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Original Paper

MicroRNA-124 Overexpression in Associated with Lymph Node Metastasis in Breast Cancer

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ABSTRACT

Breast cancer as a heterogeneous sophisticated disease includes several group with discrete clinical consequences. The disease is the most prevalent malignancy after non-melanoma skin cancers and it is also considered as the second leading cause of death after lung cancer. In fact, breast cancer is account for 23% of all cancer cases and 14% of deaths from cancer. The major cause of breast cancer deaths is actually metastasis of the tumor. As a result, it is prominent to identify the disease mechanism and diagnose molecular tools in order to predict metastasis. The specimens were collected from 30 metastatic and 30 primary tumor tissues of breast cancer patients. After that, RNA extraction was accomplished by means of GeneAll kit and then was stored in -80 degrees. Then, cDNA synthesis was carried out by miscript II RT kit from Qiagenecompany. Finally, sybergreen Real Time PCR of all samples was done for miRNA124, miRNA130a and miRNA 16 as a reference by means of Pre-designed primers of Qiagene Company. The results of molecular expression study showed that the amount of miRNA 124 in metastatic tissues has approximately increased double of primary tumor tissues. It is also revealed that the amount of miRNA has similarly increased by about 1.7 times. According to recent results, it can be possible to regard molecule as a major cause of metastasis process in breast cancer.

Keywords: MiRNA, Metastasis, Breast Cancer

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Breast cancer is the most frequent cancer and second most common cause of cancer related death in women worldwide (1). Although breast cancer is not a lethal cancer by self, metastasis to distant organs is the main cause of breast cancer mortality and is associated with poor prognosis in breast cancer patients (2-4).

Spreading of tumor to auxiliary lymph node is one of the most important factors predicting metastasis of tumor cells (5, 6). Conventional therapeutic strategies includes assessment of local lymph node

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involvement by neoplastic cells. This helps to determining of cancer stage, prognosis and survival of patients (7). So, understanding of molecular mechanisms underlying metastasis of breast cancer has a vital importance to prevention, early diagnosis and also improving of therapeutic strategies (2, 7).

Furthermore, during metastatic cell colonization in destination organ some cancerous phenotypes of cancer cells reverse to make possible metastatic cells colonization (6). Recent fast growing number of evidence has supported the role of miRNAs as an important contributor to many human complex diseases including breast cancer (1, 8).

In last decade studies have shown that MicroRNA expression signature abnormalities are associated with tumor initiation, development, progression, invasion, metastasis and response to therapy (9, 10). Therefore these molecules can be used as diagnostic, prognostic and also predictive biomarkers (10-12). MicroRNAs (miRNAs) are short non-coding RNAs of 21–25 nucleotides that regulate gene expression post-transcriptionally by binding to the 3′ untranslated region (UTR) of target mRNAs (13, 14).

Several studies have shown that miRNA expression profiling can discriminate between normal breast tissue and breast cancer (14). Furthermore evaluating of miRNAs profile can predict occurrence of tumor metastasis. These studies suggest potential applications for certain miRNAs as biomarkers for breast cancer metastasis (2, 10).

MicroRNA-124 is one of the interesting research topics in cancer development and prognosis in several cancers. This MicroRNA is known as a tumor suppressor in several cancers including breast cancer (15). cellular models show that increasing of this microRNA is associated with increasing of cell motility and invasion of tumor cells.

However, there is no information about microRNA-124 expression change during metastasis process. Decoding of these changes may help better understanding of process of metastatic cell invasion and also colonization in destination organ.

To further investigation of these processes, in this experiment we compared the expression levels of miRNA-124 in primary breast cancer tissues and metastatic tumors.

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Methods

Tissue sampling

Thirty fresh breast tumor tissue, and 30 tumor cell bearing lymph node samples were gathered from pastor-no and Qaem hospital of Mashhad, Iran. This study design was approved by local ethics committee of Mashhad University of medical sciences and all of patients signed informed consent. None of patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. Identity of tissue samples were confirmed by pathologist. Samples were snapped frozen and stored in -80°C until use.

RNA extraction and quantitative real-time PCR

miRNAs from tissue samples was extracted using Hybrid-R miRNA isolation Kit (GeneAll, South Korea) according to the manufacturer's instructions.

Then, cDNA synthesis was carried out with miscript II RT kit (Qiagen, Germany) following the manufacturer's protocol. Quantitative RT-PCR was performed using sybergreen based miScript Precursor Assays kit (Qiagen, Germany) and Prime Q-Master mix (Amplicon, Denmark) in a StepOne Real-time PCR system. MIR-16 was used as endogenous reference gene and expression changes were calculated using DDCt method.

Statistical analysis

Independent T test was performed to investigate significant difference in MIR-124 expression levels between primary breast tumor samples and metastatic tissues. $P<0.5$ was defined as significant frequency difference between the two groups.

Result

To investigate the expressional behavior of miR-124in human breast cancer metastasis we compared miR-124 expression level between breast cancer tumors and metastatic lymph nodes from 60 patients. Quantitative real-time PCR analysis showed that miR-124 expression increase in metastatic lymph nodes specimens in compare with primary breast tumor tissues (Figure 1).

Discussion

Breast cancer metastasis to distant organs is a very complicated process that significantly reduces survival rate of breast cancer patients (2, 7). Fully characterization of metastasis process has a vital importance to prevention of this process (2, 16).

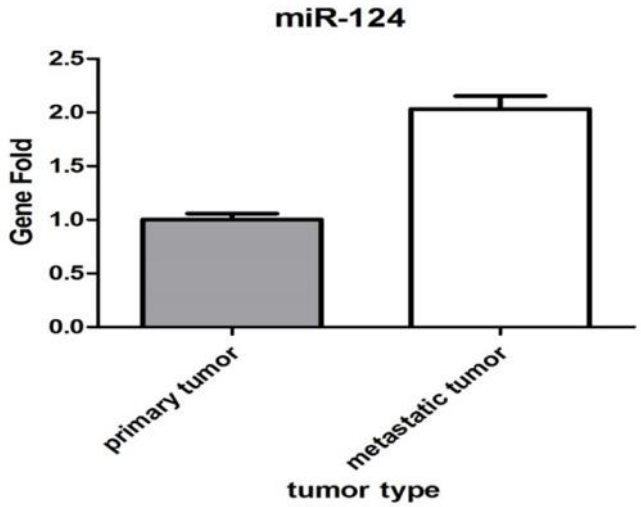

Figure 1: The expression of miR-124 was determined by quantitative real-time PCR (60 metastatic breast cancer and tumor tissues)

Colonization of metastatic calls into target tissues has a reverse order of biologic steps involving in metastasis. Mesenchymal to epithelial transition is one of the most important steps in implantation of metastatic cells into target organs. This process includes the transition from motile, multipolar or spindle-shaped mesenchymal cells to planar arrays of polarized cells called epithelial. Inhibiting of this process can help to control of tumor metastasis as a therapeutic strategy in treatment of breast cancer.

Several pathways contribute in MET process which can be candidate as therapeutic target (17-19).

MicroRNA-124 is a relatively known tumor suppressor microRNA which represses tumor growth, invasion and metastasis. microRNA-124 reduces Breast cancer epithelial to mesenchymal transition (EMT) via targeting of Slug gene (20). Furthermore, this microRNA targets TNF-α and inhibits EMT in prostate cancer cells (21).

miR-124 down regulation in breast cancer cell lines leads to increase of migration and invasion of cells. Furthermore reduction of miR-124 in primary breast tumor tissues is associated with increase of tumor metastasis (18, 22).

Conclusions

Surprisingly, our results obtained by comparing of miR-124 expression level in primary breast cancer cells and metastatic cells show increasing of miR-124 expression level in lymph node metastasis samples versus primary tumors.

In conclusion this results suggest that miR-124 can be an important player in orchestrate of metastasis.

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