MINI-REVIEW

Role of miRNAs in Breast Cancer

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Abstract

miRNAs belong to an important class of endogenous molecules which are present in a wide range of organisms including animals, plants and viruses. They are involved in regulating expression of several genes inside a cell due to presence of complementary region against specific mRNA molecules. Altered expression patterns cause progression of multiple diseases inside an organism. They have also been confirmed to be involved in different cancers including breast cancer. In this review, we discuss role of miRNAs with respect to uncontrolled division of cells, promotion, progression and metastasis in breast cancer.

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Introduction

MicroRNA (miRNA) molecules have recently been revealed as active regulators of gene expression. At post transcriptional level, expression profiles of almost 10-30% of total genes is regulated by miRNAs in eukaryotes. They belong to a class of non-coding RNA molecules, typically 22-25nt long and are transcribed from the genome in multicellular organisms as well as in viruses (Pfeffer et al., 2004). In humans, approximately ~450 miRNA molecules have been confirmed till now (Lim et al., 2003).

miRNA Roles

Biogenesis of miRNA

Biogenesis of miRNA involves transcription of a pri-miRNA precursor usually by RNA polymerase II. An endonuclease enzyme, like Drosha in the nucleus, processes the pri-miRNA and converts it into precursor miRNA (pre-miRNA). These are 80-100nt long sequences containing a stem loop secondary structure. Exportin-5 assists in the transport of pre-miRNAs from nucleus to cytoplasm, where a cytoplasmic ribonuclease dicer cleaves it into double stranded mature miRNA. RNA induced silencing complex (RISC) bound miRNA then regulates the translation of a complementary messenger RNA (mRNA). Mature miRNA recognizes its complementary sequences in the 3` untranslated regions of an mRNA via seed region, typically position 2-7 in the miRNA. Because high complementarity is not required for regulation, a single miRNA can therefore target multiple genes (Ying and Lin 2005; Bartel 2004; Lee et al., 2002; Ruby et al., 2007).

miRNAs Roles in Cellular Processing

miRNAs are involved in various cellular processes including development of heart and skeletal muscles, determination or maintenance of cell lineage. Their tissue specific expression is also observed in hematopoiesis, insulin secretion, adepocyte development, brain pattering (Harfe 2005), proliferation and apoptosis (Bushati and Cohen, 2007). Involvement of miRNAs in diverse cellular events magnifies their importance and indicates that dysregulated expression and function may also lead to development of different diseases as reported in fragile X syndrome (Caudy et al., 2002), Tourette's syndrome and cancers (Calin and Carlo, 2006).

Involvement of miRNAs in Cancer

In humans, almost 200 miRNA molecules are involved in progression of cancers. Cancer is a polygenic disease caused by alterations in tumor suppressor and oncogenes either at genetic, epigenetic or expression levels. miRNA involvement has been observed in almost every type of cancer including lung cancer (Takamizawa et al., 2006), breast cancer (Iorio et al., 2005), papillary thyroid carcinoma (He et al., 2005), gastric carcinoma (Michael et al., 2003) and colon carcinoma (Cummins and Velculescu, 2006). Altered expression profiles of miRNAs in malignant cells have also improved the classification of poorly differentiated tumors, performed using mRNA profiles. Abnormal expression of miRNAs has also been found at the premalignant stages as well. Etiology of dysfunctional expression of these miRNAs in cancers are still not clearly understood (Calin and Carlo, 2006).

Dual Nature of MiRNAs as Tumor Suppressor or Oncogenes

It has been observed that miRNAs act as tumor suppressor genes when their expression is downregulated. For example, the expression pattern of miR-15a and miR-16-1 in the case of chronic lymphocytic leukemias. Similarly, some members of the miRNA family are over-expressed and thus act as oncogenes, as in the case

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of miR17-92 cluster that target the E2F1 oncogene in lymphomas. Since breast cancer is included as one of top five most prevailing cancers across the globe so we have designed a comprehensive literature search to scroll all those miRNAs which are directly or indirectly modulating this disease.

Mechanisms of Action in Breast Cancer

Breast cancer specific miRNAs, with respect to their interactions with fundamental pathways of specific genes and other factors are categorized.

Cellular proliferation, Immunological and anti-apoptotic effects

Toll like receptors are involved in producing immunological response against specific microbial agents. They activate the NF-kB with the help of different downstream signaling molecules including TNFR-associated factor 6 (TRAF-6) and IL-1 receptor associated kinase (IRAK) (Li et al., 2001). Increase level of NF-kB is directly associated with marked reduction of miR-146a and miR-146b (Taganov et al., 2008). Apart from cell survival and increased proliferation such cells compromise immunological traps and increases their metastatic potential (Bhaumik et al., 2008). So antiapoptotic activity of this molecule is also very well addressed in this regard. Up-regulation of Bmi-1 due to miR-200c and miR-128 also renders these cells resistant to apoptosis (Hoenerhoff et al., 2009; Yu et al., 2009).

miR-17-5p and miR-20a target CCND1 (a molecule involved in cell cycle). Expression of miR-17-5p and miR-20a are both downregulated in breast cancer. Cyclin D1 is a protein encoded by the CCND1 gene. It has been suggested that miR-17-5p is involved in suppressing the synthesis of cyclin D1. In breast cancer, this miRNA is down-regulated therefore the control over Cyclin D1 synthesis is not achieved and cell proliferation occurs. p27Kip1, which expresses at high level when the cells are not dividing, is an inhibitor of CDK1. Down-regulation of CDK1 p27Kip1 is important for cells to move into S-phase. miR-221 and miR-222 are up-regulated in breast cancer, which leads to low levels of p27Kip1 and continuous cellular proliferation (Aktas et al., 1997; Lesage et al., 2007). Upregulation of CCND1 leads to hyperactivation of Wnt signaling pathway frequently observed in many types of cancer (Polakis, 2000; Brown 2001).

PI3K/AKT pathway is involved in mediating responses such as inhibition of apoptosis and stimulation of cell proliferation, hence important for the survival of a cell. PTEN is a tumor suppressor gene which functions as a negative regulator of AKT signaling (Ramaswamy et al., 1999). It inhibits cell migration, spreading and focal adhesion formation. Its role as a tumor suppressor gene is also related to it lipid phosphatase activity. An up-regulated miR-21 down-regulates the PTEN. Another target to the same miRNA is the Programmed Cell Death 4 (PDCD4) gene. PDCD4 induces the expression of p21 that acts as an inhibitor of CDK (Frankel et al., 2008). As the name indicates, it has a major role in the process of apoptosis. Altered function of miR-21 inhibits PDCD4 and therefore aids the uncontrolled cellular growth and cancer progression. As a single miRNA may interact with range

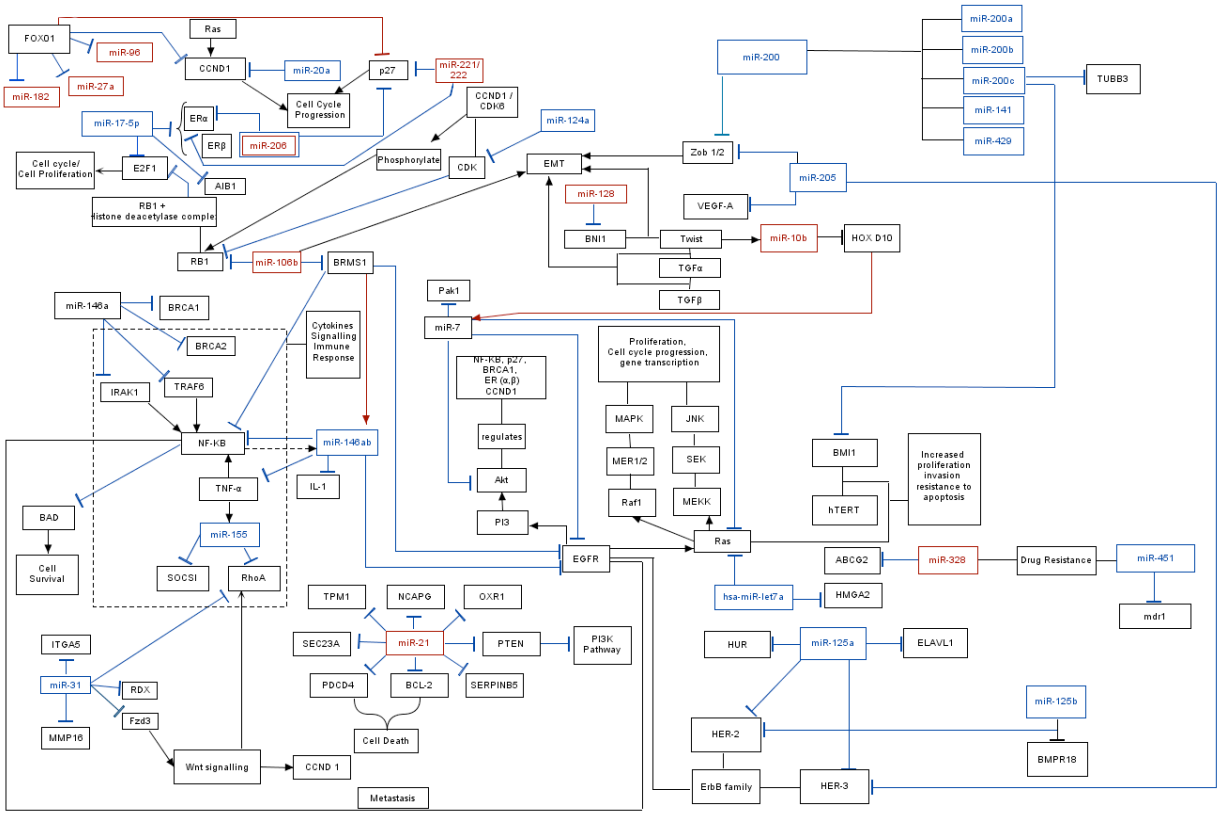

Figure 1. A Pathway Diagram Showing Various Up- and Down-Regulated miRNAs Involved in Breast Cancer Associate Pathways

of mRNAs belonging to different proteins so miR-21 is also involved in targeting various genes other than the ones involved in AKT pathway. Serpin peptide inhibitor 5 (SEPINB5), B-Cell lymphoma 2 (Bcl2), tropomyocin 1 (TPM1), non-SMC condensin I complex subunit G (NCAPG), oxidative-stress responsive 1 (OXSR1), and Sec23 homolog A (SEC23A) are among those genes (Si et al., 2007). Bcl2 has an anti-apoptotic effect and its up-regulation would aid the cells to avoid death. TPM1 is associated with stabilizing the microfilaments. NCAPG is a regulatory subunit of condensing complex.

In canonical Wnt pathway, β-catenin is the key component and its activation leads to transactivation of the downstream target genes (Mohinta et al., 2007). miR-31 is involved in targeting the human frizzled transmembrane receptor, Frizzled-3 (fzd3) and RhoA (Valastyan wt al., 2009). RhoA belonging to Ras family acts as a key regulator of cell adhesion, migration, endocytic trafficking, cytokinesis, gene transcription, and cell proliferation through control of the actin cytoskeleton remodeling and other cellular responses to external stimuli (Katoh, 2005). RHOA is involved in WNT/PCP signaling. Abnormal WNT/PCP signaling pathway in human cancer leads to more malignant phenotypes, such as abnormal tissue polarity, invasion, and metastasis. Aberrant activation of this signaling pathway also leads to poor prognosis of human cancer patients through the induction of invasion and metastasis (Katoh, 2005). Therefore, the role of miR-31 as a tumor suppressor emerges. It is being down-regulated in all different types of interactions in the Wnt pathway. Other pro-metatstatic genes regulated by miR-31 include the ITGA5, MMP16 and RDX (Valastyan et al., 2009).

Mutations in genes controlling cell growth and differentiation are the key players in causing cancers. ras is one such family of genes whose members have been found to be frequently mutated in case of human cancers, including breast cancer. The main function of this family is the regulation of signal transduction pathways that lead to the growth and differentiation of cells. It activates different downstream effectors when in GTP-bound state. After a signal is transferred, GTP is converted into GDP and ras is inactivated (Scheffzek et a., 1997). In case of mutation, the conversion into GDP does not occur and cell is continuously signaled to continue the cycle. Ras phosphorylates the inactivated Retinoblastoma (Rb) gene to activated state and enables cell to progress into the S phase from G1 phase. Deactivated Rb, due to action of Cyclin dependent kinases (CDK), binds to E2F transcription factor and keeps the progression blocked (D'Abaco et al., 2002). Ras also induces the cyclin D1 and reduces the cell doubling time. miR-106b and has-let-7a are two miRNAs that interact with the ras. In case of breast cancer, miR-106b is over-expressed which leads to down-regulation of Rb and ultimately cell cycle progression keeps on (Ivanovska et al., 2008).

Breast cancer type 1 susceptibility protein (BRCA1) has been demonstrated as a negative regulator of growth and it is associated with the hypophosphorylated form of Rb (Aprelikora et al., 1999). Interactions of miR-146a with the 3`UTRs of BRCA1 and BRCA2 mRNA is involved in regulation of their expression. Sequential variations, such as presence of C:U pair instead of G:U in the precursor miR-146a molecule, increase the affinity with which it binds to 3`UTR. Up-regulation of miR-146a in breast cancer may be responsible for the altered expression patterns of BRCA1 (Shen et al., 2008). Another miRNA, miR106b, may also be involved in affecting the BRCA1 and Rb interactions, as this miRNA itself targets the Rb gene. This miRNA is up-regulated in case of breast cancer and can promote cellular proliferation (Pan et al., 2009).

Breast cancer metastasis suppressor 1 (BRMS1) is involved in reducing the metastatic potential of the cells. It keeps inhibiting the cell cycle progression until the cell is ready to divide. Higher levels of miR-106b have been observed in the cancer samples where metastasis has occurred. It is suggested that this particular miRNA is involved in controlling the expression of BRMS1. This gene is also affected by an up-regulated miR-146 (a and b), which is involved in inhibiting the migration as well as invasion of cells under normal conditions (Hurst et al., 2009).

Transcriptional Impairment/Growth

Mitogen-activated protein kinases (MAPKs) are another set of molecules involved in growth, cytokine signaling etc. Epidermal growth factor receptor (EGFR) family, activator of this pathway, has four related members including EGFR/ErbB1, HER-2/ErbB2, HER-3/ErbB3 and HER-4/ErbB4. These have an extracellular domain for interaction with ligands, a helical transmembrane segment, and an intracellular protein tyrosine kinase (TK) domain. Activated receptors, normally after binding to EGF, form homodimer, heterodimer or even oligodimers. The dimerized receptors indicate a phosphorylated TK domain which in turn becomes a docking site for several adaptor proteins and signaling enzymes. Excessive signaling from ErbB is associated with the development to different types of tumors (Atalay et al., 2003). Two de-regulated miRNAs including miR-146a/b and has-let-7a are involved in malfunctioning of this pathway in breast cancer patients. The normal function of miR-146a/b is to reduce the expression of epidermal growth factor receptor (EGFR) and hence the suppression of metastasis (Hurst et al., 2009).

Another oncogenic miRNA, miR-10b, is involved in the tumor invasion and metastasis. A transcription factor known as Twist, is involved inducing this miRNA. This leads towards the expression of RHOC, a pro-metastatic gene, as the expression of miR-10b inhibits HOXD10.

Forced expression of FOXO factors can counteract the proposed antiapoptotic and proliferative effect of PI3K/PKB pathway by triggering either apoptotic responses or a cell cycle arrest (Schmidt et al., 2002). In cells with dividing ability, the members of FOXO family function by promoting the cell cycle arrest at the G1/S boundary (Greer & Brunet, 2005).

FOXO1 transcription factor coordinately maintains the regulation of genes that are involved in apoptotic responses, cell cycle checkpoints, and cellular metabolism. It is a target of miR-182, miR-27a and miR-96 and is a putative tumor suppressor. The expression of miR-182,

miR-27a and miR-96 is up-regulated in breast cancer and the levels of FOXO1 are reduced in breast cancer samples as compared to normal samples. When miRNA silencing enzymes were used, then the levels of FOXO1 were raised. The raised levels of FOXO1 were seen to decrease the cell viability because of inhibition of cell cycle traverse and induction of cell death (Guttilla & White, 2009). It can be concluded that these three miRNA (miR-182, miR-27a and miR-96) act as oncogenes in breast cancer. In breast cancer, the targeting of FOXO1 by the above mentioned three microRNAs contributes to transformation or maintenance of an oncogenic state.

Estrogen binds to its two receptors namely ER-α and ER-β, which are ligand dependent nuclear transcription factors (Hossain et al., 2006). These receptors require transcription co-activators and co-repressors for positive and negative transcriptional activities respectively (McDonnell and Norris, 2002). AIB1, a transcriptional activator in case of estrogen receptors, enhances the activity of ER and E2F1 transcription factors (Louie et al., 2004). It is an oncogene and its amplified levels have been observed in breast cancer. It is suggested that miR-17-5p regulates the expression of both ER and AIB1 gene. In breast cancer, the miRNA profiles indicate significantly lower levels of miR-17-5p. Thus the regulation of the two mentioned genes cannot be achieved and they express at high levels (Hossain et al., 2006).

Epithelial-mesenchymal transitions

Epithelial-mesenchymal transition (EMT) is involved in increasing invasion and metastasis of tumor cells. These transitions allow cells to detach from each other and also increase their mobility. Transforming growth factor (TGFβ), tumor necrosis factor (TNFα) and hepatocyte growth factor act as activators of EMT. Tumor cells or the infiltrating cells are responsible for producing these factors. The main function of these factors is to target the expression of EMT-inducing transcription repressors. Functional involvement of Zinc-finger E-box binding homeobox1 (Zeb1) in regulating invasion and metastasis of human tumors is indicated by Burk et al., 2008. Zeb1 acts as an intracellular transmitter of EMT, while TGFβ and TNFα are the initiators, in breast cancer. Another transcription factor that is involved in the EMT induction is known as Twist.

MicroRNAs which are involved in activating the epithelial differentiation in breast cancer cells include mostly the members of miR-200 family. Among the family, miR-200c and miR-141 are the most important members (Park et al., 2008). Main target for miR-200c is considered to be Zeb1 and Zeb2, as scored by TargetScan (Burk et al., 2008). But it is considered that most the miR-200 family members can repress these genes due to their high similarity in the seed region (Cochrane et al., 2010). Expression of miR-200 family and miR-205 is down-regulated in breast cancer which results in the up-regulation of Zeb1 and Zeb2 genes.

Moloney murine leukemia virus insertion region-1 (Bmi-1), an oncogene and a member of polycomb group of transcription repressors, interacts with the H-Ras and dys-regulates various growth pathways to ultimately cause the induction of transformation in mammary epithelial cells. Bmi-1 is a target of down-regulated miR-128 and miR-200c, which cause the up-regulation of Bmi-1 in tumor cells (Yang et al., 2010; Saeki et al., 2009).

Human Epidermal growth factor receptor 2 (HER2), also known ErbB-2, has been found to be up-regulated in breast cancer. Down-regulation of miR-125a and miR-125b is associated with the malfunction of HER2. miR-125a also targets a stress induced HuR protein, encoded by ELAVLI1 gene. This gene is considered to be involved in tumor-maintenance. The elevated level of HuR in cytoplasm increases the invasiveness of cells during cancer. It also controls the expression of gene causing angiogenesis such as VEGF-A (Guo et al., 2009; Gubin et al., 2010).

miR-125b negatively regulates BMPR1B gene. A disease associated SNP was found in the 3' UTR of BMPR1B gene which generated C/T allele variants. miR-125b differentially regulates the C/T allelic variants BMPR1B. It down-regulates the C allele more strongly as compared to T allele. This disrupts the proper regulation of BMPR1B by miR-125b. The presence of this SNP causes disruption of regulation by miR-125b, increased BMPR1B expression and elevated disease risk. BMPR1B binds bone morphogenetic protein (BMP) and are multifunctional signaling molecules. miR-125b is down-regulated in breast cancer (Sætrom et al., 2009).

An important miRNA which is overexpressed and is involved in causing epithelial-mesenchymal transitions is the miR-10b. This overexpression causes an increase in invasiveness and metastatic ability of tumor cells. miR-10b is induced by a transcription factor Twist. Another example of such miRNA is the miR-128.

Future Prospect s Regarding Drug Resistance

The role of miRNA as a prognostic and diagnostic indicator for human breast cancer is well established but their role in causing cancer drug resistance is still in the process of exploration. These molecules are also intercalated with breast cancer with the acquisition of drug resistance.

The level of miR-451 is inversely related to mdr1. In breast cancer, there is downregulation of miR-451, leading to an increase in mdr1 levels. The product of mdr1 functions as an ATP-dependant efflux transporter whose function is to protect body from environmental toxins. It functions to reduce the intracellular concentration of a wide spectrum of drugs and antibiotics (Kovalchuk et al., 2008). When it is overexpressed in breast cancer, it causes drug resistance of cancer cells to drugs given for breast cancer therapy. Thus, miR-451 functions as an important regulator of mdr1 and to prevent drug resistance by mdr1.

Another miRNA found in association with drug resistance is miR-328. It targets the ABCG2 and negatively regulates its activity. Down-regulation of miR-328 has been observed in breast cancer, which leads to an upregulation of ABCG2. ABCG2 is an ATP binding cassette membrane transporter and is associated in the control of absorption, distribution and clearance of numerous xenobiotics, including pharmaceutical

agents, dietary carcinogens and conjugated metabolites. An overexpression of ABCG2 has been found in drug resistant breast cancer tumor cells. Therefore, miR-328 dysregulation plays an important role in causing multidrug resistance (Pan et al., 2009).

Conclusions

miR-21 as a Candidate Prognostic Marker

miR-21 can be used as a biomarker for the identification cancer as it is found to deregulate many genes leading to development of breast cancer. It acts as an oncogene and is up-regulated in all pathways in breast cancer. Therefore, by targeting miR-21, correct expression of many genes crucial to various pathways can be restored and it can serve as potential target for developing biomarker for diagnosis of breast cancer.

miR-146a/b as Candidate Prognostic Marker

miR-146 a/b can also be used as a biomarker for breast cancer identification since it is deregulated in various pathways leading to the development of breast cancer. It acts as a tumor suppressor gene. Thus, by correctly targeting miR146 a/b, the proper functioning of its target genes can be restored.

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