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mRNA Expression of Peroxisome Proliferator-activated Receptor Gamma (*PPARG*) Transcription Factor in Gastric Carcinoma

Hoseini SA[1,2], Bornehdeli S[2], Abolghasemi M[3], Saei EP[3] and Dolatabadi JEN[4]*

[1]Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Tuberculosis and Lung Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran

***Corresponding author:** Dolatabadi JEN, Assistant Professor of Pharmaceutical Nanotechnology, Tabriz University of medical sciences, Associate editor of Bio Impacts (BI), Member of Iranian Society of Biochemistry, Research and Development Complex, Tabriz University of Medical Sciences, Daneshgah street, Tabriz, Iran

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Abstract

Background: Inflammation and carcinogenesis are associated in different type of cancers and genes which have a key role in inflammation usually are also dysregulated in different tumors. Peroxisome Proliferator-Activated Receptor Gamma (*PPARG*) is one of the important inflammatory transcription factors in digestive system, especially gastric. Activation of *PPARG* has been attributed to inhibition of tumor growth in several malignancies. In this study, we examined whether the *PPARG* gene is involved in the carcinogenesis of gastric cancer.

Methods: A total of 50 tumor samples and matched normal tissue margins were collected during surgery from patients with gastric cancer. After total RNA extraction and cDNA synthesis, mRNA expression levels *PPARG* were examined by Real-time polymerase chain reaction.

Results: The results showed that, the mRNA expression level of *PPARG* was significantly downregulated in the tumor tissues in comparison to the marginal tissue (P = 0.002; Fold change = 0.76). Tumor grade of the patients was associated with mRNA expression level of *PPRAG* in tumor tissues (Eta = 0.786, P = 0.027; Table 3)

Conclusions: Our results indicate that *PPARG* might be involved in the pathogenesis of gastric cancer. Therefore, molecular approaches in increasing the expression of this gene might open up horizons for controlling the progression of gastric cancer.

Keywords: *PPARG*; Gastric cancer; Gene expression; Tumor growth

Introduction

Gastric cancer is one of the most common cancers throughout the world, which is ranked as the third cause of cancer-related deaths [1]. As in other cancers, different factors such as obesity, physical inactivity, smoking cigarettes, inflammation, and genetic factors play important roles in the development of gastric cancer [1]. Several mutations on DNA of gastric cells change gene expression patterns in gastric cancer [2,3]. In fact, there are mainly two types of genes, which are involved in cancer development, some with increased level of expression known as oncogenes while some with decreased expression level known as tumor suppressor genes [4].

Peroxisome Proliferator-Activated Receptors (PPARs) are a group of nuclear receptor proteins that have significant roles in inflammatory pathways [5-9]. However, PPARs have a key role in inflammation by controlling prostaglandin and leukotriene production. Moreover, these receptors are also involved in cellular lipid and whole-body glucose homeostasis [10,11]. Three types of PPARs have been detected in deference tissues (alpha, gamma, and delta [beta]) and among them PPAR-γ or *PPARG* is the common variant in gastric tissue. More specifically, it was demonstrated that *PPARG* negatively regulates inflammatory responses in the digestive system. Its important roles in cell metabolism, inflammation, and energy homeostasis candidate *PPARG* as a potential therapeutic target in different cancers [12-16].

Recent studies have disclosed that *PPARG* is expressed in different tissues and *PPARG* ligands can stimulate the inhibition of tumor cell growth in human prostate cancer cells, colon cancer cells [2-5], and liposarcoma cells. It has also been reported that *PPARG* can trigger apoptosis in mammalian cells [6-8].

Due to its pathology, early diagnosis of gastric cancer is very important for effective therapy. Like others, in gastric cancer morphological changes are evident after molecular changes, which actually occur at early stages. As a result, by establishing new and effective biomarkers for an initial cancer screening at the molecular level, more accurate diagnosis and better treatment strategies can be achieved [1,9,10]. Different molecular approaches can also be developed to suppress of mimic the expression of such molecules that, ultimately, hinder the progression of tumor cells. Considering the important role of *PPARG* in cancer-related pathways, the main purpose of this study was to investigate the expression of *PPARG* and its role in the manifesting the pathological presentations of gastric cancer that may confer a potential for this molecule to be developed

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as biomarkers for gastric cancer diagnosis and treatment.

Patients and Methods

Study participants

Fifty tumoral and their matched marginal tissues, as the normal control group, were gathered from gastric cancer patients who had referred to Imam Reza Hospital of Tabriz University of Medical Sciences during 2014-2018. To get a pure sample population, all the patients were native Turkish of North West of Iran. Through sample gathering, patients who had undergone chemotherapy and radiation therapy were excluded. All samples were collected during surgery and then immediately frizzed by nitrogen and stored RNA extraction. The clinical data of the patients were collect and summarized in Table 1. The Human Research Ethics Committees from the Tabriz University of Medical Sciences approved the protocol of this study and written informed consent was taken by all patients or their relatives before sampling.

RNA extraction

Total RNA was extracted from tumoral and marginal tissues by Tripure isolation reagent (Roche, Cat No. 11667165001) according to the manufacturer's protocol. Quantity of RNA samples was determined by Nanodrop and quality was examined by gel electrophoreses on 1% agarose. Afterwards, RNA samples were stored in - 80°C till cDNA synthesis.

Complementary DNA syntheses and Real-time PCR quantification

We applied TAKARA complementary DNA (cDNA) syntheses kit (TAKARA, Cat No. 6130) to synthesize cDNA according to the company's instructions. In this study, 2 step Real-time PCR was used for quantitatively measuring of target gene expression. Quantitative Real-time PCR was conducted by SYBR green PCR master mix (TAKARA, Cat No. RR820W) and specific primer set for target and housekeeping genes (Table 2). Primers were adopted from the study by Zafari *et al.* [17]. For normalizing, the expression level of target genes GAPDH (housekeeping gene) expression level was used. At the end, the average of duplicated C_T values was measured and the relative expression level of target genes was determined by comparative C_T method [18].

Statistical analysis

Statistical analysis was performed using the Graph Pad Prism 7 (Graph Pad Software Inc. San Diego, CA, USA). Kolmogorov-Smirnov's normality test was applied for evaluating normality of data. Independent sample *t* test was conducted to compare target gene expression level between cancerous tissues and their paired marginal tissues. Cross tab (Eta) analysis was conducted to evaluate relationship between clinical features of the patients with relative mRNA expression of *PPRAG*. All results were expressed as mean ± Standard Deviation (SD). Statistical significance level for all *P* value was less than 0.05.

Result

Baseline specifications and clinicopathological data of gastric cancer patients are demonstrated in Table 1. The mRNA expression level of *PPARG* gene was significantly downregulated in tumor tissues compared with the marginal normal tissue ($P = 0.002$, Fold change = 0.76; Figure 1). Among the clinical date, tumor grade of the patients was associated with mRNA expression level of *PPRAG* in tumor tissues (Eta = 0.786, $P = 0.027$; Table 3)

Discussion

Early diagnose is an important factor in successful treatment of cancers and through past decades, new methods for detection of gastric cancer have been innovated. However, due to pathology of gastric cancer, most patients are in an advanced or metastatic stage at the time of diagnosis, resulting in a poor prognosis [11]. To improve the treatment rate in gastric cancer, early detection of tumor is important. So far, the conventional methods have not been able to reach to this goal [15]; thus, it is necessary to find new biomarkers that could be used for the initial screening.

Inflammation is one of the early steps in many type of cancers specially in gastric cancer. It seems by studying genes which are involved in inflammatory pathways, we could find new biomarkers for early detection of gastric cancer [13]. *PPARG* is one of the key molecules involved in inflammation [19]. Previous studies on *PPARG* expression in cancer showed disparate results. Some studies demonstrated overexpression of *PPARG* in cancer tissues [20-23], while other studies showed decreased *PPARG* mRNA expression [24-26]. For example, upregulation of *PPARG* mRNA in HER2-overexpressing breast cancer was reported [16], while it was downregulated in patients with neuroblastoma [27]. These data suggest that *PPARG* can act as a tumor suppressor or oncogene depending on the tissue type, cellular environment, and genetic background of a patient [28]. Our results showed downregulation of the mRNA expression level of *PPARG* in tumor tissues compared with the normal marginal tissues from gastric cancer patients. Given that the expression of *PPARG* in different cancer tissues can stimulate the inhibition of tumor cell growth in human cancer cells [2-5], it seems that downregulation of *PPRAG* might contribute to growth and proliferation of tumor cells in gastric cancer. The contradictory findings about the expression of *PPRAG* in different tumor cells might stem from the nature as well as the phase of tumor grad. However, considering the controversial role of *PPARG* in tumor initiation and development, and according to our results, it is still possible to consider this gene as a potential therapeutic target.

In this study, we also analyzed the possible relation between the mRNA expression level of *PPARG* and clinicopathological features of patients with gastric cancer. Our results showed significant relationships between the expression level of this gene and tumor grade of our patients. To prematurely conclude, downregulation of *PPRAG* mRNA expression contributes to the advancement of tumor progression.

Conclusion

Alterations in the expression of molecular markers during the initiation and progression of carcinogenesis can be the basis for designing more effective drugs, and may prevent cancer development in early diagnosed patients. Because of a long interval between the initiation of gastric cancer and appearance of symptoms, a panel of molecular markers can be used in screening and early detection of it. Our results indicate that the *PPARG* gene may be used within this context. However, because a small sample size was used in this study,

further studies are required to confirm the application of *PPARG* in screening and diagnosing gastric cancer.

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