

# Expression of microRNA-370 in human breast cancer compare with normal samples

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## Abstract

**Background:** Breast cancer is the second leading cause of deaths from cancer in the woman. MicroRNAs (miRNAs) are endogenous noncoding RNAs that are known critical player in carcinogenesis. The role of miR-370 in malignancies remains controversial because of its levels varying in different cancers according to its targets while the role of miR-370 in breast cancer has not been addressed so far. The aim of this study was to identify the expression pattern of miR-370 in human breast cancer tissue compared to adjacent healthy tissue.

**Materials and Methods:** Twenty-two fresh frozen tissues (normal and malignant) from patients with breast cancer were examined for miR-370 by quantitative real-time polymerase chain reaction method at 2013.

**Results:** We observed up-regulation (six-fold higher) of miR-370 in breast cancer tissue compared with normal adjacent tissue. Tumor samples in stage III, invasive ductal type, larger tumor size, human epidermal growth-factor receptor 2+, estrogen receptor/progesterone receptor-, P53 - status showed significantly increased expression in miR-370.

**Conclusion:** Together, miR-370 may acts as an onco-miRNA, and it may have a novel role in breast cancer. Detection of miR-370 and its targets could be helpful as a diagnostic biomarker and therapeutic target.

**Key Words:** Biomarker, breast cancer, microRNAs

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## INTRODUCTION

Cancer is a subtype of diseases that a group of cells grown out of control and has the potential to invade or spread to other parts of the body.<sup>[1]</sup> Based on the results obtained from American Cancer Society in 2013, breast cancer in women and prostate cancer in men are the highest rates of new cases of cancer.<sup>[2]</sup> In addition breast, cancer is the second leading cause of

deaths from cancer after lung and respiratory tract cancer in woman.<sup>[3]</sup> The number of breast cancer is rising about 3–4% in developing countries. Breast cancer is one of the most common cancer types that affect 21.4% of the woman in Iran.<sup>[4]</sup> On the other hand, breast cancer is generally classified into different categories based on the gene expression profile, phenotype and susceptibility to therapy. Dietary, lactation and environmental factors, which it can be

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put a person at risk for cancer. Unfortunately, up to now, the causes of breast cancer are not completely understood of latest factors, the most important factor that cause cancer is the genetic changes in main gene such as suppressor gene, growth regulators, and oncogenes.<sup>[3,5]</sup>

Some studies has been shown that MicroRNAs(miRNAs) are highly conserved small (18–24-nucleotide) and single-stranded noncoding RNAs that mediate posttranscriptional gene regulation and leading to degradation or translation repression of target messenger RNAs (mRNAs) by recognition of 3' untranslated region of mRNAs.<sup>[6,7]</sup> About 3.0% of human genes encode for miRNAs, and up to 30.0% of human protein coding genes may be regulated by miRNAs. miRNAs play a key role in diverse biological processes including development, cell proliferation, differentiation and apoptosis. Therefore, altered miRNA expression is likely to contribute to human disease, including cancer.<sup>[6,8]</sup>

In cancer, miRNAs function as regulatory molecules, acting as oncogenes by down-regulate tumor suppressors or other genes involved in cell differentiation or they acting as tumor suppressors by down-regulate different proteins with oncogenic activity.<sup>[8-11]</sup> Several miRNA are associated with breast cancer. Studies indicated that miRNA could play a role as a tumor suppressor or inhibit breast cancer cell migration, as well as invasion.<sup>[12,13]</sup> In addition, it has been shown that there are differences not only between normal and breast cancer tissue, but also between different breast cancer subtypes.<sup>[14-17]</sup>

MicroRNA-370 is involved in several biologic processes. The role of miR-370 in malignancies remains controversial; it has been verified as a tumor suppressor in papillary thyroid carcinoma, colorectal cancer,<sup>[18]</sup> oral squamous carcinoma cells<sup>[19]</sup> and malignant cholangiocytes.<sup>[20,21]</sup> In contrast, substantial evidence demonstrates that overexpression of miR-370 in gastric carcinoma,<sup>[22]</sup> prostate cancer<sup>[23]</sup> and acute myeloid leukemia that contributes to the progression of malignancies.<sup>[24]</sup>

The role of miR-370 in breast cancer has not been addressed so far. Hence, this study aimed to identify first analysis of tumor-specific miR-370 expression in human breast cancer tissue compare to adjacent normal tissue to confirm proposed potential of miR-370 expression level as a diagnostic biomarker of breast cancer in these patients. This could be serve a basis for functional studies of the role of miR-370 in the etiology or even diagnostic and treatment of breast cancer.

## MATERIALS AND METHODS

### Patients and samples

The study was approved by the Ethic Committee of Isfahan Medical University. Necessary information such as the chemotherapy, age, menopausal status, disease history was collected using the information contained in the medical record and histopathological data including stage of disease, human epidermal growth factor receptor 2 (HER2)/estrogen receptor (ER)/progesterone receptor (PR) status were prepared with pathological report. Twenty-two fresh frozen tissues (normal and malignant) from patients with breast cancer were obtained from the National Tumor Bank of Iran at 2013.

### RNA extraction

A volume of 50 mg of tissue homogenized by liquid nitrogen in a sterile mortar and pestle and the resulting powder transferred to 1.5 mL sterile micro tubes. Small RNA (<200 nucleotides) was extracted from tissue powder using the hybrid-R™ miRNA kit (GeneAll®, Korea) according to manufacturer instructions. Quality and quantity of small RNAs were checked on 1.0% agarose gels and a nanodrop spectrophotometer (NANO SPEC CUBE biophotometer, German). RNA samples were dissolved in RNase-free water and stored at –80°C.

### cDNA synthesis and real-time polymerase chain reaction

RNA (1.5 µg) was used for cDNA synthesis in a poly-A tail manner using Parsgenome miR-Amp kit (Parsgenome, Iran) following the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out in an ABI7000 sequence detector (Applied Biosystems, Foster City, CA, USA).

The RT-PCR Syber Green master mix was made on ice in a final volume of 10 µl consisting of primers (10 pmol/µl). Then cDNA (20 ng/µl) were added to it following the manufacturer's protocol (Parsgenome, Iran). All experiments were performed in triplicate. Thermal cycling conditions were: Initial denaturation at 95°C for 30 s, followed by 40 cycles of amplification at 95°C for 5 s and 20 s at 65°C and then 30 s at 72°C. The primer sequences used are available upon request (Parsgenome, Iran) [Table 1]. 5s rRNA was used as a reference to normalize RT-PCR data for miRNA analysis.<sup>[25]</sup>

### Statistical analysis

The mean difference of the miR-370 expression between normal and malignant tissue was determined using the REST 2009 software (version 2.0.13-Qiagen) by Corbett Life Science, a QIAGEN Company. For

all tests, a  $P < 0.05$  was considered statistically significant.

## RESULTS

### MicroRNA expression

The miR-370 expression pattern was performed on twenty-two normal and malignant tissues from patient with breast cancer by qRT-PCR method. Expression data of miR-370 were normalized with corresponding mean value of 5s rRNA as an endogenous gene.<sup>[25]</sup> Tissue samples were fresh frozen and collected from National Tumor Bank of Iran. All of the patients were female with a mean age 46.9 years old (range: 32–77). Patient demographic characteristics are shown in Table 2.

MicroRNA-370 expression level was significantly elevated in breast cancer tissue compare with normal adjacent tissue; Amplification plot is shown in Figure 1. The median level of miR-370 expression

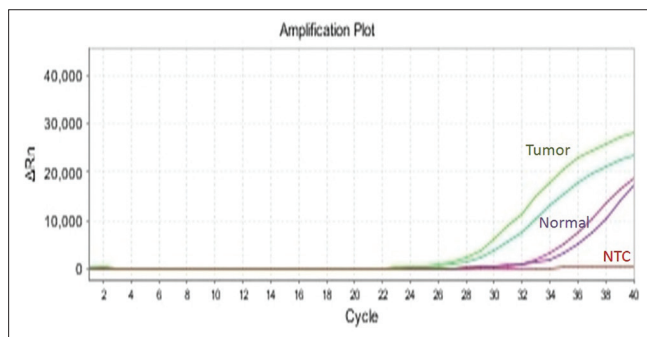
**Table 1: Primers sequence**

Primers name	Primers sequences (5'-3')
hsa-miR-370	F: GCCTGCTGGGGTGGAACTGGTAA R: GCGAGCACAGAATTAATACGAC
5S rRNA	F: GCCCGATCTGTCTGATCTC R: CCTGACCCTGCTTAGCTTCC

miR-370: Microrna-370

**Table 2: Patient demographic characteristics**

Demographics	Number of samples (%)
Age (years)	
Mean	46.9
Range	32-77
Menopausal status	
Post	6 (27.3)
Pre	16 (72.7)
Family history	
Negative	10 (45.5)
Positive	12 (54.5)



**Figure 1:** Amplification plot of microRNA-370 in tumor and normal breast tissues, nontemplate control that the control sample without DNA was seen in bottom of the curve

in tumor samples was six-fold higher than the median level of the control group and is shown in Figure 2 ( $P = 0.011$ ). We classified the tumor samples as ductal *in situ* carcinoma (DCIS) ( $n = 7$ ) and invasive ductal carcinoma (IDC) ( $n = 15$ ). miR-370 was found to be differentially expressed in the tumor subtypes. Significantly difference was observed between IDC group and normal group ( $P = 0.042$ ) whereas no significant difference were observed between DCIS and control groups ( $P = 0.19$ ).

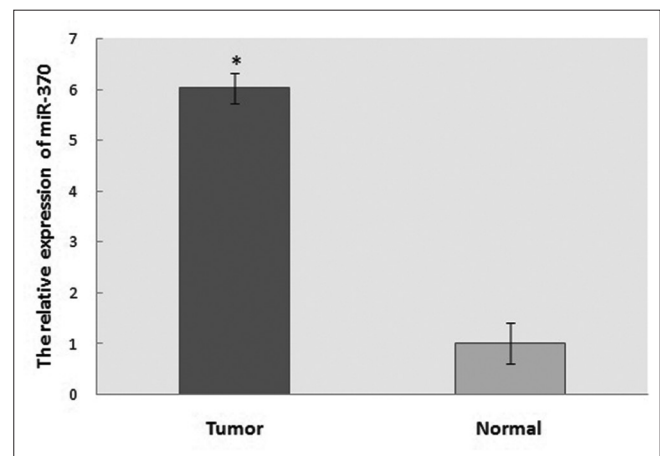
### Tumor characteristics

Tumor characteristics such as stage, tumor size, lymphatic invasion, ER/PR status, P53 status and HER2 status are summarized in Table 3. With regard to clinical stage, tumor samples in stage III, results were showed that expression of miR-370 significantly increased ( $P = 0.004$ ). While despite increased expression in the sample at stage I–II, increased expression was not significant ( $P = 0.297$ ) [Table 3].

Moreover, miR-370 overexpression was correlated with larger tumor size, HER2+, ER/P– status, P53– sample and which it was significantly expressed higher in these sample ( $P < 0.001$ ,  $P < 0.001$ ,  $P = 0.001$ ). In turn, we could not find any strong associations between expression of miR-370 in the sample with or without lymphatic invasion ( $P = 0.054$ ,  $0.071$ ).

## DISCUSSION

Cancer is a most important public health problem in the world,<sup>[2]</sup> and many miRNAs have been found to have links with some types of cancer.<sup>[26-28]</sup> Deregulated miRNA expression has been documented in a wide range of cancers. Thus many researchers tend to have a profile of miRNA in tumor tissues in recent



**Figure 2:** The relative expression of microRNA-370 (miR-370) in tumor and normal breast tissues, miR-370 expression was normalized against an endogenous control 5s rRNA. \* $P < 0.05$

**Table 3: miR-370 expression ratio in groups according to the clinical and pathologic parameters**

Tumor characteristics	Number of samples (%)	miR-370 fold change	P
Histology			
DCIS	7 (31.82)	5.551	0.19
IDC	15 (68.18)	4.661	0.042
Stage			
I-II	17 (77.27)	2.248	0.297
III-IV	5 (22.72)	171.73	0.004 UP
Tumor size (cm)			
≤2	2 (9.09)	8.056	0.18
>2	20 (90.91)	5.58	0.013 UP
Distant metastasis (M0/M1)			
Positive	0	-	-
Negative	22 (100)	6.023	0.011 UP
LN			
Positive	11 (50)	7.621	0.054
Negative	11 (50)	4.76	0.071
Hormone receptor status			
Positive (ER+ and/or PR+)	7 (31.82)	3.875	0.41
Negative (ER- and PR-)	7 (31.82)	21.727	<0.001 UP
Missing	8 (36.36)	0.347	0.393
HER2-IHC status			
Positive	7 (31.82)	97.006	<0.001 UP
Negative	7 (31.82)	0.868	0.918
Missing	8 (36.36)	2.883	0.379
P53			
Positive	7 (31.82)	1.359	0.818
Negative	6 (27.27)	100.775	0.001 UP
Missing	9 (40.91)	2.93	0.313

DCIS: Ductal carcinoma *in situ*, IDC: Invasive ductal carcinoma, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, IHC: Immunohistochemistry, P53: Tumor protein p53, LN: Regional lymph nodes invasion, miR-370: MicroRNA-370

years.<sup>[16]</sup> Research into the causes, prevention, and treatment of breast cancer is being done in many medical centers throughout the world. Paradoxical roles of miR-370 had shown in various cancers, but its role (as onco-miRNA or tumor suppressor) in breast cancer has not been shown so far. In order to investigate whether miR-370 are differentially expressed in breast cancer tissue versus normal breast tissues, we analyzed miR-370 expression pattern in twenty-two human breast cancer tissue compare to adjacent normal tissue by qRT-PCR method.

In the present study, in agreement with other studies<sup>[29]</sup> we showed that Iranian patient with breast cancer were at the lower mean age (mean 46.9 ± 11.2 years) in compared with 64 years in the USA and 48–50 years in China.<sup>[30]</sup>

In addition, an average of six-fold up-regulation observed in tumor tissue compared with normal tissue [Figure 2]. Our findings are largely in accordance with the previous findings for a number of malignancies,

including prostate cancer,<sup>[23]</sup> gastric carcinoma,<sup>[22]</sup> acute myeloid leukemia,<sup>[24]</sup> and Wilms tumor<sup>[31]</sup> which in these studies miR-370 up-regulated and acts as an onco-miRNA. Therefore, we suggest this miR-370 could be invented in different cancer, and it does important to be considering as a main factor for diagnosis or treatment issue.

Regarding tumor characteristics such as clinical stage, subtype and tumor size, tumor samples with larger tumor size, higher clinical stage (III) and invasive type, a significantly increase expression in miR-370 has been found. Previous evidence showed up-regulation of miR-370 was correlated with higher stage (III) and tumor progression.<sup>[22-24,31]</sup> Controversy, other research showed that miR-370 was down-regulated in some malignancies such as hepatocellular carcinoma,<sup>[32]</sup> cholangiocarcinoma,<sup>[20,21]</sup> colorectal cancer cell line,<sup>[18]</sup> central nervous system tumor-derived cell line,<sup>[33]</sup> oral squamous cell carcinoma.<sup>[19]</sup> Hence, we suggest that up-regulation of miR-370 may possibly associate with an aggressive disease phenotype and/or function as an onco-miRNA in breast cancer.

Different studies reported miR-370 targets such as MAP3K8 and WNT10B (human cholangiocarcinomas),<sup>[20,21]</sup> FOXO1 (prostate cancer),<sup>[23]</sup> WTX (Wilms tumor)<sup>[31]</sup> transforming growth factor beta receptor II (gastric carcinoma),<sup>[22]</sup> insulin receptor substrate 1 (oral squamous cell carcinoma),<sup>[19]</sup> neurofibromatosis 1 and FOXM1 (acute myeloid leukemia),<sup>[24,34]</sup> BAX and AKT1 in colorectal cancer cell line,<sup>[18]</sup> thus, it suggests that miR-370 targets might have different role in progression or inhibition of cancer; some of them act as tumor suppressor and others as oncogenes. Therefore, the contradictory effect of miR-370 on malignancy may be due to the ability of miRNA to regulate the expression of multiple target genes that have different function. However, it might be the dominant event depends on cell type or cellular contexts.

Moreover, expression levels of ER, PR, and HER2 receptors use for classification of breast cancer and selection of adjuvant treatment.<sup>[35,36]</sup> In addition, their expression could have an effect on chemotherapy sensitivity and change during disease progression and have an effect on chemotherapy sensitivity. Now researchers have particular interest to classification and predict disease progression of breast cancer according to the miRNA expression profiling.<sup>[16]</sup> Our work seems the first findings of an association between miR-370 overexpression and HER2 positivity, ER/PR- negative status, P53- negative samples. However, we did not find any strong associations between miR-370 expression and nodal involvement.

Previous miRNA profiling studies in breast cancer have identified associations between the sets of miRNAs with HER2 or ER/PR status.<sup>[37]</sup> As well as miR-30 expression and miR-520 g down-regulation correlated with ER and PR status, miR-342 up-regulate in ER and HER2 positive status and let-7f-1, let-7a-3, let-7a-2 in lymph node metastasis.<sup>[16,38]</sup> Hence, the association of specific miRNAs with ER, PR and HER2/neu status indicates a role for these miRNAs in disease classification of breast cancer and regulation of ER and HER2 by miRNAs, known to be of prognostic significance in breast cancer, has been demonstrated.<sup>[16]</sup>

Overall, this research showed up-regulation of miR-370 in breast cancer. Hence, it suggests another mechanism involved in the pathology of breast cancer and demonstrated that miR-370 up-regulation exhibits clinical relevance as it is linked to advanced breast cancer. Since higher levels of miR-370 are found in higher tumor stages (III), we propose a novel role of the miR-370 in development the cancer. Detection of miR-370 and its association with tumor stages may be useful as a diagnostic biomarker. Further investigations with larger sample sizes are still required to provide more evidence regarding its role in the development and progression of breast cancer, as well as in their potential use for diagnosis. Research to identify and validate its specific downstream mRNA targets and its targeting *in vivo* should be evaluated to determine if miR-370 has potential as a novel effective therapeutic tool against breast cancer.

## CONCLUSION

Collectively, the present study showed that miR-370 may act as an onco-miRNA, Hence, the onco-miR370 and its association with tumor stages may be useful as a diagnostic biomarker. More researches need to be done for evaluating a novel effective therapeutic tool in defeating breast cancer.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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