

MicroRNAs and Their Clinical Importance in Triple Negative Breast Cancer

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Abstract

Triple negative breast cancer (TNBC) represents a challenging disease due to its absence of estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (HER2/neu). The available therapies do not always exert the desired clinical effects in the patient and TNBC also has a high rate of recurrence and poor prognosis. Determination of the molecular profile of TNBC using gene expression assays including microRNAs (miRNAs) analysis has conferred a clear understanding of the heterogeneous nature of these tumors. MiRNAs are small nucleotide molecules with the ability to regulate the expression of our genes, as we know, in cancer this gene regulation is lost, by oncogenes up-regulation or tumor suppressor genes down-regulation; multiple miRNAs have been described to have a critical role in the regulation of these genes and have been named as oncomir's and tumor suppressor miRNAs, furthermore the availability of technology to synthesize miRNAs and their antagonists (antagomirs) and recent promising Nano techniques that allows us to deliver miRNAs to a specific target have considerably increased research and expectation for possible new treatments or monitoring protocols, moreover, its detection in serum or other fluids by noninvasive procedures could be used for detection of this disease and correlate with the prognosis or to consider treatment adjustments. In this review we discuss recent molecular evidence involving miRNAs and its impact as diagnostic or prognostic markers and as therapeutic targets in TNBC.

Keywords: MicroRNAs; Triple negative breast cancer; Biomarkers; Regulation; Prognostic Diagnostic

Abbreviations

ABC: ATP Binding Cassette Transporter; ATP: Adenosine Triphosphate; BRCA 1: Breast Cancer Susceptibility Gene 1; DNA: Deoxyribonucleic Acid; ER: Estrogen Receptor; GTP: Guanosine Triphosphate; HER2/neu: Epidermal Growth Factor Receptor 2; MiRNAs: MicroRNAs; Oncomir: Oncogenic MiRNA; PCR: Polymerase Chain Reaction; PDCD4: Programmed Cell Death 4; PR: Progesterone Receptor; RNAs: Ribonucleic Acids; TNBC: Triple Negative Breast Cancer; TPM 1: Tropomyosin 1

Introduction

Triple negative breast cancer represents a highly invasive clinical subtype of breast cancer, characterized by the absence of estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor 2 (HER2), this cancer shows a high recurrence rate and worse prognosis if compared with other subtypes, which could partially be explained by the lack of available target therapies [1]. Actually there are no standard treatment guidelines for this disease. Recently was demonstrated that cell signaling pathways in both normal and tumor tissues are regulated by different miRNAs. Determination of the molecular profile of TNBC using gene expression assays has conferred a clear understanding of the heterogeneous nature of these tumors and several studies have demonstrated that miRNAs are responsible for a large proportion of TNBC heterogeneity [2,3].

MiRNAs are one class of small noncoding RNAs that were first identified in *Caenorhabditis elegans* [4]; they are ~22 nucleotides in length and function as antisense regulators of other RNAs [5] mediating regulatory processes.

MiRNAs are synthesized in the cell nucleus, processed by Drosha complex and then exported to the cytoplasm by GTP dependent exportin-5, where they are additionally processed by the Dicer enzyme complex to yield mature miRNAs(Figure 1).

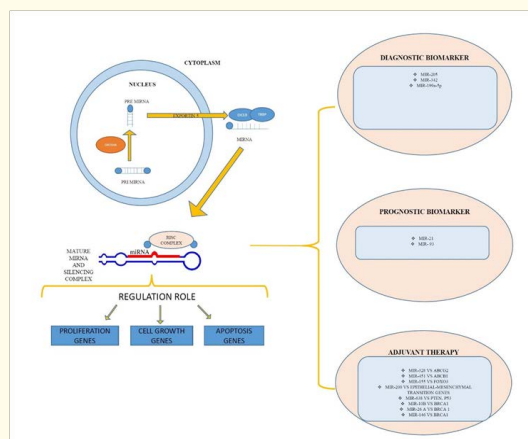


Figure 1: Biogenesis and function of miRNAs in Cancer.

Currently ~ 2,588 mature human sequences have been catalogued by the online repository miRBase [6] but the functionality of most of them is yet to be discovered.

In cancer, miRNAs play a crucial role by promoting uncontrolled cell division and blocking apoptosis. During cancer development, progression and metastasis, miRNAs are subdivided in two main categories: tumor suppressor and oncogenic miRNAs (oncomir’s), some of them can be correlated with the prognosis of the disease or detected in serum for diagnostic purposes. Multiple studies have revealed that several miRNAs target specifically the three missing receptors ER, PR, and HER2 as well as the breast cancer susceptibility gene BRCA1 (Breast Cancer Susceptibility Gene 1) in TNBC development (Figure 1).

The aim of this review is to show molecular evidence regarding miRNAs and its impact as diagnostic, prognostic and predictive markers and therapeutic targets in TNBC.

Potential microRNAs as diagnostic biomarkers in TNBC

The discovery in 2008 of cell-free miRNAs in blood [7-9] and other body fluids such as tears, urine and pleural effusions exhibiting distinctive regulation patterns in TNBC respect to non-TNBC and healthy controls [10-12] has yielded a potential source of non-invasive biomarkers for cancer that could be routinely measured in easily accessible samples. A great advantage involving analysis of miRNAs is their high stability in biological samples and high resistance to changing environmental conditions, such as freezing, thawing cycles or enzymatic degradation [13]. This remarkable stability is partially explained by miRNAs association with protein complexes and their containment in circulating microvesicles called exosomes. In addition, due to their short sequence length and end-region sequence variation, measurement is easily obtained. Common measurement methods for miRNAs include: hybridization-based methods as microarrays and reverse transcription quantitative PCR assays, and next generation sequencing-based methods.

Savad., *et al.* [11] suggested that miR-205 and miR-342 could be used as potential biomarkers for TNBC diagnosis, showing evidences of downregulation in tumor samples. Previous reports have demonstrated that these miRNAs had different targets such as Tropomyosin 1 (TPM 1), Programmed Cell Death 4 (PDCD 4), and some component of P53 [14,15]. On the other hand, Eichelser., *et al.* [10] demonstrated that up regulation in serum levels of exosomal miR-373 is linked to TNBC, suggesting that miR-373 could be able to downregulate the

protein expression of ER. Finally, studies of Shin., *et al.* [12] showed a plasma downregulated level of miR-199a-5p in TNBC when compared with non-TNBC samples and healthy controls, these findings suggest that this miRNA could be a predictive marker for diagnosis of TNBC. There is only one study regarding this miRNA which demonstrated that miR-199a-5p is a regulator of autophagy and its regulation sensitized breast cancer cells to irradiation [16].

The table 1 summarizes recent experimental evidence regarding the role of miRNAs as diagnostic biomarkers in TNBC.

MiRNA-based prognostic and predictive biomarkers in TNBC

Biomarkers can be classified in two types: prognostic and predictive biomarkers; Prognostic biomarkers can assess the patient's outcome, aspects like recurrence after treatment and predictive biomarkers can assess the likelihood of benefit from a specific treatment or intervention [17]. As we already mentioned, one of the most important aspects to consider is the invasiveness of the sampling procedure, recently several miRNAs have been identified as potential biomarkers even in cell free fluids [18].

Detection of upregulated levels of miR-21 was correlated with a poor prognosis of TNBC, also its upregulation promoted TNBC cell proliferation *in vitro* [19], as we can see, both effects are closely linked.

Additionally, as described in other study, miR-93 regulation level in TNBC tissues was significantly higher than in non-TNBC tissues and ectopic transfection of mir-93 promoted cell proliferation, invasion, and metastasis [20], therefore miR-21 and miR-93 could be used as prognostic biomarkers in patients.

Some other miRNAs, listed in Table 1, have the ability to promote the exit of some drugs through ATP binding cassette transporters (ABC transporters) in the cell, these could be used as predictive biomarkers, since we actually lack available miRNA-based therapies we could adjust the treatment: a higher dose may be required to obtain a certain effect or, if available, use drugs exempt from the ABC transporter pump-out effect, other miRNAs like miR-638 and its enhanced radiation and chemotherapy sensitivity in TNBC cells, could predict the clinical response to radiotherapy and chemotherapy, considering both effects, miR-638 may serve as a potential prognostic biomarker and therapeutic target for breast cancer as well [21].

As discussed above, miRNAs have the potential to be used as biomarkers in TNBC and help the clinician obtain a better assessment and treatment, improving the patient's outcome and life expectancy.

Potential role of miRNAs in TNBC therapy

TNBC represents a challenge for current therapies and other options must be considered, patients often do not respond to radiation or chemotherapy, being the latter one the most common; four mechanisms are implicated in pharmacological resistance: increased drug efflux through ABC transporters, alteration of drug targets, alteration of DNA repair pathways and evasion of apoptosis, some miRNAs are implicated in these mechanisms and replacement or blocking therapies could be used [22].

Mirna-based therapeutic strategies for breast cancer is part of nucleic acids-based strategies used to restore the normal activity of miRNAs, they are classified in two main categories: anti-miRNA therapy and miRNA replacement therapy [23].

Anti-miRNA therapy includes miRNA sponges and antagomirs, these are miRNA antagonists that affect miRNA-related pathways by binding and blocking oncogenic miRNAs[23-25], antagomirs bind one miRNA at a time while miRNA sponges can bind several miRNAs from one family. This specific approach restores the normal expression of genes and reduces the progression of cancer or sensitizes cells to conventional therapies.

Mirna-replacement therapy impacts on drugs that inhibit ATP binding (e.g., imatinib, nilotinib, gefitinib, erlotinib, and others) as it can sensitize cells, and combinatorial application with other drugs may reduce resistance. MiR-328 expression, for example, has been shown to increase mitoxantrone sensitivity by targeting ABCG2, restoring miR-328 downregulated expression as a therapy could improve the outcome since ABCG2 pumps out mitoxantrone and doxorubicine, miRNAs can be used as adjuvants to conventional therapies [22], not only as a stand-alone therapy (Table 1).

Mirna	Target	Regulation in TNBC	Prospects	Reference
Diagnostic Biomarker				
mir-205 mir-342	Multiple targets in epithelial-mesenchymal transition (pre metastatic state)	Down-regulated	Significantly downregulated compared with normal breast tissue and other breast cancer groups	Savad., <i>et al.</i> [11] 2012
mir-373	Estrogen Receptor (ER)	Up-Regulated	Its association with ER/PR negative tumors helps confirmation of TNBC diagnosis	Eichelser., <i>et al.</i> [10] 2014
mir-199a-5p	Multiple targets	Down-regulated	Correlates with tumour stage, proportionally decreasing as the stages advance.	Shin., <i>et al.</i> [12] 2015
Prognostic biomarker				
mir-21	Multiple tumor suppressor genes	Up-Regulated	Use as biomarker, it correlates with poor prognosis	Dong., <i>et al.</i> [19] 2014
mir-93	Multiple cell proliferation genes	Up-Regulated	Use as biomarker, it correlates with poor prognosis	Hu., <i>et al.</i> [20] 2015
Therapy Adjuvant				
mir-328	ABCG2	Down-regulated	Restoring its regulation improves mitoxantrone and doxorubicine sensitivity.	Garofalo and Croce [22]
mir-451	ABCB1	Up-Regulated	miR-451 mimics may be an effective approach for sensitizing cells to certain drugs, such as tamoxifen and irinotecan.	Garofalo and Croce[22]
mir-146	BRCA1	Down-regulated	BRCA1 may down-regulate the expression of the EGFR gene by increasing the miR-146a level in breast cancer-derived cells	Kumaraswamy <i>et al.</i> 2014, M'hamed <i>et al.</i> 2015
mir-155	FOXO3a	Up-Regulated	miR-155 mimics may be an effective approach for sensitizing cells to paclitaxel and doxorubicine.	Gyparaki <i>et al.</i> 2014
mir-638	PTEN, p53	Down-regulated	Enhances radiation and chemotherapy sensitivity in TNBC cells by regulating BRCA1 expression via DNA repair pathways	Tan <i>et al.</i> 2014
MRX34 (mir-34 mimic)	SIRT 1	Therapy	Current drug under clinical trials for multiple cancers	Devulapally and Paulmurugan [27]
mir-200 family	Multiple targets in epithelial-mesenchymal transition (pre metastatic state)	Down-regulated	Restoring its regulation could improve the outcome in aggressive cases.	Kaboli <i>et al.</i> (2015)
mir-10b mir-26a	BRCA1	Up-Regulated	Using mimics to repress its activity could restore DNA damage repair mechanisms	M'hamed <i>et al.</i> 2015

Table 1: Evidences of altered levels of miRNAs and its potential function in TNBC.

Specific delivery of miRNAs to their targets is a common concern, several strategies are subject of current research such as nanoparticles, they can target cells with no accumulation on other organs or tissues, they can be fused with other particles to facilitate en-

docytosis in cancerous cells and have shown longer in vivo half-life compared with bare miRNAs [26]. Only two candidate miRNAs have reached clinical trials: SPC3649 (Santaris Pharma, Horsholm, Denmark), a miR-122 anti-sense LNA, and MRX34 (Mirna therapeutics, Inc.), a liposomal miR-34 mimic. MiR-34 is a tumor suppressor miRNA that is down-regulated in metastatic breast cancer [23,27].

Based on the evidence shown in this minireview, we consider miRNAs represent a new therapeutic tool with enough potential to significantly improve current therapies.

Conclusion

Relatively recent discovery of microRNAs and the elucidation of their function in our genome has opened new fields of research, as we discussed above it has opened the possibility to obtain a better and more accurate diagnosis, prognosis and, in the near future, more specific therapies that could reduce the high rate of mortality that TNBC involves; it's important to highlight that this molecules not necessarily substitute the available therapies or diagnostic protocols, but they have great potential to improve both aspects, and not only as a stand-alone option, as we mentioned, research areas such as nanomaterials and nanoparticles also bring new ways to considerably increase the specificity and effectiveness of every possible use of miRNAs bringing us closer to the possibility to offer TNBC patients an earlier diagnosis, a better prognosis, and a personalized treatment.

Conflict of Interest

We declare that no financial interest or conflict of interest exists among the authors.

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