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The evolving role of microRNA subtypes 21, 155 and 10b as oncogenes and biomarkers in breast cancer in a decade: A systematic review article.

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Key words

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ABSTRACT

Breast cancer is a malignant disease with a high incidence worldwide in women- and to less extent in men-, thus remains a health burden. miRNA plays a key role in the development and prognosis of breast cancer; as a single miRNA has the ability to regulate multiple gene targets and adversely alter their expression. In this review, we attempt to systematically analyze the rapidly accumulating body of research regarding the role of miRNAs subtypes particularly as oncogenes and biomarkers in breast cancer, and their clinical implications. A total of 11 pertinent articles in the past 10 years were extracted from high-rank reviews, analyzed, and summarized. Amongst many (eight miRNA subtypes), three subtypes (miR21, 155, and 10b) were selected in order to evaluate the validity of their oncogenicity as well as their diagnostic value as biomarkers.

INTRODUCTION

Breast Cancer:

According to the American Society, breast cancer is the most common cancer among women (1). It is a highly heterogeneous complex neoplastic disease with diverse intertumoral and intratumoral non-

uniformity that occurs due to abnormal proliferation of cells or tissues lining the mammary glands and ducts. Various factors play a role in the development and promotion of breast cancer by changes in

the expression of certain genes, amongst which the noncoding RNAs (miRNAs) are worth mentioning (2). Breast cancer can be classified based on its unique phenotype, tumor grade, and molecular characterization (3). It has been reported that the differential expression of miRNAs in breast cancer. Consequently, an increasing number of studies have suggested that miRNAs are closely associated with BC occurrence and development. Undoubtedly, TNM staging is of great prognostic value. However, considering all the limitations of the currently available prognostic strategies, it is generally recognized that new, affordable, more accurate methods indicative of molecular characteristics of tumors are needed to achieve personalized treatment. Still, it remains difficult to achieve these goals, because of the absence of refined (sensitive and specific) biomarkers for disease monitoring and for addressing breast cancer on an individual basis (4). MicroRNAs are small single-stranded, non-coding RNA molecules that span 19 to 25 nucleotides in length (5). The first discovery of microRNAs (miRNA), *lin-4*, in 1993 by the Ambros and Ruvkun groups in *Caenorhabditis elegans*, (6) has developed the field of molecular biology. miRNAs are transcribed from DNA sequences into primary miRNAs (primiRNAs) and processed into precursor

miRNAs (pre-miRNAs) and mature miRNAs. In most cases, miRNAs interact with the 3' UTR of target mRNAs to interrupt gene expression (6). miRNAs are critical for post-transcriptional gene regulators of various biological functions. They regulate ample cellular and signaling pathways, including cell development and differentiation, cell proliferation, and apoptosis as well as cell migration (7). It is believed that miRNA genes constitute about 1–2% of the known genes in eukaryotes. miRNAs also downregulate gene expression by binding to their specific target mRNAs 3'-untranslated regions (3'UTRs) leading to either mRNA degradation, translational repression, mRNA decay and deadenylation depending on the degree of complementarity with target mRNA sequences. Many genuine studies on miRNA have highlighted their role in the etiopathogenesis of a variety of human diseases, such as neurodegenerative disorders, diabetes, cardiac hypertrophy, and respiratory diseases, and there is a long history of regulatory relationships between miRNAs and cancer hallmarks, particularly breast cancer (12). miRNAs have been reported to play many important roles as oncogenes (oncomiRNAs), tumor suppressors and biomarkers. MiRNA abnormal expressions are also considered to be possible biomarkers of BC because they are stably found in tumor tissues as

well as in patients' body fluids such as blood, plasma, and saliva. miRNAs in body fluids, also called circulating miRNAs, are remarkably stable, packaged into extracellular microparticles or bound with lipoproteins, which protect them from RNase digestion. In this review, we attempt

Biogenesis and Maturation:

Winter *et al* viewed that the biogenesis of mature miRNA involves a series of biological processes. Firstly, the primary miRNA (pri-miRNA) is transcribed by RNA polymerase II or III in the nucleus, forming an elongated RNA hairpin structure that is subsequently cleaved by Drosha into a small stem-loop structure of ~70 nt, (pre-miRNA). The pre-miRNA is exported from the nucleus into the cytoplasm by exportin-5 and the loop is cleaved after the pre-miRNA is loaded onto Dicer, producing a double-stranded

to scientifically analyze the rapidly accumulating research regarding the emerging role of miRNAs subtypes, particularly as oncogenes and biomarkers in breast cancer, and their clinical implications (7).

structure of miRNA and antisense miRNA. The latter is normally degraded, however the long (~22 nt) mature miRNA strand is integrated into the miRNA-induced silencing complex (mRISC), resulting in mRNA degradation or translational repression. Mature miRNA levels are regulated via binding to ceRNAs such as circular (c) RNAs, pseudogenes, and lncRNAs, which act as sponges to prevent miRNA binding to target mRNA (8). See (Figure1)

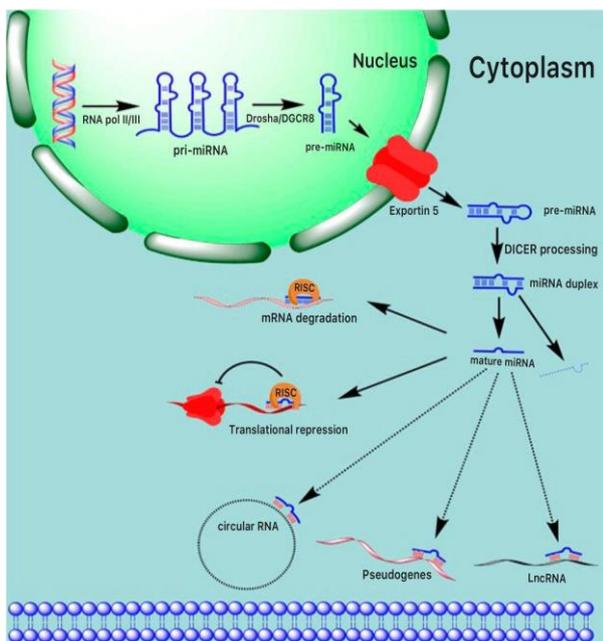


Figure1: Schematic representation of miRNA biogenesis and function. The primary miRNA transcript (pri-miRNA) is transcribed in the nucleus by RNA polymerase II/III, creating an elongated hairpin structure of RNA that is then split into a small stem-loop structure of ~70 nt by Drosha (pre-miRNA). Pre-miRNA is exported by exportin-5 from the nucleus into the cytoplasm and the loop is cleaved after loading the pre-miRNA onto Dicer, creating a double-stranded miRNA and antisense miRNA* structure. The latter is usually degraded, while in the miRNA-induced silencing complex (mRISC), the long (~22 nt) mature miRNA strand is integrated, leading to mRNA degradation or translational repression. Via binding to ceRNAs such as circular (c) RNAs, pseudogenes, and lncRNAs, which function as sponge, mature miRNA levels are regulated to prevent miRNA binding to target mRNAs.

miRNAs subtypes as oncogenes

The first proof of the link between miRNA and cancer derived from studies on B cell chronic Lymphocytic leukemia (CLL), particularly in an attempt to identify a translocation induced deletion on chromosome 13q14 that is frequently detected in CLL (9). The mechanisms of oncomiRNA can be explained by a certain number of proteins. Some of these proteins are integral components of the RNA induced silencing complex (RISC) that delivers those small RNAs to complementary sites within mRNA. miRNAs exert their silencing function usually through interactions with the 3' untranslated region (3' UTR) of the target gene through imperfect base pairing. Moreover, miRNAs can function as master gene regulators, impacting a number of cellular pathways important for normal cellular functions as well as cancer development and progression. These alterations culminate in an increased expression of the oncomiR such as miR-21, miR-155 and miR-10b and decreased tumor suppressors such as Let-7a (2). MiR-10b was shown to be upregulated in breast cancer cells. Overexpression of miR10b is a target for the transcription factor Twist, which causes interruption of homeobox D10 (HOXD10) mRNA translation. This leads to increased expression of Ras

homolog gene family member C (RhoC), which promotes cell invasion and metastasis (10). Similarly, miR-21 plays an important role in many carcinomas, especially breast cancer development and progression. Through increased cell growth in vitro, tumor growth in vivo and inhibited expression of multi-tumor suppressor proteins such as TIMP3, PDCD4 and tropomyosin 1 (alpha). In addition, it has a major role in invasion and metastasis by targeting multiple anti-metastatic genes. Finally, miR-155 acts as an oncogene/upregulate factor in breast cancer cells, resulting in suppressing the expression of protein Suppressor of Cytokine Expression 1 (SOCS1) both in vitro and in vivo. Pro-tumorigenesis is inversely associated to SOCS1 inhibition.. The miR-155 also represses tumor suppressor for head box O3a expression in breast cancer (11).

miRNAs as Biomarkers

Lawrie et al. conducted the first study of miRNA quantification in serum.. who found that serum levels of miR-21 were associated with relapse-free survival in patients with diffuse large B-cell lymphoma. After that, several studies have assessed the potential biomarker use of serum or plasma miRNAs in breast cancer and other different types of cancer. Current challenges in the management of breast

cancer include a continuing search for sensitive, minimally invasive markers that can be exploited to detect early neoplastic changes, thus facilitating the detection of breast cancer at an early stage, as well as monitoring the progress of patients with breast cancer and their response to treatments. MicroRNAs (miRNAs or miRs) have been proposed as promising biomarkers in breast cancer because they can be easily detected in tumor biopsies (non-circulating miRNAs) as well as blood, plasma, serum, and saliva (circulating miRNAs). Furthermore, circulating miRNAs bind to lipoproteins such as HDL, are linked to the Argonaute 2 (Ago2) protein, or are bundled into exosome-like microparticles, microvesicles, and apoptotic bodies. There is also a great need for the identification of sensitive, reliable, and acceptable markers of response to neoadjuvant and adjuvant therapies (12).

METHOD

A search of PubMed Medline has yielded thousands of papers. The search databases were filtered to identify articles published from 2010 to 2020 using the following search strategy: (microRNA OR miRNA) AND (oncogenes and biomarkers) AND (breast cancer). This systematic review was conducted in accordance with the PRISMA guidelines. The Real Time RT-PCR

technique was used to identify the miRNA expression level of selected miRNAs. Also, the statistical analysis of miRNA expression profiles of the BC and its stages was performed using the program SPSS. For the inferential analysis of data, parametric data from the T-test and non-parametric data from the Mann-Whitney method were used. A P value of less than 0.05 is considered as a significant value.

RESULTS

The search strategy retrieved 48 articles. 16 of these articles were duplicates, 6 were not freely available, 2 were not in English, 11 were irrelevant, 2 were not pertinent, 1 was earlier than 2010, and 11 were eligible. The above-mentioned steps concerning the selection of studies are illustrated in detail in (**Figure2**). Therefore, a total of 11 articles were included in this systematic review, and the role of 3 miRNAs molecules as oncogenes & biomarkers is described in (**Table1**). According to the results presented in Table1, 81.8% of published studies have not taken into account statistical analysis to assess the role of miRNAs in breast cancer. Since (45.4%) of the studies focused on oncogenic miRNAs, and their associated genes in BC, 27.3% of their main goal is breast cancer, its classification, and tumor grades, 9.1% of the articles addressed miRNAs as

biomarkers, and the rest studied the processes of miRNAs biogenesis), the remaining 18.2% were quantitatively analyzed for the role of miRNAs as oncogenes and biomarkers in breast cancer.

Expression levels of miR-10b were correlated with breast cancer tumor stages. Generally, the miR-10b expression levels are significantly elevated when the tumor grade is increased (p -value ≤ 0.05). There were no statistically significant changes observed between Stage II and III (p -value ≥ 0.05). Stage III miR-10b expression level is significantly lower than Stage IV (p -value ≤ 0.05). Significant expression level changes were also observed between stages I-II and stages II-IV (p -value ≤ 0.05).

The expression levels of circulating miR-21 in breast cancer patients were significantly higher (p -value ≤ 0.05). There was no significant difference in the

histopathological level of miR-21 expression in patients with breast cancer Grade II and Grade III differentiation, nor Grade III and Grade IV (p -value ≥ 0.05). Also, there was no significant difference in the expression of miR-21 according to tumor sizes and breast cancer subtypes. Circulating miR-155 was significantly higher in patients with tumors larger than 5 cm (p -value ≤ 0.05). MiR-155 expression levels were significantly different according to various tumor grades, and clinical stages (p -value ≤ 0.05). No significant difference was observed between any of the studied miRNAs regarding lymph node metastasis. Mir-21 was significantly over-expressed in ER-/PR- cases. Specific miRNAs (miR-10b, miR-21, and miR-155) are associated with tumor metastasis and other clinical characteristics for BC, facilitating the identification of individuals who are at risk.

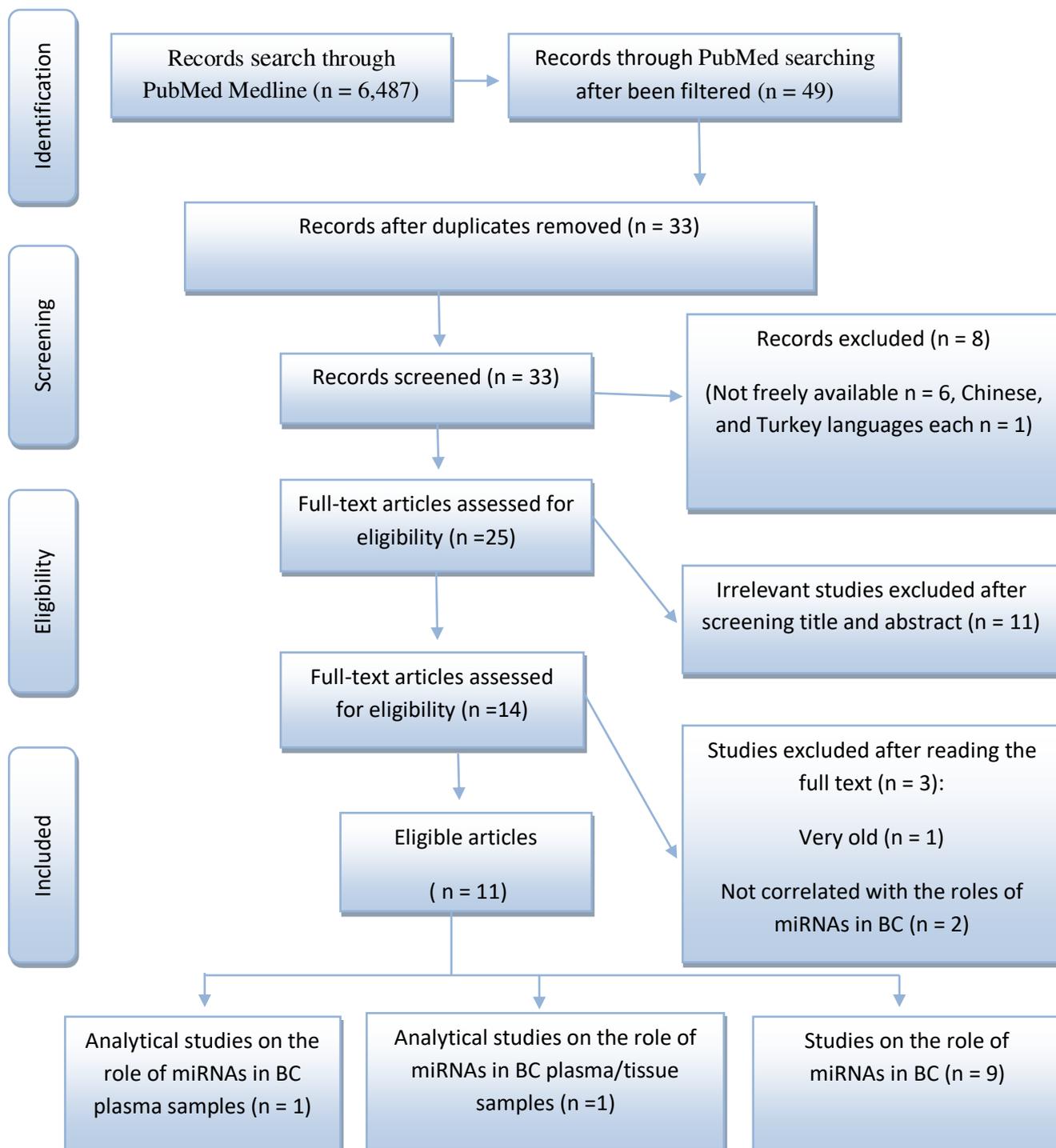


Figure2: Flow diagram represents the methodology of this study and its process.

| microRNA subtype | Receptor | Detection method | Role | Target gene | Biological sample, and Tumor stage | P- value | References |
|------------------|-------------------|------------------|--------------------------|---------------------|------------------------------------|----------|------------|
| miR-10b | ER+,PR+, and Her- | qRT-PCR | Oncogenes, and biomarker | HOXD10 | Plasma, | 0.5969 | (13) |
| | | | | | Tissue | 0.3689 | (13) |
| | | | | | Stage II, III | 0.7400 | (14) |
| | | | | | Stage III, IV | 0.0040 | (14) |
| miR-21 | ER+,PR+, and Her- | qRT-PCR | Oncogenes, and biomarker | MASPIN, TPM1, PDCD4 | Plasma, | 0.7684 | (13) |
| | | | | | Tissue | 0.7516 | (13) |
| | | | | | Stage II, III | 0.0900 | (14) |
| | | | | | Stage III, IV | 0.5400 | (14) |
| miR-155 | ER+,PR+, and Her- | qRT-PCR | Oncogenes, and biomarker | SOCS1 | Plasma, | 0.8804 | (13) |
| | | | | | Tissue | 0.2644 | (13) |
| | | | | | Stage II, III | 0.0078 | (14) |

Table1: List of role of miRNAs in breast cancer.

DISCUSSION

The expression levels of miR-21, miR-10b, and miR-155 were derived from patients' tissue (breast cancer biopsy) and plasma samples of BC patients. The results showed a comparable expression pattern among the different samples, with no significant discrepancy. Matamala *et al* represent an exception to this rule. (15). Nevertheless, Svoronos *et al.* concluded a matched expression level of the miRNAs subtypes between breast tumor tissues and serum. In our analysis, miR-21, and miR-155 were significantly over expressed in tumor specimens in comparison to the control group. (16). In addition, the expression of miR-21, miR-155, and some other types of miRNAs was powerfully correlated with the clinicopathologic features of breast cancer, such as tumor grades and sex hormone receptor expression (16, 17). We also analyzed the results of the previous miRs expression in the plasma to see whether there was a correlation between the expression level of the miRs and the various clinic-pathologic features or not. For example, these studies found the expression of the estrogen receptor (ER) and progesterone receptor (PR) is associated with miR-21 and miR-155. The ER is recognized as a key factor in motivating mammary cell proliferation, which can lead to up-regulation during the

early stages of tumorigenesis and tumor development (18). Likewise, Al-Khanbashi *et al.* observed the expression patterns of the miRs related to Her2/neu/ER/PR in breast tumors. In this context, two other independent studies have also shown the expression of miR-21 in breast cancer patients compared to healthy ones is increased. In addition, the discovered plasma levels of miR-10b and miR-155 between breast cancer patients and the healthy, as well as the serum level of miR-155 in PR+ tumors have exposed a significant dissimilarity in comparison to PR- cases (19). Wang *et al.* (2010) explain the clinicopathological features of breast tumors such as histological grades and the sex hormone receptor were connected with the expression of miR-21 and miR-155 (20). Additionally, we have stratified the patients to look at the relationship between the plasma levels of the selected miRs and the stage II/III of the disease based on the TNM staging. In 30 cases of breast cancer, miR-21, miR-10b, and miR-155 did prove a significant difference in the staging compromised control. Furthermore, there was a significant difference in miR-155 expression in the plasma between stages II and III. Wang *et al.* (2010) in a comparative between the higher malignancy grades and control breast tissues, showed that miR-21, miR-106a, and miR-155 were significantly up-regulated in malignancy grades (20).

The comparative expression of MiR-155 expression was not changed in the benign tumors but was increased 2 fold and 5 fold in grade II and III. The miR-21 was not changed in the benign tumors but increased 2 fold in the grade II tumors and 4.5 fold in the grade III tumors. Similarly, in both grades, miR-21, miR-126, miR-155, miR-199a, and miR-335 were strongly linked to ER or PR. Additional studies have also shown that miR-21 can up-expression of the oncogene proteins and down expression of the tumor suppressor proteins. Likewise, the miR-21 in breast tumor tissues has been shown to increase expression to be directly linked to the incidence of the disease, tumor size, and staging.

CONCLUSIONS

In summary, the expression of miR-21, miR-155, and miR-10b are downregulated in healthy individuals and upregulated in breast cancer. As well, the results showed a similar expression between the tissue and the plasma samples and noted that there were no significant changes. Furthermore, after operations, it was found that the chemotherapy and radiotherapy led to more reductions in the oncomiRs and increased the tumor suppressor genes during treatment. This can be considered as a good diagnostic tool for the process of perfection and suitable response to standard treatment. Generally, the difference between the expressions of the miRs could be due to differences in the sample sources, age, stage of tumor cancer, different analytical methods, and/or different platforms in the studies. Therefore, it seems that an increasing or decreasing expression of miRs can be related for various reasons.

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