ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ ΝΟΣ. ΑΛΕΞΑΝΔΡΑ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

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MSc: "Clinical Trials: Design and Conduct"

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Ευάγγελος Τέρπος, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ

Τίτλος ΜΔΕ *«Ο ρόλος των microRNAs στην πρόγνωση στον καρκίνο του μαστού»*

"The role of microRNAs in breast cancer prognosis"

Όνομα: Ελένη Ζωγράφου

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Επάγγελμα/ή Ιδιότητα: Βιολόγος

Επιβλέπουσα καθηγήτρια: Φλώρα Ζαγουρή, Αν. Καθηγήτρια Ιατρικής Σχολής ΕΚΠΑ

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Πίνακας περιεχομένων και ευρετήριο πινάκων και σχημάτων

Πρόλογος

Αισθάνομαι ιδιαίτερη υποχρέωση να εκφράσω τις θερμές μου ευχαριστίες και την ευγνωμοσύνη μου στον Διευθυντή και Επιστημονικό Υπεύθυνο του ΠΜΣ: «Κλινικές Μελέτες: Σχεδιασμός και Εκτέλεση» της Ιατρικής Σχολής ΕΚΠΑ, Καθηγητή κ. Ευάγγελο Τέρπο για την ενθάρρυνσή του να ασχοληθώ με την έρευνα στο πεδίο των κλινικών μελετών και για την πολύτιμη καθοδήγηση καθ' όλη τη διάρκεια του μεταπτυχιακού προγράμματος σπουδών.

Επιθυμώ επίσης να εκφράσω θερμές ευχαριστίες και προς την Αναπληρώτρια Καθηγήτρια κα. Φλώρα Ζαγουρή, επιβλέπουσα της παρούσας μεταπτυχιακής διατριβής, για την έμπρακτη υποστήριξη και για την πολύτιμη συμβολή της στο σχεδιασμό και στην εκτέλεση της παρούσας εργασίας.

Θερμές ευχαριστίες θα ήθελα να εκφράσω και προς το εκλεκτό μέλος της ορισθείσας τριμελούς επιτροπής, Επίκουρη Καθηγήτρια Θεραπευτικής της Ιατρικής Σχολής, κα. Μαρία Γαβριατοπούλου, για την επίβλεψη της διατριβής μου και τη συνολική βοήθεια στη διεκπεραίωση αυτού του πονήματος.

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Τέλος, ένα μεγάλο ευχαριστώ οφείλω στην οικογένειά μου, που με ανιδιοτελή και ουσιαστικό τρόπο στάθηκαν δίπλα μου και κατέστησαν δυνατή την επίτευξη αυτού του σκοπού.

Περίληψη

Τα microRNAs (miRNAs) είναι μια πολυπληθής οικογένεια μικρών νουκλεοτιδίων που δεν κωδικοποιούν πρωτεΐνες, αλλά δρουν ως αρνητικοί ρυθμιστές γονιδίων και συμμετέχουν σε πληθώρα βιολογικών διεργασιών. Πρόσφατα δεδομένα συσχετίζουν την απορρύθμιση της έκφρασης διαφόρων miRNAs με την ανάπτυξη καρκίνου, λειτουργώντας είτε ως ογκογονίδια είτε ως ογκοκατασταλτικά γονίδια, αλλά ο ρόλος τους στην πρόγνωση των ασθενών με καρκίνο μαστού παραμένει ασαφής. Σκοπός της παρούσας μελέτης είναι η διερεύνηση της προγνωστικής αξίας των miRNAs στον καρκίνο του μαστού. Πραγματοποιήθηκε συστηματική ανασκόπηση στη βάση δεδομένων PubMed για να προσδιοριστούν οι κατάλληλες μελέτες. Μετά την έρευνα και την ανασκόπηση της βιβλιογραφίας εντοπίστηκαν 117 σχετικές μελέτες, με βάση τα κριτήρια εισαγωγής και σχετικότητας των ερευνών. Διαπιστώσαμε ότι 110 διαφορετικά microRNAs έχουν συσχετιστεί με την πρόγνωση στον καρκίνο του μαστού. Συμπερασματικά, τα miRNAs θα μπορούσαν να χρησιμεύσουν ως νέα προγνωστικά εργαλεία στον καρκίνο του μαστού, ενώ η κλινική εφαρμογή αυτών των ευρημάτων δεν έχει ακόμη επαληθευτεί.

Λέξεις κλειδιά: καρκίνος μαστού, microRNAs, πρόγνωση, βιοδείκτες

Abstract

Breast cancer is a heterogeneous disease that differs greatly among patients and even within each individual tumor. MicroRNAs (miRNAs) have been found to play an important role in the occurrence and development of human cancers, functioning either as potential oncogenes or tumor suppressor genes, but their role in the prognosis of breast cancer patients remains unclear. The aim of the present review study is to highlight recent preclinical and clinical studies performed on both circulating and tissue-specific miRNAs and their potential role as prognostic markers in breast cancer. We performed a systematic review to explore the prognostic value of miRNAs in breast cancer. We systematically searched the PubMed database to identify eligible studies. After performing the literature search and review, 117 relevant studies were identified. We found that 110 aberrantly expressed miRNAs have been associated with prognosis in breast cancer. In conclusion, miRNAs could serve as novel prognostic tools in breast cancer, while the clinical application of these findings has yet to be verified.

Keywords: breast cancer, microRNAs, prognosis, biomarkers

1. INTRODUCTION

1.1 Breast cancer epidemiology

Breast cancer is the most frequent cancer among women. An estimated 1.67 million new cancer cases were diagnosed in 2012 (25% of all cancers) while 521,900 deaths were reported the same year (Ferlay et al., 2015). Breast cancer incidence among different countries presents variations. The slight majority of cases is occurring in women from less developed countries, and this is considered to be associated with a number of reasons, including degree of organization of operational screening activities and differential distribution of pivotal risk factors, such as parity (Ferlay et al., 2018).

Mortality from breast cancer is in part decreasing over the last decades; this may be due to a. the well-recognized improvements in diagnosis and treatment of the disease, b. to the constantly increasing breast cancer awareness leading to earlier detection and c. to a reported decrease in breast cancer incidence (Tarone 2017; Siegel et al., 2017). In total, European breast cancer mortality rates declined from 17.9 in 2002 to 15.2 per 100,000 in 2012 (15.3%) (Carioli et al., 2017). Carioli et al., predicted further appreciable declines in breast cancer mortality to 2020 across Europe, and an overall rate around 13.5/100,000 women in the EU as compared to over 20 in the early 1990's (Bosseti et al., 2013). Major risk factors for sporadic breast cancer are considered to be reproductive, menstrual, and nutritional (Winters et al., 2017); these factors do not seem to have an impact on these favorable trends. On the other hand, this trend most likely reflects the overall recent advancement in breast cancer management, although a single therapeutic breakthrough has not been presented (Cuzick et al., 2011; Negri et al., 2014; Cardoso et al., 2017).

This is a major achievement that emphasizes the importance of improved diagnosis and various aspects of integrated and personalized treatment for a common cancer. The presence of a large number of subsequent therapeutic improvements has contributed to this major success in Europe (Davies et al., 2013). Trends differ in central and eastern Europe, where they are less favorable, and this finding is supporting a need to implement further interventions to improve breast cancer diagnosis and management in those countries (Carioli et al., 2017). According

to the GLOBOCAN 2018 worldwide estimates of cancer incidence and mortality produced by the International Agency for Research on Cancer, in 2018, about 2,088,849 new cases were diagnosed and approximately 626,679 women were predicted to die from the disease (Bray et al., 2018). These data further support the need to improve the existing treatment modalities and to develop better preventive, diagnostic, and prognostic strategies addressing breast cancer.

1.2 Breast cancer prognosis

Breast cancer is a distinctively heterogeneous disease characterized by distinguishing molecular and morphological traits that inform prognosis and determine the proper therapeutic approach. Firstly, identification of novel approaches is needed for the management of patients at earlier stages. To this day, the biological features that are routinely used for the diagnosis and prognosis of patients with breast cancer and for determining the therapy are histological grade (Rakha et al., 2008), lymph node status, hormone receptor status, and human epidermal growth factor receptor type 2 (HER2) status (Viale 2012). Some of these factors have been associated with the survival rate of patients and even with their clinical outcome after treatment (Singletary et al., 2002).

Breast cancer is classified into five subtypes based on these distinct gene expression profiles: luminal A, luminal B, basal, human epidermal growth factor receptor 2 (HER2/ERBB2)-positive (+), and normal breast-like tumors (Perou et al., 2000). The majority of tumors are classified as luminal (Cho, 2016) and express estrogen receptor (ER), progesterone receptor (PR), and the luminal cytokeratins (CK) 8 and 18. Luminal B tumors have higher Ki67 expression than the luminal A subtype (Cheang et al., 2009). Ki67 is a protein-histological marker associated with cellular proliferation, disease recurrence, and poor survival in breast cancer patients (Voduc et al., 2010). ER+ tumors are associated with smaller, low grade tumors and are often lymph node negative (Tao et al., 2015). HER2+, basal and 'normal breast-like' tumors are ER/PR negative (ER-/PR-). HER2 is constitutively active and predicts reduced disease-free survival (Arteaga et al., 2011). Triple negative breast cancer (TNBC) is a highly diverse group of breast cancers with an overall poor prognosis (Judes et al., 2016). The HER2+, basal-like, and TNBC tumors are associated with a

more advanced stage at presentation and patients have reduced disease-free survival (DFS) (Bauer et al., 2007). However, some patients with a similar combination of breast cancer features, have been found to have different clinical outcomes. Thus, the role of these factors in determining diagnosis and prognosis and in predicting therapeutic outcomes in breast cancer still remains limited (Schnitt, 2010).

In an effort to provide accurate diagnostic and prognostic tools for the effective management of the disease, several tests have been developed for breast cancer detection and prognosis. Mammography overall remains the primary screening test for breast cancer detection. However, this important screening test is not recommended for women younger than 40 years of age, as they tend to have denser breast tissue (Checka et al., 2012). Aside to pain and discomfort that it might cause to patients, mammography is also associated with increased rates of false positives ranging from 12% to 65% and is correlated with over-diagnosis (Nassar et al., 2017).

Additionally, various multi-gene expression-based tests are utilized for breast cancer diagnosis and prognosis such as MammaPrint, MapQuant Dx, Oncotype Dx, PAM50 Breast Cancer Intrinsic Subtype Classifier (Prosigna), and Theros Breast Cancer Index. For instance, MammaPrint measures the mRNA expression levels of 70 different genes and categorizes ER+ cancer patients into low-risk or high-risk prognostic groups while it classifies all ER− cancers as high-risk (Tian et al., 2010). Another diagnostic test is Prosigna that quantifies the mRNA expression of 50 genes and predicts the risk of distant recurrence of ER+ breast cancer in postmenopausal women treated with adjuvant endocrine therapy (Wallden et al., 2015). A 21-gene assay called Oncotype DX is also used for computing a recurrence score for ER+ breast cancer (McVeigh, et al., 2017). However, all these tests mainly require formalin fixed paraffin embedded (FFPE) or fresh frozen tissue biopsies and are therefore invasive, while they are only useful for hormone receptor-positive or invasive breast cancer (Nassar et al., 2017). Furthermore, their use is limited by higher cost and its failure to predict recurrence beyond 5 years (Wen et al., 2017). None of these tests currently includes miRNAs. To date, there is one study correlating miRNA expression with recurrence scores (RS) from Oncotype DX on 23

human BC tumors (Emmadi et al., 2015). That study reported reduced expression of Let-7 family members in cases with high RS and high expression of miR-377-5p, miR-663b and miR 3648 were associated with high RS scores.

Moreover, circulating antigens such as carcinoembryonic antigen (CEA) and cancer antigen 153 (CA153), have been identified as prognostic tools for breast cancer since they were reported to be elevated in the serum of breast cancer patients, especially those with HER2+ and ER− subtypes respectively. However, the use of CEA and CA153 as prognostic serum markers still remains controversial due to conflicting results of different studies and their reported low sensitivity and specificity (Shao et al., 2015).

Considering all the limitations of the currently available prognostic strategies, it is overall recognized that new affordable methods are needed to further help diagnosis and prognosis and to indicate which is the optimal treatment for patients with breast cancer on an individual basis. Still, it remains difficult to achieve these goals because of the absence of sensitive and specific biomarkers for early detection and for disease monitoring.

The first evidence of a different from normal expression of miRNAs in human cancers was reported in B-cell chronic lymphocytic leukemia (Visone and Croce, 2009), where chromosomal deletion of the 13q14 locus resulted in loss of expression of two miRNAs. This was an important discovery for the genomic profile of the disease and prompted research on expression of miRNAs in human tumors (Visone and Croce, 2009). Furthermore, more genomic alterations in miRNA loci have been found in 227 patients with cancers of the breast, ovaries and melanoma by Zhang et al (Zhang et al., 2006).

Particularly in breast cancer, microRNAs (miRNAs or miRs) have been proposed as promising biomarkers because they can be readily detected in tumor biopsies (non-circulating miRNAs) and can also be identified in blood, plasma, serum, and saliva (circulating miRNAs) (Bertoli et al., 2015). Furthermore, circulating miRNAs are being bound to lipoproteins such as HDL, associated with Argonaute 2 (Ago2) protein, or packaged into exosome-like microparticles, micro-vesicles, and apoptotic

bodies (Arroyo et al., 2011). Therefore, they are protected from endogenous RNAase activity, and hence they are reliable.

In conclusion miRNAs are increasingly recognized as promising biomarkers, given the fact that they are easy to isolate, and they maintain their structural stability under different conditions of sample processing and isolation. In a recent study, miRNA profiling has been found to improve breast cancer classification and to differentiate patients with breast cancer as responding or not responding to therapies (Cava et al., 2014). These results are promising and suggest that these diagnostic tools have the potential to be used as new diagnostic, prognostic, and predictive biomarkers for breast cancer, and eventually make a great impact on the clinical management of patients with breast cancer (Bertoli et al., 2015).

1.3 Introduction to microRNAs

Cancer is a complex process in the genomic level, as it results in multiple genomic alterations and progresses through proliferation, invasion and metastasis. The molecular pathways that are altered during cancer progression include both protein - coding genes and noncoding genes, according to newer data. These noncoding RNAs include groups such as “small RNAs” and microRNAs (miRNAs or miRs), that subsequently regulate mRNA translation and post-transcriptionally control gene expression (Visone and Croce, 2009).

MicroRNAs are a small class of endogenous, evolutionarily conserved, single-stranded noncoding RNAs, with a length of approximately 19–24 nucleotides (Wang et al., 2014). Interaction between miRNAs and mRNAs, within the 3’untranslated region of the target genes, leads to the degradation or inhibition of mRNA translation (Yoruker et al., 2015). Their mechanism of action is through binding to the 3’ untranslated region (3’UTR) of mRNAs to repress translation and/or promote mRNA degradation (Iorio and Croce, 2012). The first report of free nucleic acids in the serum of patients with cancer was published in 1977 by Leon et al (Leon et al., 1977).

A relatively new database, the “primary repository for miRNA sequences and annotations”, miRBase (www.mirbase.org), launched in 2006 with just 218 miRNA

loci (Kozomara and Griffiths-Jones, 2014) and since then miRNA analysis has allowed the discovery of more than 38589 mature miRNAs. An estimated 2588 miRNAs are transcribed from intragenic or intergenic regions of the human genome (June 2014; http://www.mirbase.org) (Kozomara and Griffiths-Jones, 2014). Finally, the discovery of microRNAs had a profound impact on the understanding of many gene regulation processes in the past years, including regulation of cell proliferation, differentiation, angiogenesis, migration, and apoptosis (Bertoli et al., 2015).

1.3.1 miRNA biogenesis and mechanisms of action

Although many steps to the miRNA pathogenesis pathway and repressive mechanisms are not well defined, there are some key steps of the miRNA pathogenesis that are overall recognized (Fig. 1). The miRNA precursor molecules, called pri-miRNAs are formed as multiple “hairpin structures” (of -70 nucleotides) in the nucleus. Subsequently these precursors are exported to the cytoplasm by Exportin-5 The generated pre-miRNA is exported from the nucleus to the cytoplasm by Exportin 5 (a RanGTP-dependent dsRNA-binding protein (Bohnsack et al., 2004), and are cleaved by DICER, in union with transactivation-responsive RNA-binding protein 2 (TARBP2) and AGO2 (DICER complex). The process generates a double-stranded miRNA-miRNA* duplex. The two strands are then separated: the mature miRNA is incorporated into the RNA-induced silencing (RISC) complex, whereas the passage miRNA* strand can be loaded in the RISC as well or usually degraded (Visone and Croce, 2009). Finally, miRNAs tether to the 3’ UTR of a mRNA target to repress protein synthesis.

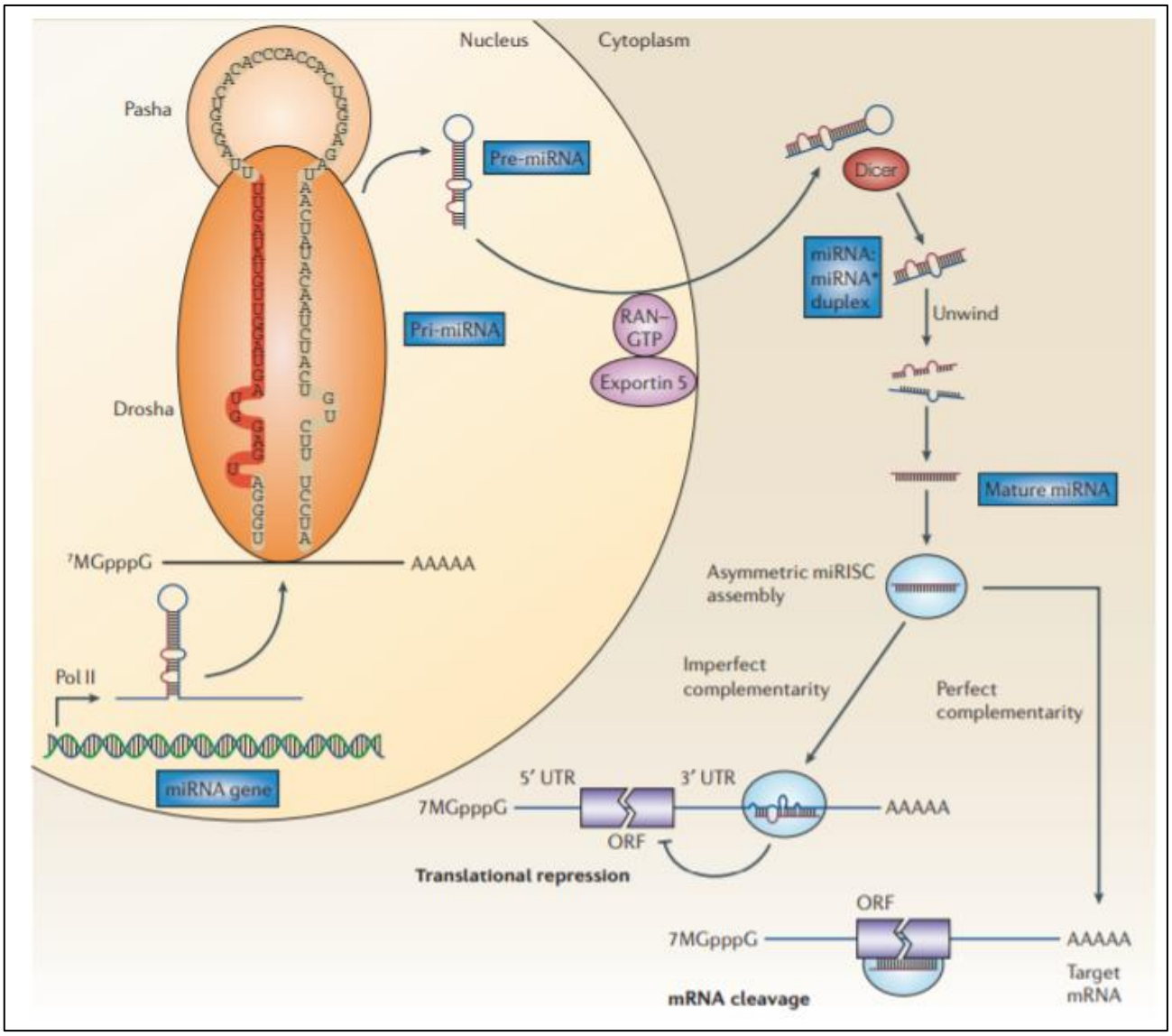

Figure 1 *The biogenesis of microRNAs. (Esquela-Kerscher and Slack, 2006)*

Regarding the mechanisms of action of miRNAs, the major factor for miRNA binding to its target mRNA is a 6-8-nucleotide sequence at the 5' end of the miRNA, the "seed" sequence. Of note, any sequence complementarity between the loaded miRNA and the seed region triggers a decrease in target mRNA expression levels. This "seed matches" can occur in any region of the mRNA but are more likely to be present in the 3' UTR of a mRNA (Qin et al. 2010). miRNAs can also bind to other regions in the target mRNA (Lytle et al., 2007). Depending on the degree of homology to the 3' UTR target sequence, miRNAs induces either the translational

repression or the degradation of mRNAs. it is important that miRNA is also capable of regulating the expression of many genes, in other words each miRNA simultaneously regulates multiple cellular signaling pathways.

The mechanism reported above is the so called “traditional” mechanism of action of miRNAs. There are other recently reported “non-canonical” mechanisms. there is some evidence that that miRNAs may a. increase the translation of a target mRNA by recruiting protein complexes at the AU-rich region of the target mRNA or b. can indirectly increase target mRNA levels by interacting and modulating repressor proteins that block the translation of the target mRNA (Eiring et al. 2010). Finally, some research may suggest that miRNAs could enhance ribosome biogenesis, and therefore modulate protein synthesis, or skip cell cycle arrest and therefore activate target gene repression (Vasudevan et al., 2007; Orom et al., 2008).

1.4 miRNAs and Breast Cancer

Important evidence has shown that miRNAs play an important role in breast cancer via their regulatory function (Negrini and Calin, 2008). Particularly the use of novel technologies, such as microarray expression data, showed that aberrant miRNA expression is present in breast cancer (Andorfer et al., 2011). The miRNA molecule can increase the control over its target gene. If the target gene is an oncogene, the cancer is suppressed (tumor suppressor-miRs); if the target gene is a tumor suppressor, the cancer is developing (onco-miRs). Also, miRNA can reduce the control over its target gene. If the target gene is an oncogene, the cancer develops (onco-miRs); if the target gene is a tumor suppressor, the cancer is suppressed (tumor suppressor-miRs) (Fig. 2) (Esquela-Kerscher and Slack, 2006).

Characteristically, Negrini et al. (2008), have mentioned in their paper three representative research teams that discussed the role of miRNAs in breast cancer metastasis in their functional and molecular studies, as well as the significance of abnormal expression of this miRNA in breast tumorigenesis: 1. The study by Ma and coworkers (2007) revealed that upregulation of miR-10b promotes invasion and metastasis in breast cancer, 2. Travazoie et al. (2008), found that miR-335, miR-126,

and miR-206 are metastasis-suppressor miRNAs, and 3. Huang and colleagues (2008), identified significant upregulation of miR-373 in samples from patients with metastatic breast cancer.

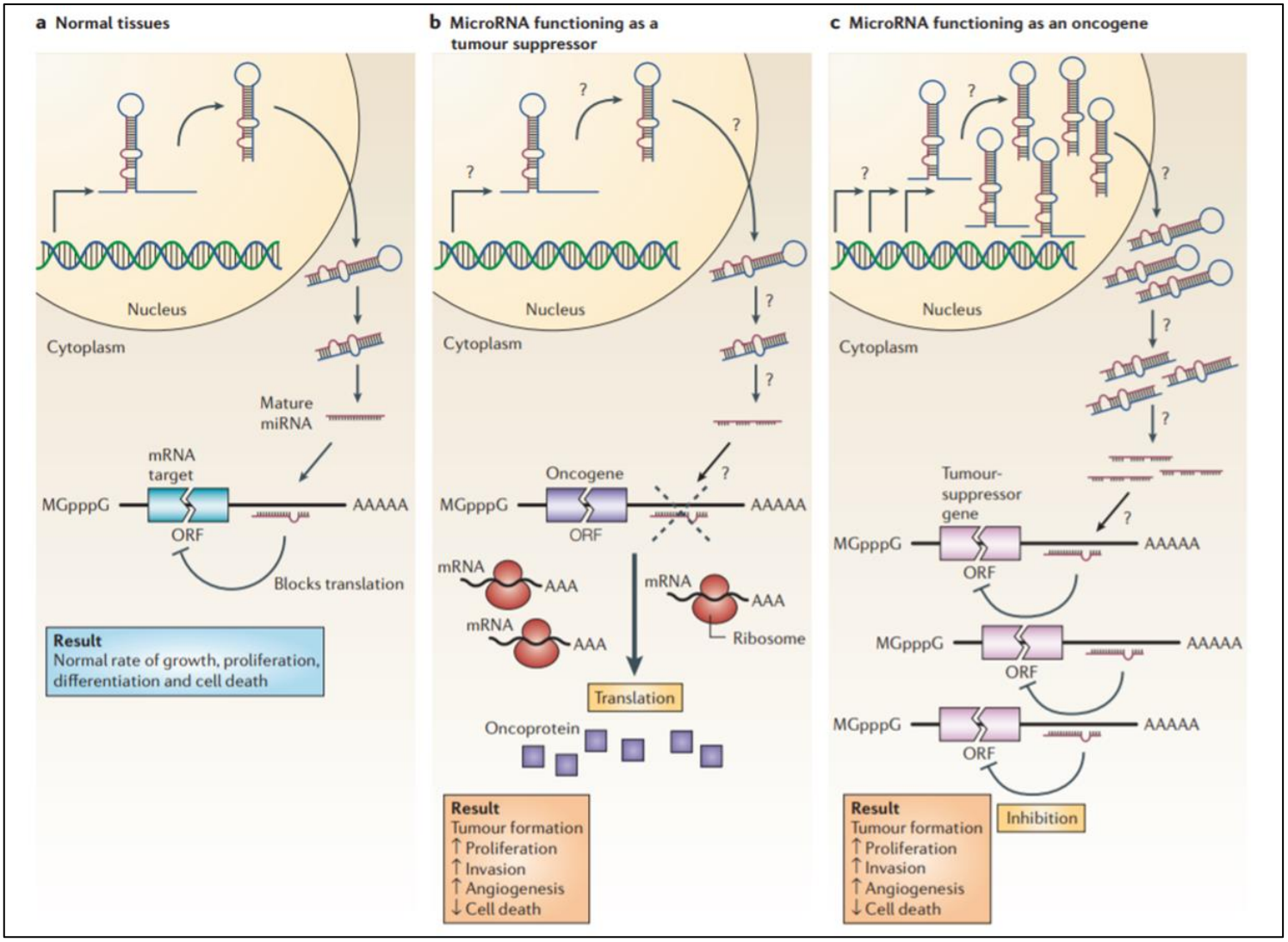

Figure 2 MicroRNAs can function as tumour suppressors and oncogenes. (Esquela-Kerscher and Slack, 2006).

1.4.1 Mechanisms altering miRNA expression levels

Accumulating experimental evidence indicates that miRNAs play a pivotal role in many cellular functions via the regulation of gene expression. According to research, as has been presented above, their dysregulation has been shown in carcinogenesis (Iorio and Croce b, 2012). Important mechanisms that can alter miRNA expression levels have been reported in the literature and are the following:

1. Epigenetic mechanisms: A large proportion of miRNA loci on the genome are associated with CpG islands, indicating regulation by methylation. Another epigenetic phenomenon altered in BC is histone acetylation. It has been shown that acetylated histones can diminish expression of anti-oncogenic miRNAs (Bertoli et al., 2015).

2. A genetic alteration as frameshift mutations resulting from microsatellite instability. It is important that half of the known miRNAs are located in cancer-associated region, such as fragile sites, minimal regions of loss of heterozygosity, minimal regions of amplification, or common breakpoint regions (Bertoli et al., 2015).

3. miRNA biogenesis pathway defects, that could affect each step of miRNA biogenesis, making the cell suitable for and vulnerable to oncogenic changes.

4. Transcriptional repression by other upstream proteins. Multiple factors can influence the expression levels of a single miRNA molecule. In fact, it seems that a. miRNAs and transcription factors work cooperatively, and b. miRNAs are involved in the functional feedback loop (where transcription factors influence miRNA expression levels and vice versa). Oncogenic miRNA expression changes could be due to the activity of tumor-related transcription factors, such as SMAD, p53 protein family, ataxia telangiectasia mutated (ATM) and Myc). We also know that BRCA1 transcription factor and the epidermal growth factor receptor (EGFR/HER1) are able to inhibit miRNA maturation and enhance cell survival and invasiveness (Bertoli et al., 2015).

1.5 Methods for Detecting miRNAs

Development of new miRNA detection methods has been one of the most popular research fields. miRNAs possess special characteristics: they are small in size, low content, and exhibit high sequence similarity, therefore accurate quantification of miRNAs is challenging. These are the methods and techniques that have been developed for miRNA detection. Each one of them has advantages and limitations (Hamam et al., 2017):

1. Northern blotting is the golden standard method since the early miRNAs study (Lagos-Quintana et al., 2001). However, this is a complex procedure and has low sensitivity limits, therefore it is not considered appropriate for wide applications.
2. Quantitative reverse transcriptase real-time (qRT-) PCR is widely used; it is a highly sensitive method that requires only small amounts of input RNA (Kroh et al., 2010) Major limitation of the qRT-PCR is that it is oftentimes used to quantify the levels of a defined set of miRNAs (usually <700);, and therefore it cannot be used for high-throughput profiling.
3. Microarray platforms are an alternative method for detecting circulating miRNA. MicroRNA microarray analysis is usually performed by fixing the high density known sequence of DNA probes on the solid support such as glass or nylon membrane, subsequently interacting with the variety of miRNA target molecules based on nucleic acid hybridization between target miRNAs and their corresponding complementary probes. As a last step, the signal intensity of the hybridization probes is detected (Cheng et al., 2018). The method is advantageous since it allows to simultaneously detect large numbers of circulating miRNAs (Hamam et al., 2016). Its disadvantages include a low dynamic range and inability to detect novel (i.e., unannotated) miRNA species; the cost of production and detection of microarray is also high (Cheng et al., 2018).
4. Most of miRNA detection methods require total RNA extracts that lack of spatial information of miRNA in cells and tissues, so development of methods to detect subcellular and tissue localization of miRNAs. This is essential for direct assessment of expression levels in tissue. In situ hybridization (ISH) is a powerful

tool that identifies the expression level and the co-localization information of specific miRNA within individual cells or in tissue.

5. Next-generation sequencing is another technology for detecting miRNAs based on deep sequencing (Wu et al., 2012). Advantages of this method is its ability to detect both annotated and unannotated miRNAs. Disadvantages are that it requires large amounts of starting material and the amount of data that must be analyzed requires complex bioinformatics tools.
6. Direct quantification of circulating miRNAs in bodily fluids has become possible using the NanoString nCounter platform (Oikonomopoulos et al., 2016). This is based on a novel digital molecular barcoding technology that enables quantification of the exact copy number of miRNA species in a biological sample (Alajez et al., 2012). Major limitation of this platform is that it can only detect up to 800 human miRNAs per slide.

Given the strengths and shortcomings of each detection approach, the choice will largely depend on availability, type of sample, and the research question being addressed. The increasing development of miRNA research, has initiated novel approaches, developed for the detection of miRNA with various levels of sensitivity, specificity, multiplicity, and imaging in situ. Particularly interesting are the nucleic acid amplification-based methods and many of detection techniques such as droplet digital PCR (ddPCR), electrochemiluminescence (ECL), surface-enhanced Raman spectroscopy (SERS), and mass spectrometry (MS) among the many methods used for highly sensitive detection of miRNA. Effective and timely miRNA detection may enhance the miRNA functional researches and may improve clinical diagnostics, since they have a great potential to be considered as diagnostic, predictive and prognostic biomarkers (Cheng et al., 2018).

1.6 Aim

The aim of the present review study is to highlight recent preclinical and clinical studies performed on both circulating and tissue-specific miRNAs and their potential role as prognostic markers in breast cancer.

In this study we will also discuss miRNA biogenesis and function and their involvement in malignancy, and particularly their putative role as oncogenes or tumor suppressors on breast cancer. We will particularly focus on the potential role of miRNAs in breast cancer prognosis, and on how miRNAs have the potential to answer actual clinical needs, such as identification of biomarkers for prognosis, in order to achieve the goal of individualized cancer treatment.

2. MATERIALS AND METHODS

2.1 Methods of Search Strategy and Study Eligibility

This systematic review was conducted in accordance with the PRISMA guidelines (Liberati et al., 2009) and in line with the a priori protocol agreed on and signed by EZ and FZ. Eligible studies were sought in PubMed without any restriction of publication language; end-of-search date was January 28, 2019. The following search algorithm was used: breast[ti] AND (carcinoma OR carcinomas OR cancer OR cancers OR neoplasm OR neoplasms) AND (microRNA[ti] OR miR[ti] OR miRNA[ti] OR microRNAs[ti] OR miRs[ti] OR miRNAs[ti]) AND (prognosis[ti] OR prognostic[ti] OR survival[ti] OR outcome[ti] OR mortality[ti]). Eligible articles included studies examining the prognostic role of microRNAs in breast cancer. Only prospective and retrospective studies as well as case reports were considered eligible. In instances where multiple (overlapping) publications stemming from the same study were identified, the larger size study and the one with longer follow-up was included, unless the reported outcomes were mutually exclusive. Authors working independently and blindly to each other in pairs (E.Z., F.Z.) performed the selection of eligible studies; in case of disagreement, consensus with the whole team was reached.

2.2 Data Extraction

The extraction of data comprised general information, including the name of the miRNA molecule, the breast cancer type in which its expression was determined, method of detection, the sample type that was used, its prognostic value in breast cancer, its function in cancer (onco-miR or tumor suppressor-miR) and the author-year of publication. Data were independently extracted and analyzed by a pair of reviewers (E.Z. and F.Z.), with 1 reviewer being blinded to the other; if needed, final decision was reached by team consensus.

Eligible literature met the following criteria: (1) measured miR expression levels in tumor or blood samples or human cell lines; and (2) only articles in English. Publications were excluded if they had one or more of the following criteria: (1) studies referring to the prognostic role of single nucleotide polymorphisms (SNPs) in miRNA genes affecting their function; (2) studies that refer to the prognostic role of

target miRNA molecules (molecules regulated by miRs); (3) studies based solely on a bioinformatics approach or a computational algorithm, with survival data originated from databases without subsequent biological validation and (4) review papers, meta-analyses, comments, letters or duplicate publications.

2.3 Definition of oncogenic miRNAs and tumor suppressor miRNAs

According to previous publications (Liu et al., 2017), miRNAs were considered as tumor suppressive or protective when they were down-regulated compared with normal counterpart, meaning that these miRNAs were associated with a hazard ratio value larger than one, otherwise, they were called oncogenic miRNAs or onco-miRs or risky miRNAs.

3. RESULTS

The search strategy retrieved 192 articles. Of these articles, 42 were irrelevant, 11 were reviews, eight (8) were meta-analyses, six (6) were retracted articles, three (3) were not in English, three (3) were duplicates, two (2) were comments and 117 were eligible. The aforementioned steps concerning the selection of studies are illustrated in detail in Fig. 3. Therefore, a total of 117 articles were eligible for this systematic review and the prognostic role of 110 miRNA molecules is described (Table 1). Furthermore, from our search we retrieved five studies, in which authors have identified six distinct microRNA signatures with prognostic value in breast cancer (Table 2). In the following sections (3.1 and 3.2), all the identified microRNAs and miRNA signatures and their potential prognostic role are presented in detail.

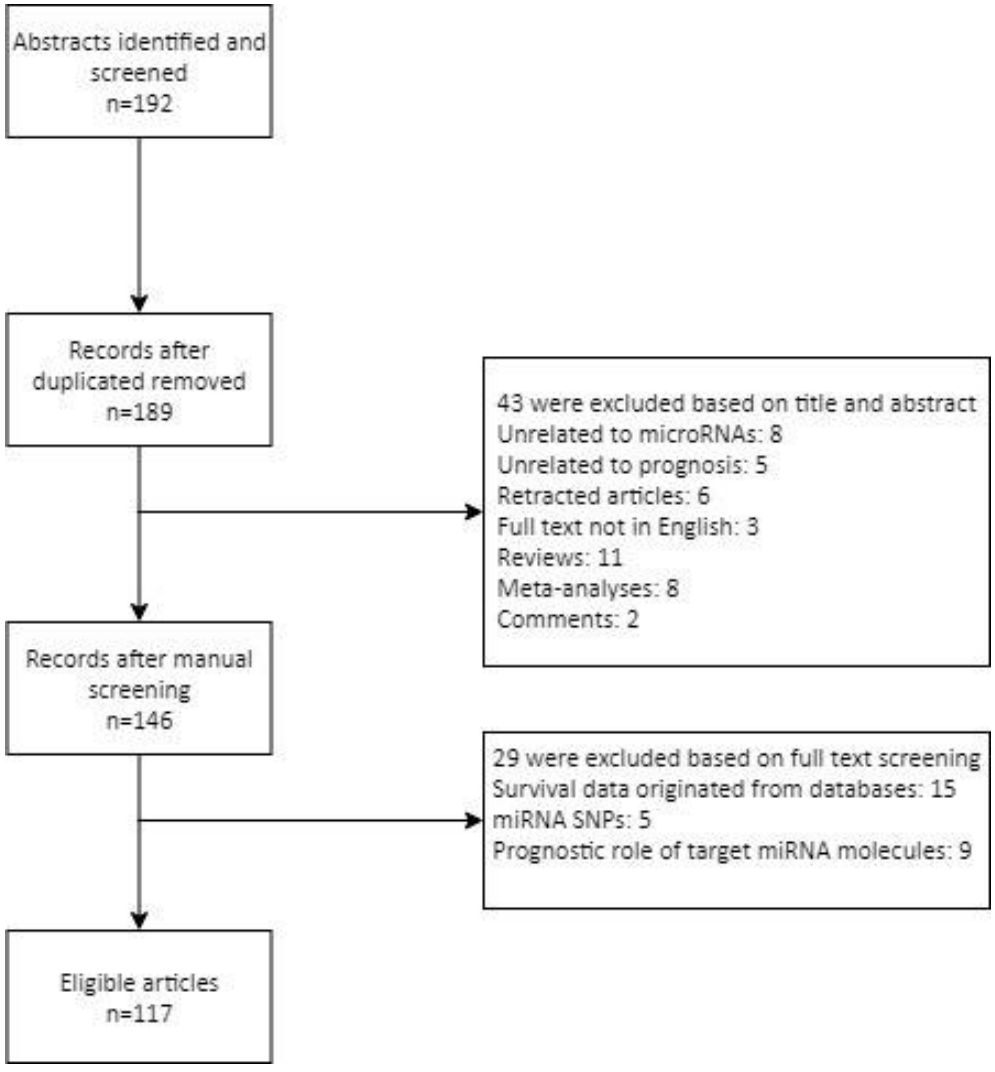

Figure 3 Flow diagram of the study selection process

3.1 miRNAs with a prognostic role in breast cancer: our search results

According to our results we have identified numerous miRNAs reported by various authors. All the identified microRNAs and their potential prognostic role are presented below (see Table 1):

miR-1ab/206/613 family member **miR-1** up-regulation has been associated with distant metastasis, in a study comparing stage IV breast carcinoma tissues to stage I-III cases (Minemura et al., 2015). In fact, in the same study, abnormal miR-1 expression was associated with an aggressive breast cancer phenotype and miR-1 status was characterized as a potent prognostic factor in human breast cancer patients. **miR-206** has be found to have a lower basal expression level in breast cancer cell lines than in normal breast cells, suggesting that up-regulation of miR-206 expression attenuates cell survival and promotes cell apoptosis (Hesari et al., 2018). According to an earlier study by Li et al. (2013), miR-206 has been downregulated in 119 (93%) tumor tissues, while decreased miR-206 has been reported to be an unfavorable prognostic factor for OS in breast cancer, significantly associated with advanced clinical stage and lymph node metastasis. On the contrary, Quan et al. (2018), have recently stated that miR-206 expression is higher in tumor tissues than in para-cancerous tissues. The same research group has reported that the 3-year survival rates of miR-206 high expression group were lower than that of miR-206 low expression group in a total of 372 cases, which may potentially have an impact on the prognosis of patients (Quan et al., 2018).

miR-124, which belongs to the miR-124/124ab/506 family, exerts a tumor suppressor effect by targeting cyclin-dependent kinase 6, and epigenetic silencing of miR-124 leads to CDK6 activation and Rb phosphorylation (Oltra et al., 2018). Our search revealed that decreased expression of **miR-124** has been correlated with tumor progression and poor prognosis in 133 breast cancer patients, using qRT-PCR (Dong et al., 2015). The correlation between miR-124 levels and the clinicopathological factors of the patients was also analyzed by the same authors, revealing an association of decreased expression with advanced TNM stage, lymph node metastasis and poorer pathological differentiation (Dong et al., 2015). Furthermore, it has been reported that miR-124-gene-hypomethylation, which leads

to higher miRNA expression, presents significantly better survival rates for older patients with breast cancers (>50 years old), identifying it as a potential specific survival biomarker in that specific age group (Oltra et al., 2018).

miR-588 has been reported as downregulated in fresh frozen breast tissue specimens and its aberrant expression has been closely associated with patients' poor prognosis and overall survival, thus suggesting a potential prognostic biomarker role (Yu 2017).

miR-711 aberrant overexpression has been associated with poor OS and DFS times, in 30 paired breast cancer and non-cancerous FFPE tissue samples (Hu 2016). Additionally, in vitro experiments have demonstrated that overexpression of miR-711 promotes proliferation, colony formation, migration and invasion of breast cancer cells. Considering the collective data, Hu et al. (2016) have characterized miR-711 as an independent prognostic factor in patients with breast cancer.

The **let-7 family** members are often cited as the model tumor- suppressing miRNAs, since they negatively regulate the expression of the RAS oncogene, an oncogene that contributes to the pathogenesis of several types of human tumors (Elghoroury et al., 2017). Our search revealed a study performed on 125 serum samples of patients with breast cancer, reporting that levels of miRNA let-7 expression negatively correlate with metastases (Elghoroury et al., 2017). The authors state that there is a probable association between decreased levels of miRNA let-7 and metastases risk, implying a noteworthy role of miRNA let-7 in breast cancer progression and prognosis. **let-7b** expression has been associated with the luminal subtype in a thorough analysis performed on 2919 formalin-fixed paraffin-embedded (FFPE) archival breast tumors, while according to Quesne et al (2012), it is an independent positive prognostic factor in this particular group (Quesne et al., 2012). **let-7c/miR-99a/miR-125b** cluster has been identified as a group of miRNAs that regulate HER2 protein expression and when lost may lead to worse outcome for patients in the luminal A subset (Bailey et al., 2015). Bailey at al. (2015), have discussed that patients with luminal A tumors, who express higher levels of these miRNAs, have significantly better survival than those expressing lower levels. **miR-125b** expression

has been reported as significantly increased in 221 breast cancer tissues compared to 49 non-cancerous tissues, and high miR-125b expression has been implicated in poor breast cancer prognosis (Luo et al., 2017). In addition, high miR-125b expression has been significantly correlated with tumor size and TNM stage in the HER2-positive patients, along with a poor prognosis (Luo et al., 2017). Notably, targeting miR-125b in Res-Let cells has been associated with a reduction in letrozole resistance (Vilquin et al., 2015). In serum, altered expression of miR-125b has also been associated with chemotherapy response and disease-free survival (DFS) (Liu et al., 2017). **miR-99a** downregulation has been associated with poor prognosis, in an analysis of serum collected from 72 patients with breast cancer and 40 healthy volunteers, suggesting a tumor suppressive role in breast cancer (Li et al., 2016).

miR-205 has been associated with tumors of ductal morphology in a large cohort of FFPE tissue samples and therefore is of significant positive prognostic value within these tumors (Quesne et al., 2012). Another analysis performed on tissues from 84 early breast cancer patients and a long follow-up, has revealed that downregulation of miR-205 is significantly associated with reduced DFI and OS in early breast cancer, and therefore miR-205 has been named an independent prognostic factor associated with early disease relapse (Markou et al., 2014).

miR-21 expression has been found to be elevated in sera of cancer patients compared to healthy controls, in multiple studies (Toraih et al., 2015; Usmani et al., 2015). Higher levels of serum miR-21 have been correlated with high grade tumors, extensive nodal involvement, distal metastasis, advanced clinical stages and poor survival of breast cancer patients (Toraih et al., 2015, Yadav et al., 2016). Interestingly, Usmani et al., showed higher expression of serum miR-21 in the daughters of stage III invasive ductal carcinoma patients compared to healthy controls, indicating disease inheritability (Usmani et al., 2015). Serum miR-21 has also been reported as a potential prognostic factor for trastuzumab therapy in HER2-positive metastatic breast cancer patients. (Badr et al., 2018). Lastly, miR-21 decreased serum expression has been linked to improved chemotherapy response and DFS, following a survival analysis of 118 breast cancer serum samples (Liu et al., 2017). In breast cancer tissue, high level expression of miR-21 has been significantly

correlated with features of aggressive disease, including advanced clinical stage, lymph node metastasis, and reduced overall survival (Yan et al., 2008, Qian et al. 2009, Lee et al., 2011). An analysis performed on FFPE breast tissue samples from 84 patients with early breast cancer, has demonstrated that miR-21 is an independent factor associated with early DFS (Markou et al., 2014), which is in accordance with other studies (Qian et al., 2009). Additionally, miR-21 expression has been found to be up-regulated in TNBC tissue specimens, posing a correlation with poor prognosis in TNBC as well (Dong et al., 2014; Medimegh et al., 2014). Further results, derived from cell lines, have demonstrated that the upregulated miR-21 promotes tumor proliferation and inhibits cell apoptosis in vitro (Dong et al., 2014), while miR-21 knockdown suppresses cell growth, migration and invasion (Yan et al., 2016). Collectively, the aforementioned findings emphasize the oncogenic role of miR-21 and indicate that its high expression may serve as a molecular prognostic marker for breast cancer.

miR-29b has been shown to act as a suppressive microRNA in breast cancer and as a marker for recurrence and metastasis of the disease (Shinden et al., 2015). Specifically, miR-29b decreased expression in 94 primary breast tumors has been significantly associated with poorer DFS and multivariate analysis has indicated that miR-29b expression is an independent prognostic factor for OS (Shinden et al., 2015). Similarly, elevated miR-29b levels have been correlated with significantly longer DFS and a lower risk to relapse in 121 malignant and 56 benign breast tissue samples (Papachristopoulou et al., 2018). Consequently, miR-29b levels constitute a promising biomarker of prognosis for patients with invasive lobular and ductal breast carcinoma.

miR-155 levels are related to clinical features of breast cancer, although reports are contradictory. An analysis of all 231 patients' tissues demonstrated a statistically significant negative correlation between OS and miR-155 expression level, while elevated miR-155 was significantly associated with late stage (stage III/IV) and high-grade tumors, lymph node metastasis and triple-negative breast cancer (Kong 2014). On the other hand, Jang et al. showed that high level of miR-155 expression was associated with better DMFS, in 190 formalin-fixed paraffin-embedded TNBC

specimens (Jang et al., 2017), possibly due to a different functional involvement in this molecular subset.

miR-9 has been suggested as a prognostic marker in TNBC; high level of miR-9 expression has shown significant association with poor DFS and distant metastasis-free survival (DMFS) in the triple-negative subtype (Jang et al., 2017). These results are confirmed by a later study, reporting that increased levels of miR-9 in breast tissue portended a significantly elevated risk of progression to malignancy with respect to a lower OS and shorter DFS. Subsequent experiments on breast cancer cell lines have shown that miR-9 can enhance the generation of cancer stem cells to yield an invasive phenotype (Cheng 2018).

miR-221 belongs to the miR-221/222/222ab/1928 family, and its overexpression has been reported on breast tumor cells (Cheng et al., 2018); increased expression of miR-221 in 206 breast tissue samples has been prognostic of shorter OS and DFS associated with larger tumor size, poor differentiation, late-stage evolution, and lymph-node metastasis (Cheng et al., 2018). In another study, high miR-221 expression has been presented as an independent poor prognostic factor in 76 breast tissue specimens, where the RFS of patients with positive miR-221 was significantly shorter than of those with negative miR-221 (Eissa 2015). In vitro, miR-221 overexpression strongly increased cell proliferation and invasion (Falkenberg et al., 2013). Downregulation of **miR-221-3p,** deriving from the 3' prime end of the hairpin, likely contributes to the poor outcome of TNBC patients, through inhibition of *PARP1*, thus affecting the prognosis of TNBC patients (Deng et al., 2017). **miR-222** has been associated with the occurrence of distant metastases in tumor tissues. In particular, high levels of miR-222 strongly increased cell proliferation and invasion in vitro (Falkenberg et al., 2013). Of note, overexpression of **miR-222-3p** has also been reported as an independent prognostic factor for shorter DFS, when quantified in the serum of patients postoperatively (Wang et al., 2018).

miR-200 family miRNAs (miR-141, miR-200a, miR-200b, miR-200c, miR429, miR-203 and miR-375) expression levels have been associated with survival and metastasis in a number of studies. **miR-200a** has been identified as a potential prognostic factor

for OS and PFS, associated with circulating tumor cells status (Madhavan et al., 2012) and with a potential to detect the onset of metastasis in the plasma of patients, as early as 2 years prior to clinical diagnosis (Madahavan et al., 2016). **miR-200b** has been found to be elevated in plasma samples (Madahavan et al., 2012; Madahavan et al., 2016); however, reports have shown a tumor suppressive role as well, since it has exhibited significant down-regulation in both breast cancer tissues and cell lines, and its low expression has been correlated with poor outcome, late TNM stage, ER- and HER-2+ status, indicating that it may act as an independent prognostic predictor for breast cancer patients (Ye 2014, Yao et al., 2015). **miR-200c** high expression has been associated with shortened relapse-free survival in PR-negative tissues, with increased recurrence and more frequent distant metastasis, providing a refined predictor of outcome (Tuomarila et al., 2014). **miR-200c/141 cluster** overexpression in TNBC has been found to promote metastasis and has been deemed as a poor prognostic factor in TNBC, after a series of experiments on paraffin tissues, cell lines and xenograft animal models (Jin et al., 2017). **miR-203** has been reported as a potential prognostic factor for OS and PFS (Madhavan et al., 2012; Madhavan et al., 2016) associated with EMT in cell lines (Fisher et al., 2015), although this miRNA has also been found to be significantly higher in triple positive breast tissues, suggesting a potentially tumor suppressive effect on cancer progression of ER positive breast cancers (Yu et al., 2012). **miR-203a** downregulation may be a potential prognostic marker associated with increased stage in invasive lobular carcinomas (Gomes et al., 2016). Elevated **miR-203-5p** expression has been significantly associated with decreased OS for TNBC, in a next generation sequencing study (Turashvili et al., 2018). **miRNA-375** expression has been found to be significantly higher in the serum of patients with pre/postmenopausal breast cancer and benign tumors, associated with receptors used for the prognosis of breast cancer (Ali et al., 2018). On the other hand, in a de novo analysis, miR-375 prevalence in circulation has appeared to reflect better clinical outcome, including NCT response and relapse with metastatic disease (Wu et al., 2012).

miR-548c-5p has been emphasized as a new independent prognostic factor in TNBC, since a combination of the tumoral expression of miR-548c and three other known

prognostic parameters (tumor size, lymph node invasion and CK 5/6 expression status) allowed for relapse prediction (Boukerroucha et al., 2015)

miR-320a low expression levels have been correlated with shorter OS time, with analysis performed on 145 FFPE breast tissue samples revealing that miR-320a is a potential independent prognostic biomarker for invasive breast cancer (Yang et al., 2014).

miR-199b-5p down-regulation in breast cancer patients has been associated with malignant clinical characteristics. Analysis results on 131 snap frozen tissue samples and cell lines have shown that breast cancer patients with high levels of miR-199b-5p had a better OS than those with low levels, considering miR-199b-5p as a potential prognostic biomarker for breast cancer (Fang et al., 2016).

miR-22 elevated expression levels have been associated with poor OS, while it has been associated with epithelial-mesenchymal transition (EMT), a key alteration in progression of cancer cells, after functional experiments on breast cancer cell lines (Pandey et al., 2015). In contrast, miR-22 has been reported to function as a tumor suppressor in 122 FFPE tissue specimens, and as such, it has been significantly correlated with TNM stage, local relapse, distant metastasis, and survival of breast cancer patients (Chen et al., 2016).

miR-218 elevated expression has been observed in clinical breast cancer specimens compared to normal tissues; however, after stratifying of patients according to their clinicopathological features in the same study, lower expression was associated with lymph node metastases, higher grades, and poorer prognosis of patients (Ahmadinejad et al., 2017).

miR-127 has been found to be significantly downregulated in 110 breast cancer tissues, and low miR-127 expression has been significantly correlated with lymph node metastasis, advanced clinical stage and poorer overall survival. Additional functional analyses in the same study showed that upregulation of miR-127 significantly inhibited growth, enhanced apoptosis, and reduced migration and invasion in breast cancer cells (Wang et al., 2014).

miR-644a expression and its gene signature have been implicated in tumor progression and distant metastasis-free survival. In fact, according to Raza et al., breast cancer patients with high miR-644a signature have significantly longer distant-metastasis-free survival, suggesting miR-644a as a novel tumor suppressor involved in progression and metastasis of breast cancer (Raza et al., 2016).

miR-361-5p overexpression has been involved in a significantly better clinical outcome and DFS, holding an important prognostic value, especially for patients with TNBC. These findings from 375 female patients may therefore highlight the prognostic value of miR-361-5p expression in breast cancer. (Cao et al., 2016)

miR-183 family includes miR-183, miR-96, and miR-182; aberrant expression of the **miR-183/-96/-182 cluster** in breast cancer tissues has been associated with aggressiveness in multiple cancers, including breast cancer, since an increased miR-183/182/96 cluster level has been correlated with local relapse, distant metastasis and poor clinical outcomes (Song et al., 2016). **miRNA 182** expression has been found to be higher in the serum of patients with pre/postmenopausal breast cancer and benign tumors, significantly associated with receptors used for the prognosis of breast cancer (Ali et al., 2018). miR-182 has also been reported to be significantly over expressed in TNBC, associated with lymph node metastases occurrence and strongly correlated with patients' genico-obstetric history in non TNBC, in a study performed on 60 triple-negative and non-TNBC cases, along with corresponding healthy samples from adjacent tissues (Medimegh et al., 2014). **miR- 96** has been reported as a potential prognostic factor for OS, associated with the key EMT phenomenon and with the regulation of growth factors involved in G1- to S-phase transition (Fisher et al., 2015).

miR-145 loss of expression has been related to the development of breast cancer and Liu et al. (2016), has shown on 257 female patients, that low miR-145 expression might be an adverse prognostic factor. These results are confirmed by Quan et al. (2018), who have recently reported that the 3-year survival rates of miR-145 low expression group are lower compared to those exhibiting high miR-145 expression.

miR-493 high expression has been correlated with better disease-free survival and further analysis has revealed that miR-493 expression levels have been significantly prognostic in 382 TNBC patients (Yao et al., 2018).

miR-30 family members (miR-30abcdef/30abe-5p/384-5p) seem to hold a prognostic value, according to our search results. **miR-30a** decreased levels have been associated with breast cancer progression in a survival analysis (Cheng et al., 2012), where overexpression suppressed the migration and invasiveness phenotypes of breast cancer cell lines. Moreover, reduced tumor expression of miR-30a in breast cancer patients have been associated with an unfavorable outcome, including late tumor stage, lymph node metastasis, and worse progression (mortality and recurrence) (Cheng et al., 2012). Additionally, low expression of miR-30a has been suggested as an independent predictor of decreased OS and RFS in TNBC (Turashvili et al., 2018). Low **miR-30a-3p**, and **miR-30a-5p** expression, have been significantly associated with decreased overall survival (OS) and with shorter relapse-free survival (RFS) in TNBC (Turashvili et al., 2018). **miR-30c-5p** has also been suggested as a prognostic factor for RFS in TNBC, following validation by qRT-PCR in 51 tissue samples (Turashvili et al., 2018). **miR-30e*** expression has been identified as a protective prognostic marker in breast cancer, mainly in the ESR1+/ERBB2− subtype (D'Aiuto et al., 2015).

miR-4653-3p high tissue expression level has been presented as a potential predictor for favorable DFS, in 400 HR+ breast cancer patients receiving tamoxifen adjuvant therapy (Zhong et al., 2016)

miR-148a low expression has been linked to diagnosis of high-grade primary tumors and poor prognosis of breast cancer patients, particularly for patients with Basal and Luminal B subtypes. Importantly, reduced miR-148a expression has been detected in higher-grade tumor samples and correlated with increased likelihood to develop metastases and poor prognosis in subsets of breast cancer patients, particularly those with TNBC. Therefore, low expression of miR-148a has been significantly associated with worse overall survival in patients classified as triple negative. (Xu et al., 2016)

miR-10b has been presented as a potential biomarker that could play a predictive role in lymph node metastases occurrence across TNBC and in the incidence of high-grade tumors in non-TNBC cases (Medimegh et al., 2014). Eleveted expression of miR-10b in 108 pairs of tumor and non-tumor breast tissue samples has been associated with adverse outcome, which is further supported from data derived from in vitro studies (Chang et al., 2014). miR-10b expression has also been associated with clinical outcome in a prospective cohort of paired breast tumor and normal specimens (n=150). Finally, a survival analysis of 230 breast tissue samples has shown that high levels of miR-10b result to a short relapse free survival (RFS) of breast cancer, acting as an independent prognostic factor of RFS (Eissa et al., 2015).

miR-34a/b/c expression has been examined in plasma collected from 173 TNBC patients and from 75 age-matched healthy women, revealing that reduced **miR-34a** and **miR-34c** expression is highly associated with tumor progression and indicates worse prognosis (Zeng et al., 2017). miR-34a expression activation has also been proposed as a marker for a lower risk of recurrence or death from breast cancer, in a study performed on a large cohort of breast tumors (n=1,172) on TMAs (Peurala et al., 2011). However, there is a study stating that the overexpression of miR-34a in FFPE tissue samples of breast cancer patients is linked to poor responses (Chen et al., 2016). miR-34c, has also been presented as an independent risk factor for OS in TNBC patients (Zeng et al., 2017). Expression levels of **miR-34b** have been shown to negatively correlate with disease free survival (DFS) and (OS) of 39 TNBC patients. (Zvoboda et al., 2012).

miR-601 has been found to be significantly down-regulated in breast cancer tissues compared with matched adjacent non-cancerous breast tissues (30 pairs). Moreover, it has been demonstrated, in a larger cohort (n=150), that down-regulation of miR-601 has been closely associated with distant metastasis and poor distant metastasis-free survival in breast cancer, with miR-601 levels inversely correlated with metastatic potential of human breast cancer cell lines. (Hu et al., 2016)

miR-638 decreased expression has been significantly correlated with lymph node metastasis TNM stage, and shorter overall survival, after analysis of tissues collected

from 125 breast cancer patients (Li et al., 2018). Notably, in cell lines, downregulation of miR-638 has been capable of promoting cell proliferation, migration, and invasion (Li et al., 2018). Additionally, in TNBC, high levels of miR-638 and abnormal BRCA1 detection have been significantly associated with a better overall survival (Zavala et al., 2016)

miR-146a high levels and abnormal BRCA1 detection in TNBC have been significantly associated with a better overall survival for patients with BRCA1-deficient TNBC tumors (Zavala et al., 2016).

miR-374a levels have been found to be lower in breast cancer tissues than in normal tissues and miR-374a to be differentially distributed in breast cancer, leading to a distinct variation in breast cancer prognosis (Li et al., 2013). It has been suggested that miR-374a may function as a tumor oncogene and contribute to breast cancer development, from data obtained from a pan-cancer tissue microarray (Zhang et al., 2018).

miR-409-3p expression in breast cancer specimens has been observed to be decreased compared with matched normal breast tissues. Results obtained from 190 pairs of BC tissues and adjacent nontumor tissues, have revealed that miR-409-3p may be related to the prognosis of patients with breast cancer and might be a promising predictor of recurrence (Cao et al., 2016).

miR-125a-5p low expression has been associated with lower survival rates and expression of miR-125a-5p has been found to be relatively lower in patients with shorter survival compared to long-term survivors. It has been shown that miR-125a-5p exerts a tumor suppressive function, both in vitro and in vivo and it has been proposed as a useful prognostic biomarker in breast cancer (Hsieh et al., 2015).

miR-874 expression has been found to be downregulated in 47 pairs of breast cancer tissues. Zhang et al. have suggested that miR-874 expression may be a prognostic biomarker of OS in breast cancer patients, mediated through DNA methylation. (Zhang et al., 2017)

The miR-15abc/16/16abc/195/322/424/497/1907 family consists of multiple miRs. **miR-15a** low expression in primary tumors has been significantly correlated with shorter disease-free survival and overall survival compared to the high miR-15a expression in TNBC cases, with low miR-15a expression acting as an independent prognostic factor for overall survival (Shinden et al., 2015). **miR-16** overexpression has been shown to remarkably inhibitE2 induced cell proliferation of breast cancer cells, while further studies have shown that this miRNA is significantly higher in triple positive compared with triple negative breast tissues (Yu et al., 2012). **miR-497** expression levels have been significantly lower in HER2-positive and TNBC tissues, while patients with a higher miR-497 expression had a relatively better 5-year survival rate (Liu et al., 2016). In addition, low miR-497 expression has been correlated with poor prognosis of breast cancer patients in a recent study (Zhong et al., 2018).

miR-129-5p down-regulation has been correlated with advanced clinical stage and poor prognosis in 200 tissue specimens from patients with breast cancer. The same researchers have also noted that miR-129-5p down-regulation fosters EMT in breast cancer, after a series of experiments in cell lines (Yu et al., 2015).

miR-370 high levels of expression have been related with lymph node metastasis, advanced stage, and frequent perineural invasion in 60 primary breast cancer tissues. Moreover, patients with high miR-370 expression have presented poor disease-free survival compared to the low-expression group. Therefore, upregulation of miR-370 in breast cancer has correlated with breast cancer progression. (Sim et al., 2015)

miR-301a belongs to the miR-130ac/301ab/301b/301b-3p/454/721/4295/3666 family and has been upregulated in cancer tissues compared with adjacent noncancerous tissues (Yu et al., 2014). Furthermore, high miR-301a expression has been significantly associated with larger tumor size and LNM (Yu et al., 2014) and a decreased OS (Zheng et al., 2018). Analyzing miR-301a expression in breast tissue biopsies at the time of diagnosis could potentially identify candidates for active surveillance, acting as an independent prognostic factor for the survival of patients

with breast cancer (Zheng et al., 2018, Yu et al., 2014). **miR-454** high expression has been indicative of worse disease-free survival (DFS) in 534 stage I-III breast cancer FFPE tissues from female patients (Cao et al., 2016). In addition, miR-454 was positively correlated with worse clinical outcome in the TNBC subtype in the same study. Interestingly, patients in the low miR-454 expression cohort had better response to anthracycline compared to non-anthracycline chemotherapy, suggesting that miR-454 may act as a potential predictor of prognosis and chemotherapy response in TNBC (Cao et al., 2016). **miR-454-3p** has been identified as a potential prognostic marker for DFS, in tumor interstitial fluid of breast tumors (Halvorsen et al., 2016).

miR-24-3p has been upregulated in patients with metastases, both in plasma and in breast cancer tissues (Khodadadi-Jamaryan et al., 2018). Furthermore, patients whose primary tumors expressed high levels of miR-24-3p have had a significantly lower survival rates, in results obtained through an in-silico analysis (Khodadadi-Jamaryan et al., 2018). **miR-24-2*** has been associated with tumor suppressive activity, since according to Martin et al. (2014), overexpression results in suppression of cell survival. Of note, a similar biological change has been observed in vivo (Martin et al., 2014).

miR-940 downregulation has been detected in breast cancer patients compared with healthy controls, while decreased miR-940 expression has also been found in 128 TNBC serum samples, suggesting that serum downregulated miR-940 may serve as a prognostic biomarker in breast cancer patients. (Liu et al., 2018)

miR-329 down-regulation has been proposed as an effective diagnostic and prognostic biomarker through analysis of 134 breast cancer tissues and 70 healthy volunteers, while in silico analysis confirmed the initial results obtained from biological experiments (Li et al., 2017).

miR-1247-5p low expression in breast cancer tissues has been significantly associated with the advanced TNM stage, lymph node metastasis, poorer pathological differentiation and molecular subtype (Zhang et al., 2018). Patients in the low miR-1247-5p group have presented shorter disease-free survival and overall

survival than those in the high miR-1247-5p group, highlighting its potential role as a tumor suppressor (Zhang et al., 2018). Moreover, functional studies have shown that overexpression of miR-1247-5p inhibits proliferation and induces apoptosis in breast cancer cells (Zeng et al., 2018). In silico analysis, including 839 breast cancer patients has further demonstrated that miR-1247-5p is an independent prognostic indicator for overall survival and recurrence-free survival (Zeng et al., 2018).

miR-204 (miR-204/204b/211 family) low expression has been significantly associated in 129 breast tissue samples with TNM stage, metastasis and a poorer overall survival and disease-free survival time than those with high miR-204, while it has been also correlated with chemotherapeutic resistance (Li et al., 2014).

miR-494 has exhibited prognostic value for patients with invasive breast carcinoma. Specifically, among node-negative disease, reduced levels of miRNA-494 have predicted 8.5-fold risk of breast cancer death (Gurvits et al., 2018).

miR-27a high expression has been associated with poor overall survival in 102 patients with breast cancer (Tang et al., 2012). miR-27a promotes tumor growth and metastasis which suggests that miR-27a could be a valuable marker of breast cancer progression (Tang et al., 2012). **miR-27b-3p** has been characterized as an independent predictor of poor prognosis for TNBC, according to a prediction model developed based on independent clinicopathological and miRNA covariates (Shen et al., 2014).

miR-133a reduced expression has been observed in cancerous tissues and in cell lines, and has been associated with lymph nodes metastasis, high clinical stages, and shorter relapse-free survivals of patients with breast cancer (Wu et al., 2012). Furthermore, in cell lines, transfection of miR-133a oligonucleotides significantly decreased migration and invasion capacity of breast cancer cells, while knockdown of miR-133a expression induced breast cancer cell migration and invasion (Wu et al., 2012).

miR-19a high serum levels have been associated with inflammatory breast cancer (IBC), since patients with metastatic IBC have exhibited significantly higher serum

miR-19a median levels than patients with metastatic non-IBC (Anfossi et al., 2014). Finally, high serum miR-19a levels have been associated with longer progression-free survival time and longer overall survival time in patients with metastatic HER2(+) IBC (Anfossi et al., 2014). Upregulated **miR-19b** expression has been observed in breast cancer tissues and cells compared to controls and it has been associated with distant metastasis, poor overall survival and TNM stage, as an independent prognostic factor (Li et al., 2018).

miR-339-5p reduced expression has been associated with an increase in metastasis and with high-stage, while patients with miR-339-5p expression have showed better OS and relapse-free survivals compared with those without miR-339-5p expression (Wu et al., 2010).

miR-143 has been found to be significantly higher in triple positive breast tissues, suggesting a potentially tumor suppressive effect on cancer progression of ER positive breast cancers (Yu et al., 2012). A functional analysis performed using cell lines, has shown that miR-143 inhibits breast cancer cell proliferation (Yu et al., 2012).

miR-187 expression in breast cancer has been found to lead to a more aggressive, invasive phenotype and act as an independent predictor of outcome. Utilizing a comprehensive bioinformatics approach, Mulrane et al. have discovered that miR-187 is associated with poor outcome in two independent breast cancer cohorts. (Mulrane et al., 2012).

miR-597 low expression has been observed to be closely associated with positive lymph node metastasis, higher TNM stage, poorer pathological differentiation and a shorter overall survival time. Therefore, miR-597 has been presented as an independent prognostic indicator of overall survival, with decreased miR-597 expression suggesting unfavorable prognosis for breast cancer patients (Zhang et al., 2018).

miR-210 expression has been associated with tumor proliferation and differentiation. Furthermore, miR-210 has been associated with poor clinical

outcome in ER-positive, tamoxifen-treated breast cancer patients (Rothé et al., 2011). miR-210 expression in TNBC has been found to be significantly higher than in estrogen receptor-positive/HER2-negative breast cancers, whereas patients that showed low miR-210 expression experienced significantly better disease-free and overall survival than those with high miR-210 expression (Toyama et al., 2012). Functional analyses in breast cancer cell lines have revealed that miR-210 involvement in cell proliferation, migration and invasion (Rothé et al., 2011), and its high prognostic power for DFS when transferred into the clinical setting of primary breast cancer (Bleckmann et al., 2015). Notably, one of the earliest studies states that, miR-210 overexpression is induced by hypoxia and its expression levels in breast cancer samples are an independent prognostic factor (Camps et al., 2008). **miR-210-3p** expression levels have also been associated with a better prognosis in terms of overall survival, through EMT regulation (Fisher et al., 2015)

miR-1179 expression has been found to be downregulated in breast cancer tissues and cell lines, with low miR-1179 expression correlated with lymph node metastasis, advanced clinical stage and shorter overall survival (Li et al., 2018). miR-1179 has been presented as a tumor suppressor and an independent prognostic factor of overall survival in breast cancer patients (Li et al., 2018).

miR-7 expression has been associated with tumor size, tumor grade, ER and PR status and according to Uhr et al. (2018), it appears to be associated with a generally more aggressive tumor type; miR-7 expression has also been related to prognosis in ER-positive tumors, associated with more aggressive features (Uhr et al., 2018).

miR-574 overexpression in FFPE tissue samples of breast cancer patients with poor responses has suggested that this specific miRNA could serve as a potential candidate used for detection and optimal chemotherapeutic choices for breast cancer patients (Chen et al., 2016). **miR-574-3p** has also been identified as a potential novel prognostic marker for breast cancer, with miR-574-3p being down-regulated in tumor samples. Independent validation of signatures (for OS) further strengthened the study findings (Krishnan et al., 2015).

miR-590-3p overexpression has significantly induced apoptosis in breast cancer cell lines; on the contrary, knockdown of miR-590-3p in these cells has led to a significantly higher viability (Abdolvahabi et al., 2018).

miR-330-3p expression level has been significantly higher in 233 breast cancer specimens than that in corresponding noncancerous tissues and high levels of miR-330-3p have been correlated with shorter 5-year overall survival of breast cancer patients. miR-330-3p upregulation may be associated with prognosis in patients with breast cancer. (Wang b et al., 2018)

miR-451 overexpression has been linked to an increase in apoptosis, and, importantly, restoration of the growth-inhibitory effectiveness of SERMs in endocrine-resistant cells. Opposite effects have been reported by miR-451 knockdown (Bergamaschi et al., 2012).

miR-122, member of the miR-122/122a/1352 family, has exhibited strong correlations with clinical outcomes. Higher levels of circulating miR-122 specifically predicted metastatic recurrence in stage II-III breast cancer patients, while it has been reported that miR-122 prevalence in the circulation predicts BC metastasis in early-stage patients (Wu et al., 2012).

3.2 miRNA signatures with a prognostic role in breast cancer: our search results

According to our search, we retrieved five studies, in which authors have identified six distinct microRNA signatures with prognostic value in breast cancer, based on miRNA expression levels in tissue or serum samples (see Table 2):

1. A 10-miRNA classifier incorporating miR-21, miR-30c, miR-181a, miR-181c, miR-125b, miR-7, miR-200a, miR-135b, miR-22 and miR-200c has been developed in order to predict distant relapse free survival (DRFS). With this classifier, HR+HER2- patients are scored and classified into high-risk and low-risk disease recurrence, which is significantly associated with 5-year DRFS of the patient. The patients with high-risk recurrence determined by this classifier benefit more from chemotherapy. (Gong et al., 2016)

2. Four miRNAs have been used to construct a miRNA signature, after analysis of 159 breast cancer tissue samples. According to the analysis, miR-191-5p increases, whereas miR-214-3p, miR-451a, and miR-489 inhibits cell proliferation, migration, and invasion abilities. Risk scores derived from the 4-miRNA signature are calculated to stratify the patients into high- or low-risk groups. Patients with high-risk scores have poorer overall survival and disease-free survival. The miRNA signature has been presented as an independent prognostic factor. (Chen et al., 2018)
3. A 4-miRNA signature has been identified given by miR-155, miR-493, miR-30e and miR-27a expression levels, that allows subdivision of TNBCs into high risk and low risk groups. This signature has both diagnostic and prognostic value, predicting outcomes of patient treatment with the two most commonly used chemotherapy regimens in TNBC. (Gasparini et al., 2014)
4. Two miRNA signatures predictive of overall survival and distant-disease free survival for patients 50 yrs of age or younger have been identified. In particular, the expression levels of three “risk-associated” (miR-125b, 655, 421) and four “protective” miRNAs (miR-16, 374a/b, 497) are being used. (Cascione et al., 2013)
5. A tissue microRNA (miRNA) signature has been identified, that predicts prognosis in young breast cancer patients. Three candidate miRNAs (miR-183-5p, miR-194-5p, and miR-1285-5p) have been detected, that could be used as prognostic biomarkers in young breast cancer patients (Hironaka-Mitsuhashi et al., 2017)

4. DISCUSSION

In the past few years, miRNAs have attracted considerable attention in the cancer research field, due to their regulatory actions in multiple levels. Specifically, according to numerous studies, miRNAs are involved in the regulation of key biological processes implicated in breast cancer initiation, progression and metastasis, including cell proliferation, cell death, apoptosis, immune response, cell cycle energetics, metabolism, replicative immortality, senescence, invasion (McGuire et al., 2015), and in angiogenesis (Goh et al., 2016).

Several lines of evidence have proven that in breast cancer, alterations in the expression levels of miRNAs are due to a number of mechanisms, such as epigenetic control, transcription factors, or the effect of mutated proteins. miRNAs are emerging as novel prognostic biomarkers for breast cancer (Bertoli et al., 2015). This is due to the immense need for early determination of breast prognosis, which is essential for defining the proper treatment regimen of patients. Depending on the target gene that they regulate, miRNAs can either serve as “tumor suppressor miRs” by repressing oncogenes or as “onco-miRs” by targeting tumor suppressor genes. However, a number of miRNAs play both tumor suppressor and onco-miR roles depending on the cellular context and tumor type (Muluhngwi et al., 2017).

In this context, miRNAs can serve as prognostic biomarkers in breast cancer. A prognostic biomarker should indicate a patient’s outcome, for example, disease recurrence or disease progression, independent of the treatment regimen that was followed. The aim of the present review has been to highlight recent preclinical and clinical studies performed on circulating and tissue-specific miRNAs and therefore identify their potential role as prognostic markers in breast cancer. We have identified several studies that investigate the potential correlation between miRNA profile expression in breast cancer tissue, in the circulation and in breast cancer cell lines and their possible use as prognostic factors. Although various miRNAs were found to be associated with prognosis in breast cancer, most of these miRNAs were assessed in only a single study. Six miRs (miR-10b, miR-200b, miR-21, miR-203, miR-373, and miR-210) were evaluated in at least 4 studies.

MiR-21 is one of the most extensively studied cancer-related miRNAs and its aberrant expression and deregulation may play an pivotal role in the majority of cancers (Pfeffer et al., 2015) miR-21 may serve as a key regulator of oncogenic processes, including tumor growth, migration, and invasion (Selcuklu et al., 2009), through targeting the pro-apoptotic phosphatase and tensin homolog (PTEN) and promoting tumor cell proliferation (Dong et al., 2014). According to our initial search results, we retrieved 12 studies (Table 1) and four meta-analyses (Pan et al., 2014; Wang et al., 2015; Tang et al., 2015; Jinling et al., 2017) focusing on the prognostic value of miR-21, which collectively provide robust evidence that miR-21 up-regulation is associated with poor outcomes in cancer patients.

Mir-210 has multiple functions in cancer cells and is involved in angiogenesis, cell cycle regulation, DNA damage repair, mitochondrial metabolism, and immune response (Qin et al., 2014). According to our search results, including 7 studies (Camps et al., 2008; Rothé et al., 2011; Toyama et al., 2012; Madhavan et al., 2012; Bleckmann et al, 2015; Boukerroucha et al., 2015, Madhavan et al., 2016), high expression of miR-210 has been significantly associated with poor survival in patients with breast cancer. Notably, single miR-210 assay has been proposed as an independent prognostic factor in this disease.

Concerning miR-10b, our findings, further elaborated in section 3.1., emphasize the oncogenic role of miR-10b and indicate that its high expression may be correlated with poor survival in breast cancer, while a metanalysis derived from our initial search further strengthens our findings (Wang et al., 2016).

miR-200b and miR-203 have both been characterized as tumor suppressors in multiple tumor types (Liu et al., 2017). However, there seems to be an inconsistency in the existing literature, since we retrieved two studies that have found that higher expression of circulating miR-200b and miR-203 is associated with worse outcome (Madhavan et al., 2012; Madhavan et al., 2016). However, other studies on tissue samples and cell lies presented inverse results (Ye et al., 2014; Yao et al., 2015; Yu et al., 2014), potentially highlighting the diverse regulatory roles of miRNA molecules depending on the cellular context and biological sample (blood VS tissue).

In addition, our search retrieved five studies that have found six miRNA signatures to be useful for predicting the outcome of breast cancer (Cascione et al., 2013; Gasparini et al., 2014; Gong et al., 2016; Hironaka-Mitsuhashi et al., 2017; Chen et al., 2018). Coordinated regulation of multiple miRNAs of potential prognostic value, has helped researchers identify panels of prognostic microRNAs for breast cancer. The discovery of microRNA expression signatures shows considerable promise for determining the prognosis of individuals with breast cancer. Similar miRNA signatures have been identified in a variety of other cancers, including acute myeloid leukemia, chronic lymphocytic leukemia, colon cancer, pancreatic cancer, and non-small cell lung cancer (Grady et al., 2010). These reports highlight that this class of RNA molecules is showing substantial potential to be used as prognostic biomarkers for cancer.

Among the limitations of this effort, it should be stressed that this process was essentially driven by the search algorithm, which focused mainly on titles of the published literature, in an effort to provide more relevant results. Furthermore, clear heterogeneity was observed in our results, due to differences in patient characteristics (ethnicity, age, tumor stage, and grade) and the use of different isolation and detection methods, cut-off values for miRNA expression levels, sample preparation methods and sample types (i.e., paraffin-fixed, formalin-fixed, freshly frozen tumors, plasma or serum).

Conclusions and future perspectives

Based on the results of this systematic review, we believe that miRNA detection may be a useful tool in the prognosis of breast cancer. Prognosis plays a vital role for medical oncologists in patient management and in making clinical decisions that are aligned with their patients' needs and goals of care. Prognostic studies can address important questions that are relevant to patient outcomes, though they must be rigorously and carefully designed to ensure that we obtain reliable results (Halabi and Owzar, 2010). The thorough validation of prognostic factors is a necessary and unavoidable process in order to minimize uncertainty in predicting outcome in future breast cancer patients. Therefore, extensive validation

studies focusing on particular miRNAs or miRNA panels should be performed to relate baseline clinical and experimental covariables to outcome. Eventually, all the reviewed molecular studies may help in bringing prognostic miRNAs closer to clinical practice.

5. REFERENCES

Abdolvahabi, Z., Nourbakhsh, M., Hosseinkhani, S., Hesari, Z., Alipour, M., Jafarzadeh, M., ... & Golpour, P. (2018). MicroRNA-590-3P suppresses cell survival and triggers breast cancer cell apoptosis via targeting sirtuin-1 and deacetylation of p53. Journal of cellular biochemistry.

Ahmadinejad, F., Mowla, S. J., Honardoost, M. A., Arjenaki, M. G., Moazeni-Bistgani, M., Kheiri, S., & Teimori, H. (2017). Lower expression of miR-218 in human breast cancer is associated with lymph node metastases, higher grades, and poorer prognosis. Tumor Biology, 39(8), 1010428317698362.

Alajez, N. M., Shi, W., Wong, D., Lenarduzzi, M., Waldron, J., Weinreb, I., & Liu, F. F. (2012). Lin28b promotes head and neck cancer progression via modulation of the insulin-like growth factor survival pathway. Oncotarget, 3(12), 1641.

Ali, O. S., Shabayek, M. I., Seleem, M. M., Abdellateif, H. G., & Makhlouf, D. O. (2018). MicroRNAs 182 and 375 sera expression as prognostic biochemical markers in breast cancer. Clinical breast cancer, 18(6), e1373-e1379.

Andorfer, C. A., Necela, B. M., Thompson, E. A., & Perez, E. A. (2011). MicroRNA signatures: clinical biomarkers for the diagnosis and treatment of breast cancer. Trends in molecular medicine, 17(6), 313-319.

Anfossi, S., Giordano, A., Gao, H., Cohen, E. N., Tin, S., Wu, Q., ... & Hortobagyi, G. N. (2014). High serum miR-19a levels are associated with inflammatory breast cancer and are predictive of favorable clinical outcome in patients with metastatic HER2+ inflammatory breast cancer. PloS one, 9(1), e83113.

Arroyo, J. D., Chevillet, J. R., Kroh, E. M., Ruf, I. K., Pritchard, C. C., Gibson, D. F., ... & Tait, J. F. (2011). Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proceedings of the National Academy of Sciences, 108(12), 5003-5008.

Arteaga, C. L., Sliwkowski, M. X., Osborne, C. K., Perez, E. A., Puglisi, F., & Gianni, L. (2012). Treatment of HER2-positive breast cancer: current status and future perspectives. Nature reviews Clinical oncology, 9(1), 16.

Badr, M., Said, H., Louka, M. L., Elghazaly, H. A., Gaballah, A., & Atef Abd El Mageed, M. (2018). MicroRNA-21 as a predictor and prognostic factor for trastuzumab therapy in human epidermal growth factor receptor 2-positive metastatic breast cancer. Journal of cellular biochemistry.

Bailey, S. T., Westerling, T., & Brown, M. (2014). Loss of estrogen-regulated microRNA expression increases HER2 signaling and is prognostic of poor outcome in luminal breast cancer. Cancer research.

Bauer, K. R., Brown, M., Cress, R. D., Parise, C. A., & Caggiano, V. (2007). Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer, 109(9), 1721-1728.

Bergamaschi, A., & Katzenellenbogen, B. S. (2012). Tamoxifen downregulation of miR-451 increases 14-3-3ζ and promotes breast cancer cell survival and endocrine resistance. Oncogene, 31(1), 39.

Bertoli, G., Cava, C., & Castiglioni, I. (2015). MicroRNAs: new biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. Theranostics, 5(10), 1122.

Bleckmann, A., Leha, A., Artmann, S., Menck, K., Salinas-Riester, G., Binder, C., ... & Klemm, F. (2015). Integrated miRNA and mRNA profiling of tumor-educated macrophages identifies prognostic subgroups in estrogen receptor-positive breast cancer. Molecular oncology, 9(1), 155-166.

Bohnsack, M. T., Czaplinski, K., & GÖRLICH, D. (2004). Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. Rna, 10(2), 185-191.

Bosetti, C., Bertuccio, P., Malvezzi, M., Levi, F., Chatenoud, L., Negri, E., & La Vecchia, C. (2013). Cancer mortality in Europe, 2005–2009, and an overview of trends since 1980. Annals of oncology, 24(10), 2657-2671.

Boukerroucha, M., Josse, C., ElGuendi, S., Boujemla, B., Frères, P., Marée, R., ... & Bours, V. (2015). Evaluation of BRCA1-related molecular features and microRNAs as prognostic factors for triple negative breast cancers. BMC cancer, 15(1), 755.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 68(6), 394-424.

Camps, C., Buffa, F. M., Colella, S., Moore, J., Sotiriou, C., Sheldon, H., ... & Ragoussis, J. (2008). hsa-miR-210 Is induced by hypoxia and is an independent prognostic factor in breast cancer. Clinical cancer research, 14(5), 1340-1348.

Cao, G. H., Sun, X. L., Wu, F., Chen, W. F., Li, J. Q., & Hu, W. C. (2016). Low expression of miR-409-3p is a prognostic marker for breast cancer. Eur Rev Med Pharmacol Sci, 20(18), 3825-3829.

Cao, Z. G., Huang, Y. N., Yao, L., Liu, Y. R., Hu, X., Hou, Y. F., & Shao, Z. M. (2016). Positive expression of miR-361-5p indicates better prognosis for breast cancer patients. Journal of thoracic disease, 8(7), 1772.

Cao, Z. G., Li, J. J., Yao, L., Huang, Y. N., Liu, Y. R., Hu, X., ... & Shao, Z. M. (2016). High expression of microRNA-454 is associated with poor prognosis in triple-negative breast cancer. Oncotarget, 7(40), 64900.

Cardoso, F., Costa, A., Senkus, E., Aapro, M., André, F., Barrios, C. H., ... & Carey, L. (2017). 3rd ESO–ESMO international consensus guidelines for advanced breast cancer (ABC 3). Annals of Oncology, 28(1), 16-33.

Carioli, G., Malvezzi, M., Rodriguez, T., Bertuccio, P., Negri, E., & La Vecchia, C. (2017). Trends and predictions to 2020 in breast cancer mortality in Europe. The Breast, 36, 89-95.

Cascione, L., Gasparini, P., Lovat, F., Carasi, S., Pulvirenti, A., Ferro, A., ... & Shapiro, C. L. (2013). Integrated microRNA and mRNA signatures associated with survival in triple negative breast cancer. PloS one, 8(2), e55910.

Cava, C., Bertoli, G., Ripamonti, M., Mauri, G., Zoppis, I., Della Rosa, P. A., ... & Castiglioni, I. (2014). Integration of mRNA expression profile copy number alterations, and microRNA expression levels in breast cancer to improve grade definition. PloS one, 9(5), e97681.

Chang, C. H., Fan, T. C., Yu, J. C., Liao, G. S., Lin, Y. C., Shih, A. C. C., ... & Yu, A. L. T. (2014). The prognostic significance of RUNX2 and miR-10a/10b and their inter-relationship in breast cancer. Journal of translational medicine, 12(1), 257.

Cheang, M. C., Chia, S. K., Voduc, D., Gao, D., Leung, S., Snider, J., ... & Perou, C. M. (2009). Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. JNCI: Journal of the National Cancer Institute, 101(10), 736-750.

Checka, C. M., Chun, J. E., Schnabel, F. R., Lee, J., & Toth, H. (2012). The relationship of mammographic density and age: implications for breast cancer screening. American Journal of Roentgenology, 198(3), W292-W295.

Chen, B., Tang, H., Liu, X., Liu, P., Yang, L., Xie, X., ... & Wei, W. (2015). miR-22 as a prognostic factor targets glucose transporter protein type 1 in breast cancer. Cancer letters, 356(2), 410-417.

Chen, X., Lu, P., Wang, D. D., Yang, S. J., Wu, Y., Shen, H. Y., ... & Tang, J. H. (2016). The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues. Gene, 595(2), 221-226.

Chen, X., Wang, Y. W., Zhu, W. J., Li, Y., Liu, L., Yin, G., & Gao, P. (2018). A 4-microRNA signature predicts lymph node metastasis and prognosis in breast cancer. Human pathology, 76, 122-132.

Cheng, C. W., Wang, H. W., Chang, C. W., Chu, H. W., Chen, C. Y., Yu, J. C., ... & Shen, C. Y. (2012). MicroRNA-30a inhibits cell migration and invasion by downregulating

vimentin expression and is a potential prognostic marker in breast cancer. Breast cancer research and treatment, 134(3), 1081-1093.

Cheng, C. W., Yu, J. C., Hsieh, Y. H., Liao, W. L., Shieh, J. C., Yao, C. C., ... & Shen, C. Y. (2018). Increased cellular levels of microRNA-9 and microRNA-221 correlate with cancer stemness and predict poor outcome in human breast cancer. Cellular Physiology and Biochemistry, 48(5), 2205-2218.

Cheng, Y., Dong, L., Zhang, J., Zhao, Y., & Li, Z. (2018). Recent advances in microRNA detection. Analyst, 143(8), 1758-1774.

Cho, N. (2016). Molecular subtypes and imaging phenotypes of breast cancer. Ultrasonography, 35(4), 281.

Cuzick, J., DeCensi, A., Arun, B., Brown, P. H., Castiglione, M., Dunn, B., ... & Vogel, V. (2011). Preventive therapy for breast cancer: a consensus statement. The lancet oncology, 12(5), 496-503.

D'aiuto, F., Callari, M., Dugo, M., Merlino, G., Musella, V., Miodini, P., ... & Daidone, M. G. (2015). miR-30e* is an independent subtype-specific prognostic marker in breast cancer. British journal of cancer, 113(2), 290.

Deng, L., Lei, Q., Wang, Y., Wang, Z., Xie, G., Zhong, X., ... & Bu, H. (2017). Downregulation of miR-221-3p and upregulation of its target gene PARP1 are prognostic biomarkers for triple negative breast cancer patients and associated with poor prognosis. Oncotarget, 8(65), 108712.

Dong, G., Liang, X., Wang, D., Gao, H., Wang, L., Wang, L., ... & Du, Z. (2014). High expression of miR-21 in triple-negative breast cancers was correlated with a poor prognosis and promoted tumor cell in vitro proliferation. Medical oncology, 31(7), 57.

Dong, L. L., Chen, L. M., Wang, W. M., & Zhang, L. M. (2015). Decreased expression of microRNA-124 is an independent unfavorable prognostic factor for patients with breast cancer. Diagnostic pathology, 10(1), 45.

Eiring, A. M., Harb, J. G., Neviani, P., Garton, C., Oaks, J. J., Spizzo, R., ... & Becker, H. (2010). miR-328 functions as an RNA decoy to modulate hnRNP E2 regulation of mRNA translation in leukemic blasts. Cell, 140(5), 652-665.

Eissa, S., Matboli, M., Sharawy, A., & El-Sharkawi, F. (2015). Prognostic and biological significance of microRNA-221 in breast cancer. Gene, 574(1), 163-167.

Eissa, S., Matboli, M., Shehata, H. H., & Essawy, N. O. (2015). MicroRNA-10b and minichromosome maintenance complex component 5 gene as prognostic biomarkers in breast cancer. Tumor Biology, 36(6), 4487-4494.

Elghoroury, E. A., ElDine, H. G., Kamel, S. A., Abdelrahman, A. H., Mohammed, A., Kamel, M. M., & Ibrahim, M. H. (2018). Evaluation of miRNA-21 and miRNA Let-7 as Prognostic Markers in Patients with Breast Cancer. Clinical breast cancer, 18(4), e721-e726.

Emmadi, R., Canestrari, E., Arbieva, Z. H., Mu, W., Dai, Y., Frasor, J., & Wiley, E. (2015). Correlative analysis of miRNA expression and Oncotype Dx recurrence score in estrogen receptor positive breast carcinomas. PloS one, 10(12), e0145346.

Esquela-Kerscher, A., & Slack, F. J. (2006). Oncomirs—microRNAs with a role in cancer. Nature reviews cancer, 6(4), 259.

Falkenberg, N., Anastasov, N., Rappl, K., Braselmann, H., Auer, G., Walch, A., ... & Atkinson, M. J. (2013). MiR-221/-222 differentiate prognostic groups in advanced breast cancers and influence cell invasion. British journal of cancer, 109(10), 2714.

Fang, C., Wang, F. B., Li, Y., & Zeng, X. T. (2016). Down-regulation of miR-199b-5p is correlated with poor prognosis for breast cancer patients. Biomedicine & Pharmacotherapy, 84, 1189-1193.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., ... & Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer, 136(5), E359-E386.

Ferlay, J., Colombet, M., Soerjomataram, I., Dyba, T., Randi, G., Bettio, M., ... & Bray, F. (2018). Cancer incidence and mortality patterns in Europe: estimates for 40 countries and 25 major cancers in 2018. European Journal of Cancer.

Fisher, J. N., Terao, M., Fratelli, M., Kurosaki, M., Paroni, G., Zanetti, A., ... & Goodall, G. J. (2015). MicroRNA networks regulated by all-trans retinoic acid and Lapatinib control the growth, survival and motility of breast cancer cells. Oncotarget, 6(15), 13176.

Gasparini, P., Cascione, L., Fassan, M., Lovat, F., Guler, G., Balci, S., ... & Huebner, K. (2014). microRNA expression profiling identifies a four microRNA signature as a novel diagnostic and prognostic biomarker in triple negative breast cancers. Oncotarget, 5(5), 1174.

Goh, J. N., Loo, S. Y., Datta, A., Siveen, K. S., Yap, W. N., Cai, W., ... & Dharmarajan, A. M. (2016). microRNAs in breast cancer: regulatory roles governing the hallmarks of cancer. Biological Reviews, 91(2), 409-428.

Gomes, B. C., Martins, M., Lopes, P., Morujão, I., Oliveira, M., Araújo, A., ... & Rodrigues, A. S. (2016). Prognostic value of microRNA-203a expression in breast cancer. Oncology reports, 36(3), 1748-1756.

Gong, C., Tan, W., Chen, K., You, N., Zhu, S., Liang, G., ... & Li, Z. (2016). Prognostic value of a BCSC-associated microRNA signature in hormone receptor-positive HER2-negative breast cancer. EBioMedicine, 11, 199-209.

Grady, W. M., & Tewari, M. (2010). The next thing in prognostic molecular markers: microRNA signatures of cancer. Gut, 59(6), 706-708.

Gurvits, N., Autere, T. A., Repo, H., Nykänen, M., Kuopio, T., Kronqvist, P., & Talvinen, K. (2018). Proliferation-associated miRNAs-494,-205,-21 and-126 detected by in situ hybridization: expression and prognostic potential in breast carcinoma patients. Journal of cancer research and clinical oncology, 144(4), 657-666.

Halabi, S., & Owzar, K. (2010, April). The importance of identifying and validating prognostic factors in oncology. In Seminars in oncology (Vol. 37, No. 2, pp. e9-e18). WB Saunders.

Halvorsen, A. R., Helland, Å., Gromov, P., Wielenga, V. T., Talman, M. L. M., Brünner, N., ... & Haakensen, V. D. (2016). Profiling of microRNAs in tumor interstitial fluid of breasttumors–a novel resource to identify biomarkers forprognostic classification and detection of cancer. Molecular Oncology, 11, 220-234.

Hamam, R., Ali, A. M., Alsaleh, K. A., Kassem, M., Alfayez, M., Aldahmash, A., & Alajez, N. M. (2016). microRNA expression profiling on individual breast cancer patients identifies novel panel of circulating microRNA for early detection. Scientific reports, 6, 25997.

Hamam, R., Hamam, D., Alsaleh, K. A., Kassem, M., Zaher, W., Alfayez, M., ... & Alajez, N. M. (2017). Circulating microRNAs in breast cancer: novel diagnostic and prognostic biomarkers. Cell death & disease, 8(9), e3045.

Hata, A., & Kashima, R. (2016). Dysregulation of microRNA biogenesis machinery in cancer. Critical reviews in biochemistry and molecular biology, 51(3), 121-134.

Hesari, Z., Nourbakhsh, M., Hosseinkhani, S., Abdolvahabi, Z., Alipour, M., Tavakoli-Yaraki, M., ... & Yarahmadi, S. (2018). Down-regulation of NAMPT expression by mir-206 reduces cell survival of breast cancer cells. Gene.

Hironaka-Mitsuhashi, A., Matsuzaki, J., Takahashi, R. U., Yoshida, M., Nezu, Y., Yamamoto, Y., ... & Shimizu, C. (2017). A tissue microRNA signature that predicts the prognosis of breast cancer in young women. PloS one, 12(11), e0187638.

Hong, L., Yang, J., Han, Y., Lu, Q., Cao, J., & Syed, L. (2012). High expression of miR-210 predicts poor survival in patients with breast cancer: a meta-analysis. Gene, 507(2), 135-138.

Hsieh, T. H., Hsu, C. Y., Tsai, C. F., Long, C. Y., Chai, C. Y., Hou, M. F., ... & Tsai, E. M. (2015). miR-125a-5p is a prognostic biomarker that targets HDAC4 to suppress breast tumorigenesis. Oncotarget, 6(1), 494.

Hu, J. Y., Yi, W., Wei, X., Zhang, M. Y., Xu, R., Zeng, L. S., ... & Chen, J. S. (2016). miR-601 is a prognostic marker and suppresses cell growth and invasion by targeting PTP4A1 in breast cancer. Biomedicine & Pharmacotherapy, 79, 247-253.

Hu, J. Y., Yi, W., Zhang, M. Y., Xu, R., Zeng, L. S., Long, X. R., ... & Wang, H. Y. (2016). MicroRNA-711 is a prognostic factor for poor overall survival and has an oncogenic role in breast cancer. Oncology letters, 11(3), 2155-2163.

Huang, Q., Gumireddy, K., Schrier, M., Le Sage, C., Nagel, R., Nair, S., ... & Gimotty, P. A. (2008). The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nature cell biology, 10(2), 202.

Iorio, M. V., & Croce, C. M. (2012). MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO molecular medicine, 4(3), 143-159.

Iorio, M. V., & Croce, C. M. (2012). microRNA involvement in human cancer. Carcinogenesis, 33(6), 1126-1133.

Jang, M. H., Kim, H. J., Gwak, J. M., Chung, Y. R., & Park, S. Y. (2017). Prognostic value of microRNA-9 and microRNA-155 expression in triple-negative breast cancer. Human pathology, 68, 69-78.

Jin, T., Kim, H. S., Choi, S. K., Hwang, E. H., Woo, J., Ryu, H. S., ... & Moon, W. K. (2017). microRNA-200c/141 upregulates SerpinB2 to promote breast cancer cell metastasis and reduce patient survival. Oncotarget, 8(20), 32769.

Jinling, W., Sijing, S., Jie, Z., & Guinian, W. (2017). Prognostic value of circulating microRNA-21 for breast cancer: a systematic review and meta-analysis. Artificial cells, nanomedicine, and biotechnology, 45(6), 1216-1221.

Judes, G., Rifaï, K., Daures, M., Dubois, L., Bignon, Y. J., Penault-Llorca, F., & Bernard-Gallon, D. (2016). High-throughput «Omics» technologies: New tools for the study of triple-negative breast cancer. Cancer letters, 382(1), 77-85.

Khodadadi-Jamayran, A., Akgol-Oksuz, B., Afanasyeva, Y., Heguy, A., Thompson, M., Ray, K., ... & Zeleniuch-Jacquotte, A. (2018). Prognostic role of elevated mir-24-3p in breast cancer and its association with the metastatic process. Oncotarget, 9(16), 12868.

Kong, W., He, L., Richards, E. J., Challa, S., Xu, C. X., Permuth-Wey, J., ... & Cheng, J. Q. (2014). Upregulation of miRNA-155 promotes tumour angiogenesis by targeting VHL and is associated with poor prognosis and triple-negative breast cancer. Oncogene, 33(6), 679.

Kozomara, A., & Griffiths-Jones, S. (2013). miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic acids research, 42(D1), D68-D73.

Krishnan, P., Ghosh, S., Wang, B., Li, D., Narasimhan, A., Berendt, R., ... & Damaraju, S. (2015). Next generation sequencing profiling identifies miR-574-3p and miR-660-5p as potential novel prognostic markers for breast cancer. BMC genomics, 16(1), 735.

Kroh, E. M., Parkin, R. K., Mitchell, P. S., & Tewari, M. (2010). Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods, 50(4), 298-301.

Lagos-Quintana, M., Rauhut, R., Lendeckel, W., & Tuschl, T. (2001). Identification of novel genes coding for small expressed RNAs. Science, 294(5543), 853-858.

Lee, J. A., Lee, H. Y., Lee, E. S., Kim, I., & Bae, J. W. (2011). Prognostic implications of microRNA-21 overexpression in invasive ductal carcinomas of the breast. Journal of breast cancer, 14(4), 269-275.

Leon, S. A., Shapiro, B., Sklaroff, D. M., & Yaros, M. J. (1977). Free DNA in the serum of cancer patients and the effect of therapy. Cancer research, 37(3), 646-650.

Levi, F., Lucchini, F., Negri, E., & La Vecchia, C. (2004). Trends in mortality from major cancers in the European Union, including acceding countries, in 2004. Cancer: Interdisciplinary International Journal of the American Cancer Society, 101(12), 2843-2850.

Li, C., Zhang, J., Ma, Z., Zhang, F., & Yu, W. (2018). miR-19b serves as a prognostic biomarker of breast cancer and promotes tumor progression through PI3K/AKT signaling pathway. OncoTargets and therapy, 11, 4087.

Li, J. Y., Zhang, Y., Zhang, W. H., Jia, S., Kang, Y., & Tian, R. (2013). Effects of differential distribution of microvessel density, possibly regulated by miR-374a, on breast cancer prognosis. Asian Pacific Journal of Cancer Prevention, 14(3), 1715-1720.

Li, J., Song, Z. J., Wang, Y. Y., Yin, Y., Liu, Y., & Nan, X. (2016). Low levels of serum miR-99a is a predictor of poor prognosis in breast cancer. Genet Mol Res, 15(3).

Li, M., Wang, J., & Liu, H. (2018). Downregulation of miR-638 promotes progression of breast cancer and is associated with prognosis of breast cancer patients. OncoTargets and therapy, 11, 6871.

Li, P., Dong, J., Zhou, X., Sun, W., Huang, H., Chen, T., ... & Lu, M. (2017). Expression patterns of microRNA-329 and its clinical performance in diagnosis and prognosis of breast cancer. OncoTargets and therapy, 10, 5711.

Li, W. J., Xie, X. X., Bai, J., Wang, C., Zhao, L., & Jiang, D. Q. (2018). Increased expression of miR-1179 inhibits breast cancer cell metastasis by modulating Notch signaling pathway and correlates with favorable prognosis. European review for medical and pharmacological sciences, 22(23), 8374-8382.

Li, W., Jin, X., Zhang, Q., Zhang, G., Deng, X., & Ma, L. (2014). Decreased expression of miR-204 is associated with poor prognosis in patients with breast cancer. International journal of clinical and experimental pathology, 7(6), 3287.

Li, Y., Hong, F., & Yu, Z. (2013). Decreased expression of microRNA-206 in breast cancer and its association with disease characteristics and patient survival. Journal of International Medical Research, 41(3), 596-602.

Li, Y., Ma, X., Zhao, J., Zhang, B., Jing, Z., & Liu, L. (2013). microRNA-210 as a prognostic factor in patients with breast cancer: meta-analysis. Cancer Biomarkers, 13(6), 471-481.

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P., ... & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS medicine, 6(7), e1000100.

Liu, A., Yu, Q., Peng, Z., Huang, Y., Diao, S., Cheng, J., ... & Hong, M. (2017). miR 200b inhibits CD133+ glioma cells by targeting the AKT pathway. Oncology letters, 13(6), 4701-4707.

Liu, B., Su, F., Chen, M., Li, Y., Qi, X., Xiao, J., ... & Zhang, J. (2017). Serum miR-21 and miR-125b as markers predicting neoadjuvant chemotherapy response and prognosis in stage II/III breast cancer. Human pathology, 64, 44-52.

Liu, H. T., Xu, Y. T., Li, H. Y., Zhao, J., Zhai, H. Y., & Chen, Y. (2016). Loss of microRNA Expression is Involved in the Development and Prognosis of Breast Cancer Complicated by Type 2 Diabetes Mellitus. The International journal of biological markers, 31(4), 368-374.

Liu, J., Zhou, Y., Shi, Z., Hu, Y., Meng, T., Zhang, X., ... & Zhang, J. (2016). microRNA-497 modulates breast cancer cell proliferation, invasion, and survival by targeting SMAD7. DNA and cell biology, 35(9), 521-529.

Liu, W., Xu, Y., Guan, H., & Meng, H. (2018). Clinical potential of miR-940 as a diagnostic and prognostic biomarker in breast cancer patients. Cancer Biomarkers, (Preprint), 1-7.

Liu, Y., Zhang, Y., Li, Q., Li, J., Ma, X., Xing, J., ... & Jia, L. (2017). MiRNAs predict the prognosis of patients with triple negative breast cancer: A meta-analysis. PloS one, 12(1), e0170088.

Luo, Y., Wang, X., Niu, W., Wang, H., Wen, Q., Fan, S., ... & Zeng, Z. (2017). Elevated microRNA-125b levels predict a worse prognosis in HER2-positive breast cancer patients. Oncology letters, 13(2), 867-874.

Lytle, J. R., Yario, T. A., & Steitz, J. A. (2007). Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. Proceedings of the National Academy of Sciences, 104(23), 9667-9672.

Ma, L., Teruya-Feldstein, J., & Weinberg, R. A. (2007). Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature, 449(7163), 682.

Madhavan, D., Peng, C., Wallwiener, M., Zucknick, M., Nees, J., Schott, S., ... & Sohn, C. (2016). Circulating miRNAs with prognostic value in metastatic breast cancer and for early detection of metastasis. Carcinogenesis, 37(5), 461-470.

Madhavan, D., Zucknick, M., Wallwiener, M., Cuk, K., Modugno, C., Scharpff, M., ... & Benner, A. (2012). Circulating miRNAs as surrogate markers for circulating tumor cells and prognostic markers in metastatic breast cancer. Clinical Cancer Research.

Markou, A., Yousef, G. M., Stathopoulos, E., Georgoulias, V., & Lianidou, E. (2014). Prognostic significance of metastasis-related microRNAs in early breast cancer patients with a long follow-up. Clinical chemistry, 60(1), 197-205.

Martin, E. C., Elliott, S., Rhodes, L. V., Antoon, J. W., Fewell, C., Zhu, Y., ... & Beckman, B. S. (2014). Preferential star strand biogenesis of pre-miR-24-2 targets PKC-alpha and suppresses cell survival in MCF-7 breast cancer cells. Molecular carcinogenesis, 53(1), 38-48.

McGuire, A., Brown, J. A., & Kerin, M. J. (2015). Metastatic breast cancer: the potential of miRNA for diagnosis and treatment monitoring. Cancer and metastasis reviews, 34(1), 145-155.

McVeigh, T. P., & Kerin, M. J. (2017). Clinical use of the Oncotype DX genomic test to guide treatment decisions for patients with invasive breast cancer. Breast Cancer: Targets and Therapy, 9, 393.

Medimegh, I., Omrane, I., Privat, M., Uhrhummer, N., Ayari, H., Belaiba, F., ... & Elgaaied, A. B. (2014). MicroRNAs expression in triple negative vs non triple negative breast cancer in Tunisia: interaction with clinical outcome. PloS one, 9(11), e111877.

Minemura, H., Takagi, K., Miki, Y., Shibahara, Y., Nakagawa, S., Ebata, A., ... & Suzuki, T. (2015). Abnormal expression of miR-1 in breast carcinoma as a potent prognostic factor. Cancer science, 106(11), 1642-1650.

Mulrane, L., Madden, S. F., Brennan, D. J., Gremel, G., McGee, S. F., McNally, S., ... & Gallagher, W. M. (2012). miR-187 is an independent prognostic factor in breast cancer and confers increased invasive potential in vitro. Clinical cancer research, clincanres-1420.

Muluhngwi, P., & Klinge, C. M. (2017). Identification of miRNAs as biomarkers for acquired endocrine resistance in breast cancer. Molecular and cellular endocrinology, 456, 76-86.

Nassar, F. J., Nasr, R., & Talhouk, R. (2017). MicroRNAs as biomarkers for early breast cancer diagnosis, prognosis and therapy prediction. Pharmacology & therapeutics, 172, 34-49.

Negri, E., Zambelli, A., Franchi, M., Rossi, M., Bonifazi, M., Corrao, G., ... & La Vecchia, C. (2014). Effectiveness of trastuzumab in first-line HER2+ metastatic breast cancer after failure in adjuvant setting: a controlled cohort study. The oncologist, 19(12), 1209-1215.

Negrini, M., & Calin, G. A. (2008). Breast cancer metastasis: a microRNA story. Breast Cancer Research, 10(2), 303.

Oikonomopoulos, A., Polytarchou, C., Joshi, S., Hommes, D. W., & Iliopoulos, D. (2016). Identification of circulating microRNA signatures in Crohn's disease using the Nanostring nCounter technology. Inflammatory bowel diseases, 22(9), 2063-2069.

Oltra, S. S., Peña-Chilet, M., Vidal-Tomas, V., Flower, K., Martinez, M. T., Alonso, E., ... & Ribas, G. (2018). Methylation deregulation of miRNA promoters identifies miR124-2 as a survival biomarker in Breast Cancer in very young women. Scientific reports, 8(1), 14373.

Ørom, U. A., Nielsen, F. C., & Lund, A. H. (2008). MicroRNA-10a binds the 5' UTR of ribosomal protein mRNAs and enhances their translation. Molecular cell, 30(4), 460-471.

Pan, F., Mao, H., Deng, L., Li, G., & Geng, P. (2014). Prognostic and clinicopathological significance of microRNA-21 overexpression in breast cancer: a meta-analysis. International journal of clinical and experimental pathology, 7(9), 5622.

Pandey, A. K., Zhang, Y., Zhang, S., Li, Y., Tucker-Kellogg, G., Yang, H., & Jha, S. (2015). TIP60-miR-22 axis as a prognostic marker of breast cancer progression. Oncotarget, 6(38), 41290.

Papachristopoulou, G., Papadopoulos, E. I., Nonni, A., Rassidakis, G. Z., & Scorilas, A. (2018). Expression Analysis of miR-29b in Malignant and Benign Breast Tumors: A Promising Prognostic Biomarker for Invasive Ductal Carcinoma With a Possible Histotype-Related Expression Status. Clinical breast cancer, 18(4), 305-312.

Parrella, P., Barbano, R., Pasculli, B., Fontana, A., Copetti, M., Valori, V. M., ... & Coco, M. (2014). Evaluation of microRNA-10b prognostic significance in a prospective cohort of breast cancer patients. Molecular cancer, 13(1), 142.

Perou, C. M., Sørlie, T., Eisen, M. B., Van De Rijn, M., Jeffrey, S. S., Rees, C. A., ... & Fluge, Ø. (2000). Molecular portraits of human breast tumours. nature, 406(6797), 747.

Peurala, H., Greco, D., Heikkinen, T., Kaur, S., Bartkova, J., Jamshidi, M., ... & Bützow, R. (2011). MiR-34a expression has an effect for lower risk of metastasis and associates with expression patterns predicting clinical outcome in breast cancer. PloS one, 6(11), e26122.

Pfeffer, S. R., Yang, C. H., & Pfeffer, L. M. (2015). The Role of miR-21 in Cancer. Drug development research, 76(6), 270-277.

Qian, B., Katsaros, D., Lu, L., Preti, M., Durando, A., Arisio, R., ... & Yu, H. (2009). High miR-21 expression in breast cancer associated with poor disease-free survival in early

stage disease and high TGF-β1. Breast cancer research and treatment, 117(1), 131-140.

Qin, Q., Furong, W., & Baosheng, L. (2014). Multiple functions of hypoxia-regulated miR-210 in cancer. Journal of Experimental & Clinical Cancer Research, 33(1), 50.

Qin, W., Shi, Y., Zhao, B., Yao, C., Jin, L., Ma, J., & Jin, Y. (2010). miR-24 regulates apoptosis by targeting the open reading frame (ORF) region of FAF1 in cancer cells. PloS one, 5(2), e9429.

Quan, Y., Huang, X., & Quan, X. (2018). Expression of miRNA-206 and miRNA-145 in breast cancer and correlation with prognosis. Oncology letters, 16(5), 6638-6642.

Quesne, J. L., Jones, J., Warren, J., Dawson, S. J., Ali, H. R., Bardwell, H., ... & Caldas, C. (2012). Biological and prognostic associations of miR-205 and let-7b in breast cancer revealed by in situ hybridization analysis of micro-RNA expression in arrays of archival tumour tissue. The Journal of pathology, 227(3), 306-314.

Rakha, E. A., El-Sayed, M. E., Menon, S., Green, A. R., Lee, A. H., & Ellis, I. O. (2008). Histologic grading is an independent prognostic factor in invasive lobular carcinoma of the breast. Breast cancer research and treatment, 111(1), 121-127.

Raza, U., Saatci, Ö., Uhlmann, S., Ansari, S. A., Eyüpoğlu, E., Yurdusev, E., ... & Doğan, H. T. (2016). The miR-644a/CTBP1/p53 axis suppresses drug resistance by simultaneous inhibition of cell survival and epithelial-mesenchymal transition in breast cancer. Oncotarget, 7(31), 49859.

Rothe, F., Ignatiadis, M., Chaboteaux, C., Haibe-Kains, B., Kheddoumi, N., Majjaj, S., ... & Piccart, M. (2011). Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. PloS one, 6(6), e20980.

Schnitt, S. J. (2010). Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. Modern Pathology, 23(S2), S60.

Selcuklu, S. D., Donoghue, M. T., & Spillane, C. (2009). miR-21 as a key regulator of oncogenic processes. Biochem Soc Trans, 37(Pt 4):918-25.

Shao, Y., Sun, X., He, Y., Liu, C., & Liu, H. (2015). Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. PloS one, 10(7), e0133830.

Shen, S., Sun, Q., Liang, Z., Cui, X., Ren, X., Chen, H., ... & Zhou, Y. (2014). A prognostic model of triple-negative breast cancer based on miR-27b-3p and node status. PLoS One, 9(6), e100664.

Shinden, Y., Akiyoshi, S., Ueo, H., Nambara, S., Saito, T., Komatsu, H., ... & Takano, Y. (2015). Diminished expression of MiR-15a is an independent prognostic marker for breast cancer cases. Anticancer research, 35(1), 123-127.

Shinden, Y., Iguchi, T., Akiyoshi, S., Ueo, H., Ueda, M., Hirata, H., ... & Sugimachi, K. (2015). miR-29b is an indicator of prognosis in breast cancer patients. Molecular and clinical oncology, 3(4), 919-923.

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. CA: a cancer journal for clinicians, 67(1), 7-30.

Sim, J., Ahn, H., Abdul, R., Kim, H., Yi, K. J., Chung, Y. M., ... & Jang, K. (2015). High MicroRNA-370 expression correlates with tumor progression and poor prognosis in breast cancer. Journal of breast cancer, 18(4), 323-328.

Singletary, S. E., Allred, C., Ashley, P., Bassett, L. W., Berry, D., Bland, K. I., ... & Hughes, L. L. (2002). Revision of the American Joint Committee on Cancer staging system for breast cancer. Journal of clinical oncology, 20(17), 3628-3636.

Song, C., Zhang, L., Wang, J., Huang, Z., Li, X., Wu, M., ... & Xie, X. (2016). High expression of microRNA-183/182/96 cluster as a prognostic biomarker for breast cancer. Scientific reports, 6, 24502.

Svoboda, M., Sana, J., Redova, M., Navratil, J., Palacova, M., Fabian, P., ... & Vyzula, R. (2012). MiR-34b is associated with clinical outcome in triple-negative breast cancer patients. Diagnostic pathology, 7(1), 31.

Tang, W., Zhu, J., Su, S., Wu, W., Liu, Q., Su, F., & Yu, F. (2012). MiR-27 as a prognostic marker for breast cancer progression and patient survival. PloS one, 7(12), e51702.

Tang, Y., Zhou, X., Ji, J., Chen, L., Cao, J., Luo, J., & Zhang, S. (2015). High expression levels of miR-21 and miR-210 predict unfavorable survival in breast cancer: a systemic review and meta-analysis. The International journal of biological markers, 30(4), 347-358.

Tarone, R. E. (2017). Birth cohort trends for breast cancer among women in Europe and North America. Epidemiology, Biostatistics and Public Health, 14(1).

Tao, Z., Shi, A., Lu, C., Song, T., Zhang, Z., & Zhao, J. (2015). Breast cancer: epidemiology and etiology. Cell biochemistry and biophysics, 72(2), 333-338.

Tavazoie, S. F., Alarcón, C., Oskarsson, T., Padua, D., Wang, Q., Bos, P. D., ... & Massagué, J. (2008). Endogenous human microRNAs that suppress breast cancer metastasis. Nature, 451(7175), 147.

Tian, S., Roepman, P., van't Veer, L. J., Bernards, R., De Snoo, F., & Glas, A. M. (2010). Biological functions of the genes in the mammaprint breast cancer profile reflect the hallmarks of cancer. Biomarker insights, 5, BMI-S6184.

Toraih, E. A., Mohammed, E. A., Farrag, S., Ramsis, N., & Hosny, S. (2015). Pilot study of serum microRNA-21 as a diagnostic and prognostic biomarker in Egyptian breast cancer patients. Molecular diagnosis & therapy, 19(3), 179-190.

Toyama, T., Kondo, N., Endo, Y., Sugiura, H., Yoshimoto, N., Iwasa, M., ... & Yamashita, H. (2012). High expression of microRNA-210 is an independent factor indicating a poor prognosis in Japanese triple-negative breast cancer patients. Japanese journal of clinical oncology, 42(4), 256-263.

Tuomarila, M., Luostari, K., Soini, Y., Kataja, V., Kosma, V. M., & Mannermaa, A. (2014). Overexpression of microRNA-200c predicts poor outcome in patients with PR-negative breast cancer. PLoS One, 9(10), e109508.

Turashvili, G., Lightbody, E. D., Tyryshkin, K., SenGupta, S. K., Elliott, B. E., Madarnas, Y., ... & Nicol, C. J. (2018). Novel prognostic and predictive microRNA targets for triple-negative breast cancer. The FASEB Journal, fj-201800120R.

Uhr, K., Sieuwerts, A. M., de Weerd, V., Smid, M., Hammerl, D., Foekens, J. A., & Martens, J. W. M. (2018). Association of microRNA-7 and its binding partner CDR1-AS with the prognosis and prediction of 1 st-line tamoxifen therapy in breast cancer. Scientific reports, 8(1), 9657.

Usmani, A., Shoro, A. A., Memon, Z., Hussain, M., & Rehman, R. (2015). Diagnostic, prognostic and predictive value of MicroRNA-21 in breast cancer patients, their daughters and healthy individuals. American journal of cancer research, 5(8), 2484.

Vasudevan, S., Tong, Y., & Steitz, J. A. (2007). Switching from repression to activation: microRNAs can up-regulate translation. Science, 318(5858), 1931-1934.

Viale, G. (2012). The current state of breast cancer classification. annals of Oncology, 23(suppl_10), x207-x210.

Vilquin, P., Donini, C. F., Villedieu, M., Grisard, E., Corbo, L., Bachelot, T., ... & Cohen, P. A. (2015). MicroRNA-125b upregulation confers aromatase inhibitor resistance and is a novel marker of poor prognosis in breast cancer. Breast Cancer Research, 17(1), 13.

Visone, R., & Croce, C. M. (2009). MiRNAs and cancer. The American journal of pathology, 174(4), 1131-1138.

Voduc, K. D., Cheang, M. C., Tyldesley, S., Gelmon, K., Nielsen, T. O., & Kennecke, H. (2010). Breast cancer subtypes and the risk of local and regional relapse. Journal of clinical oncology, 28(10), 1684-1691.

Wallden, B., Storhoff, J., Nielsen, T., Dowidar, N., Schaper, C., Ferree, S., ... & Vickery, T. (2015). Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. BMC medical genomics, 8(1), 54.

Wang, H., Chen, S. H., Kong, P., Zhang, L. Y., Zhang, L. L., Zhang, N. Q., & Gu, H. (2018). Increased expression of miR-330-3p: a novel independent indicator of poor prognosis in human breast cancer. European review for medical and pharmacological sciences, 22, 1726-1730.

Wang, J., Zhang, K. Y., Liu, S. M., & Sen, S. (2014). Tumor-associated circulating microRNAs as biomarkers of cancer. Molecules, 19(2), 1912-1938.

Wang, N., Chen, P., Huang, L. P., & Wang, T. Z. (2016). Prognostic significance of microRNA-10b overexpression in breast cancer: a meta-analysis. Genet Mol Res, 15(2).

Wang, S., Li, H., Wang, J., Wang, D., Yao, A., & Li, Q. (2014). Prognostic and biological significance of microRNA-127 expression in human breast cancer. Disease markers, 2014.

Wang, Y., Yin, W., Lin, Y., Yin, K., Zhou, L., Du, Y., ... & Lu, J. (2018). Downregulated circulating microRNAs after surgery: potential noninvasive biomarkers for diagnosis and prognosis of early breast cancer. Cell death discovery, 5(1), 21.

Wang, Y., Zhang, Y., Pan, C., Ma, F., & Zhang, S. (2015). Prediction of poor prognosis in breast cancer patients based on microRNA-21 expression: a meta-analysis. PloS one, 10(2), e0118647.

Wen, H. Y., Krystel-Whittemore, M., Patil, S., Pareja, F., Bowser, Z. L., Dickler, M. N., ... & Brogi, E. (2017). Breast carcinoma with an Oncotype Dx recurrence score< 18: Rate of distant metastases in a large series with clinical follow-up. Cancer, 123(1), 131-137.

Winters, S., Martin, C., Murphy, D., & Shokar, N. K. (2017). Breast Cancer Epidemiology, Prevention, and Screening. In Progress in molecular biology and translational science (Vol. 151, pp. 1-32). Academic Press.

Wu, X., Somlo, G., Yu, Y., Palomares, M. R., Li, A. X., Zhou, W., ... & Wang, J. (2012). De novo sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. Journal of translational medicine, 10(1), 42.

Wu, X., Somlo, G., Yu, Y., Palomares, M. R., Li, A. X., Zhou, W., ... & Wang, J. (2012). De novo sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. Journal of translational medicine, 10(1), 42.

Wu, Z. S., Wang, C. Q., Xiang, R., Liu, X., Ye, S., Yang, X. Q., ... & Wu, Q. (2012). Loss of miR-133a expression associated with poor survival of breast cancer and restoration of miR-133a expression inhibited breast cancer cell growth and invasion. BMC cancer, 12(1), 51.

Wu, Z. S., Wu, Q., Wang, C. Q., Wang, X. N., Wang, Y., Zhao, J. J., ... & Xu, X. C. (2010). MiR-339-5p inhibits breast cancer cell migration and invasion in vitro and may be a potential biomarker for breast cancer prognosis. BMC cancer, 10(1), 542.

Xu, X., Zhang, Y., Jasper, J., Lykken, E., Alexander, P. B., Markowitz, G. J., ... & Wang, X. F. (2016). MiR-148a functions to suppress metastasis and serves as a prognostic indicator in triple-negative breast cancer. Oncotarget, 7(15), 20381.

Yadav, P., Mirza, M., Nandi, K., Jain, S. K., Kaza, R. C. M., Khurana, N., ... & Saxena, A. (2016). Serum microRNA-21 expression as a prognostic and therapeutic biomarker for breast cancer patients. Tumor Biology, 37(11), 15275-15282.

Yan, L. X., Huang, X. F., Shao, Q., Huang, M. Y., Deng, L., Wu, Q. L., ... & Shao, J. Y. (2008). MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. Rna.

Yan, L. X., Liu, Y. H., Xiang, J. W., Wu, Q. N., Xu, L. B., Luo, X. L., ... & Mei, P. (2016). PIK3R1 targeting by miR-21 suppresses tumor cell migration and invasion by reducing PI3K/AKT signaling and reversing EMT, and predicts clinical outcome of breast cancer. International journal of oncology, 48(2), 471-484.

Yang, H., Yu, J., Wang, L., Ding, D., Zhang, L., Chu, C., ... & Liu, X. (2014). miR-320a is an independent prognostic biomarker for invasive breast cancer. Oncology letters, 8(3), 1043-1050.

Yao, L., Liu, Y., Cao, Z., Li, J., Huang, Y., Hu, X., & Shao, Z. (2018). MicroRNA-493 is a prognostic factor in triple-negative breast cancer. Cancer science, 109(7), 2294.

Yao, Y., Hu, J., Shen, Z., Yao, R., Liu, S., Li, Y., ... & Yue, L. (2015). MiR-200b expression in breast cancer: a prognostic marker and act on cell proliferation and apoptosis by targeting Sp1. Journal of cellular and molecular medicine, 19(4), 760-769.

Yoruker, E. E., Aydoğan, F., Gezer, U., Saip, P., & Dalay, N. (2015). Analysis of circulating microRNAs during adjuvant chemotherapy in patients with luminal A breast cancer. Molecular and clinical oncology, 3(4), 954-958.

Yu, H., Li, H., Qian, H., Jiao, X., Zhu, X., Jiang, X., ... & Huang, J. (2014). Upregulation of miR-301a correlates with poor prognosis in triple-negative breast cancer. Medical oncology, 31(11), 283.

Yu, M., Zhang, X., Li, H., Zhang, P., & Dong, W. (2017). MicroRNA-588 is downregulated and may have prognostic and functional roles in human breast cancer. Medical science monitor: international medical journal of experimental and clinical research, 23, 5690.

Yu, X., Zhang, X., Dhakal, I. B., Beggs, M., Kadlubar, S., & Luo, D. (2012). Induction of cell proliferation and survival genes by estradiol-repressed microRNAs in breast cancer cells. BMC cancer, 12(1), 29.

Yu, Y., Zhao, Y., Sun, X. H., Ge, J., Zhang, B., Wang, X., & Cao, X. C. (2015). Down-regulation of miR-129-5p via the Twist1-Snail feedback loop stimulates the epithelial-mesenchymal transition and is associated with poor prognosis in breast cancer. Oncotarget, 6(33), 34423.

Zavala, V., Pérez-Moreno, E., Tapia, T., Camus, M., & Carvallo, P. (2016). miR-146a and miR-638 in BRCA1-deficient triple negative breast cancer tumors, as potential biomarkers for improved overall survival. Cancer Biomarkers, 16(1), 99-107.

Zeng, B., Li, Y., Feng, Y., Lu, M., Yuan, H., Yi, Z., ... & Ren, G. (2018). Downregulated miR-1247-5p associates with poor prognosis and facilitates tumor cell growth via DVL1/Wnt/β-catenin signaling in breast cancer. Biochemical and biophysical research communications, 505(1), 302-308.

Zeng, Z., Chen, X., Zhu, D., Luo, Z., & Yang, M. (2017). Low expression of circulating microRNA-34c is associated with poor prognosis in triple-negative breast cancer. Yonsei medical journal, 58(4), 697-702.

Zhang, J., He, Y., Yu, Y., Chen, X., Cui, G., Wang, W., ... & Ren, Z. (2018). Upregulation of miR-374a promotes tumor metastasis and progression by downregulating LACTB and predicts unfavorable prognosis in breast cancer. Cancer medicine.

Zhang, L., Huang, J., Yang, N., Greshock, J., Megraw, M. S., Giannakakis, A., ... & Yao, G. (2006). microRNAs exhibit high frequency genomic alterations in human cancer. Proceedings of the National Academy of Sciences, 103(24), 9136-9141.

Zhang, L., Yan, D. L., Yang, F., Wang, D. D., Chen, X., Wu, J. Z., ... & Xia, W. J. (2017). DNA methylation mediated silencing of microRNA-874 is a promising diagnosis and prognostic marker in breast cancer. Oncotarget, 8(28), 45496.

Zhang, P., Fan, C., Du, J., Mo, X., & Zhao, Q. (2018). Association of miR-1247-5p expression with clinicopathological parameters and prognosis in breast cancer. International journal of experimental pathology, 99(4), 199-205.

Zhang, X. Y., Liu, D. J., Yuan, R. B., Zhang, D. H., Li, S. R., Zhang, S. H., & Zhang, L. Y. (2018). Low expression of miR-597 is correlated with tumor stage and poor outcome in breast cancer. European review for medical and pharmacological sciences, 22, 456-460.

Zheng, J. Z., Huang, Y. N., Yao, L., Liu, Y. R., Liu, S., Hu, X., ... & Shao, Z. M. (2018). Elevated miR-301a expression indicates a poor prognosis for breast cancer patients. Scientific reports, 8(1), 2225.

Zhong, H., Yang, J., Zhang, B., Wang, X., Pei, L., Zhang, L., ... & Wang, C. (2018). LncRNA GACAT3 predicts poor prognosis and promotes cell proliferation in breast cancer through regulation of miR-497/CCND2. Cancer Biomarkers, (Preprint), 1-11.

Zhong, X., Xie, G., Zhang, Z., Wang, Z., Wang, Y., Wang, Y., ... & Zheng, H. (2016). MiR-4653-3p and its target gene FRS2 are prognostic biomarkers for hormone receptor positive breast cancer patients receiving tamoxifen as adjuvant endocrine therapy. Oncotarget, 7(38), 61166.

6. TABLES

Table 1 *List of prognostic microRNAs in breast cancer*

PROGNOSTIC microRNA	BREAST CANCER TYPE	DETECTION METHOD	PROGNOSTIC VALUE	ROLE	BIOLOGICAL SAMPLE	REFERENCES
let-7	breast cancer not classified	qRT-PCR	potential prognostic biomarker as altered levels of miR-let-7 are associated with metastases risk	tumor suppressor	serum	Elghoroury et al., 2017
let-7-3p	TNBC	NGS, qRT-PCR	independent prognostic factor for OS, DFS	onco-miR	FFPE	Turashvili et al., 2018
let-7b	luminal subtype	qRT-PCR, LNA-ISH, TMAs	independent prognostic factor for OS associated with luminal tumors	tumor suppressor	FFPE	Quesne et al., 2012
let-7c/miR-99a/miR-125b cluster	estrogen-dependent human breast cancer cell line	Nanostring, qRT-PCR, luciferase report assay	potential prognostic factor for OS in the luminal A subtype	tumor suppressor	cell lines	Bailey et al., 2015
miR-1	ER-positive, stage IV breast	microRNA PCR array, microarray, ISH, IHC	independent worse prognostic factor of DFS and breast cancer-specific survival associated with stage, lymph node metastasis, distant metastasis,	onco-miR	FFPE	Minemura et al., 2015

	cancer		histological grade, ER status, PR status and Ki-67			
miR-10b	breast cancer not classified, TNBC (Medimegh et al., 2014)	qRT-PCR	independent prognostic factor for DFS associated with distant metastasis, occurrence in TNBC, associated with genico-obstetric history	onco-miR	FFPE, fresh frozen tissue, cell lines	Parrella et al., 2014; Chang et al., 2014; Medimegh et al., 2014; Eissa et al., 2015
miR-122	breast cancer not classified (stage II-III)	qRT-PCR, NGS	potential prognostic factor for disease relapse, predictor of metastasis	onco-miR	serum	Wu et al., 2012
miR-124	breast cancer not classified, (>50 years old)	qRT-PCR	prognostic factor for OS associated with advanced TNM stage, lymph node metastasis and poorer pathological differentiation, associated with age at diagnosis (>50 years old)	tumor suppressor	FFPE, fresh frozen tissue	Dong et al., 2015; Oltra et al., 2018
miR-1247-5p	breast cancer not classified	qRT-PCR	independent prognostic indicator for DFS, OS	tumor suppressor	FFPE, fresh frozen tissue, cell lines	Zeng et al., 2018; Zhang et al., 2018

miR-125a-5p	breast cancer not classified	microarray, qRT-PCR, luciferase assay, ISH, IHC	potential prognostic factor for OS, progression-free survival (PRS)	tumor suppressor	serum, cell lines	Hsieh et al., 2015
miR-125b	HER2 positive breast cancer (Luo et al., 2017), stage II/III breast cancer (Liu et al., 2017)	qRT-PCR, ISH	prognostic factor for OS, DFS, associated with aromatase inhibitor esistant breast cancers	onco-miR	FFPE, serum, cell lines	Vilquin et al., 2015; Luo et al., 2017; Liu et al., 2017
miR-1260	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS	onco-miR	plasma	Madhavan et al., 2016
miR-126-5p	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-127	breast cancer not	qRT-PCR	prognostic factor of OS	tumor suppressor	fresh frozen tissue, cell lines	Wang et al., 2014

	classified					
miR-1274a	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS	onco-miR	Plasma	Madhavan et al., 2016
miR-1274b	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-128-3p	TNBC	qRT-PCR	prognostic factor for RFS	tumor suppressor	FFPE	Turashvili et al., 2018
miR-129-5p	breast cancer not classified	qRT-PCR, luciferase report assay	potential prognostic factor for OS, DFS, associated with EMT	tumor suppressor	FFPE, fresh frozen tissue, cell lines	Yu et al., 2015
miR-133a	breast cancer not classified	qRT-PCR, TMA, ISH, Luciferase assay	potential prognostic factor for DFS associated with migration and invasion	tumor suppressor	FFPE, fresh frozen tissue, cell lines	Wu et al., 2012
miR-140	breast cancer not classified	qRT-PCR, microarray	asocciated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-141	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS associated with circulating tumor cells status	onco-miR	plasma	Madhavan et al., 2012; Madhavan et al., 2016
miR-143	triple possitive breast	qRT-PCR, Western blot,	potentially tumor suppressive effect on cancer progression of ER positive breast cancers, impairment of cell	tumor suppressor	FFPE	Yu et al., 2012

	cancer	luciferase report assay, MTS assay	proliferation			
miR-144	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS	miR-144 tumor suppressor	PLASMA	Madhavan et al., 2016
miR-145	breast cancer not classified	qRT-PCR	potential prognostic factor for DFS, OS (3-year survival rate)	tumor suppressor	fresh frozen tissue	Liu et al., 2016; Quan et al., 2018
miR-146a	BRCA1-deficient TNBC tumors	qRT-PCR	potential prognostic factor for OS	tumor suppressor	FFPE, cell lines	Zavala et al., 2016
miR-148a	TNBC	qRT-PCR, microarray	potential prognostic factor for OS associated with metastasis	tumor suppressor	Cell lines, mouse models	Xu et al., 2016
miR-155	TNBC, breast cancer not classified	qRT-PCR, microarray, luciferase report assay	prognostic factor of DMFS, associated with lymph node metastasis	tumour suppressor (Jang 2017), onco-miR (Kong 2014)	FFPE, fresh frozen tissue, cell lines	Kong et al., 2014; Jang et al., 2017
miR-15a	TNBC	qRT-PCR	prognostic factor for OS, DFS	tumor suppressor	fresh frozen tissue	Shinden et al., 2015
miR-16	triple possitive breast	qRT-PCR, Western blot,	potentially tumor suppressive effect on cancer progression of ER positive breast cancers, impairment of cell	tumor suppressor	FFPE	Yu et al., 2012

	cancer	luciferase report assay, MTS assay	proliferation			
miR-182	breast cancer not classified (premeno pausal, postmeno pausal, benign), TNBC	qRT-PCR	potential prognostic factor to predict lymph node metastases occurrence in TNBC, associated with genico-obstetric history, related with hormonal receptors	onco-miR	FFPE, serum	Medimegh et al., 2014; Ali et al., 2018
miR-1825	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-183/182/96 cluster	breast cancer not classified	qRT-PCR, ISH	potential prognostic factor for OS, DFS	onco-miR	breast tissues not classified, cell lines	Song et al., 2016
miR-187	breast cancer not classified	TMA, ISH	independent prognostic factor FOR breast cancer–specific survival (BCSS)	onco-miR	FFPE, cell lines	Mulrane et al., 2012
miR-193b	breast cancer not	microRNA arrays, qRT-	potential prognostic factor for OS, PFS	onco-miR	PLASMA	Madhavan et al., 2016

	classified	PCR				
miR-195-5p	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-199a-5p	TNBC	NGS	prognostic factor for OS	tumor suppressor	FFPE	Turashvili et al., 2018
miR-199b-5p	breast cancer not classified (TNM I-II stage)	qRT-PCR, assays in vitro	potential prognostic factor for OS associated with TNM stage and lymph node metastasis	tumor suppressor	fresh frozen tissue and cell lines	Fang et al., 2016
miR-19a	newly diagnosed IBC stage III, IBC stage IV, non-IBC stage II-IV and HER2+ breast cancer	qRT-PCR	potential prognostic factor for OS, DFS in patients with metastatic HER2(+) IBC.	tumor suppressor	serum, cell lines	Anfossi et al., 2014
miR-19b	breast cancer not classified	qRT-PCR	prognostic factor for OS associated with distant metastasis and TNM stage	onco-miR	fresh frozen tissue, cell lines	Li et al., 2018

miR-200a	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS, associated with circulating tumor cells status, potential to detect the onset of metastasis	onco-miR	Plasma	Madhavan et al., 2016, Madhavan et al., 2012
miR-200b	breast cancer not classified	qRT-PCR, microRNA arrays, ISH, TMA, luciferase report assay	potential prognostic factor for OS (independent), PFS associated with advanced clinical stage, metastasis, cell proliferation, apoptosis, cell cycle distribution and circulating tumor cells status, potential to detect the onset of metastasis	tumor suppressor, onco-miR (Madhavan 2012, Madhavan 2016)	FFPE, plasma, cell lines	Madhavan et al., 2012; Ye et al., 2014; Yao et al., 2015; Madhavan et al., 2016
miR-200c	breast cancer not classified	qRT-PCR, microRNA arrays	prognostic factor of OS, DFS, potential to detect the onset of metastasis, associated with circulating tumor cells status	onco-miR	fresh frozen tissue, plasma	Madhavan et al., 2012; Tuomarila et al., 2014; Madhavan et al., 2016
miR-200c/141 cluster	breast cancer not classified, TNBC	qRT-PCR, CAT reporter assay, siRNA transfection Western blot	poor prognostic factor in TNBC, promoting metastasis	onco-miR	FFPE, cell lines, xenograft animal model	Jin et al., 2017
miR-203	breast cancer not	microRNA arrays, qRT-	potential prognostic factor for OS, PFS associated with EMT and circulating	onco-miR, tumor suppressor	FFPE, plasma, cell lines	Yu et al., 2012; Madhavan et al., 2012; Fisher et al., 2015;

	classified, ER positive breast cancer (Yu et al., 2012)	PCR, Western blot, luciferase report assay, MTS assay	tumor cells status	(Yu et al., 2014)		Madhavan et al., 2016
miR-203-5p	TNBC	NGS	prognostic factor for OS	onco-miR	FFPE	Turashvili et al., 2018
miR-203a	ductal in situ, invasive ductal and lobular carcinoma	qRT-PCR	potential prognostic marker associated with increased stage in invasive lobular carcinomas	tumor suppressor	FFPE	Gomes et al., 2016
miR-204	breast cancer not classified	qRT-PCR	potential prognostic factor for OS, DFS, correlated with chemotherapeutic resistance	tumor suppressor	FFPE	Li et al., 2014
miR-205	breast cancer not classified	qRT-PCR, LNA-ISH, TMAs, IHC	potential prognostic factor for OS associated with tumours of ductal morphology, for OS and DFS in early breast cancer	tumor suppressor	FFPE	Quesne et al., 2013; Markou et al., 2014
miR-206	breast cancer not classified	qRT-PCR, luciferase report assay	potential prognostic factor for OS	onco-miR (Quan et al., 2018), tumor supressor (Li	fresh frozen tissue, cell lines	Li et al., 2013; Hesari et al., 2018; Quan et al., 2018

				et al., 2013, Hesari 2018)		
miR-20b-5p	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS, correlated with the presence of breast tumor interstitial fluid	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-21	stage II/III breast cancer HER2 positive (Liu et al., 2017), TNBC (Dong et al., 2014; Medimegh et al., 2014)	qRT-PCR, microarray, luciferase report assay	independent prognostic factor of OS, DFS, prognostic biomarker for resistance to trastuzumab, to predict lymph node metastases occurrence in TNBC, to predict high grade in non TNBC possible, prognostic factor in daughter of patients, associated with genico-obstetric history	onco-miR	FFPE, serum, fresh frozen tissue, cell lines	Yan et al., 2008; Qian et al., 2009; Lee et al., 2011; Dong et al., 2014; Markou et al., 2014; Medimegh et al., 2014; Toraih et al., 2015; Usmani et al., 2015; Yan et al., 2016; Liu et al., 2017; Badr et al., 2019; Yadav et al., 2016;
miR-210	early first primary breast cancer, TNBC	qRT-PCR, microarrays	independent prognostic factor for OS, DFS, associated with poor clinical outcome in ER-positive, tamoxifen-treated BC patients, involved in cell proliferation, migration and invasion, Potential to detect the onset of metastasis prior to clinical diagnosis,	onco-miR	FFPE, fresh frozen tissue, plasma, cell lines (Breast cancer and tumor-educated macrophages)	Camps et al., 2008; Rothé et al., 2011; Toyama et al., 2012; Madhavan et al., 2012; Bleckmann et al, 2015; Boukerroucha et al., 2015, Madhavan et al., 2016

			associated with circulating tumor cells status			
miR-210-3p	breast cancer cell lines	qRT-PCR	potential prognostic factor for OS associated with EMT and regulation of growth factors involved in G1- to S-phase transition	onco-miR	cell lines	Fisher et al., 2015
miR-215	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS, Potential to detect the onset of metastasis prior to clinical diagnosis	miR-215 tumor suppressor	plasma samples	Madhavan et al., 2016
miR-218	breast cancer not classified	qRT-PCR	prognostic factor for OS associated with lymph node metastases, higher grades,	tumor suppressor, increased expression leads to prognosis	fresh frozen tissue	Ahmadinejad et al., 2017
miR-22	breast cancer not classified	qRT-PCR, ISH, luciferase report assay	potential prognostic factor for OS, DFS, associated with EMT/metastasis	onco-miR (Pandley et al., 2015), tumor suppressor (Chen et al., 2016)	FFPE, cell lines	Pandey et al., 2015; Chen et al., 2016
miR-221	breast cancer not classified	qRT-PCR	prognostic factor for DFS, OS, RFS	onco-miR	FFPE (Falkenberg et al., 2013), fresh frozen tissue (Cheng et al., 2018, Eissa et al., 2015), cell lines	Falkenberg et al., 2013; Eissa et al., 2015; Cheng et al., 2018

					(Cheng et al., 2018)	
miR-221-3p	TNBC	qRT-PCR	prognostic factor for DFS	tumour suppressor	FFPE, cell lines	Deng et al., 2017
miR-222	breast cancer not classified	qRT-PCR, TMA	potential prognostic factor related to lymph node metastasis, down-regulation of the estrogen receptor, EMT, tumor progression, poor response and chemotherapy resistance	onco-miR	FFPE, fresh frozen tissue, cell lines	Falkenberg et al., 2013; Chen et al., 2016
miR-222-3p	breast cancer not classified (pre/posto peratively)	qRT-PCR, microarray	independent prognostic factor for DFS postoperatively	onco-miR	Serum	Wang et al., 2018
miR-24-2*.	breast cancer cell lines	qRT-PCR	associated with tumor suppressive activity through the suppression of cellular survival	tumor suppressor	cell lines, fresh frozen mouse tissue	Martin et al., 2014
mir-24-3p	stage I-III breast cancer	Nanostring technology	potential prognostic biomarker of occult metastasis	onco-miR	plasma	Khodadadi-Jamaryan et al., 2018
miR-27a	breast cancer not classified	ISH, IHC	independent prognostic factor for OS, DFS	onco-miR	FFPE	Tang et al., 2012
miR-27b-3p	TNBC	qRT-PCR	independent prognostic factor for OS,	onco-miR	FFPE	Shen et al., 2014

			DMF survival			
miR-29a	breast cancer not classified	qRT-PCR, microarray	asocciated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-29b	lobular and ductal subtypes of breast cancer	qRT-PCR	prognostic factor for OS (Papachristopoulou 2018, Shinden 2015) , DFS (Shinden 2015)	tumor suppressor	fresh frozen tissue	Shinden et al., 2015; Papachristopoulou et al., 2018
miR-301a	breast cancer not classified (Zheng 2018), TNBC (Yu 2014)	qRT-PCR, microarray, ISH	prognostic factor for DFS, OS	onco-miR	FFPE	Zheng et al., 2018, Yu et al., 2014
miR-30a	TNBC	NGS, qRT-PCR, microarray, luciferase report assay	independent prognostic factor for OS, DFS	tumor suppressor	FFPE, cell lines	Turashvili et al., 2018, Cheng et al., 2012
miR-30a-3p	TNBC	qRT-PCR	prognostic factor for OS, RFS	tumor suppressor	FFPE	Turashvili et al., 2018
miR-30a-5p	TNBC	NGS	prognostic factor for OS, RFS	tumor suppressor	FFPE	Turashvili et al., 2018

miR-30c-5p	TNBC	qRT-PCR	prognostic factor for RFS	tumor suppressor	FFPE	Turashvili et al., 2018
miR-30e*	ESR1-/ERBB2-tumours	miRNA microarrays hybridisation	prognostic factor for DFS	tumor suppressor	fresh frozen tissue	D'Aiuto et al., 2015
miR-3178	breast cancer not classified	qRT-PCR, microarray	asocciated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-320a	breast cancer not classified	chromogenic in situ hybridization	potential prognostic factor for OS for invasive breast cancer	tumor suppressor	FFPE	Yang et al., 2014
miR-324-5p	TNBC	NGS	prognostic factor for OS	onco-miR	FFPE	Turashvili et al., 2018
miR-329	breast cancer not classified	qRT-PCR	independent prognostic factor for OS	tumor-suppressor	serum, fresh frozen tissue, cell lines	Li et al., 2017
miR-330-3p	breast cancer not classified	qRT-PCR	potential prognostic factor for OS	onco-miR	fresh frozen tissue	Wang et al., 2018
miR-339-5p	breast cancer not classified	qRT-PCR, TMA, ISH	independent prognostic factor for OS, DFS	tumor suppressor	FFPE, cell lines	Wu et al., 2010
miR-34a	breast cancer not	qRT-PCR, TMAs	prognostic factor for OS, asocciated with response and chemotherapy	tumor suppressor, onco-miR	FFPE, plasma, cell lines	Peurala et al., 2011; Chen et al., 2016; Zeng et al., 2017

	classified TNBC		resistance	(Chen 2016)		
miR-34b	TNBC	qRT-PCR	prognostic factor for OS, DFS	onco-miR	FFPE	Svoboda et al., 2012
miR-34c	TNBC	qRT-PCR	independent risk factor for OS	tumor suppressor	Plasma	Zeng et al., 2017
miR-361-5p	breast cancer not classified, TNBC	TMAs, ISH	prognostic factor for DFS	tumor suppressor	FFPE	Cao et al., 2016
miR-365	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS	miR-365, onco-miR	Plasma	Madhavan et al., 2016
miR-370	breast cancer not classified	qRT-PCR, TMA	potential prognostic factor for DFS	onco-miR	FFPE	Sim et al., 2015
miR-374a	breast cancer not classified (Zhang 2018), invasive ductal carcinoma stage II (Li 2013)	qRT-PCR, TMAs, Luciferase activity assay, MTT assays, IHC	potential prognostic factor for DFS, contributes to tumorigenicity and progression	onco-miR	FFPE, fresh frozen tissue, cell lines, xenograft mouse models	Li et al., 2013; Zhang et al., 2018

miR-375	breast cancer not classified, stage II-III locally advanced and IBC patients (Wu et al., 2016)	qRT-PCR, microRNA arrays, NGS	potential prognostic factor for OS, PFS associated with circulating tumor cells status, related to hormonal receptors	onco-miR, tumor suppressor (Wu et al., 2013)	serum, plasma	Madhavan et al., 2012; Wu et al., 2013; Madhavan et al., 2016; Ali et al., 2018
miR-409-3p	breast cancer not classified	qRT-PCR	independent prognostic factor for OS associated with advanced TNM stage, lymph node metastasis, and poorer pathological differentiation	tumor suppressor	fresh frozen tissue	Cao et al., 2016
miR-423	breast cancer not classified	qRT-PCR, microarray	asocciated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-429	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS	miR-429 onco-miR	Plasma	Madhavan et al., 2016
miR-451	breast cancer cell lines	qRT-PCR	potential factor associated with cell survival and endocrine resistance	tumor suppressor	cell lines	Bergamaschi et al., 2012

miR-454	breast cancer not classified (stage I-III)	TMA, ISH	potential prognostic factor for OS (especially in TNBC), DFS, associated with response to anthracycline	onco-miR	FFPE	Cao et al., 2016
miR-454-3p	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-4653-3p	HR+ BC women (stage I~III) treated with adjuvant tamoxifen	qRT-PCR	potential prognostic biomarker for DFS	tumor suppressor	FFPE	Zhong et al., 2016
miR-486-5p	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, Potential to detect the onset of metastasis prior to clinical diagnosis	miR-486-5p tumor suppressor	Plasma	Madhavan et al., 2016
miR-493	TNBC	TMAs, in situ hybridization	prognostic factor for DFS	tumour suppressor	FFPE	Yao et al., 2018
miR-494	node-negative	in situ hybridizatio	8.5-fold risk of breast cancer death (association trend-not clinical	tumour suppressor	fresh frozen tissue	Gurvits et al., 2018

	breast cancer	n	significance)			
miR-497	breast cancer not classified, TNBC	qRT-PCR, luciferase report assay	potential prognostic factor for OS	tumor suppressor	fresh frozen tissue, cell lines, orthotopic mouse models	Liu et al., 2016; Zhong et al., 2018
miR-548c-5p	TNBC	qRT-PCR, ISH	independent prognostic factor for OS, DFS	onco-miR	FFPE	Boukerroucha et al., 2015
miR-574	breast cancer not classified	qRT-PCR, microarray	asocciated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-574-3p	breast cancer not classified	qRT-PCR, NGS	potential prognostic factor for OS, DFS	tumor suppressor	FFPE	Krishnan et al., 2015
miR-588	breast cancer not classified	qRT-PCR	prognostic factor of OS	tumour suppressor	fresh frozen tissue, cell lines	Yu et al., 2017
miR-590-3p	breast cancer cell lines	qRT-PCR, luciferase report assay	associated with breast cancer cells viability, growth and apoptosis	tumor suppressor	cell lines	Abdolvahabi et al., 2018
miR-597	breast cancer not classified	qRT-PCR	prognostic factor of OS	tumor suppressor	fresh tissue	Zhang et al., 2018
miR-601	breast	qRT-PCR	prognostic factor for DFS associated	tumor suppressor	FFPE, cell lines	Hu et al., 2016

	cancer-not classified		with cell proliferation and metastasis			
miR-638	breast cancer not classified (Li et al., 2018), BRCA1-deficient TNBC tumors (Zavala et al., 2016)	qRT-PCR	independent prognostic factor for OS associated with lymph node metastasis and TNM stage	tumor suppressor	FFPE, fresh frozen, cell lines	Zavala et al., 2016; Li et al., 2018
miR-644a	breast cancer cell lines	qRT-PCR, luciferase report assay	associated with tumor progression and distant metastasis-free survival	tumor suppressor	cell lines	Raza et al., 2016
miR-660-5p	breast cancer not classified	qRT-PCR, NGS	potential prognostic factor for OS, DFS	onco-miR	FFPE	Krishnan et al., 2015
miR-6780b	breast cancer not classified	qRT-PCR, microarray	associated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-7	breast cancer not	qRT-PCR	potential prognostic factor for OS, DFS predictive of an adverse response to	onco-miR	fresh frozen tissue, cell lines	Uhr et al., 2018

	classified		tamoxifen therapy			
miR-711	breast cancer not classified	qRT-PCR	independent prognostic factor for OS, DFS, associated with breast cancer cells' proliferation, colony formation, invasion	onco-miR	FFPE, cell lines	Hu et al., 2016
miR-744	breast cancer not classified	qRT-PCR, microarray	associated with poor response and chemotherapy resistance	onco-miR	FFPE, cell lines	Chen et al., 2016
miR-801	breast cancer not classified	microRNA arrays, qRT-PCR	potential prognostic factor for OS, PFS associated with circulating tumor cells status	onco-miR	plasma	Madhavan et al., 2012; Madhavan et al., 2016
miR-874	breast cancer not classified	qRT-PCR	prognostic factor for OS	tumour suppressor	fresh frozen tissue, cell lines	Zhang et al., 2017
miR-9	TNBC, breast cancer not classified	qRT-PCR	prognostic factor of DFS and DMFS, OS	onco-miR	FFPE, fresh frozen tissue, cell lines	Jang et al., 2017; Cheng et al., 2018
miR-93-5p	breast cancer not classified	microRNA arrays	potential prognostic factor for DFS, correlated with the presence of breast tumor interstitial fluid	onco-miR	FFPE, interstitial breast tumor fluids, serum	Halvorsen et al., 2016
miR-940	invasive ductal	qRT-PCR	prognostic factor for OS	tumor suppressor	Serum	Liu et al., 2018

	carcinoma, TNBC					
miR-95-3p	TNBC	qRT-PCR	prognostic factor for OS, RFS in patients treated with anthracycline-based chemotherapy	onco-miR	FFPE	Turashvili et al., 2018
miR-96	breast cancer cell lines	qRT-PCR	potential prognostic factor for OS associated with EMT and regulation of growth factors involved in G1/S-phase transition	onco-miR	cell lines	Fisher et al., 2015
miR-99a	breast cancer not classified	qRT-PCR	potential prognostic factor for OS, independent risk factor for breast cancer	tumor suppressor	Serum	Li et al., 2016
miR-1179	breast cancer not classified	RT-PCR	independent prognostic factor for OS	tumor suppressor	breast tissue not classified, cell lines	Li et al., 2018

Abbreviations: quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR), In situ hybridization (ISH), locked nucleic acid probe in situ hybridization (LNA-ISH), Immunohistochemistry (IHC), epithelial-mesenchymal transition (EMT), formalin-fixed paraffin embedded (FFPE), Next Generation Sequencing (NGS), overall survival (OS), relapse free survival (RFS), disease free survival (DFS), progress free survival (PFS), triple negative breast cancer (TNBC), Inflammatory breast cancer (IBC), estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor type 2 (HER2)

Table 2 List of prognostic microRNA signatures in breast cancer

miRNA SIGNATURE	BREAST CANCER TYPE	DETECTION METHOD	PROGNOSTIC VALUE	ROLE	BIOLOGICAL SAMPLE	REFERENCES
miR-183-5p, miR-194-5p, miR-1285-5p signature	breast cancer not classified (age <35 years)	microarrays, qRT-PCR	potential prognostic factor for OS in young breast cancer patients.	miR-183-5p onco-miR miR-194-5p onco-miR miR-1285-5p tumor suppressor	FFPE	Hironaka-Mitsuhashi et al., 2017
miR-21, miR-30c, miR-181a, miR-181c, miR-125b, miR-7, miR-200a, miR-135b, miR-22 and miR-200c signature	HR+HER2- patients	qRT-PCR	potential prognostic factor for DRFS	10-miRNA-based classifier as a prognostic model	FFPE	Gong et al., 2016
miR-155, miR-493, miR-30e and miR-27a signature	TNBC	qRT-PCR, IHC	potential prognostic factor for OS associated with taxanes resistance	miR-155 tumor suppressor miR-493 tumor suppressor miR-30e onco-miR miR-27a onco-miR	FFPE	Gasparini et al., 2014
miR-16, 155, 125b,	TNBC	qRT-PCR	potential	miR-16 tumor suppressor	FFPE	Cascione et

374a signature			prognostic factor for OS	miR-155 tumor suppressor miR-125b onco-miR miR-374a tumor suppressor		al., 2013
miR-16, 125b, 374a, 374b, 421, 655, 497 signature	TNBC	qRT-PCR	potential prognostic factor for DDFS	miR-16 tumor suppressor miR-125b onco-miR miR-374a tumor suppressor miR-374b tumor suppressor miR-421 onco-miR miR-655 onco-miR miR-497 tumor suppressor	FFPE	Cascione et al., 2013
miR-191-5p, miR-214-3p, miR-451a, and miR-489 signature	breast cancer not classified	qRT-PCR, microarray	independent prognostic factor for OS, DFS	miR-191-5p onco-miR miR-214-3p tumor suppressor miR-451a tumor suppressor miR-489 tumor suppressor	FFPE, cell lines	Chen et al., 2018

Abbreviations: quantitative reverse transcriptase real-time polymerase chain reaction (qRT-PCR), formalin-fixed paraffin embedded (FFPE), overall survival (OS), distant disease-free survival (DDFS), distant recurrence free survival (DRFS)