breast cancer .[49] miR-141, miR-200b and miR-200c target the B lymphoma Mo-MLV insertion region 1 homolog (Bmi1) gene to suppress the p53 leading to the programmed cell death via the ARF tumor suppressor (p19Arf) gene. Lerner et. al in 2012 revealed that miR-200c would induce apoptosis by targeting Noxa pro-apoptotic gene (Bcl-2 family) and Fimbriae associated protein 1 (FAP-1) (inhibitor of CD95).

MicroRNA expression in breast cancer cells is associated with the levels of the estrogen, progesterone and Her2/neu receptors in breast cancer patients . [50]The correlation of the expression of miRNAs to the level of hormone receptor can serve as a prognostic indicator of the patient response to breast cancer treatment. The miRNAs suppress the estrogen receptor alpha (ERα) expression by binding with the 3’ UTR of estrogen receptor 1 (ESR1) mRNA. The activation of ERα enhance the transcription of target gene that control the cancer formation including cell proliferation, differentiation, and survival. Most of the downregulated miRNAs in estrogen receptor positive (ER^+) breast cancer are tumor suppressor gene miRNAs suppressing the cell proliferation and upregulation of the miRNAs can be used to prevent proliferation breast cancer cells.[51][52] MiR-145 are downregulated in breast cancer .[22] This could be used as a diagnostic marker for an early cancer detection. Spizzo et. al in 2010 demonstrated the pro-apoptotic effect of miR-145 with the activation of TP53 stimulating the expression of miR-145 and enhancing the pro-apoptotic effects. MiR-145 targets the ER-α gene to promote apoptosis and induce p53 transcription of the miR-145. MiR-145 targets and controls N-RAS and VEGF-A at the post-transcriptional level to inhibit the neovascularization, growth, and cancer cell invasion.

The overexpression or silencing of miRNAs can be useful in determination of the metastatic and invasive characteristic of breast cancer. This regulatory role of the miRNA’s expressions on the breast cancer stem cells can be a potential therapeutic target for the destruction and the eradication of the cancer cells through the metastatic and self-renewal pathway by preventing the formation of the mammosphere .[30] They are involved in the progression of breast cancer.

Cyclin D1 induces secretion of miRNAs controlling cancer immune response and oncogenic miRNAs by binding to the Toll-like receptor 8 to activate the pro inflammatory metastatic response. Cyclin D1 regulate the secretion of P- element induced Wimpy testis (PIWI) by interacting with the RNAs to control the expansion of the stem cells and increasing the levels of PIWI. Thus, they interact with the RNAs (piRNAs) to control the expansion of stem cells and increase the level of PIWI member of Argonaute family in breast cancer .[53]

Hypoxia was found to promote cancer aggressiveness by increasing the resistance of cancer to treatment . Hypoxia promotes cancer cells invasiveness and migration C-MET gene expression, which reprogram and expand the cancer stem cells colonies. The implication of the hypoxia in the breast cancer progression with increased incidence of death among patients . Hypoxia inducible factor 1 alpha (HIF-1α) mediated hypoxic response and regulates the transcription of the genes in response to hypoxia . HIF-1α promote cell survival by the activation of transcription gene suppressing the expression of E-cadherin resulting in the loss of cell-cell interaction and EMT . HIF-1α promotes apoptosis by increasing the expression of pro-apoptotic gene BCL2 or by the increase in the level of p53.

The exposure of breast cancer cells to hydrogen peroxide (H_2O_2) free radicals resulted in an increase in the level of expression of ESA^+, $CD44^+$ and $CD24^-$ resulting in the loss of stem cells ability to mammosphere and the ability to generates cancer colony . Hydrogen peroxide free radicals triggered by the p53 activation and promoting the p21 expression indicate the role of p53/p21 signaling pathway in the free radical induced senescence in the BCSCs. Increase levels of HIF-1α expression is associated with poor prognosis and more aggressive high grade poorly differentiated breast cancer. Many transcriptional genes are deregulated by hypoxia resulting in the cancer aggressiveness and some of these deregulated genes include VEGF-A, WNT1, SMAD4, NDRG2 and EGFR.[13][29].

The maintenance of low level of free radicals was found to be important for the cancer stem cells survival. Thus, it is critical to avoid generation of free radicals in the breast cancer stem cells to prevent loss of function and this findings suggest the therapeutic benefit of the reactive oxygen species in the development of novel drug that can be used to eradicate drug resistant cancer stem cells via induction of premature cell death. The maintenance of undifferentiated stem cells is associated with hypoxia to enhance self-renewal property of the stem cells and inhibit the stem cells differentiation.

MiR-21, miR-126, miR-155, miR-199a and miR-335 were found to be associated with the histologic tumor grading of breast cancer and expression status of the sex hormone receptors . Research study revealed that miR-21 concentrations can be used to distinguish normal healthy stem cells from the breast cancer stem cells. Thus, this can be useful in distinguishing the local regional spread from the distant metastasis. Upregulated levels of miR-214 and miR-218 will distort the cell cycle by suppressing the proliferation of cancer cells and promoting the programmed cell death. [54] overexpression of miR-621 inhibit the expression of F-box protein 11 (FBXO11), which is its targeting gene, and increase the activity of p53 by promoting apoptosis in the breast cancer stem cells and increase the responsiveness to the chemotherapy .[54] MiR-621 can be used as a prognostic indicator in the treatment of breast cancer. Thus, the higher the expression of miR-621 the better the prognosis. MiR-621 increases the sensitivity to paclitaxel plus carboplastin (PTX/CBP) chemotherapy by inhibiting the FBXO11 to increase the activity of p53 and promoting apoptosis .[54]

The miR-128 are downregulated and reduced in the BCSCs when polycomb ring finger oncogene BMI-1 and the ABCCS (ATP binding cassette sub-family C member) genes were targeted. MiR-128 expression associated with the resistance to cancer therapy and can be used to predict the survival following treatment. The overexpression of miR-128 decreased the levels of BMI-1 and ABCCS genes in the BCSCs and promoted the apoptosis and DNA damage following treatment with doxorubicin while the under-expression of miR-128 in the BCSCs was associated with the resistance to chemotherapy. [54]

The miR-495 upregulated in the breast cancer stem cells by targeting the REDD1 gene and overexpression of miR-495 promote the colony formation. Study with the associated clusters of breast cancer stem cells using $PROCR^{+/}ESA^{+}$ instead of $CD44^{+}/CD24^{-/low}$ cells revealed overexpression of miR-495 would down-regulate the expression of E-cadherin and result in an increase cancer invasion. MiR-495 targets the REDD1 to enhance the hypoxia resistant cell proliferation through the post-transcription mechanism.

Research study revealed a variation in the expression of BCSCs at primary tumor site from the secondary metastatic region .[2] MiR-33b were found to be targeting HMGA2, SALL4 and Twist1 for the inhibition of cancer cells migration, invasion, and metastasis . MiR-199a promote the cancer cells initiation, propagation, and metastasis but inhibiting the FOXP2 expression .[54] [55]56] miR-20a downregulate the ligands of NKG2D (MICA and MICB) which are the activators of NK (Natural killer) cell receptor in the BCSCs. Wang et. al in

2014 revealed NKG2D promotes the cancer cells metastasis by increasing the BCSCs resistance to natural killer cell cytotoxicity. [55]56]

The p53 tumor suppressor gene was identified as the target for the miRNA-34 (Choi et. al, 2011). The downregulation of the miR-34c in the breast cancer stem cells target the Notch 4 gene and the genetic ablation of the miR-34a was noticed to promote the generation of induced pluripotent stem cells (IPSC) without compromising the proliferative and the differentiative properties of the IPSCs. [55]56] MiR- 34c inhibits cancer cell invasion, proliferation and promotes apoptosis. The reduced expression and downregulation of the miR-34c through hyper-methylation of the promoter region of BCSCs would result in an increase proliferation and EMT. [55]56]

miR-7 was found to prevent the metastasis of breast cancer stem cells by targeting the SETDB1and blocked the expression of c-myc, Twist, and miR-9 by targeting the STAT3 pathway. [3]Metastatic breast cancer cells expressed high level of osteoblast related genes and increase the chances of breast cancer metastasis to the bone . [55] Croset et al in 2015 revealed that the miRNAs regulation of the differentiation of osteoblast and their action as a bone metastasis regulator. Reduced expression of miR-340 resulted in the rapid progression and metastasis of the breast cancer. Thus, increase level of expression of miR-340 can be useful in the suppression of breast cancer progression and metastasis.[54] [55][56]

9. FUTURE PERSPECTIVES

There are currently limited studies related to the regulatory roles of miRNAs on the BCSCs in breast cancer. The source of circulating miRNAs in the body fluid are still obscured to the scientists, it is difficult to determine whether the circulating miRNAs expressions in the body fluid is tumor specific or product of the dead cells. Thus, experimental reliability assessment is required before circulating miRNAs can be used as a marker. More broad and extensive studies are needed to be conducted in establishing the significance of the regulatory expressions of microRNAs on BCSCs in the management of breast cancer. The better understanding of the mechanism of microRNAs expression and the regulatory roles on BCSCs can be applied in determining the prognosis and responsiveness of breast cancer to a novel treatment. An efficient breast cancer therapy can be developed aiming at breast cancer

stem cells destruction by using miRNA effective delivery and avoiding normal progenitor stem cells destruction. The miRNAs expression can be used to determine the metastatic BCSCs for an efficient targeting mechanism and the identification of these metastatic BCSCs can be useful in the determination of the breast cancer stage, prognosis and the responsiveness to the novel treatment regimen for the breast cancer.

10. CONCLUSION

Studies have shown the relationship between the expression of miRNAs and cancer behaviors. MicroRNAs have been shown to have association with cancer metastasis and aggressiveness. Breast cancer stem cells are responsible for the initiation, dissemination, and breast cancer resistance to the available treatment options. MicroRNAs are major regulators of breast cancer stem cells and they would be an extremely useful for the treatment of breast cancer by targeting the BCSCs.

MicroRNAs regulatory effect on neovascularization, drug resistance, and metastasis microRNAs can be used as the predictive indicators in determining the prognosis of breast cancer following treatment. The miRNAs expression can be used to determine the metastatic BCSCs for an efficient targeting mechanism and identification of these metastatic BCSCs can be useful in the determining the cancer stage, prognosis, and the responsiveness to therapy.

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