ORIGINAL ARTICLE

The Emerging Role of microRNAs as Novel Diagnostic and Therapeutic Tools For Breast Cancer: a systematic review

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ABSTRACT

Objective: A systematic review was conducted to evaluate recent studies performed on miRNAs as a diagnostic and therapeutic biomarker in breast cancer.

Study Design: Online standard databases like PubMed, EMBASE, Web of Science and COCHRANE library were searched to identify research articles relavant to the topic.

Place and Duration of Study: All online published studies between 2010-2020, in the scientific electronic databases were analyzed.

Materials and Methods: This review was performed according to PRISMA guidelines. Databases were systematically analyzed to explore the diagnostic and therapeutic potentials of miRNAs in breast cancer.

Results: We identified twenty-seven studies after literature search. We found six studies focused on diagnosis, sixteen on therapeutics and five on both diagnosis and therapeutics of miRNAs in breast cancer.

Conclusion: The combined data obtained in the present systematic review specify that miRNAs could serve as novel diagnostic and therapeutic biomarkers in breast cancer, whereas the practical relevance of the current results has yet to be established.

Keywords: *Breast Cancer, Diagnostics, miRNA, Molecular Biomarkers, Therapeutics.*

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Introduction

Female breast cancer has surpassed lung cancer as the cancer that is most frequently diagnosed in the world. Breast cancer was estimated to have caused 2.3 million new cases in 2020, accounting for 11.7% of all new cancer cases, and 684,996 of those cases were fatal.[1] The current conventional treatment modalities comprise chemotherapy as systemic treatments, surgery or radiation as local therapies, immunotherapy and targeted therapy.[2] Although these therapeutic approaches have significantly increased the survival of breast cancer patients, their effects may have varied since some patients responded less favourably while others did, based on the specific subtype of cancer.[3,4] Additionally, there are few therapeutic options for some subtypes of breast cancer, including triple-negative breast cancer (TNBC), which is resistant to hormone therapy. High recurrence rates and distant metastases to the major organs such as the lungs and brain with poor outcomes are the major challenges associated with breast cancer. These results highlight the need for effective alternative methods for breast cancer prevention, prognosis prediction, and treatment.[5]

MicroRNAs (miRNAs) are conserved non-coding single-stranded RNAs comprising roughly 19 to 24 nucleotides in length.[6] Over the last few years, miRNAs have gained major attention in cancer research due to their imperative role in tumor initiation and progression which has created the new prospect for early cancer diagnosis and therapies.[7] These miRNAs can serve as “tumor suppressor” or as “onco-gene” or could play a dual role depending on

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the tumor type and targeted gene.[8] More evidence suggests that specific miRNAs could make excellent options for the detection and treatment of breast cancer patients. Furthermore, miRNAs may be considered therapeutic targets, with suppression or restoration of a specific miRNA capable of causing an in-vivo response.[9] Several biological pathways such as transcription factors and the effect of mutated proteins have been demonstrated to alter the expression levels of miRNAs in breast cancer.[10] The objective of this systematic review is to collect and highlight the information on miRNAs in breast cancer, in order to assess its potential role as diagnostic or therapeutic biomarkers.

Materials and Methods

The current systematic review was conducted according to the guideline of the PRISMA 2020 statement (Table 1 – Supplementary table for PRISMA 2020 checklist).[11] The search was performed using a combination of terms related to miRNA-based diagnostic and therapeutic biomarkers for breast cancer. All published studies, in the scientific electronic databases of PubMed, EMBASE, Web of Science and COCHRANE library, were analyzed on 5th May 2021.

Inclusion Criteria

The databases were searched to identify research articles published between 2010-2020. An inclusion criterion was based on the following keywords: miRNAs, molecular biomarker, breast cancer, diagnostic and therapeutic. All research articles published in English were included only.

Exclusion criteria (1) studies not performed on breast cancer (2) breast cancer metastasize to other organs (3) studies that focus on the prognostic role of miRNAs (4) studies based on bioinformatics and nano-theranostic approaches (5) meta-analysis, review papers, conference abstract, clinical trials, letter to editors, comments, case reports, and duplicated publications.

Data Extraction

In the data extraction, we included the miRNA name, its diagnostic and therapeutic significance in breast cancer, role in cancer as tumor suppressor or oncogene, its biological effect, miRNA replacement therapy or inhibition therapy and the reference. The most important factor for the data extraction was miRNA and their diagnostic and therapeutic role in breast cancer. All data processing and screening was done manually. Throughout the assessment processes, the authors and reviewers remained unbiased.

Results

The detail selection process, done by using four databases (EMBASE, PubMed, COCHRANE Library and Web of Science) is depicted in Figure 1.

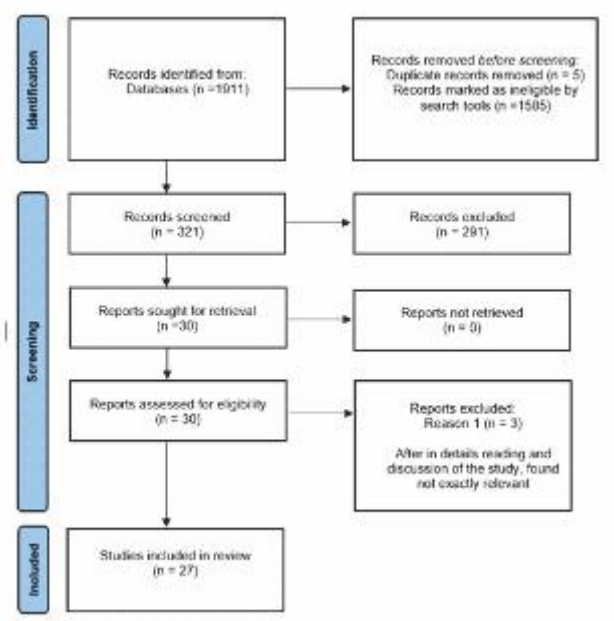

Fig 1: PRISMA flow diagram of study selection process

A total of 1911 papers were identified from 2010 to 2020. In these 1911 articles, 5 articles were found duplicates and 1585 were found irrelevant, as per the exclusion criteria of the study. The excluded articles include reviews (640), conferences (135), book chapters (281), clinical trials (11), non-English literature articles (2), correspondence (1), meta-analysis (1), and others (514). Then, 321 articles were further screened based on their abstract and paucity of adequate information, of which 290 were removed because they didn't fulfill the inclusion criteria. The remaining 30 articles were shortlisted for the present study in which 27 articles were includes after excluding three studies based on full text read (Table 1).

In this review we focused on the breast cancer types, patient stage of cancer, ethnicity, signaling pathways and the expression of miRNAs as oncogenes or tumour suppressors.

Overall 16 miRNAs (miR-125, miR-205, miR-424, miR-489, miR-200a, miR-203, miR-542-3p, miR-

1179, miR-3188, miR-4306, miR-362-5p, miR-203b-3p, miR-203a-3p, miR-34a, miR-127, miR-429) were identified with therapeutic application. A total of six miRNAs (let-7, miR-195, miR-10b, miR-34a, miR-125b, miR-99a-5p) were reported to have diagnostic significance. The remaining five miRNAs (miR-105, miR-502-5p, miR-370, miR-25-3p, miR-30d) were reported to have miRNAs application as both diagnostic and therapeutic biomarkers (Table 1).

Table 1: List of miRNAs and associated biological functions in breast cancer

S.No	miRNA	Breast cancer type	Biological role	References
1	let-7	TNBC and luminal tumors	Tumor suppressor	[12]
2	miR-195	TNBC and luminal tumors	Tumor suppressor	[12]
3	miR-10b	Unclassified BC type	onco-miR	[13]
4	miR-34a	Unclassified BC type	Tumor suppressor	[14]
5	miR-125b	Unclassified BC type	Tumor suppressor	[14]
6	miR-99a-5p	Unclassified BC type	Tumor suppressor	[15]
7	miR-125b	Unclassified BC type	Tumor suppressor	[16]
8	miR-205	Unclassified BC type	Tumor suppressor	[16,17]
9	miR-424	Unclassified BC type	Tumor suppressor and onco-miR	[16]
10	miR-489	Unclassified BC type	Tumor suppressor	[18]
11	miR-200a	TNBC	Tumor suppressor	[19]
12	miR-203	Unclassified BC type	Tumor suppressor	[20]
13	miR-542-3p	Unclassified BC type	Tumor suppressor	[20]
14	miR-1179	Unclassified BC type	Tumor suppressor	[21]
15	miR-3188	Unclassified BC type	onco-miR	[22]
16	miR-4306	TNBC	Tumor suppressor	[23]
17	miR-362-5p	TNBC	onco-miR	[24]
18	miR-203b-3p	Unclassified BC type	Tumor suppressor	[25]
19	miR-203a-3p	Unclassified BC type	Tumor suppressor	[25]
20	miR-34a	TNBC	Tumor suppressor	[26]
21	miR-127	TNBC	Tumor suppressor	[27]
22	miR-429	TNBC	Undefined	[28]
23	miR-105	Unclassified type	Undefined	[29]
24	miR-502-5p	Unclassified type	Tumor suppressor	[30]
25	miR-370	Unclassified type	Undefined	[31]
26	miR-25-3p	TNBC	Tumor suppressor	[32]
27	miR-30d	Unclassified BC type	Tumor suppressor and oncogene	[33]

TNBC, Triple negative breast cancer; miRNA, microRNA; BC, Breast Cancer

Diagnostic Potential of miRNAs in Breast Cancer

Among the selected studies concentrated on the diagnostic potential of miRNA in breast cancer, the older one was published in 2017. Here, Qattan et al., recognized let-7 and miR-195 as tumor suppressor circulating miRNAs in TNBC.[12] They measured the plasma levels of miRNAs in disease-free individuals (34), TNBC (36) and luminal tumors (57) using RT-qPCR. Interestingly, their findings discovered raised levels of let-7 in luminal breast cancer patients and high levels of miR-195 in TNBC patient plasma.

Monroig-Bosque et al. designed a strategy using small molecules that affect the overexpression of miR-10b (SMIRs).[13] They demonstrated that linifanib (multi-tyrosine kinase inhibitor) might inhibit the expression of miR-10a significantly and inverse its oncogenic role *in vitro* and *in vivo* setting of breast cancer. Moreover, high levels of miR-10a reduced the effectiveness of linifanib by reducing its kinase inhibitory effect in breast cancer.[13]

Some studies were focused on miRNA role as a diagnostic biomarker with chemotherapy, such as the work done by Kassem et al. on 39 newly diagnosed Egyptian breast cancer patients.[14] They evaluated the plasma levels of miR-34a and miR-125b to anticipate the effect of neoadjuvant chemotherapy and found that miR-34a expression level was significantly higher in patients compared to controls. However, miR-125b expression level was insignificantly higher in responsive patients, suggesting miR-34a and miR-125b could be potentially non-invasive diagnostic biomarkers for breast cancer.

More recently, Garrido-Cano et al. analysed expression levels of miR-99a-5p in primary tumors and plasma of breast cancer patients and found, considerably, its lower levels in breast cancer tissues than in healthy breast tissues.[15] On the other hand,

they found that in the plasma miR-99a-5p levels were significantly higher than healthy controls, suggesting it is a non-invasive diagnostic biomarker for early detection of breast cancer.

Therapeutic Potential of miRNAs in Breast Cancer

With a collective assessment of shortlisted articles, it was found that miRNAs can serve as replacement therapy against other therapeutic targets, it can inhibit or suppress tumor growth and can cause combined effects with other miRNAs that affect the cancer growth and metastasis. Details of all shortlisted articles on miRNAs and their potential are summarized in Table 1.

In the oldest research, Vilquin et al. showed that aromatase inhibitor resistance was caused by upregulation of miR-125b using the miRNA microarray experiments.[16] They found that either miR-205 or miR-125b can be ectopically overexpressed in MCF-7aro cells line to produce resistance to anastrozole and letrozole and to activate the AKT/mTOR pathway. Also, in MCF-7aro cell line estrogen-independent growth properties can be conferred by increasing miR-125b expression levels. The authors also found that targeting miR-125b can overcome letrozole resistance and is a potential therapeutic target in aromatase inhibitors resistant breast cancers.

In 2016, Huo and his group identified expression profile of miR-205 in inflammatory breast cancer. They showed that this miRNA was reduced in tumor tissue only. Similar results were found in inflammatory breast cancer compared with non inflammatory breast cancer tissue.[17] In this study, low levels of miRNA-205 were related to worse distant metastasis free and overall survival.

Patel et al. identified the potential role of miR-489, which was dysregulated miRNAs causing breast cancer progression and metastasis using the molecular studies.[18] They specifically noted that miR-489 was downregulated in HER2+ breast cancer by HER2 downstream signaling through MAP pathway and was associated with aggressive tumor phenotype. In another work by Kim et al. the miR-200a was reported as a promising therapeutic target for the treatment of TNBC.[19] It was identified in this research article that insulin like growth factors, which are mRNA binding protein, IMP 2/3 were overexpressed in TNBC and caused epithelial-mesenchymal transition (EMT) and metastasis. IMP2/3 were direct targets of miR-200a via progesterone receptor (PR) mRNA destabilization and represents a novel double-negative feedback loop that suppresses the pro-metastatic activities in TNBC. Similarly, Lyu and colleagues studied that overexpression of HER3 diminished the expression levels of two *Survivin*-targeting miRNAs, miR-542-3p and miR-203.[20] While, its specific knockdown enhanced the expression of miR-542-3p and miR-203 in breast cancer cells. Consistently, a mimic of both miRNAs showed that miR-542-3p exhibited better results not only in *Survivin* inhibition, but also increased paclitaxel induced apoptosis especially in overexpressing breast cancer cells (HER2). Furthermore, the results designated that HER3 signaling upregulated *Survivin* by elimination of miR-203 and miR542-3p, with the better inhibition efficacy of miR-542-3p, due to its three binding sites on *Survivin* mRNA.

Li et al, further, investigated that overexpression of miR-1179 affects breast cancer cells proliferation, migration and invasion by targeting Notch signaling pathway.[21] Mechanistically, it was found that upregulation of miR-1179 inhibited Notch 1, Notch 4 and Hes1 expression, thus indicating a therapeutic role of miR-1179 in breast cancer cells.

Chen and Chen intended to investigate the role of miR-3188 in breast cancer development, which targets TUSC5 gene.[22] They showed that miR-3188 was upregulated and downregulated in breast cancer tissues and cell lines than in normal tissues and cell lines respectively. Furthermore, they transfected MCF-cells with miR-3188 inhibitor showed inhibition of cell proliferation, migration and stimulation of apoptosis. They also concluded that there is a direct association between overexpression of this miRNA and p-p38 expression. miRNA suppression significantly decreased the p-p38 expression and was used as a therapeutic agent.

Zhao et al. studied the transcriptional downregulation of miR-4306 and their effects on three main factors; estrogen receptor alpha (ER-α), human epidermal growth factor receptor 2 (HER2) and PR of TNBC.[23] Functional assay was performed using in vitro analysis. The molecular mechanism with their therapeutic potential was also studied using the orthotopic mouse model. They found that

miR-4306 was transcriptionally regulated by ER-α, HER2 and PR and knocking down of these factors caused downregulation of miR-4306. Thus, low expression levels of miR-4306 were strongly associated with lymph node metastasis and poor survival rate of patients.

Zhang et al. emphasized on the biological role of miR-362-5p and found a substantially elevated levels of miR-362-5p in TNBC cells as compared to HER-2 overexpressing cells.[24] Further analysis was done on the miR-362-5p and its target gene Sema3A, and an inverse relationship between miR-362-5p and Sema3A were observed. In another study, focusing on miR-203a-3p and miR-203b-3p, by Aakko et al., They suggested that these miRNAs are among the c-Myc–regulated elements that can control the expression of Bcl-xl and thus can effect tumor cell sensitivity and paclitaxel therapy.[25]

Furthermore, Weng et al. identified miR-34a as a promoter in M1 polarization in TNBC, emphasizing that this miRNA modifies tumor microenvironment and heterogeneity. It was also reported by Weng and his colleagues that a ribosome binding protein (MCT-1), encoded by MCTS1 gene, coordinates ribosomal recycling with translation initiation and tissue growth.[26] MCT-1 antagonist in combination with expression of miR-34a can change the polarity and immune cells activation for improving the efficacy of TNBC. Overexpression of MCT-1 stimulates EMT and matrix metalloproteinase (MMP) activation in TNBC cells.

Latest work on miR-127, by Umeh-Garcia and colleagues, shown that its downregulation was associated with a decrease in patient survival.[27] The authors used a novel approach of miR-127 prodrug (miR-127^{PD}). This prodrug is converted to functional miR-127-3p in TNBC cells, where it decreases the cells viability, motility and sensitizing cells to chemotherapy. Additionally, systemic delivery of miR-127^{PD} suppresses tumor growth and spontaneous metastasis of TNBC cells. In a nutshell, this study revealed that miR-127 plays important role in suppression of tumor growth and metastasis of TNBC.

Finally, Cava and colleagues in 2020 found miR-429 was upregulated and functioned as an oncogene. It acts as a regulator of migration and invasion of cancer cells, which is required for HER2+ cell proliferation, one of the major factors in breast cancer proliferation.[28] Also, they showed that miR-429 regulated HIF1α pathway via directly targeting VHL mRNA; an important molecule for HIF1α degradation. Moreover, they showed that silencing of miR-429 delayed tumor growth and might be used a therapeutic probe in HER2+ breast cancer.

miRNA Potential as Both Diagnostic and Therapeutic Biomarker in Breast Cancer

Metastasis prediction and therapy are essential for improving the longevity of breast cancer patients, as metastatic breast cancer remains a heterogeneous disease with poor prognosis. Around 30% of women identified at an early stage have secondary progression. In this section, five original articles were discussed, and the selected miRNAs details are listed in Table 1.

As early as 2014, Zhou et al. used MDA-MB-231 and the MCF-10A cell lines for studying the role of miRNAs.[29] The group worked on miRNAs which function as gene regulation, and found that miRNAs were differentially secreted among the two lines. Amongst different miRNA, the major focused was miR-105, predicted by several algorithms (TargetScan, miRDB, and PicTar) to target TJP1 (tight junction protein 1; also recognized as zonula occludens 1 or ZO-1), a migration-related gene. They reported that miR-105 typically expressed and secreted by metastatic breast cancer cells, is an effective regulator of migration via targeting the tight junction protein ZO-1.

Sun et al. Studied different breast cancer cells and found that miR-502-5p was significantly downregulated in MCF-7 and MDA-MB-231 breast cancer cell lines.[30] Furthermore, they found that overexpression of TRAF2 abrogates miR-502-5p and causes death of breast cancer cells. Hence, their results suggested the role of miR-502-5p as a tumor suppressor and thus can be considered as a potential diagnostic and therapeutic biomarker for breast cancer.

Mollainezhad et al. addressed the expression of miR-370 in human breast cancer, using twenty-two fresh frozen tissues samples of normal and malignant tissues. An examination was carried out by using a quantitative real-time polymerase chain reaction method.[31] They observed an upregulated miR-370 in breast cancer tissues compared to normal adjacent tissues.

In another study, scientists elucidated the role of miR-25-3p in TNBC as diagnostic and therapeutic biomarker.[32] They showed that miR-25-3p is involved in tumor proliferation both *in vitro* and *in vivo*. For that, they measured miR-25-3p differential expression using qRT-PCR and found that miRNA was upregulated in TBNC and its suppression induced cell apoptosis. Interestingly, Han et al., demonstrated the potential importance of miR-30d and recommended it as a diagnostic biomarker and therapeutic target.[33] They worked on miR-30d-KLF-11-STAT3 pathway and found the biological function of this pathway in cell proliferation and metastasis of breast cancer. Their experimental outcomes demonstrated that the levels of miR-30d were enhanced in the breast cancer cell lines as compared to the non-tumor mammary gland cell line. Moreover, the miR-30d mimic showed increased survival rates in breast cancer cells, by promoting migration, invasion, mediating EMT and inhibiting apoptosis.

Discussion

Most of the updated research studies concluded that miRNAs play vital roles in multiple biological processes, including cell apoptosis, invasion, migration and proliferation. Ultimately, adding tumor suppressor genes or oncogenes in different cancers.[34-37] Deregulated miRNAs can serve as diagnostic and therapeutic targets, as they are found to have imparting crucial roles in carcinogenesis of the breast cancer. Several studies are being carried out to evaluate the aforementioned properties of miRNAs. Here, we have studied comprehensively the role of miRNAs in diagnostics and therapeutics, through a systemic literature search. The study showed that miRNAs have strong diagnostic potential. Secondly, miRNAs have significance as therapeutics targets and thirdly, miRNAs levels can be used to predict the outcomes of chemotherapy.

Overall, we have identified 27 articles that encompass the diagnostic and/or therapeutic role of miRNAs in breast cancers. Interestingly, the studies that highlighted the role of miRNA as therapeutics were more in number as compared to studies that evaluated them as diagnostic marker. We found that 16 studies demonstrated the significance of miRNAs in therapeutics while 6 studies recommended it as diagnostic markers. The results were remarkable and suggested a path to improve the diagnostic and therapeutic strategies using miRNAs, in context to breast cancer. Herein, we will discuss all these molecules stated above, but as we consider that twin role of miRNA is of more value, the miRNAs with dual potential will be discussed in more detail.

One important miRNA, miR-105, plays role in a variety of cancers, including colorectal cancer with its oncogenic character [38], esophageal [39], and non-small lung cancer.[40] The significance of miR-105 has been documented in breast cancer by Li et al.[41] Zhou and colleagues in 2014 have demonstrated that miR-105 plays a vital role in destroying the barriers of vascular endothelium by targeting the tight cellular junctions at pre metastatic stage and later facilitating metastasis. It is also indicated that the miRNA possesses the diagnostic as well as therapeutic potential. As an anti-miR-105 treatment, it abolishes the niche adaptation of tumor derived miR-105 systemic effect and in monolayers of endothelium, it damages and destroys cellular integrity against metastasis as a natural barrier.[29] Another miRNA, a potential regulator of TRAF2, is miR-502-5p, which possesses dual role in breast cancer progression.[30] TRAF2 enhance tumor cell proliferation and promotes cell metastasis with an increased survival rate of cancer. Many studies reported that TRAF2 is also a critical mediator of NF-κB cell by directly interacting with NF-κB pathway.[42] It was also proposed by Sun et al. that miR-502-5p may also target oncogenic TRAF2 in breast cancer and thus act as a tumor-suppressor gene[30].

Another type of miRNA, miR-370 had a controversial role in malignancies due to its varying levels, in multiple cancers.[43,44] Previous, studies documented that miR-370 targets critical proteins such as MAP3K8 and WNT10B (human cholangiocarcinomas)[45], FOXO1 (prostate cancer)[46], and transforming growth factor beta receptor II (gastric carcinoma).[47] Interestingly, miR-370 has been proposed as marker with dual value (diagnosis and therapeutic) in breast cancer by Mollainezhad et al.[31] Furthermore, our search showed that miR-25-3p is also a new diagnostic and therapeutic target in breast cancer. Chen and co-workers also indicated that it is oncogenic and exerts a crucial effect on TNBC progression.[32] miR-25-3p imparts the dual effects via targeting tumor suppressor B-cell

translocation gene 2 (BTG2). The BTG2, is reported to play important roles in cell cycle progression, proliferation, DNA damage repair and apoptosis.[48,49] miR-30d is among other miRNAs documented to act as both diagnostic biomarker and treatment targets in breast cancer. Han et al. reported that depending on krüppel-like factor 11 (KLF-1) and pSTAT3levels, miR-30d plays pivotal roles in breast cancer progression[33]. It has been well documented that KLF-11 and STAT3 are involved in multiple cancers proliferation and progression.[50]

Fuethermore, there were few studies which either just emphasized on the diagnostic value of miRNAs or them as therapeutic targets. miR-99a-5p, miR-34a and miR-125b, miR-10b, hsa-miR-195 and let-7miR were highlighted as diagnostic makers by Garrito-Cano et al., Kassem et al., Monroig-Bosque et al. and Qattan et al., respectively.[12-15] Using different strategies, the authors concluded that each miRNA, as a biomarker, facilitates the detection of breast cancer. Some of them were recomended for early diagnosis while others were suggested for later stage usage. For example, hsa-miR-195 and let-7miR and miR-99a-5p were proposed for earlier detection of breast cancer. Among them, miR-99a is already been confirmed as a tumor suppressor and its overexpression is linked to inhibition of invasion, migration and proliferation in breast cancers. While, for the first time, Garrito et al. proposed that circulating miR-99a-5p is used as diagnostic biomarker in plasma of breast cancer patients.[15] Similarly, circulating levels of hsa-miR-195 andlet-7 in plasma were found to be significantly higher than those of healthy individuals and thus proposed as diagnostic biomarker.[12] miRNAs, miR-34a and miR-125b also exhibited their potential to be used as diagnostic biomarker for breast cancer patients.[14] Many studies showed that both of these miRNAs are tumor suppressors and with breast cancer, their expression is down-regulated leading to inhibition of cell growth, invasion and migration. Kassem and his coworker found that miR-34a expression levels were comparatively higher in breast cancer patients as compared to their controls with p value <0.001 while miR-125b expression levels were insignificantly higher in breast cancer patients on contrary to their controls.[14] However, they further performed ROC curve analysis for diagnostic value of miR-125b evaluation and found that its sensitivity was 66.7%, specificity was 70.0%, PPV was 90.6%, NPV was 41.2% and accuracy was 73.5%. Finally, the study concluded that miR-34a and miR-125b are significantly correlated and are non-invasive diagnostic biomarkers for breast cancer. Moreover, miR-10b is the first oncogenic miRNAs that impart a crucial role in tumor progression and metastasis with its oncogenic activity via downstream targets interactions, including HOXD10, NF1, KLF4, and PTEN.[51-53] Monroig-Bosque laboratory presented that miR-10b can be used for selecting breast cancer patients for linifanib treatment.[13]

Notably, many studies demonstrated the significance of miRNAs in breast cancer therapeutics. Our search retrieved 16 studies reporting the therapeutic values of miR-125b, miR-205, miR-424, miR-205, miR-489, miR-200a, miR-203, miR-542-3p, miR-3188, miR-1179, miR-4306, miR-362-5p, miR-203b-3p, miR-203a-3p, miR-34a, miR-127, miR-429. Mechanistically, some of the miRNAs are found involved in the signaling mechanism such as AKT/mTOR pathway (miR-125b or miR-205, HER2-SHP2-MAPK (miR-489), HER3 signaling (miR-203 and miR-542-3p), Notch signaling pathway (miR-1179). Alterations of downstream gene networks are associated with miRNAs deregulation and is a promising feature for underpinning different disease effects. Some miRNAs can target both tumor suppressors and oncogenes such as miR-205. Studies have shown that it has anti-metastatic and anti-tumoral effects, thus it has double therapeutic efficacy. Its oncogenic role was targeted using a miRNA-inhibition approach[54], while its tumor-suppressive activity was exploited by a miRNA-replacement approach.[55] So, a context dependent role could be accepted for some miRNAs. This is a critical concern that must be carefully examined to derive an appropriate selection of miRNAs for therapeutic purposes and build reliable and safe miRNA-based treatment. In addition, miR-34a was reported as both therapeutic agent and as diagnostic biomarker.[14,26] Thus, miR-34a is also a member with dualistic application in breast cancer. The results exhibited that miRNAs exhibit great potential as the therapeutic agent, however, for RNA based therapeutic development, stability and delivery must be addressed to overcome main challenges.[56]

The accurate target delivery of miRNAs with more efficiency should be endorsed crossing multiple barriers such as enzyme degradation, blood clearance, and poor bioavailability. Also, miR-34a emerged as a tumor suppressor in our systematic review of literature. Studies have showed that it downregulates the oncogene expression in various cancer triggering pathways.[57] In vivo breast cancer models have shown an enhanced antitumoral immune response, increased antitumor effects, suppression in breast cancer cell migration and promoted antimetastatic activity by miR-34a replacement therapy.[58] Another therapeutic role of miR-34a is illustrated in Figure 2. The receptor CD95 also known as Fas, is a member of tumor necrosis factor (TNF) receptor superfamily, which has been shown as extracellular anti-apoptotic targets of miR-34a, resulting in decreased cancer cell growth and inhibited apoptotic signaling.[59] The miR-34-apoptotic associated pathways in metastatic breast cancer involved upstream apoptotic signals, such as the Wnt and TGF- cascade.[60] With the advancement in cancer biology research, numerous cell cycle related gene targets of miR-34s, such asMDM4, and CDK6, have been discovered.[61,62] miR-10b was one of the first oncogenic miRNAs reported to play an important role in promoting tumor metastasis.[51] miR-10b was shown to be induced by the TWIST1 pathway, which then inhibits HOXD10 (a tumour suppressor) and promotes upregulation of RhoA and RhoC. This leads to Rhokinase activation and cytoskeleton reorganization, all of which are involved in promoting breast cancer invasion.[63]

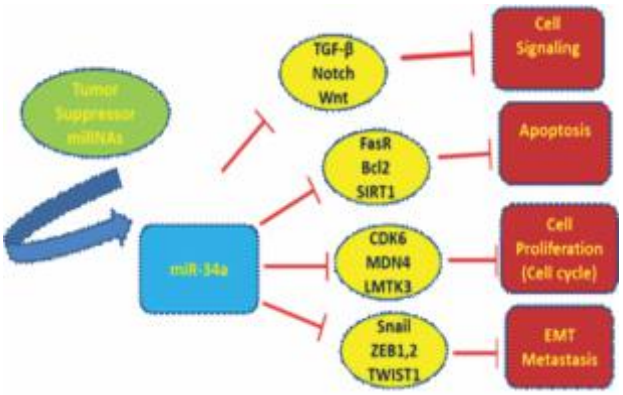

Fig 2: Role of miR-34a as a tumor suppressor. miR-34 affect important cellular activities by regulating key genes involved in cell signaling, apoptosis, proliferation and EMT metastasis

The pathway is illustrated in Figure 3, including a few other targets/regulators which have been depicted. When compared to paired primary tumors, miR-10b is overexpressed in metastatic patients, particularly in lymph node metastasis.[64] Furthermore, the oncogenic activity of miR-10b was largely induced by direct interactions with downstream targets such as PTEN12 KLF4, HOXD10 and NF1.[53,65] Studies on breast cancer models have shown that inhibiting the expression of miR-10b can efficiently suppress cancer cells proliferation, migration and invasion.[51] Thus, miR-10b emerges as a worthwhile therapeutic target in breast cancer treatment. A recent analysis showed that miRNAs have the potential to give important information in a clinical environment, exhibiting the ability to operate as both screening tools for the classification of high-risk patients and guiding the treatment decision-making process. Because of their capacity to control many genes in molecular pathways, miRNAs are critical prospects for new molecular targeted treatments. Our findings suggest that miR-34a and miR-125b expression levels might be useful non-invasive indicators for breast cancer diagnosis. Because of their unexpected stability and noninvasive detection, circulating miRNAs have gained popularity as cancer biomarkers in recent years. Furthermore, analysis by Chan et al., suggested that four miRNAs were found as important diagnostic indicators by comparing miRNA patterns between blood samples from breast cancer patients and healthy individuals.[66] As a result, miRNAs appear to be potential candidate biomarkers for the early diagnosis of breast cancer;

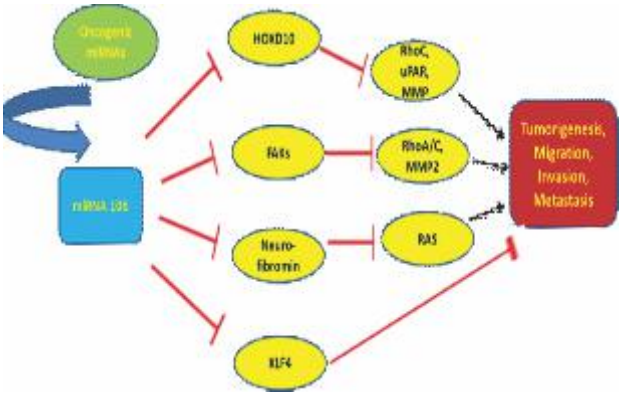

Fig 3: Role of miR-10b in oncogenic signaling. miR-10b is involved in regulating tumorigenesis and metastasis. It initially downregulates various tumor suppressors, which eventually leading to cancer development

However, further research is required to enhance the sensitivities and specificities of detection methods for circulating miRNAs. A growing body of data suggests that some miRNAs may potentially aid in the detection of breast cancer patients. Furthermore, certain miRNAs may be considered therapeutic targets, with suppression or restoration of a specific miRNA capable of generating an in vivo response. Among the limitations of the current endeavor, it must be highlighted that the search algorithm was largely in charge of the process, which focused mainly on the titles or headings of published articles in an attempt to provide more applicable results. Furthermore, because of variations in patient characteristics (race, age or tumor), as well as the use of different detection and isolation methods of miRNA expression varies, we found significant heterogeneity in our results. According to the findings of this comprehensive review, it is evident that the detection and targeting of miRNA might be able to meet the demand for independent, easily available diagnostic and therapeutic molecular markers for breast cancer therapy. The miRNAs chosen in this study can be utilized as a starting point for future studies on breast cancer.

Conclusion

We summarized the diagnostic and therapeutic roles of twenty-seven miRNAs. Given the heterogeneity and complexity of breast cancer, the use of diagnosis and therapy based on miRNA appears intriguing since they are involved in controlling numerous dysregulated genes or pathways concurrently. This systematic review found strong evidence for the use of miRNAs as diagnostic and therapeutic biomarkers in breast cancer not only in early detection, but could be utilized to increase the survival rate of patients. Future investigations are required specifically to develop these biomarkers in a clinical setting. However, the key challenge for the forthcoming development of these therapeutic biomarkers is to broaden the research on miRNAs based on a better understanding of their targets to give a more inclusive picture of the biochemical pathways that they potentially control.

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