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Review

Exosomal microRNAs in breast cancer and their potential in diagnosis, prognosis and treatment prediction

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ABSTRACT

The significance of exosomal microRNAs (EmiRs) in breast cancer (BC) diagnosis has been widely addressed over the past decades. However, little information is still available regarding these reliable biomarkers' impacts on BC early diagnosis, prognosis, and treatment outcome predictions, but their great potential in spotting BC early and their predictive essence in BC prognosis and treatment results are promising against this common cancer. The present review focuses on the most recent findings and advancements of EmiRs applications in BC early diagnosis and treatment prediction and identifies current helpful EmiRs that are widely used in this regard.

1. Introduction

Breast cancer (BC), the most common cancer among women, is considered to be the cause of almost 25% of cancer cases among females worldwide, and it is one of the leading causes of cancer-related deaths among women [1, 2]. It has been demonstrated that about 20–30% of BC patients experience metastatic malignancies, which exhibit an only five-year survival rates of 22%. Meanwhile, up to 90% of BC deaths are associated with patients with metastatic BC [3,4].

It has been claimed that BC early detection is very important for successful treatment [5], and the outcome of BC treatment, especially in metastatic cases, strongly relies on diagnosis time and method. However, the limitations of common diagnostic tools are a serious drawback in BC treatment efforts. Currently, the most common and available methods of BC medical diagnosis are mammography, ultrasound and radiation exposure, CT or MRI scans of the breast, pathologic examinations, puncture biopsy, and radical resection from tumor tissue [6–8]. All these measures have their own limitations, and in many cases, BC is diagnosed too late and after deep metastasis. False-negative results, the necessity of supplementary tests, and sensitivity inadequacy are other important disadvantages of common BC detection methods, which can be applied in many methods such as mammography [9,10].

Obviously, despite remarkable advancements in BC diagnosis and therapies, early detection still remains a serious challenge which justifies the exploration for alternative or complementary novel diagnostic and prognostic tools [11]. Thus, numerous studies have recently attempted to distinguish patients with higher risk of BC using molecular methods. In this regard, during last decades, studies have demonstrated the modification of some microRNAs (miRNAs) level in cancer patients [12,13], which gives a clue for BC early detection.

MiRNAs, which are small (21–23 nucleotides) sequences of non-coding RNAs, are functional molecules derived from introns or long non-coding RNAs (lncRNAs) that are able to regulate the expression levels of specific genes in the human body [14]. It is known that thousands of miRNAs play an important regulatory role in a wide range of biological events, including cell proliferation, differentiation, and death [14–16]. Studies have revealed the diagnostic value of specific miRNAs such as exosomal microRNAs (EmiRs) in patients' serum or plasma for early BC detection and treatment [17]. Exosomes are a group of small extracellular vesicles ranging from 30 to 150 nm in diameter and secreted by various cell types such as stroma, epithelial, and cancer cells.

In addition to long non-coding RNAs, lipids, proteins, and enzymes, miRNAs are one of the main functional molecules that exosomes usually contain. Since exosomes spread through body fluids such as blood,

Abbreviations: BC, Breast cancer; miR/miRNA, microRNA; EmiR, exosome/exosomal miRNA; TNBC, triple-negative breast carcinoma.

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saliva, and urine [18–23], exosomes carrying miRNAs (as the most abundant circulating miRNAs in the human body) are worth monitoring in various human cancers such as breast, colon, colorectal, gastrointestinal tract, ovarian, and prostate cancers [24–29]. It is now universally accepted that EmiRs show significant up- and down-regulation in BC patients and even influence BC proliferation, growth, and metastasis [16,23,30,31]. At the moment, it seems promising—and, therefore, necessary—to explore EmiRs' different aspects and applicability in BC early detection and treatment prediction. Thus, several studies have investigated and addressed EmiRs' significance in predicting BC diagnosis, prognosis, and treatment results, which are fully described in the following sections.

Although numerous EmiRs have already been explored, few have been applied to the clinical trials yet. Hence, this study tries to illustrate the most promising and practical investigations performed during last years and emphasizes on clinical aspects of the recently discovered EmiRs which may assist BC diagnosis and prognosis along with treatment outcome prediction. Unquestionably, many of them can potentially open new windows for future experiments. The present review will pave the road for scientists who aim to gather comprehensive information and improve the current knowledge regarding EmiRs' role in BC diagnosis and treatment.

2. The importance of EmiRs in BC diagnosis

Even though little is known about the precise molecular mechanisms underlying the sorting and packaging of miRNAs into exosomes [31], previous studies have found that EmiRs experience a significant change in BC patients. Their up- and down-regulation in BC cells compared to healthy cells have been reported. For instance, it has been disclosed that EmiR-134 exhibits a lower expression level in breast tumor tissues than in other tissues [32]. Such changes in EmiR levels provide a clue for BC early detection. The diagnostic values of miR-21, miR-1246 [5], and miR-134 [32] have already been noticed in this regard.

To dive into more depth of recent attempts, several studies deserve more attention. In 2016, a study involving 16 healthy plasma and 16 BC plasma samples showed higher expression levels of specific plasma EmiRs (including miR-21 and miR-1246) in patients with BC; thus, their diagnostic role was emphasized in BC detection [5]. Later on, the significance of miR-1246 was confirmed again in this regard [33]. Earlier, Eichelser et al. [34] analyzed the serum concentrations of circulating cell-free and EmiRs in BC patients, reporting significantly different serum concentrations of cell-free miR-101 and miR-373 between patients suffering from BC and benign tumors. The same pattern was observed for the level of exosomal (but not of cell-free type) miR-372 in BC patients. Their results clearly indicated the diagnostic potential of EmiR-373 as a biomarker in patients' serum [34].

Among the most recent studies, Ni et al. [35] reported high levels of EmiR-16 in some BC patients and different levels of its expression between primary and recurrent BC patients and some other histological statuses of BC. They suggested that EmiR-16 is a reliable biomarker candidate for BC diagnosis. Also, the prognostic value of EmiR-16 and some other EmiRs were confirmed [35]. Based on another comprehensive investigation, a cluster of 12 miRNAs from EmiR-106a–363 in 400 and 406 plasma and serum samples, respectively, confirmed that several plasma- and serum-derived EmiRs experience significant up-regulation in BC patients compared to healthy individuals. It has been observed that miR-92a-2–5p, miR-20b-5p, miR-106a-3p, and miR-106a-5p from plasma samples and miR-19b- 3p, miR-20b-5p, miR-92a-3p, and miR-106a-5p from serum samples can be considered as novel biomarkers in BC early detection [36].

Around the same time, Stevic et al. [37] published an inclusive study in which they explored a large cohort containing 435 BC patients for specific EmiR expression levels and found modified expression patterns for some of them. By analyzing 45 miRNAs, they found a link between clinicopathological parameters of BC subtypes and EmiR expression. Regardless of the difference in the exosomal expression levels between HER2-positive and triple-negative breast cancer (TNBC) patients, the results showed that miR-27a/b, miR-335, miR-365, miR-376c, miR-382, miR-422a, miR-433, and miR-628 had different expression patterns when compared with healthy women. Also, a significant up-regulation of EmiR-27a only in HER2-positive patients and EmiR-27b in both BC subtypes were reported when compared with healthy women. The increased occurrence of miR-335 and the higher expression levels of EmiR-376c and EmiR-382 were shown in TNBC patients. Interestingly, the researchers also observed a down-regulation of EmiR-422a and an up-regulation of EmiR-433 in HER2-positive and TNBC patients, respectively [37]. Their comprehensive data suggest several EmiRs' potential for BC diagnosis.

Recently, different expression levels of several EmiRs in 27 BC patients compared to three healthy controls were reported [38]. Patients were followed up with for two years and were investigated for specific miRNAs expression in subjects with and without recurrence. The results showed that miR-150–5p, miR-576–3p, and miR-4665–5p were expressed differently in patients with and without recurrence. After suggesting these novel prognostic biomarkers, the researchers concluded that the mentioned miRNAs had predictive value and could be applied to distinguish recurrent from non-recurrent TNBC. They also observed that 54 EmiRs were differentially expressed in TNBC patients compared to healthy controls, of which 20 miRNAs showed up-regulation and 34 experienced down-regulation (we have opted not to list them here). Their study clearly reflected the diagnostic and prognostic potential of EmiRs in TNBC treatment [38]. During another study conducted in 2020, 13 urine-derived biomarkers were analyzed in BC patients in order to investigate the diagnostic potential of mRNAs. The results indicated 98.6% sensitivity and 100% specificity of BC non-invasive diagnosis using four urinary miRNAs (miR-424, miR-423, miR-660, and let7-I) [39].

Another investigation in 2020 showed that the serum content of EmiR-148a had promising biomarker value in BC diagnosis, prognosis, and prediction of the treatment clinical outcome among BC patients [40]. It was found that the down-regulation of miR-148a in BC patients reflects unfavorable clinical responses to the therapies and reveals worse overall survival of patients compared to those with higher miR-148a level. Moreover, the diagnostic value of miR-148a level in BC patients and also its biomarker significance for identification of tumor-node-metastasis (TNM) stage of BC were reported [40].

Not only are EmiRs useful for BC early detection, but their specific expression patterns can also be applied in BC metastasis prediction. For instance, miR-411, miR-215, and miR-299–5p are well-known biomarkers for BC metastasis identification [41]. It has been reported that metastatic BC cells over-secrete miR-105. Studies have clearly revealed the miR-105's destructive function against the endothelial barriers of hosts and have suggested monitoring circulating miR-105 in patients as an early-stage BC metastasis prediction biomarker. Other researchers concluded that miR-105 can be considered a valuable prognostic biomarker for the metastatic potential of BC [42]. Furthermore, the results of a study conducted by Ding et al. [43] indicated that the EmiR-222 levels in advanced BC patients reflect the cancer's aggressiveness. Thus, it has been implicitly proposed to have a diagnostic value in early metastatic BC detection [43].

In 2019, the diagnostic, prognostic, and therapeutic impacts of five different EmiRs were investigated in BC patients. In this regard, miR-21 was shown to distinguish localized BC from distant metastases cases, and miR-105 showed different levels in localized BC patients than in healthy individuals. Thus, these small RNAs were reported to have important diagnostic applications in BC identification. Interestingly, a positive correlation between miR-21 and BC tumor size was also found in this study. Additionally, positive correlations of miR-21, miR-155, and miR-222 levels with BC circulating tumor cells and cancer dissemination were found in this investigation [44]. Table 1 summarizes the most promising EmiRs recommended for early BC diagnosis.

Table 1
Diagnostic, prognostic and treatment outcome predictive impacts of EmiRs.

Exo-miRNA	Index	Relevance	Sample type	Sample size	References
miR-148a	Down-regulation	Diagnosis, Prognosis Treatment outcome prediction	Serum	125 BC patients & 50 patients with benign breast tumors	[40]
miR-134	Down-regulation	Diagnosis	Breast tumor	77 breast tumor specimens & 17 normal breast tissue	[32]
miR-21 and miR-1246	Up-regulation	Diagnosis	Plasma	16 healthy & 16 BC samples	[5]
miR-1246	Up-regulation	Diagnosis	Serum and tissue	56 cancer & 19 healthy serum/tissues	[33]
miR-101, miR-372 and miR-373	Up-regulation	Diagnosis	Serum	50 BC patients & 12 healthy women	[34]
miR-16	Up-regulation	Diagnosis, Prognosis	Plasma	111 BC patients, 42 DCIS patients and 39 healthy women	[35]
miR-92a-2–5p, miR-20b-5p, miR-106a-3p and miR-106a-5p	Up-regulation	Diagnosis	Plasma	400 plasma samples from 200 BC patients	[36]
miR-19b- 3p, miR-20b-5p, miR-92a-3p and miR-106a-5p	Up-regulation	Diagnosis	Serum	406 serum samples from 204 BC patients	[36]
miR-27a/b, miR-335, miR-365, miR-376c, miR-382, miR-433, and miR-628	Up-regulation	Diagnosis	Plasma	435 BC patients & 20 healthy women	[37]
miR-422a	Down-regulation	Diagnosis	Plasma	435 BC patients & 20 healthy women	[37]
miR-150–5p, miR-576–3p and miR-4665–5p and several others	Up-regulation & Down-regulation	Diagnosis Prognosis	Plasma	27 BC patients & 3 control	[38]
miR-424, miR-423, miR-660, and let7-I	Up-regulation & Down-regulation	Diagnosis	Urine	69 BC & 40 healthy women	[39]
miR-215, miR-299–5p, miR-411	Down-regulation	(Metastasis) diagnosis	Serum	75 BC patients & 20 healthy individual	[41]
miR-105	Up-regulation	(Metastasis) diagnosis	Serum	38 stage II and III BC patients	[42]
miR-222	Up-regulation	(Metastasis) diagnosis	Plasma	38 BC patients & 19 normal controls	[43]
miR-21, miR-105, miR-155 and miR-222	Up-regulation	Diagnosis Prognosis	Serum & circulating tumor cells	53 BC patients	[44]
miR-150–5p, miR-576–3p and miR-4665–5p	Up-regulation	Prognosis	Plasma	27 BC patients & 3 healthy controls	[38]
miR-221	Down-regulation	Prognosis	Serum	53 BC patients	[44]
miR-148b-3p	Down-regulation	Diagnosis Prognosis	Serum	Two cohorts including 28 BC patients & 27 controls- 59 BC patients & 35 controls	[50]
miR-374, miR-185, miR-376a, miR-382, miR-410, miR-433, and miR-628	Up-regulation	Prognosis	Plasma	435 BC patients & 20 healthy women	[37]
miR-16, miR-30b and miR-93	Up-regulation & Down-regulation	Prognosis	Plasma	111 BC patients, 42 DCIS patients and 39 healthy women	[35]
miR-338–3p, miR-340–5p, and miR-124–3p	Up-regulation	Prognosis	Serum & tumor tissues	384 miRNAs	[48]
miR-29b-3p, miR-20b-5p, miR-17–5p, miR-130a-3p, miR-18a-5p, miR-195–5p, miR-486–5p, and miR-93–5p	Down-regulation	Prognosis	Serum & tumor tissues	384 miRNAs	[48]
miR-105	Up-regulation	Prognosis	Serum	38 stage II and III BC patients	[42]
miR-34a, miR-93 and miR-373, miR-17 and miR-155	Up-regulation	Diagnosis Prognosis	Serum	120 primary & 32 metastatic BC patients & 40 healthy women	[51]
miR-210	Up-regulation	Treatment outcome prediction	Plasma	29 BC patients & 28 healthy control	[52]
miR-155 and miR-301	Down-regulation	Treatment outcome prediction	Plasma	435 BC patients & 20 healthy women	[37]
miR-21	Down-regulation	Treatment outcome prediction	Serum	53 BC patients	[44]
miR-503	Up-regulation	Treatment outcome prediction	Plasma	17 BC patients that received neoadjuvant chemotherapy & 12 control	[53]

3. The importance of EmiRs in BC prognosis

In addition to their diagnostic importance, EmiRs have been widely explored in BC prognosis. Prognostic EmiRs are able to predict the patterns of BC progression or remission. TRPC5 [45], GSTP1 [46], UCH-L1 [47], miR-340–5p, miR-17–5p, miR-130a-3p, miR-93–5p [48], HER2, KDR, CD49d, CXCR4, and CD44 [49] are some exosomal biomarkers recently applied in BC prognosis studies using molecular and serological tests such as PCR, Western blot, or FCM. It has been established that these prognostic exosomal biomarkers are indicators of

patients' survival rates, BC recurrence, and its distant metastasis. Nonetheless, limited information is available regarding exosome biomarkers' impacts on BC prognosis.

To date, several important EmiRs with prognostic value have been explored, which illustrate overall BC outcomes in patients. In this regard, the prognostic potential of EmiR-150–5p, EmiR-576–3p, and EmiR-4665–5p in TNBC is aforementioned. These prognosis biomarkers are able to predict recurrence in TNBC patients [38]. It was found that the miR-221 is a valuable prognostic biomarker in BC patients [44]. Of note, the diagnostic and prognostic value of miR-148b-3p in BC treatment has also been reported [50]. Moreover, the association between EmiR-374 and tumor size in TNBC patients, as well as the associations of miR-185, miR-376a, miR-382, miR-410, miR-433, and miR-628 with HER2-positive BC patients' tumor size, has been observed [37].

Furthermore, in 2018, Ni and co-workers disclosed the association of deregulated levels of several EmiRs, including miR-16, miR-30b, and miR-93, with BC recurrence status and confirmed their prognostic value in BC patients [35]. In 2017, studies on 384 miRNAs in serum samples of patients with BC indicated the up-regulation of three miRNAs (miR-338–3p, miR-340–5p, and miR-124–3p) and the down-regulation of eight mRNAs (miR-29b-3p, miR-20b-5p, miR-17–5p, miR-130a-3p, miR-18a-5p, miR-195–5p, miR-486–5p, and miR-93–5p) in patients with cancer recurrence. However, miR-195–5p, miR-17–5p, miR-93–5p, and miR-130a-3p showed higher expression levels in tumor tissues, which contrasts with their down-regulation in serum samples [48]. These results reflected different patterns of EmiR expression levels in the serum and tumor tissues of BC patients. They also suggest that the 11 mentioned EmiRs are potential candidate biomarkers for BC prognosis.

The prognostic potential of miR-105 for later development of metastatic BC has also been reported [42]. In 2013, the expression levels of six circulating miRNAs (miR-10b, miR-17, miR-34a, miR-93, miR-155, and miR-373) were analyzed in serum samples of primary and metastatic BC patients. According to the results, in patients with primary BC, three of these six miRNAs (miR-34a, miR-93, and miR-373) showed significantly higher expression levels compared to healthy women. Also, two other miRNAs (miR-17 and miR-155) showed higher expression levels in primary BC patients than in distant metastatic cases. It was concluded that the alteration in specific miRNAs expression levels may reflect particular steps of cancer progress in BC patients [51]. These findings clearly highlight the prognostic value of circulating EmiRs in BC treatment.

As can be observed from the literature review, EmiRs role in BC prognosis and recurrence have recently attracted scientists' attention (summarized in Table 1). Nevertheless, limited information is still available regarding all aspects of EmiRs application in prediction of BC progression.

4. Treatment outcome prediction

In addition to the diagnostic and prognostic benefits of EmiRs, these small RNAs can help predict treatment responses in BC patients. A few studies have reflected on EmiR expression levels and changes as indicators of BC patients' response to common anti-cancer therapies (Table 1). For instance, miR-210 plays a role in patients' responses to chemotherapy measures [52]. Moreover, the potential predictive value of miR-155 and miR-301 and the therapeutic target significance of miR-301 in many BC patients have been noticed [37]. Meanwhile, the prognostic value of miR-21 in HER2-positive BC patients' responses to treatment with trastuzumab has been reported before [44]. They have demonstrated significantly lower levels of miR-21 in these patients during trastuzumab treatment.

Meanwhile, it has been shown that the EmiR levels are reciprocally influenced by anti-cancer therapies. For instance, based on the research conducted by Bovy et al. [53], the endothelial cells secreted miR-503, which increased in BC patients' plasma after neoadjuvant chemotherapy. Of note, miR-503 inhibits tumor growth, and its modulation affects the proliferative and invasive capacity of BC cells [53]. Thus, its alteration pattern may reflect BC treatment status. The results of another study in 2021 provided similar evidence of EmiRs' influence on anti-cancer compounds [54]. Some other aspects of EmiRs' role in BC treatment, such as their contribution to chemoresistance transmission to BC cells and their therapeutic role, have been clarified in other studies [55,56]. Thus, previous investigations of EmiRs functions and comparisons of their activities and expression levels in different stages of BC development, including pre-, post-, and mid-treatment phases, provide a meaningful pattern of BC responses to the common chemotherapies. Thus, scientists hope that EmiRs will serve BC treatment with the most precise therapeutic response predictions in the future.

5. Drawbacks and future perspective

Despite the remarkable advantages of EmiRs in BC diagnosis and prognosis, the overall theme is not devoid of drawbacks and limitations. The sensitivity, specificity, and reliability of detections have not been completely evaluated yet [57]. Taking BC into account, further investigations may partly solve the specificity issue with disclosing EmiRs change in all BC subtypes. EmiRs' application in BC diagnosis, prognosis, and treatment outcome predictions may have unknown practical differences in various BC types, including basal type BC, luminal A and B types, and human epidermal growth factor receptor 2 (HER2-overexpressing) BC, as each type has its own specific clinicopathological features. Furthermore, the exploration of better and more effective EmiR isolation methods should be considered to ensure that the most effective and qualified measures are implemented for their identification and monitoring. This way, the most possible sensitivity and reliability of detection and analysis will be guaranteed, especially since the small size and low density of exosomes in body fluids are responsible for the difficulties. Furthermore, it is important to select the best sample sites for EmiRs isolation, such as patients' plasma, serum, and tears.

Owing to microarray and next-generation sequencing techniques, meaningful advancements have been seen in EmiRs analysis in BC patients; such advancements make it necessary to provide experienced experts with the knowledge needed to analyze complicated bioinformatics data. In some investigations, however useful and pioneering they were, a larger and more inclusive cohort of patients should have been used. Thus, more comprehensive studies with more patients should be carried out in the future to validate previously reported results. Overall, due to the existence of many unknowns in the field, further explorations are mandatory to solve mentioned concerns and explain unclear aspects of EmiRs' significance in BC treatment.

To improve EmiRs' efficiency in BC diagnosis, Wang et al. [58] developed an in situ simultaneous detection construction of multiple miRNAs, which works based on a biosensor entrance into exosomes and their hybridization to the complementary miRNAs targets before activating fluorescence signals to detect exosome carrying miRNAs. They used miR-21, miR-27a, and miR-375 as model targets due to their verified significance in BC diagnosis. The authors concluded that such biosensors' application in EmiRs detection in clinical serum samples can efficiently distinguish BC patients and aid BC early detection [58]. The high sensitivity and specificity of urinary miRNA specimens' usage in BC early diagnosis [39] is another attempt in this regard.

From technical point of view, it is worth mentioning that generally the monitoring and development of new markers requires complex processes and long cycles of experiments which is time consuming in itself. Nevertheless, it is necessary to make sure that all the needed controlling considerations are taken into account like standardized and effective screening and verification strategies before a marker usage. In fact, basic investigations should be performed prior to the principle knowledge transfer to clinical trials and application. In addition, actual clinical problems, risks and drawbacks, also the ultimate goal and purpose of an exosomal marker screening and the best scenarios for its application in diagnostic and prognostic strategies should be clearly

defined prior to use. Needless to say, before screening the potential exosomal markers, it is helpful (sometimes necessary) to consider and determine the patient condition and disease type, characteristics and progress step, the best sampling size, technique and storage process, exosome detection and analysis methods as well. Moreover, it might give a clearer pattern of diagnosis and prognosis success, if final results could be compared with common clinical and gold standard methods. As previously stated, larger samples and multiple-center clinical trials can help to verify benefits of EmiRs to the BC treatment. Despite clinical considerations, also development in software background, professional research methods and bioinformatics may draw more promising horizon in this regard. It goes without saying that there is still a long way to go to reach most possible efficiency and precise in this filed.

Finally, the literature review indicates that one of the main challenges in BC treatment is its early detection and the accurate prediction of its development and responses to therapies. Along with common diagnostic methods, EmiRs show great potential in BC diagnosis and treatment prediction. As mentioned above, BC occurrence, recurrence, prognosis, and responses to common therapies are sometimes highly predictable via EmiR monitoring.

The present study gathered the most promising reports regarding the diagnostic and prognostic value of EmiRs in BC treatment. The information presented in this study confirms that EmiRs' significance in BC is not limited to their role in BC metastasis, progression, tumor growth, dormancy angiogenesis, metabolism, and drug resistance, as they are also useful in BC diagnosis, prognostic, and outcome predictions.

Declaration of Competing Interest

The authors report no declarations of interest.

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