

KMT2D and IGF2 Genes Expression in Breast Cancer Patients

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Abstract

Background: Breast cancer which is often a cancer in women is one of the most important public health problems and 1,384,155 new cases worldwide are associated with 459,000 deaths. Breast cancer is highly heterogeneous in its pathological characteristics. Current predictions and statistics suggest that both worldwide incidence of breast cancer and related mortality are on the rise. So far, many studies have been conducted on various genes in breast cancer that can help to treat this cancer.

Methods: The gene expression of KMT2D and IGF2 were investigated in 35 samples with breast cancer after genomic RNA extraction and synthesis cDNA using quantitative real-time PCR method.

Result: There was no significant difference in the expression of KMT2D and IGF2 genes in breast tumor samples compared to adjacent normal samples ($P > 0.05$). However, this study was designed as a pilot study, and further investigations are required to confirm our findings.

Keywords: Gene Expression; KMT2D; IGF2; Quantitative Real-Time PCR Method; Breast Cancer.

I. INTRODUCTION

Among all types of cancers, breast cancer, which accounts for 23% of all cancers in women, is the most common cancer and the most deadly malignancy among women, and is one of the most important causes of women's health in the world (Nafissi et al. 2012, Banegas et al. 2012). The incidence of breast cancer in the United States and Europe is twice as high as in the Asian countries and its prevalence is increasing in all countries (Porter 2008). Breast cancer incidence rates increase sharply with age, becoming substantial before age 50 years (Colditz and Rosner 2000). Approximately 182,000 women with breast cancer are diagnosed in the United States annually, accounting for roughly 26% of the incidence of cancers among women, and 40,000 women die of breast cancer every year and the second cause of death Cancer in American women after lung cancer. The lifetime risk of dying of breast cancer is approximately 3.4% (Jemal et al. 2008). Clinically, breast cancer is classified according to the morphological characteristics of the tumor to infiltrating

ductal carcinoma of no special type and a large number of 'special types' such as infiltrating lobular carcinoma, tubular, mucinous, medullary, and adenoid cystic carcinoma (Tao et al. 2015). Breast tumors have been identified based on the expression of estrogen receptors (ER) and progesterone (PR) receptors and heron oncogenes in five different subtypes. The ER positive tumors are more common than the ER-negative tumors. Also, the ER positive tumors are smaller and low grade and lymph node negative unlike the ER negative tumors (Anderson et al. 2002). Evidence suggests that tumor progression is controlled by genetic, epigenetic and environmental factors (Barrow and Michels 2014, Tao et al. 2015). In the last decade, studies confirm the presence of multiple gene and effective in breast cancer samples and some of them have been conducted on KMT2D and IGF2 genes in breast cancer (Liu et al. 2015, Ellis et al. 1998). KMT2D (lysine (K)-specific methyltransferase 2D), formerly named MLL2 (myeloid/lymphoid or mixed-lineage leukemia 2, also known as ALR/MLL4), is a histone methyltransferase that plays an important role in regulating gene transcription. In

particular, it targets histone H3 lysine 4 (H3K4), whose methylations serve as a gene activation mark (Guo et al. 2013). Different findings suggest that KMT2D acts as a tumor suppressor gene in cancer cells (Augert et al. 2017). Additional cancers that have recently been found to be driven by an aberrant KMT2D/KMT2C pathway, with frequencies ranging from 5% to 40%, include renal (Dalglish et al. 2010), prostate, bladder (Gui et al. 2011), gastric, hepatic, lung (Guo et al. 2013) and breast cancer (Kim et al. 2014). The IGF2 gene acts as an oncogene in cancer cells (Chava et al., 2012). The gene operates in normal body tissues in pathways such as the RAS/MAPK pathway, the PI3K/AKT pathway, and the AKT/mTOR pathway (Fernandez and Torres-Alemán 2012). Studies

have shown that IGF pathway involvement is present in a wide range of malignancies, including breast cancer (Shetty et al. 2011).

II. MATERIAL AND METHODS

2.1 Human specimens

Human breast cancer specimens (n = 35) and adjacent non-tumor tissues were obtained from patients at Imam Khomeini Hospital, Tehran, Iran. With informed consent from each patient. Patient demographic and clinic pathologic characteristics are shown in Table 1.

Table 1. Clinic pathologic characteristics of thirty-five breast cancer patients.

Variable	Value (n = 35)
Gender	
Female	35 (100%)
Smoking history	
Never-smoker	28 (80%)
Smoker	7 (20%)
Age	
30-40	5 (14.2%)
40-50	10 (28.6%)
50-60	12 (34.3%)
60 < n	8 (22.9%)
Stage	
I/II	19 (54.3%)
III	16 (45.7%)

2.2 RNA extraction

Total RNA was isolated from each tumor tissue and adjacent non-tumor tissue by using RiboEx (Gene All, Korea) according to the manufacturer's specifications. The concentration of total RNA in the final eluate was determined by spectrophotometry and the absorbance 260/280 ratio was controlled between 1.8 and 2.0.

2.3 cDNA preparation

The synthesis of cDNA (240 ng of total RNA per 20 µL reaction mixture) was performed using the Prime Script RT reagent kit (Perfect Real Time) RR037A (Takara, Japan) according to the manufacturer's specifications. The obtained cDNAs were stored in -80°C until use.

2.4 Real-time quantitative PCR

Real-time PCR was performed using an StepOnePlus™ Real-Time PCR Systems (ABI Applied Bio-systems, Thermo Fisher Scientific, USA) in a 15-µl reaction containing 7.5-µl of RealQ Plus 2x Master Mix Green High ROX™ (Ampliqon, Denmark), 1-µl of cDNA, 5.5-µl of H₂O and 1-µl of mixed forward and reverse primers (3 Pmol/µl concentration). Real-time PCR amplifications were done as follows: for two selected genes, PCR amplification was set to an initial 95°C for 15 min and then for KMT2D, and IGF2 genes, a total of 40 cycles, 95°C for 15 seconds and 60°C for 1 min (step and hold). All samples were analyzed in duplicate. GAPDH was used as an internal control. Gene expression was calculated using the comparative threshold cycle ($2^{-\Delta\Delta CT}$) method. The primers

used for real-time PCR are listed in Table 2 (Ahmadi et al. 2018).

Table 2. Primer sequences of three selected genes and GAPDH.

Target genes		Sequences (5' → 3')	Product length
KMT2D	Forward	GCCTGGCTTTGGTGGTTTCA	96bp
	Reverse	CCATCCCCACTCAACACCTC	
IGF2	Forward	CCGACTTCCAGACACCAATG	183bp
	Reverse	CGGTCCTGCTGAAGTAGAAG	
GAPDH	Forward	CATCAAGAAGGTGGTGAAGCA	120bp
	Reverse	GCGTCAAAGGTGGAGGAGTG	

2.5 Statistical Analysis

Statistical analysis was performed using the GraphPad Prism v7.03 (GraphPad Software Inc., USA) and T-test. For all tests, a *P* value <0.05 was considered statistically significant.

III. RESULTS

3.1 The status of KMT2D gene expression in breast cancer tissues with qPCR method

In this research, we investigated the gene expression of KMT2D in breast cancer tissues by using quantitative

polymerase chain reaction (qPCR) which included 35 tumor samples and 35 adjacent normal samples. This study showed that there is no significant difference between expression of this gene in tumor samples compared to normal samples, so that KMT2D showed a decreased expression in 17 (48.5%) tumor samples in comparison with the normal adjacent sample and also showed an increased expression in 18 (51.5%) tumor samples compared with the normal samples (*P*=0.4), (Fig. 1 and 3A show the results of the KMT2D gene).

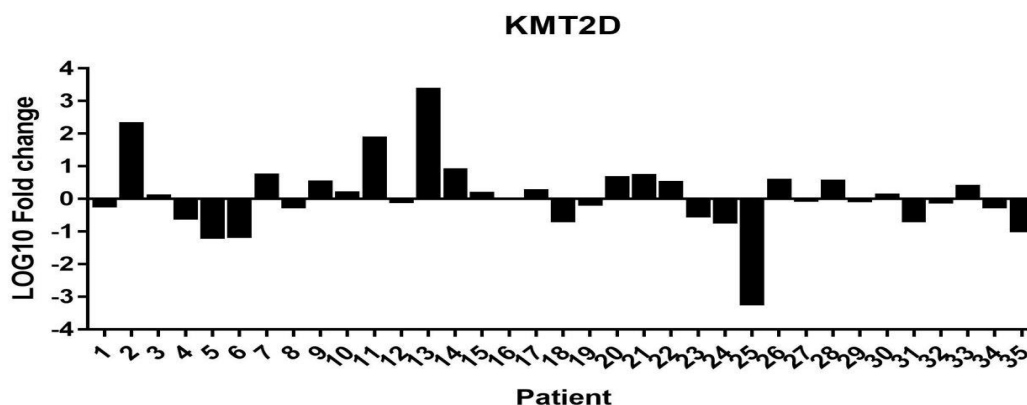


Fig. 1. The graph related to KMT2D gene expression

3.2 The status of IGF2 gene expression in breast cancer tissues with qPCR method

The gene expression of IGF2 was investigated in 35 breast tumor samples and 35 adjacent normal samples. We found that the expression of IGF2 in breast cancer samples

has no significant difference compared with adjacent normal samples. The expression of this gene in 16 (45.7 %) tumor samples was reduced in comparison to the adjacent normal sample, but increased in 19 (54.3%) tumor samples

compared to adjacent normal samples ($P=0.6$). (Fig. 2 and

3B show the results of the IGF2 gene).

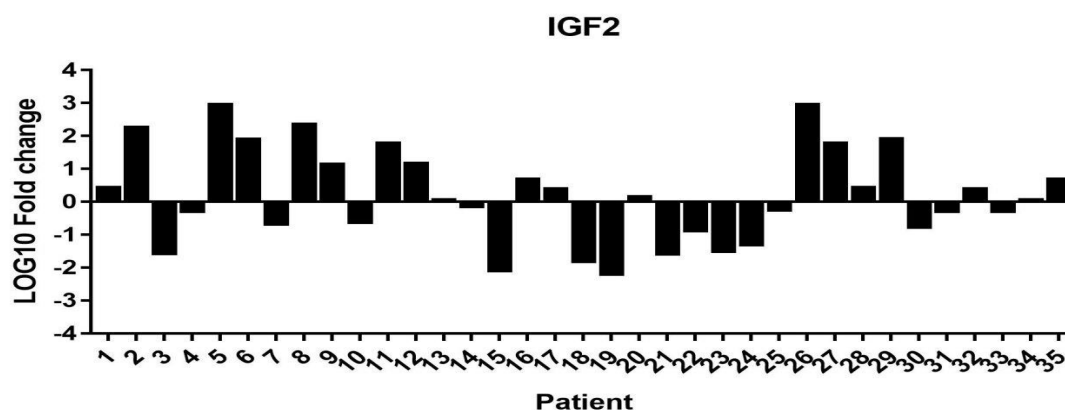


Fig. 2. The graph related to IGF2 gene expression.

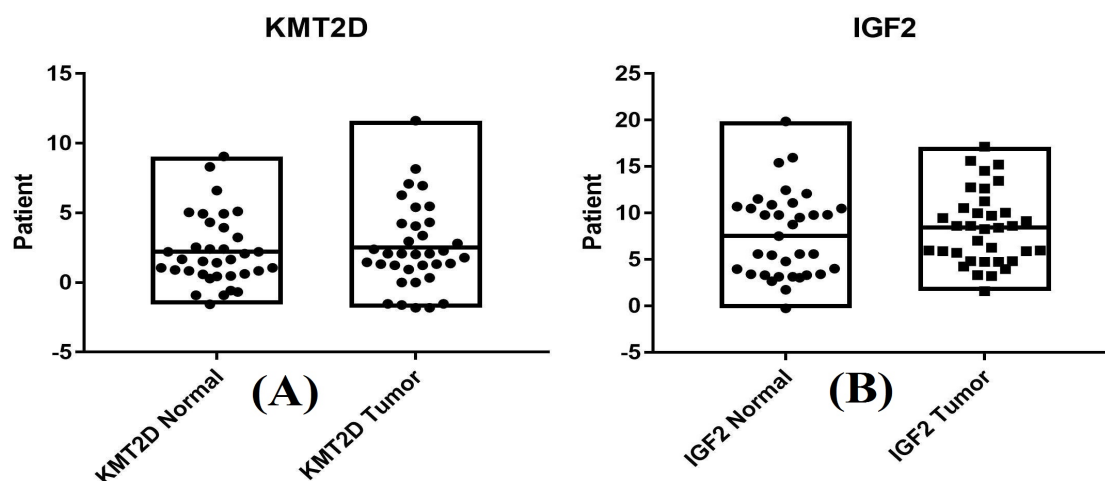


Fig. 3. The graphs related to (A) Comparison of KMT2D gene expression in tumor samples with adjacent normal samples (B) Comparison of IGF2 gene expression in tumor samples with adjacent normal samples.

IV. DISCUSSION

Over the last few years, a great number of studies have reported aberrant patterns of gene expressions in various cancers including breast cancer. Despite the many advances in treating of breast cancer, the survival rate of this patients are still poor. Therefore, understanding of the different mechanisms involved in the onset and progression of breast cancer can provide the basis for better treatment. In this study, we have analyzed the expression level of IGF2 and

KMT2D genes in thirty-five breast cancer patients by real-time quantitative PCR.

Aberrant gene expressions of KMT2D have been reported in several human cancers. Lv et al. demonstrated that high expression of KMT2D in prostate cancer patients is associated with recurrent mutations and leads to poor prognosis (Lv et al. 2017). In another study, Dawkins et al. showed that decreased KMT2C/D expression correlates with favorable outcome in Pancreatic ductal adenocarcinomas (Dawkins et al. 2016). Considering the mentioned studies

and the roles of this gene in normal breast tissues, we expect the overexpression of KMT2D gene occurs in these patients, but in this case, overexpression of KMT2D gene was observed only in 18 breast cancer patients (51.5%).

In this study, we didn't see any significant difference in expression level between tumor and normal breast tissues for this gene but the IGF2 expression was reduced in 16 out of 35 (45.7%) breast tumor tissues compared to adjacent normal tissues. We expect the gene expression difference between normal and tumor samples were significant but our expectation was not fulfilled. However, the roles of the IGF2 and KMT2D genes in the evolution of breast cancer are complicated and require more research and a larger population.

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