

## New perspective on the role of microRNAs (miRNAs) in breast cancer

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### A B S T R A C T

MicroRNAs (miRNAs) are non-coding small RNA molecules with 21–25 nucleotides. It plays significant role in control of gene expression at posttranscriptional/translational level. They are commonly dysregulated in human cancers, whereas they may behave either as oncogenes or as tumor-suppressor genes. Both genetic and epigenetic events in deregulation of miRNA can lead to cancer development. Genetic events like deletion, amplification or translocation of miRNA can lead to chromosomal abnormalities. Also breast cancer is the most common cancer in women, containing approximately one third of all illness in women. Identification of micro RNAs and target molecules have provided clear perspective for understanding paths lead to breast cancer. Also, these compounds can be used as potential biological markers in the diagnosis, prediction and treatment of breast cancer. Therefore, this paper provides an overview of evaluation of microRNAs, mechanism and relation with other biomarkers in breast cancer.

**Key words:** micro RNA, breast cancer, drug resistance.

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## Introduction

**B**reast cancer is the most common cancer in women, containing approximately one third of all illness in women<sup>1</sup>. It affects one of every 8 women in the United States. Also, it is one of the most frequent malignancies among Iranian women. Intervention of genetic changes in breast cancer has been well documented. Among the eventual changes, mutation and variation in the product of various genes have been documented<sup>1-3</sup>. Also current treatment depends on tumor characteristics including estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)<sup>4</sup>. A class of short, single-stranded and non-protein-coding RNA molecules with 21–25 nucleotides is called microRNAs (miRNAs)<sup>5-6</sup>. Micro RNA plays important role in variety of disease such as tumor initiation, progression<sup>5</sup> migration, invasion and interaction with microenvironment<sup>7</sup>, so that relation between miRNA and cancer have been seen from 0.002% in 2002 to 2% at present<sup>5</sup>. Another study showed the role of miRNA in signaling pathway of cancer progression and specialized metastasis<sup>8</sup>. About 50% of miRNA genes have been located in the introns of genes coding protein or non-coding RNAs transcripts<sup>8</sup>. Some studies showed that both genetic and epigenetic events in deregulation of miRNA can lead to cancer development<sup>9</sup>. Genetic events like deletion, amplification or translocation of miRNA can lead to chromosomal abnormalities<sup>8</sup>. For example, chromosomal deletions or mutations at the 13q13.4 loci due to miR-15a and miR-16-1 have been seen in 70% of patients with chronic lymphocytic leukemia (CLL)<sup>8</sup>. Synthesis of miRNA begins by Pol II of a long primary transcript (pri-miRNA)<sup>8</sup>. Biogenesis of miRNA, due to other shorter non-coding RNAs: tRNAs, 5S ribosomal RNA and U6 snRNA can be mediated pol III, but other reports showed that pri-miRNAs

with their own promoters must be Pol II products<sup>10-13</sup>. These mechanisms are explained in below.

### • miRNA biogenesis and processing

The multiple events is started from nucleus whereas the RNase III enzyme Drosha, coupled with its binding partner DGCR8, cleaves nascent miRNA transcripts (pri-miRNA) into 70 nucleotide precursors (pre-miRNA). Then exporting of Pre-miRNA from nucleus to cytoplasm is performed. In the cytoplasm, the hairpin precursors are cleaved by Dicer and its binding partner the transactivator RNA-binding protein TRBP into a small, imperfect dsRNA duplex (miRNA:miRNA\*) that contains both the mature miRNA strand and its complementary strand. The miRNA strand is incorporated into the miRNP complex and targets complementary mRNA sequences, exerting its functionality via mRNA cleavage or translational repression<sup>14</sup>. Fig 1 shows miRNA biogenesis and processing.

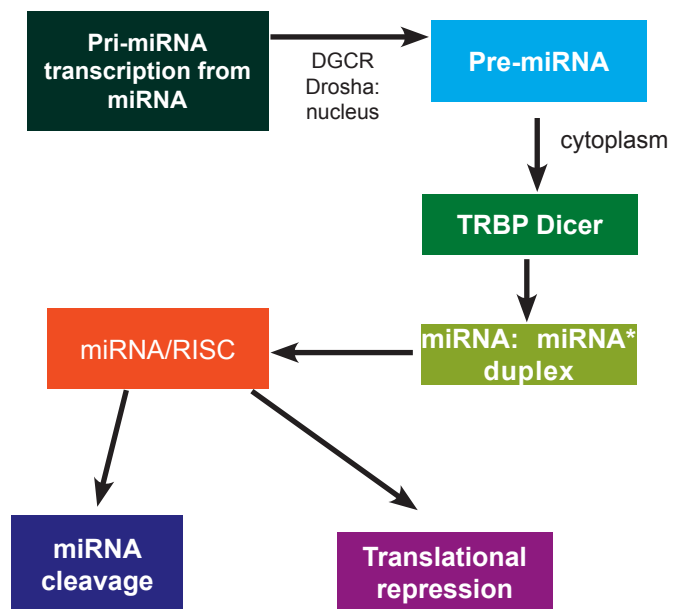


Fig.1: miRNA biogenesis and processing<sup>14</sup>

## Regulation of cell cycle factors by microRNAs in human cancer

Cell cycle regulators frequently act as oncogenes or tumor suppressors. When cell exposed to carcinogen component, P27 by binding to Cdk2-cyclin E prevents transition of G1 to S. Direct target of miRNA 221- and 222 in glioblastomas<sup>15,16</sup> and prostate cancer cells is p27 mRNA. There is an inverse relation between P27 expression and micro RNA-221 and 222. They can act by binding to target sites in the 3'UTR of p27kip1 and inhibit its translation<sup>15</sup>. Up regulation of miR-221 and miR-222 causes competition for initiation of S phase that stimulate cell proliferation. When miRNA expression (target cell cycle proteins) disrupts, it lead to the progression of the malignant phenotype in human tumors. Also other cell cycle proteins including Cdk6, Cdc25A, Cnd2 (cyclin D2), Cdk4, Rb-family protein are regulated by miRNA. Also dysfunction of the cell cycle has been seen in overexpression or inhibition of some miRNAs<sup>8</sup>. P53 as tumor suppressor gene can induce miR-34 family of miRNAs. It participates in DNA damage response. MiR-34 can cause cell cycle arrest via decreasing CDK4 and Cyclin E/CCNE2. Some miRNA with oncogenic properties may act via inhibition of cell cycle inhibitors such as members of the INK4 or Cip/Kip families. miR-24 regulated p16INK4a, a CDK4/6 specific inhibitor<sup>8</sup>. A transcriptionally represses cell cycle transcription factors is retinoblastoma (pRB). It can result to proliferative arrest. Over expression of miR-106a in different human cancers cause down regulated pRB. miR-209 controls P130/RBL2, another member of pRB family that regulates the expression of DNA methyltransferases. P130/RBL2 is also targeted by the oncogenic miR-17-92 and result proper differentiation of adipocytes. miR34-a down regulates protein levels of cyclin D1/ CCND1, CDK6 and induces important G1 cell cycle arrest

in the A549 cell line. Also miR34- via decreasing CDK4 and Cyclin E/CCNE2 protein levels promotes cell cycle arrest<sup>17</sup>. Therefore, disruption of miRNAs expression that target cell cycleproteins, can lead to the progression of the malignancy in tumors of human.

## MicroRNAs in breast cancer

Among expressed microRNAs in breast cancer, some were extensively studied because of initial discovery and significant role in breast cancer. miR-21 is the most important micro RNA over expressed in breast cancer. It mediates cell proliferation via targeting oncosuppressor genes PTEN, PDCD4 and TPM1. Also over expression of it can be associated with advanced clinical stage, lymph node metastasis, poor prognosis, poor survival and poor therapeutic outcome. Studies showed that increased level of expression of miR-21 is found in bone marrow of breast carcinoma. miRNA-21 expression in breast cancer patients is associated with radioresistance acting via compromising cell cycle progression (radiation-induced G2/M arrest)<sup>5</sup>. The association between miR-21 expression and sensitivity of breast cancer cells to doxorubicin has been seen<sup>5</sup>. miRNA-205 is capable to participate in HER receptor family mediated survival pathway via directly targeting HER3 receptor and thus inhibiting its downstream mediator Akt<sup>7</sup>. Over-expression of miR-205 is associated with inhibition of MCF7 cell migration and invasiveness. Also it via apoptosis reduces growth and colony formation of MCF-7 cells<sup>18</sup>. Reduced expression of miR-125b has been seen in breast cancer tissue<sup>8</sup>. miR-191 is over expressed in breast cancer patients. Another study showed that over expression of miR-221 and mi-222 are associated with resistance to anti-estrogenic therapies, as tamoxifen and fulvestran. Oncogenic function of miR-221 and miR-222 is done via targeting the cell cycle inhibitor of P27/Kip1. A relation between increased tamoxifen

resistance and reduced level of P27/Kip1 have been seen via increasing miR-221/miR-222 expression<sup>4</sup>. Over expression of miR-10b, reduced expression of miR-126 and miR-335 are associated with development of breast cancer metastasis and invasion. Endothelial function during embryogenesis and post-natal neo-angiogenesis are done in the presence of miRNA-126. Nygaard et al. identified eight different types of expressed miRNA including miR-200b, miR-200c, miR-21, miR-378, let-7a, miR-320, miR-23a, and miR-22 are deregulated in breast cancer tissue compared to normal breast tissue<sup>19</sup>. Expression of miRNA-145 was down regulated in breast cancer patients<sup>7</sup>. Tumor suppressor micro RNA detected in *C. elegans* is called Let-7. It acts as new regulator of renewal and tumorigenicity of breast cancer cells. Tumor development in mouse model of breast cancer can be suppressed by let-7 miRNA family<sup>7</sup>. Some studies reported that miRNA expression of let-7 is downregulated in lung cancer, but not in breast cancer<sup>20,21</sup>. Another study reported that let-7, miR-34 and miR-17-5p as tumor suppressors down-regulate the expression of the amplified-in-breast-cancer 1 (AIB1) gene and proliferation of cells in breast cancer. The level of miRNA-34 is correlated with metastasis in breast cancer patients<sup>22</sup>.

The role of miR-451 and miR-27 has been proven in resistance to chemotherapeutic agent doxorubicin via MDR-1 in MCF-7 breast cancer<sup>23,24</sup>. Deregulation of miRNA-200c is associated with drug resistance in breast cancer patients<sup>5</sup>. Assessment of miRNA-200c expression in tumors of patients with chemotherapy showed that miRNA-200c was down-regulated in non-responders than responders<sup>25</sup>. Also miRNA-30c have

significant role in chemo resistance through interaction with actin binding protein twinfilin 1<sup>26</sup>. Li et al. showed that decreased expression of miR-720 leads to lymph node metastasis<sup>27</sup>. Over-expression of miR-301a is associated with breast cancer cell migration, invasion and metastasis<sup>5</sup>. It through activated Wnt/b-catenine signal by direct targeting of PTEN causes migration, invasion and metastasis of breast cancer cells<sup>5</sup>. Comparison expression of miRNA-19 in multi drug resistance cell line (MDR) and MCF-7 showed that over-expression of miRNA-19 in MDR cell line modulate chemo resistance through targeting PTEN. Expression of miRNA-155 is associated with breast cancer progression, metastasis, higher tumor grade and stage<sup>28</sup>. Zaleska and et al. in a study showed that the level of miR-155 up-regulated in breast cancer patients<sup>5</sup>. Also they reported that sensitivity of breast cancer cell to irradiation is increased by antisense targeting miR-155. miR-155 can impel epithelial migration, invasion and epithelial mesenchymal transformation<sup>22</sup>. The level of miRNA-31 is lower in breast cancer patients with metastasis<sup>22</sup>. This component can inhibit multiple steps of metastasis. One miRNA that act as tumor suppressor is miR-206. It inhibits cell growth, migration and invasion and induces apoptosis. Therefore, decreased level of miR-206 in breast cancer patients causes advanced clinical stage and shorter overall survival<sup>22</sup>. Over-expression of miR-210 causes aggressiveness and poor prognosis of tumor<sup>29</sup>. miR-373 acts as oncogenes and is expressed in breast cancer with higher lymph node metastasis than primary breast cancer<sup>22</sup>. Also miR-520 is over-expressed in vivo; it acts via promotion of migration<sup>22</sup>. miR20-a as

oncogene can be involved in cell proliferation and invasion<sup>22</sup>. miR92- and miR106- as oncogene through the myelocytomatosis viral related oncogene (MYCN) can be played in malignant transformation. Also miR-127a via s phase increases cell progression. miRNA-132 promotes neovascularization of endothelial cells by inhibiting p120 Ras GAP expression<sup>22</sup>. HOXD-10 can positively regulate miR-7 expression. Its expression is associated with suppression of motility and invasiveness in breast cancer cells<sup>22</sup>. miR-661 expression is con-

trolled by transcription factor c/EBPa. It can act via repressing metastatic tumor antigen 1 (MTA1). Invasion and migration can be inhibited by miR-146a and miR-146b via down regulating of the nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB)<sup>22</sup>. miR-27b via downregulating of cytochrome P4501B1(-CYP1B1) causes activation of procarcinogens<sup>5</sup>. miR-22 can perform metastatic process via silencing antimetastatic miR-200<sup>22</sup>. Table 3- 1 shows summary of most important mi-RNA expression in breast cancer patients.

**Table 3-1: The list of miRNA expression in breast cancer**

miRNA	Expression in breast cancer	Ref
miRNA-21	over-expression	5
miRNA-191	over-expression	30
miRNA-206	decreased-expression	22
miRNA-10b	over-expression	22
miRNA-126	decreased-expression	29
miRNA-335	decreased-expression	29
	over-expression	22
miRNA-125b	decreased-expression	5
miRNA-145	decreased expression	30
miRNA-9	over-expression	22
miRNA-155	over-expression	5
miRNA-301a	over-expression	5
miRNA-193	decreased-expression	22
miRNA-224	decreased-expression	22
Let-7	decreased-expression	30
miRNA-720	decreased-expression	27
miRNA-221	over-expression	4
miRNA-222	over-expression	4
miRNA-342	decreased-expression	30
miRNA-22	over-expression	30
miRNA-23	over-expression	30
miR-181	over-expression	30
miRNA-26a	over-expression	8

miRNA-210	over-expression	30
miRNA-143	decreased-expression	30
miRNA-661	decreased-expression	22
miRNA-31	decreased-expression	30
miRNA-34family	decreased-expression	30
miRNA-182	over-expression	30
miR-27a	over-expression	30
miRNA-520	over-expression	22
miRNA-205	decreased expression	22
miRNA-335	decreased-expression	36
miRNA-448	decreased-expression	22
miRNA-30e	decreased expression	22
miRNA-224	decreased-expression	22
miRNA-200c	decreased-expression	22
miRNA-17-5p	decreased-expression	30
miRNA-196a	over-expression	22
miRNA-132	over-expression	22
miRNA-7	decreased-expression	38
miRNA-92	over-expression	37
miRNA-106a	over-expression	35
miRNA-29	over-expression	22
miRNA-146	decreased-expression	22
miRNA-34a	over-expression	22

## Relation between miRNA and p53, Her-2/neu tumor markers in breast cancer

### • MicroRNAs and p53

Changes in p53 gene are prevalent in many cancers, so that more than 50% of all cancers including breast cancer contain changes in the p53 gene. P53 is known tumor suppressor gene placed on chromosome 17. The p53 gene codes a 53 kDa nuclear phosphoprotein that plays an important role in many critical cellular events related to human aging and cancer including DNA damage, telomere shortening, and oxidative stress<sup>31</sup>. Several crosstalks between p53 and miRNA at multiple levels have been seen. Post-transcriptional maturation of a number of miRNAs including miR-16-1, miR-143 and miR-145 is facilitated by p53. Also p53 promotes transcription expression of many micro RNA like miR-107, miR-192 and miR-215. P53 is down regulated by some micro RNA like miR-504 and miR-125b. Also p53 protein indirectly is regulated by miR-29 with repression of some specific factors. Also expression of miR-34a in p53-pathway is induced by Ionizing radiation<sup>6</sup>.

### • MiRNAs and HER2/neu

Human epidermal growth factor receptor-2 (Her-2/neu) is over expressed in approximately 15-25% of breast cancers<sup>32,33</sup>. It appears that HER-2/neu over expression can be correlated with resistance to hormonal therapy, sensitivity to anthracycline-based chemotherapy and resistance to CMF. Her-2/neu encodes a 185-kDa transmembrane glycoprotein with intracellular tyrosine kinase activity that belongs to the epidermal growth factor receptor family<sup>34</sup>. Studies showed that there is a relation between miR-21 and HER-2/neu up regulation. Regulation of mir-21 is done via mitogen-activated

protein kinase (MAPK) pathway upon stimulation of HER-2/neu signaling in breast cancer<sup>6</sup>.

## Conclusion

Dysregulation of miRNA has been seen in development of breast cancer disease, while they may act as oncogenes or tumor-suppressor genes. Also they play significant role in control of gene expression at posttranscriptional /translational level, but more research is needed to clarify regulatory mechanisms of miRNA biogenesis and their role in initiation, progression and metastasis of cancer. Detection of miRNA molecule targets and their interactions with molecular pathways will help to better understand of cancer mechanisms.

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