

Assessing Expression of TGF- B2 and PCDH9 Genes in Breast Cancer Patients

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Abstract

Background: Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. The evolution of breast cancer is a multistep process involving genetic, epigenetic, and environmental factor interactions that result in the dysregulation of key oncogenes and tumor suppressor genes, culminating in activation of cancer-related signaling pathways. It has been broadly recognized that cancer is a disease caused by molecular alterations in either proto-oncogenes or tumor suppressor genes. In order to make an impact on breast cancer, we must understand the molecular abnormalities to target better therapeutics.

Methods: The expression status of PCDH9 and TGF- β 2 genes were checked in 35 patients with breast cancer tissues and 35 adjacent normal tissues using the q-PCR method.

Result: This paper reported that there was no significant difference between expression level of tumor samples and 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- Quantitative Real-Time PCR Method; Breast Cancer; PCDH9; TGF-B2.

I. INTRODUCTION

Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is the leading cause of death for American women between the ages of 40 and 55 (Harris et al. 1992). Breast cancer is a major public health problem, with 1,384,155 estimated new cases from population-based cancer registries in 2008 worldwide with nearly 459,000 related deaths (Druesne-Pecollo et al. 2012, Youlden et al. 2012). It has been predicted that the worldwide incidence of female breast cancer will reach approximately 3.2 million new cases per year by 2050 (Hortobagyi et al. 2005). Breast cancer is a complex disease including clinical,

morphological and molecular very distinct entities. This heterogeneity cannot be explained only by clinical parameters such as tumor size, lymph node involvement, histological grade, age; or by biomarkers like estrogen receptor (ER), progesterone receptor (PGR) and epidermal growth factor receptor 2 (HER2) routinely used in the diagnosis and treatment of patients. During the last decade research has focused in depth on the molecular biology of this disease (Eroles et al. 2012). Five intrinsic molecular subtypes of human breast cancer include Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2/ ERBB2)-positive, basal-like, and normal-like breast cancer (Perou et al. 2000, Reis-Filho and Pusztai 2011). So far, many studies have been conducted on various genes in

breast cancer that can be mentioned to PCDH9 and TGF- β 2 genes. (Yu et al. 2008, Asiedu et al. 2011). The PCDH9 gene belongs to the protocadherins family and its role is cell adhesion and is known as a tumor suppressor gene (Redies et al. 2005). This gene has a high expression in various tissues of the body, including the breast, liver, prostate, brain, lung and pancreas. For this reason, many studies have been conducted in various researches and so far its role has been proved in a number of different diseases (Wang et al. 2014). The PCDH9 gene also plays a role in the EMT signaling pathway (Kahr et al. 2013). TGF- β 2 gene belongs to the family of polypeptide growth factors. This gene performs various functions in the natural cells and acts in the proliferation, differentiation, apoptosis, and regeneration of the extracellular matrix. TGF- β activates several proteins and signaling cascades including SMAD proteins and MAPK cascade and paths such as Erk, JNK and P38 (De Caestecker 2004). The TGF- β 2 messenger path includes ligand binding to TGF β receptors and activation of two

different signaling pathways: signaling path that dependent to SMAD and signaling path that un-dependent on SMAD (Rahimi and Leof 2007). The role of TGF- β in breast cancer progression is ambiguous, as it was shown to display both tumor-suppressing and -enhancing effects (Buck and Knabbe 2006). In this paper, we investigated the expression level of PCDH9 and TGF- β 2 genes in breast cancer patients by using Real-Time PCR method.

II. MATERIAL AND METHODS

2.1 Human specimens

Human breast cancer specimens (n = 35) and adjacent non-tumor tissues were obtained from patients at Hospital with informed consent from each patient. Patient demographic and clinicopathologic characteristics are shown in Table 1.

Table 1. Clinicopathologic 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 and cDNA preparation

Total RNA was isolated from each tumor tissue and adjacent non-tumor tissue by using RiboEx (GeneAll, 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. The synthesis of cDNA (240 ng of total RNA per 20 μ L 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.

2.3 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- μ L reaction containing 7.5- μ L of RealQ Plus 2x Master Mix Green High ROX™ (Ampliqon, Denmark), 1- μ L of cDNA, 5.5- μ L of

H₂O and 1-μl of mixed forward and reverse primers (3 Pmol/μl concentration). Real-time PCR amplifications were done as follows: for three selected genes, PCR amplification was set to an initial 95°C for 15 min and then for TGFβ2 and PCDH9 genes, a total of 40 cycles, 95°C for 15 seconds and 58°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 (Anary et al. 2018).

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

Target genes		Sequences (5' → 3')	Product length
TGF-β2	Forward	TGACCCCACATCTCCTGCAAT	135bp
	Reverse	GTGGACGTAGGCAGCAATTATC	
PCDH9	Forward	AACAGATCCTGACACAGGCTT	75bp
	Reverse	TCCAGTCCAAAAACACTCTGC	
GAPDH	Forward	CATCAAGAAGGTGGTGAAGCA	120bp
	Reverse	GCGTCAAAGGTGGAGGAGTG	

2.4 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 Results obtained of PCDH9 gene expression in breast cancer tissues with RT-PCR method

Changes in PCDH9 expression were investigated in each tumor specimen. As shown in graph 1, PCDH9 expression

increased in 16 (45.7%) tumor samples and decreased in 19 (54.3%) tumor samples. After analyzing the comparison of PCDH9 gene expression between tumor tissue samples and adjacent normal tissues, this gene showed no significant increase or decrease in tumor tissue samples compared to adjacent normal samples (*P*=0.6). (Fig. 1 and 3a show the results of the PCDH9 gene).

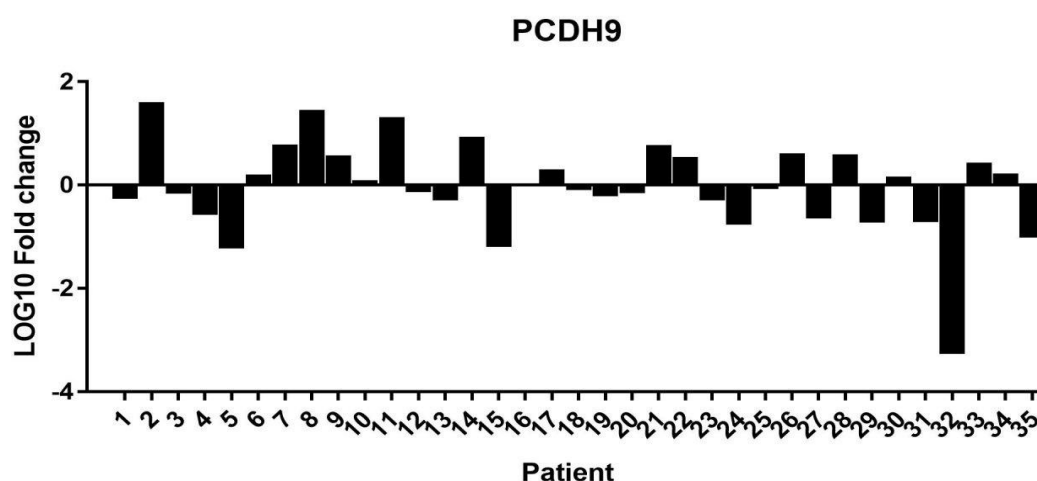


Fig.1. The graph related to PCDH9 gene expression

3.2 Results obtained of TGF- β 2 gene expression in breast cancer tissues with RT-PCR method

Investigating the expression of TGF- β 2 gene in 35 breast cancer tissue samples and 35 adjacent normal tissue samples in this study showed that 22 out of 35 (62.8%) cases of

tumor samples overexpressed in comparison with normal samples, while 13 out of 35 (37.2%) cases showed a decrease in expression levels. Therefore, there was no significant difference in expression level of this gene in the samples ($P=0.3$). (Fig. 2 and 3b show the results of the TGF- β 2 gene).

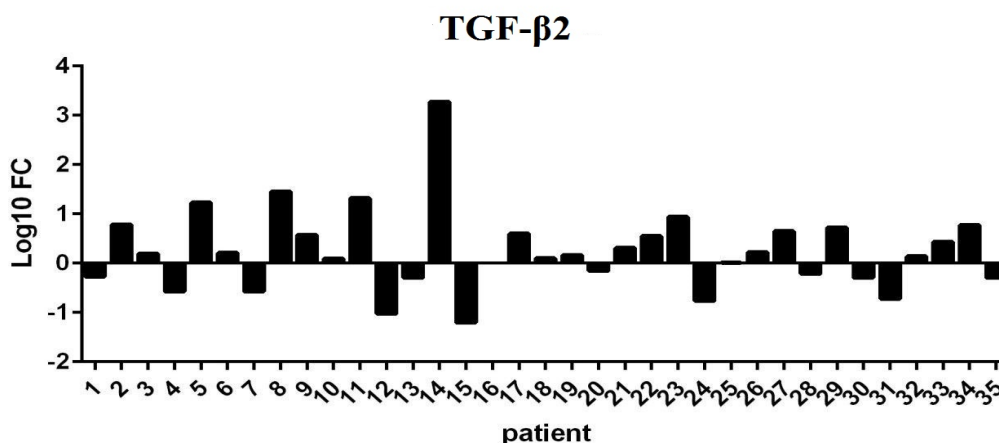


Fig.2. The graph related to TGF- β 2 gene expression

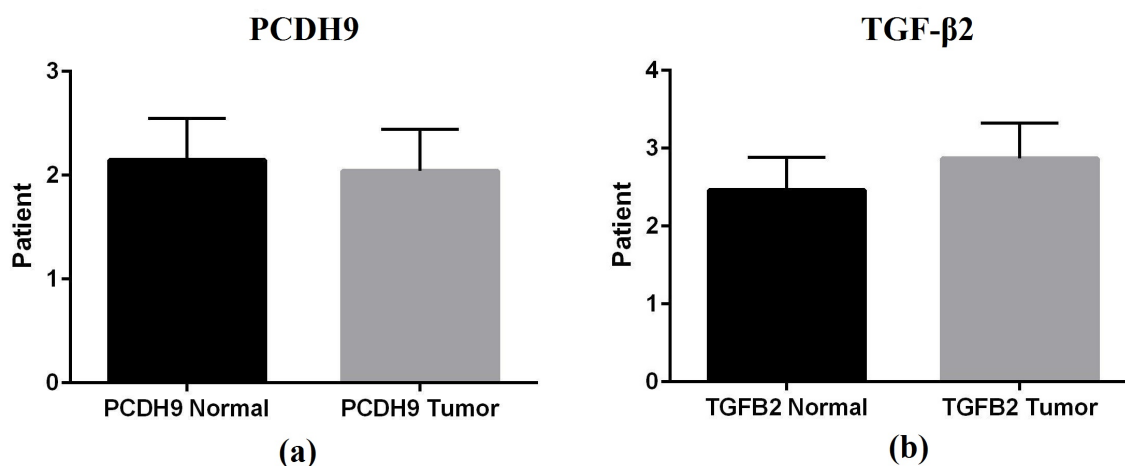


Fig.3. The graph (a) related to expression of PCDH9 gene in breast tumor samples compared to non-tumor adjacent samples and the graph (b) related to expression of TGF- β 2 gene in breast tumor samples compared to non-tumor adjacent samples.

IV. DISCUSSION

Better recognition of gene expression patterns and molecular mechanisms which involved in breast cancer, can lead to early detection of cancer and increase the survival rate for patients. For this purpose, we have analyzed the expression level of TGF- β 2 and PCDH9 genes in thirty-five breast cancer patients by real-time quantitative PCR.

Hachim et al, by studying on the expression of three TGF- β isoforms in breast cancer tissues demonstrated that TGF- β 1 and TGF- β 3, but not TGF- β 2, expression levels were increased in breast tumors compared to normal breast tissues (Hachim et al. 2018). In another study, it was found that the expression of this gene was significantly increased in the serum of patients with gastric cancer (Ma et al. 2013). According to the roles that TGF- β 2 gene plays in many

processes, it's clear that dysregulation of this gene occurs in breast tumors, but in this study, only 62.8% of samples were overexpressed for TGF- β 2 gene, and expression in 37.2% of samples have been reduced.

Another gene that was investigated in this study was PCDH9, and It has already been studied in various cancers. Loss of PCDH9 gene is associated with the differentiation of tumor cells, metastasis and predicts poor survival in gastric cancer (Chen et al. 2015). Nooshin et al. showed the methylation level of the PCDH9 gene didn't change in early stages of NSCLC tumors (Nooshin et al. 2018). In this study, we didn't see any significant difference in expression level of PCDH9 genes. In summary, we didn't see any significant difference in expression level between tumor and adjacent normal lung tissues for these two selected genes. However, this study was designed as a pilot study, and further investigations are required to confirm our findings.

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