ORIGINAL ARTICLE

Evaluation of rs1982073 polymorphism of transforming growth factor-β1 in glioblastoma

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Background: Glioblastoma (GBM) is the most common and invasive form of primary malignant brain tumors, with a survival rate of about 1 year. Transforming growth factor-β1 (*TGF-β1*) plays a very important role in tissue homeostasis and cancers. It seems that polymorphism of T29C (L10P, rs1982073, or rs1800470), which has been studied in various cancers such as breast and colon, creates the significant differences plays an important role in GBM prognosis and treatment. In this study, we evaluated the effect of T29C (rs1982073) polymorphism of TGF-β1 gene in GBM. **Materials and Methods:** This study was conducted on 100 cases of GBM including 47 paraffin-embedded brain tissue samples and 53 blood samples from another 53 GBM patients, who was under therapy, and 150 were controls. The TGF-β rs1982073 single-nucleotide polymorphism (SNP) was identified by the NCBI and genotyping was performed by high-resolution melt (HRM) assay. Melt curves from HRM which suspected to SNP were selected and subjected to direct sequencing. Finally, the collected data were entered into the SPSS software (Version. 20) and mean ± standard deviation or *n* (%) was used to show the data. **Results:** The mean age in GBM group was 51.63 ± 13.27 years. Accordingly, the two groups were matched in terms of age and gender ($P > 0.05$). The frequency of GG genotype was significantly higher in GBM patients. In contrast, although the frequency of AG genotype was higher in GBM group, it was not statistically significant. Furthermore, the presence of G allele was significantly more frequent than A allele in GBM patients. **Conclusion:** Findings of the present study supports that the Pro10Leu, rs1982073, or rs1800470 SNP in *TGF-β1* is found to be expressed significantly more in GBM patients as it was found in breast cancer.

Keywords: Glioblastoma, polymorphism, transforming growth factor-β1

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INTRODUCTION

Glioblastoma (GBM) is the most common form of malignant brain tumors with an incidence rate of 3.19/100,000 persons in the United States and survival rate of about 1 year. Risk factors include prior radiotherapy, decreased susceptibility to allergy, immune factors and immune genes, as well as some single-nucleotide polymorphisms (SNPs) detected by genomic analysis.[1,2] The most frequent location of GBM is cerebral hemispheres; with 95% of these tumors arise in the supratentorial region, while only few percent of tumors occur in the cerebellum, brain stem, and spinal cord. Low survival rates have led to creation of treatment method in order to tumor removal or increase survival in GBM[3,4] and unlike the current treatment regimens, GBM survival of 5 years has still been reported to be <10%;[3,5] this cancer has a poor prognostic[6] as well as its molecular mechanisms that are less known.[7] The transforming growth factor-β (TGF-β) superfamily encompasses around 40 secreted cytokines, including TGF-β, bone morphogenetic proteins, activins, nodal, lefty, myostatin, anti-Müllerian hormone, and growth differentiation factors. These cytokines regulate a plethora of biological functions such as cell proliferation

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and apoptosis, embryonic patterning, stem cell maintenance, cell differentiation, migration, and immune surveillance. TGF-β plays a very important role in tissue homeostasis and cancers.[8-10] This group of cytokines consists of three factors such as TGF-β1, TGF-β2, and TGF-β3, which TGF-β1 is more important.[11,12] While activating the TGF-β pathway, TGF-β was attached to its receptor that induced phosphorylation and activation of SMAD2/3 and then SMAD4, which in the following the agent would be transferred to the nucleus and regulated the expression of genes involved in cell division, migration, invasiveness, angiogenesis, and epithelial–mesenchymal transition.[9,10,13,14] In addition, TGF-β also plays a noticeable role in progression of tumors on independent paths of the SMAD.[14,15]

The role of TGF B in cancer can be considered as the both initially cell division and tumor suppressor but it may also cause tumor invasion and metastasis.[16,17] The above discussion suggests that TGF-β is a very good target for many cancer studies and has been yet evaluated in several ways.[7,10-12,16-18] Many cancer cells, such as GBM, are dependent on the TGF-β pathway,[14] and recent studies have reported the increased expression of this cytokine in GBM with less survival.[19-22] TGF-β secretion in GBM has also enhanced tumor cells.[9,10]

Malignant cancers, such as GBM, have lost selectively TGF-β capabilities in inhibiting proliferation, while other functions of this route remain intact. The TGF-β pathway plays many roles in the carcinogenesis. For example, it has effect on important activities in GBM aggressiveness such as stemness, immunosuppression, angiogenesis, and invasion/migration as well as drug-/radioresistance.[10]

Transforming growth factor-β in glioblastoma angiogenesis

The formation of microvascular environments by improvement of angiogenesis is so necessary for survival of topical tumors. Angiogenesis is one of the main characteristics of malignant glioma and is associated with vascular endothelial growth factor VF expression.[10] Therefore, anti-angiogenic treatments can be a way to control glioma malignancies. The association between angiogenesis and TGF-β was initially investigated by studies on Chinese hamsters, in which cell division and extensive angiogenesis were occurred after subcutaneous TGF-β1 injections.[23] In a recent study by Joseph *et al* it has been found that 95 genes expressed in GBM vessels and interestingly among them genes such as collagen, fibronectin, laminin, and nidogenic encoding genes, were regulated by the TGF-β pathway.[10]

Transforming growth factor-β in glioblastoma invasiveness

Malignant gliomas have many invasive properties that are related to activity of a number of cellular receptors such as receptor tyrosine kinases, G protein-coupled receptors, TGF-β isoforms to the TGF-β receptors, integrins, and tumor necrosis factor. TGF-β has a very important role in the invasive and metastatic processes of many cancers and its high expression has been reported, especially in GBM cells.[10] Proteases such as matrix metalloproteinases (MMPs) and cathepsins by extracellular matrix decomposition causes gyla invasion.[10] In addition, the role of TGF-β in inducing MMPs expression and avoiding expression of tissue inhibitor of metalloproteinase inhibitors in human glioma cells has been identified.[24,25]

Transforming growth factor-β in immunosuppression

Antitumor immune responses during various phases of malignant glioma are very complex and different. The role of TGF-β as an immunosuppressant cytokine has been important not only in GBM but also in other cancers.[10] TGF-β has pleiotropic effects on all types of immune cells. For example, inhibitory effects on adult T-cells including proliferation, cytotoxic activity, and induction of apoptosis.[26] TGF-β also stimulates immunosuppressive Tregs.[27] TGF-β1 gene is located on chromosome 19q13.1[12], and recently, several polymorphisms have been discovered in TGF-β1 gene, which are effective in its expression, and their effects have been studied on prognosis of various cancers and the response rate to chemical and radiotherapy treatments.[11,13,18,28] Meanwhile, it seems that polymorphism of T29C (L10P and rs1982073), which has been studied in various cancers such as breast and colon and create significant differences plays an important role in GBM prognosis and treatment.[11,16,18,28]

T29C polymorphism changes the proline amino acid (CCG) to leucine (CTG) in codon 10 (Pro10Leu) protein.[29] Considering the multiple roles of TGB-β in cancer suppressing or progressing and the importance of GBM among high-mortality brain tumors, in this study, we evaluated the effect of T29C (rs1982073) polymorphism of TGF-β1 gene in GBM.

MATERIALS AND METHODS

This case–control study was approved by the Research and Ethics Committee of Isfahan University of Medical Sciences. After providing sufficient information, written consent was obtained from all patients or their legal guardians before involvement in the project. This study was conducted on 100 cases of histopathologically confirmed GBM according to tumor-node-metastasis classification system of the American Joint Committee on Cancer 2010, 7th edition, including 47 paraffin-embedded brain tissue samples that was taken from the Pathology Department of Al Zahra University Hospital and 53 blood samples from another GBM patients, who was under therapy for this disease from

Milad Hospital, and 150 sex- and age-adjusted controls from population of Isfahan, Iran, from 2013 to 2015. In cases, the extension of disease, if the disease progression was defined or in the case of local recurrence or metastasis was detected (mainly in the lung, bone, and liver or combined), they were excluded.

DNA was extracted from the brain tissue samples using PFET-DNA extraction kit (Yektatajhiz Inc., Tehran, Iran) and from blood samples using Blood-DNA extraction kit (Yektatajhiz Inc., Tehran, Iran) according to the manufacturer's protocol.

The Pro10Leu, rs1982073 or rs1800470 SNP in *TGF-β1* SNP were identified by the NCBI, and ensemble databases and primers were designed by Beacon Designer 8.1 (PREMIER Biosoft International, USA) and synthesized by Bioneer (Bioneer, Korea). The forward primer was 5′-sequence-3′ and reverse primer was 5′-sequence-3′. Genotyping was performed by high-resolution melt (HRM polymerase chain reaction [PCR]) assay using a Rotor-Gene 6000 instrument (Corbett Life Science, Australia).

PCR reactions were carried out in triplicate in 10 µL of final volume using HRM kit (Qiagen, Germany) according to manufacturer protocol. The PCR program consisted of an initial denaturation – activation step at 95°C for 10 min, followed by a 40-cycle program (denaturation at 95°C for 15 s, annealing conditions 60°C for 20 s, 72°C for 20 s; an HRM step from 75°C to 95°C rising at 0.1°C/s). Curves for each triplicate were checked on the shape, melting pattern, and Tm to meet reproducibility.

Melt curves from HRM which suspected to SNP were selected and subjected to direct sequencing.

The Hardy–Weinberg equilibrium (HWE) was tested to compare the observed genotype frequencies with the expected frequencies among samples so that the genotype distribution of *TGF-β1* rs1982073 was compatible with the HWE in our patients.

Finally, the collected data were entered into the SPSS (version 20; SPSS Inc., Chicago, Ill., USA) and mean ± standard deviation or *n* (%) was used to show the data. Moreover, Fisher's exact test and independent *t*-test were applied for statistical analysis of frequency distribution of gender and mean age. To show the relationship between the genotype, *TGF-β1* rs1982073 allele and GBM disease, logistic regression was used and odds ratio (OR) was reported. Furthermore, to increase the precision of the study, variables such as age and gender were taken under control as the confounding variables. In all the analyses, $P < 0.05$ was considered statistically significant.

RESULTS

In this study, a total of 150 healthy individuals included 80 (53.3%) males and 70 (46.7%) females with the mean age of 49.67 ± 15.12-year-old were used in the control group. Furthermore, 100 patients with GBM included 55 (55%) males and 45 (45%) females with the mean age of 51.63 ± 13.27-year-old were assigned to the case group. The two groups were statistically matched in terms of age and gender ($P > 0.05$) [Table 1].

On the other hand, the frequency distribution of TGF-β1 allele G in GBM patients was 41.5% more than healthy individuals with 30.7% (OR [95% confidence interval (CI)]: 1.604 [1.104–2.330]; $P = 0.013$). Furthermore, the distribution of AG genotype in patients with GBM was 37% and in healthy individuals were 33.3%, which was not statistically significant with the incidence of GBM disease ($P = 0.192$). In contrast, a significant correlation was found between GG genotype and GBM disease (OR [95% CI]: 2.163 [1.071–4.370]; $P = 0.032$). In addition, by controlling the age and gender, the obtained results remained valid; in fact, age and gender did not play any confounding role on the results [Table 2].

DISCUSSION

Considering the multiple roles of TGB-β in cancer suppressing or progressing, and the importance of GBM among high-mortality brain tumors, in this study, we evaluated the effect of T29C (rs1982073) polymorphism of TGF-β1 gene in GBM. We analyzed the DNA sequence of the *TGF-β1* gene from blood samples taken from 100 GBM patients and 150 noncancerous controls who were adjusted for sex and age.

It is to be noted that the prevalence of GBM was higher in men and its mean age was 51.63 ± 13.27 years. Thus, it can be concluded by adding other epidemiologic results that generally, the GBM is more prevalent in men and it mostly occurs in elderlies rather than the youth. According to the National Database of Central Brain Tumor Registry of the United States, the age-adjusted GBM incidence rate is 3.97 cases/100,000 for men and 2.53 cases/100,000 for women.[30]

Table 1: Demographic characteristics of patients in two groups

Characteristics	Control (*n*=150)	Case (*n*=100)	*P*
Sex, *n* (%)			
Male	80 (53.3)	55 (55.0)	0.897
Female	70 (46.7)	45 (45.0)	
Age, year	49.67±15.12	51.63±13.27	0.281

Data shown *n* (%) or mean±SD. SD=Standard deviation

Table 2: Genotype and allele frequencies of transforming growth factor- β1 rs1982073 in two groups

Genotype/allele	Control (*n*=150), *n* (%)	Case (*n*=100), *n* (%)	OR (95% CI)	*P*
Genotype				
AA	79 (52.7)	40 (40.0)	Reference	
AG	50 (33.3)	37 (37.0)	1.461 (0.826-2.585)	0.192
			1.492 (0.837-2.661)[a]	0.175[a]
GG	21 (14.0)	23 (23.0)	2.163 (1.071-4.370)	0.032
			2.211 (1.091-4.483)[a]	0.028[a]
AG + GG	71 (47.3)	60 (60.0)	1.669 (1.00-2.787)	0.050
			1.710 (1.018-2.871)[a]	0.042[a]
Allele				
A	208 (69.3)	117 (58.5)	Reference	
G	92 (30.7)	83 (41.5)	1.604 (1.104-2.330)	0.013

[a]Adjusted for age, sex. OR=Odds ratio; CI=Confidence interval

Moreover, the analysis of association of allele and genotype of TGB-β gene with the occurrence of GBM showed that the frequency of GG genotype was significantly higher in GBM patients, but although the frequency of AG genotype was higher in GBM group, it was not statistically significant. Furthermore, the presence of G allele was significantly more frequent than A allele in GBM patients. These results remained valid by adjusting sex and age, so we may conclude that sex or age did not have a role in the presence of these alleles of genotype in these patients.

Accordingly, in the study of Pooja *et al*. who worked on 1222 samples of the Indian population, they analyzed 29C>T (Pro10Leu, rs1982073 or rs1800470) and 74G>C (Arg25Pro, rs1800471) polymorphisms in the *TGF-β1* gene. They found that these polymorphisms in the *TGF-β1* gene significantly affect breast cancer risk, which might be related to higher *TGF-β1* levels. They showed that *TGF-β1* level was significantly higher in breast cancer as compared to the control group. This higher *TGF-β1* level might be due to a higher frequency of these genotypes in breast cancer cases.[11]

In the study of Watanabe *et al*., they found 106 SNPs and 11 other types of variations in *TGF-β11* and six other genes. These genes were *TGF-β1* receptor gene *(TGF-β1R1)*, *TGF-β2* receptor gene *(TGF-β1R2)*, SMAD2, SMAD3, SMAD4, and all of which compose the *TGF-β1* signaling pathway. A total of 11 SNPs were identified in *TGF-β1*. The only SNP (TGF-B11-2, 29C>T) identified in the coding region (exon 1) was nonsynonymous and resulted in proline to leucine change (P10Leu). Hence, they suggested further evaluations of these genes in cancerous cases in order to find probable associations.[28]

In contrast, there was a study by Krippl *et al*. on 500 breast cancer cases and 500 sex- and age-adjusted controls that showed no significant association in the presence of *TGF-B11*, L10P polymorphism, and breast cancer. On the other hand, they found higher lymph node metastasis in patients carrying this allele.[31]

Finally, it might be necessary to say that, one of the limitations of this study was difficulties in gathering GBM samples, because larger sample size may lead to more reliable results. Furthermore, another weakness of our study was the lack of other clinical features and comorbid disease of these patients. Thus, the duration of disease, family history of GBM, and other comorbid diseases might affect the distribution of mentioned allele or genotypes in these patients. Hence, further study noticing these factors in a wider sample size is recommended.

CONCLUSION

In conclusion, and to the best of our knowledge, the results of the present study support that the Pro10Leu, rs1982073, or rs1800470 SNP in TGF-B1 is found to be expressed significantly more in GBM patients as it was found in the breast cancer. Thus, further evaluation regarding the effects of this polymorphism on susceptibility to other types of neoplasms and their outcome is recommended.

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Conflicts of interest

There are no conflicts of interest.

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