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The Role of Transforming Growth Factor Beta and Smad Receptors in Determining Prognosis in High-Grade Primary Brain Tumors: Glioblastoma Multiforme

O papel do fator de crescimento transformador beta e receptores smad na determinação do prognóstico em tumores cerebrais primários de alto grau: glioblastoma multiforme

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Abstract

Introduction High-grade primary brain tumors cause serious morbidity and mortality. This study aimed to investigate the role of transforming growth factor beta (TGF-β) and suppressor of mothers against decapentaplegic (Smad) receptors in high-grade primary brain tumors.

Material and Method Thirteen patients with a pathological diagnosis of glioblastoma multiforme were included in the study. Pathological preparations of each patient were analyzed retrospectively in histochemistry and immunohistochemistry laboratories. Transforming growth factor beta 1, TGF-β2, TGF-β3, Smad 1/2/3, Smad 6, and Smad 7 stainings were evaluated, and the immunoreactivity densities were examined.

Result We found out an increase in the expression of TGF-β1 and TGF-β3 protein. Regarding the inhibitin receptors, Smad 6 showed much more expression than Smad 7. Thus, we found that Smad 6 has a protective effect and role in the tissue. Immunhistochemically, TGF-β family stains, which are activated by types I-and -II

Keywords
- glioblastoma multiforme
- transforming growth factor beta
- smad proteins
- endothelial-mesenchymal transition

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receptors, and the stainless staining of the Smad family might also be showing that the TGF-β family is taking action with a secondary pathway other than the Smad family.
Conclusion In addition to Smad family receptors, Shc-GBR2, SARA, and Ras-Erk1/2 receptors should be investigated in future research. After that, the prognosis, diagnosis, and patient-based chemotherapy strategies for the treatment of glioblastoma multiforme may take a more prominent role.

Resumo

Objetivo Tumores cerebrais primários de alto grau causam morbidade e mortalidade graves. Este estudo teve como objetivo investigar o papel dos receptores fato de crescimento transformante beta (TGF-β) e mães contra homólogo decapentaplégico (Smad, na sigla em inglês) em tumores cerebrais primários de alto grau.
Métodos Treze pacientes com diagnóstico patológico de glioblastoma multiforme foram incluídos no estudo. As preparações patológicas de cada paciente foram analisadas retrospectivamente em laboratórios de histoquímica e imunohistoquímica. As colorações de TGF-β1, TGF-β2, TGF-β3, Smad 1/2/3, Smad 6, e Smad 7 foram avaliadas, e as densidades de imunorreatividade foram examinadas.
Resultados Encontramos aumento na expressão das proteínas TGF-β1 e TGF-β3. Em relação aos receptores de inibitina, o Smad 6 mostrou muito mais expressão do que o Smad 7. Assim, concluímos que o Smad 6 tem efeito e função protetores no tecido. As colorações imunohistoquímicas da família TGF-β, que são ativadas pelos receptores do tipo I e do tipo II, e as colorações menos da família Smad também podem estar mostrando que a família TGF-β está agindo com outra via secundária que não a família Smad.
Conclusão Assim como os estudos na família Smad, receptores como Shc-GBR2, SARA, Ras-Erk1/2 devem ser investigados em pesquisas futuras. Posteriormente, o prognóstico, o diagnóstico, e as estratégias de quimioterapia baseadas no paciente podem assumir um lugar mais priminente no futuro, no glioblastoma multiforme.

Palavras-chave
- glioblastoma multiforme
- fato de crescimento transformante beta
- proteínas smad
- transição endotelial-mesenquimal

Introduction

Primary high-grade tumors of the brain (glioblastoma multiforme, GBM) cause serious morbidity and mortality.[1,2] Previous studies have revealed that these cancers have drug resistance, such as BCNU (carmustine), due to differentiation in cell protein synthesis and DNA structures, and many agents, such as TGF-β, are effective at the molecular level.[3]

Epithelium-mesenchymal transition (EMT) is a morphogenic process in which epithelial cells lose their properties and acquire mesenchymal properties during the embryogenesis and progression of cancer. The EMT process is stimulated and regulated by many effectors. These effectors are growth factors (TGF-β, platelet-derived growth factor [PDGF], epidermal growth factor [EGF]), cytokines (IL-8), and extracellular matrix (ECM) components.[4,5]

In this study, the histological sections of the tumor, and the demographic data, treatment protocols, and prognoses of the patients who were operated on for an intracranial mass and had postoperative clinicopathological diagnosis of glioblastoma multiforme were retrospectively analyzed.

The TGF-β family and Smad receptors were revealed by histochemical and immunohistochemical staining from the removed tumor tissues. The clinical data were recorded with patients and/or their relatives, and their current clinical outcome, morbidity, and mortality were evaluated with the goal to examine the prognostic relationship of treatments with TGF-β and Smad receptors in tumor tissue.

Material and Method

This study was conducted with the approval of Manisa Celal Bayar University Faculty of Medicine, local ethics committee, decision number 20478486–198, dated 07.05.2014.

A total of 13 patients with a pathological diagnosis of glioblastoma multiforme operated on between November 2012 and April 2014 were included in the study. The pathological preparations of each patient were analyzed retrospectively in histochemistry and immunohistochemistry laboratories. The clinical information and treatment protocols of the patients were collected from the records of the relevant clinics, and the data about their current status and clinical course were collected by consulting the hospital records, patients, and/or patient relatives.

The samples were taken into 10% formalin solution for histological analysis. Samples taken for histological examination were routinely followed by paraffin after fixation in 10% formalin solution for 24 to 48 hours. After being left

under running water overnight, dehydration was performed in a graded series of 60%, 70%, 80%, 90% absolute alcohol. After the transparency process in xylene-alcohol and xylene, a paraffin embedding process was performed by passing through xylene-paraffin, paraffin 1, and paraffin 2. Five-µm-thick sections were taken from the blocks formed on lysine and grinded slides with a rotary microtome. The morphological evaluation was provided by routine hematoxylin-eosin staining on some of the sections.

The routine avidin-biotin indirect immunoperoxidase method was used as immunohistochemical analysis. The sections taken on lysine slides were kept at 60°C for 1 night for deparaffinization and then kept in xylene for 1 hour for chemical deparaffinization. For the rehydration process, 95%, 80%, 70%, 60% alcohol series were passed through decreasing alcohol series. Then, the samples were washed 3 times with phosphate buffer (phosphate buffer solution-PBS) for 5 minutes and incubated at room temperature with 3% H_2O_2 for 10 minutes; after that, they were washed three times with PBS for 5 minutes. For the permeabilization of the tissues, the sections kept in trypsin at 37°C for 15 minutes were washed 3 times for 5 minutes with PBS. After incubating with blocking solution for 1 hour, all primary antibodies (anti-TGF-β1, anti-TGF-β2, anti-TGF-β3, anti-Smad 1/2/3, anti-Smad 6, anti-Smad 7) were incubated at +4°C overnight. The next day, the samples were washed 3 times with PBS solution, then a biotinylated secondary antibody was added and incubated for 30 minutes. After the samples were washed 3 times for 5 minutes, streptavidin-peroxidase was added and kept at room temperature for 30 minutes. After staining with diaminobenzidine (DAB) for 2 to 5 minutes to determine the visibility of the immunohistochemical reaction, the samples were washed with distilled water. For the purpose of background staining, the samples were stained with Mayer's hematoxylin for 3 to 6 minutes, then the excess dye was washed with distilled water and covered with an immunohistochemical covering medium.

Transforming growth factor beta staining was done by antibodies numbered SC146, TGF-β2 staining SC90, TGF-β3 staining SC82, Smad ½/3 staining SC7960, Smad 6 staining SC13048, and Smad 7 staining SC11392 (Catalog No Ref 859043).

The immunoreactivity densities obtained as a result of staining were evaluated by the quantitative method and were considered as negative (-), weak (+), moderate (++), and severe (+++).

Statistical Analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 20.0 version software (IBM, New York, USA). Continuous variables were expressed as mean ± standard deviation. The chi-squared test or Fisher exact test (when the chi-squared test assumptions did not hold due to low expected cell counts), where appropriate, was used to compare these proportions in different groups. A p-value < 0.05 was considered statistically significant.

Results

Eight patients were male, and 5 were female. The lowest age was 49 and the highest was 73, while the mean age was 60.3, and the median age was 60. Although K_i-67 mitosis indexes varied between 10 and 40%, the average K_i-67 index was 22.3%, and the median K_i-67 index was 20%. Survival of 5 to 11 months was observed in 6 patients. The clinical follow-up and survival of the patients are given in ►**Table 1**.

In the morphological evaluation performed after routine hematoxylin staining in the patient samples, giant cells were observed in the normal cortex structures in the tumor tissues. Cell cytoplasms could be easily distinguished by their eosinophilic staining and pycnotic nuclei. Necrotic areas were also selected in patches. In addition to tumor tissues, histological structures belonging to normal brain tissue cortex were observed in some patient samples (►**Fig. 1 a-h**).

As a result of the evaluation of factors that play a role in TGF-β and Smad pathway in patient samples, it was found that TGF-β1 and TGF-β3 immunoreactivities were strongly positive (+++), while TGF-β2 immunoreactivity was moderately positive (++) in tumor tissue (►**Fig. 2a-b**, **3a-b**, **4a-b**). While staining for all TGF-β1 and TGF-β2 was observed in the tumor cell cytoplasm, it was observed that TGF-β2 immunoreactivity was also positive in connective tissue.

Transforming growth factor beta immunoreactivity was calculated as 3 ± 0, while TGF-β2 immunoreactivity was 2.2 ± 0.42 and TGF-β3 immunoreactivity was calculated as 3.2 ± 0.42. When the values were compared statistically, it was found that there was a significant difference between the immunoreactivities of TGF-β1 and TGF-β3 and TGF-β2 ($p < 0.001$), while there was no statistically significant difference between the TGF-β1 and TGF-β3 immunoreactivities ($p > 0.05$) (►**Table 2**, **Graphic 1**).

While Smad 1/2/3 immunoreactivity of TGF-β family receptors was intermittently positive (+/−), Smad 6 immunoreactivity was considered weak (+) and Smad 7 immunoreactivity was considered negative in all patient samples (►**Fig. 5a-b**, **6a-b**, **7a-b**) When the immunoreactivities of Smad receptors were compared statistically, it was observed that there was only significance between Smad 6 and Smad 7 ($p < 0.001$), but not among the others (►**Table 2**, **Graphic 1**).

Discussion

Glioblastoma multiforme (GBM) is the most common and most malignant glial tumor among primary brain tumors. According to the data in the USA, gliomas constitute 60% of 17,000 primary brain tumors detected every year. It creates a heterogeneous group in terms of location, age, and gender. The management includes palliative treatment regimens including surgery, radiotherapy, chemotherapy, and radiosurgery. It may occur primarily or secondary after the progression of low-grade astrocytomas. Primary GBMs are more common (60%) over the age of 50, but secondary GBMs (40%) are more common in patients under the age of 45. In their pathophysiology, loss of heterozygosity (10q

Table 1 Demographic characteristics and immunohistochemical staining patterns of the patients

Smad 7	–	–	–	–	–	–	–	–	–	–	–	–	–
Smad 6	+	+	+	+	+	+	+	+	+	+	–	+	+
Smad 1/2/3	–	–	–	–	–	–	–	–	–	–	–	–	–
TGF β3	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
TGF β2	++	++	++	++	++	++	++	++	++	++	++	++	++
TGF β1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Survival	Alive	Alive	Alive	Alive	Alive	6 months	Alive	5 months	8 months	Alive	10 months	11 months	6 months
KT	–	–	–	–	–	–	+	–	+	+	+	+	–
RT	+	+	+	+	+	+	+	+	+	+	+	+	+
K_i-67 (%)	30	25	15	30	15	40	20	30	15	10	25	15	20
Karnofsky Outcome Score	70	90	80	80	100	80	90	50	80	80	80	60	40
Karnofsky Income Score	50	80	40	60	90	70	70	40	50	70	70	50	20
Tumor Location	Frontal	Temporal	Temporal	Parietal	Temporal	Frontal	Frontal	Temporal	Parietal	Frontal	Parietal	Temporal	Frontal
Sex	M	M	F	M	M	F	M	F	M	M	M	F	F
Age	58	63	57	65	49	61	69	55	60	63	54	57	73
Patient	1	2	3	4	5	6	7	8	9	10	11	12	13

Abbreviations: KT, chemotherapy; RT, radiotherapy.

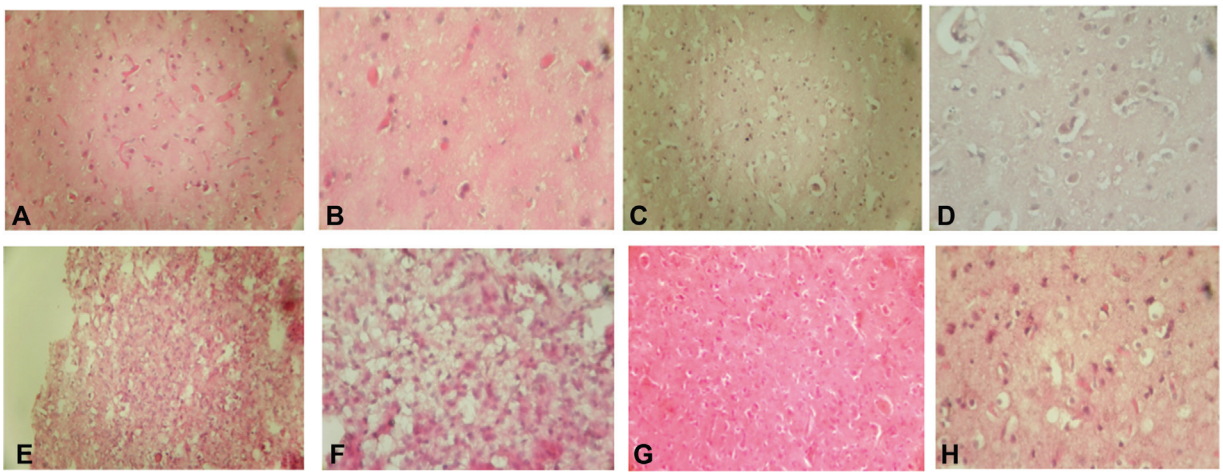


Fig. 1 (**A**) Normal brain tissue, parietal glial cells (hematoxylin & eosin × 200), (**B**) Normal brain tissue, parietal glial cells (hematoxylin & eosin × 400), (**C**) Parietally located high-grade glial tumor (GBM) (hematoxylin & eosin × 200), (**D**) Parietally located high-grade glial tumor (GBM) (hematoxylin & eosin x400), (**E**) Temporally located high-grade glial tumor (GBM) (hematoxylin & eosin × 200), (**F**) Temporally located high-grade glial tumor (GBM) (hematoxylin & eosin × 400), (**G**) Frontally located high-grade glial tumor (GBM) (hematoxylin & eosin × 400).

mutations); loss of p53, which is a tumor suppressor gene; epidermal growth factor receptor (EGFR) gene mutations; MDM2 overexpression, increased expression due to platelet derived growth factor α (PDGF-α); and 10q23.3 PTEN gene mutations. Although the temporal lobe is commonly involved (31%), it may arise from subcortical white matter almost anywhere in the brain. The brain stem, cerebellum, and spinal cord are less frequently involved. Its survival without treatment is 3 months, on average. The median survival reaches 12 months in patients who undergo surgery and, additionally, radiotherapy and chemotherapy. Less than 25% of patients can survive up to 2 years, and less than 10% of patients can survive up to 5 years. In the radiological evaluation, masses with irregular borders, nectoric areas in the central, and intensive digital edema, and peripheral contrast enhancement can be observed. –magnetic resonance spectroscopy shows an increase in the choline-creatine and lactate peaks, and a low N-acetylaspartate (NAA) peak[6].

The clinical applications of modern neurooncology depend on a consistent tumor classification. The absence of variation (variability, unusualness) provides a more accurate prediction of prognosis, and classification also forms the basis of the most important treatment advice that physicians offer to their patients. Neurooncologists use more or less the same treatment modalities for all patients with the same type of tumor. The importance of a coherent classification in neurooncology creates the need for great attention to this problem and encourages recurrent consideration of this indispensable topic.[7] Tumor classifications have been made many times since the beginning of the twentieth century, and with the help of developing technology, these classifications were either completely abandoned or revised in the light of new findings. Nowadays, it is not possible to talk about a classification with a definite reliability, which would provide the correct diagnosis and treatment selection depending on it.

As the name suggests, histological images of glioblastoma multiforme can vary. These tumors are poorly differentiated and may contain pleomorphic astrocyte cells with nuclear atypia, with increased mitotic activity. Glioblastomas show extensive cellular and molecular heterogeneity. Although the traditional view is that the tumor consists of a mass of malignant cells that grow uncontrollably, recent research shows that basic tumor cells create a microenvironment for the tumor by causing changes in normal tissues with the cytokines, chemokines, and growth factors they secrete. This microenvironment consists of many different cell groups in brain tumors. Among these, microglia, macrophage, astrocyte, oligondendrocyte, neurons, glial and neuronal progenitors, extracellular matrix, pericytes, and endothelial cells are the main ones. This neoplastic and non-neoplastic mixture determines the growth degree, invasiveness, immune response, and resistance to treatments of the tumor. Although there are many pathways associated with gliomas, TGF-β pathways play a very important role in establishing the above-mentioned features. There are several publications reporting the relationship between TGF-β levels and various advanced-stage tumors, high malignancy, and poor prognosis.[6,8]

Five isoforms of the TGFs family have been isolated so far. These are TGF-β1, TGF-β2, TGF-β3, TGF-β4, and TGF-β5. Of these, TGF-β1, TGF-β2, and TGF-β3 are expressed in mammalian tissue. The TGF-βs family participates in many functions, such as cell proliferation, differentiation, formation of extracellular matrix components, chemotaxis, immunosuppression, and cell death regulation. Transforming growth factors beta are secreted by the mesenchyme, connective tissue, endothelium, platelets, bone, and immune system cells.

Transforming growth factor beta shows its effects in the cell through many molecules (MAPKs, JNK, p38, p13K Kinases, PP'A phosphatases, Rho and Smad families). However,

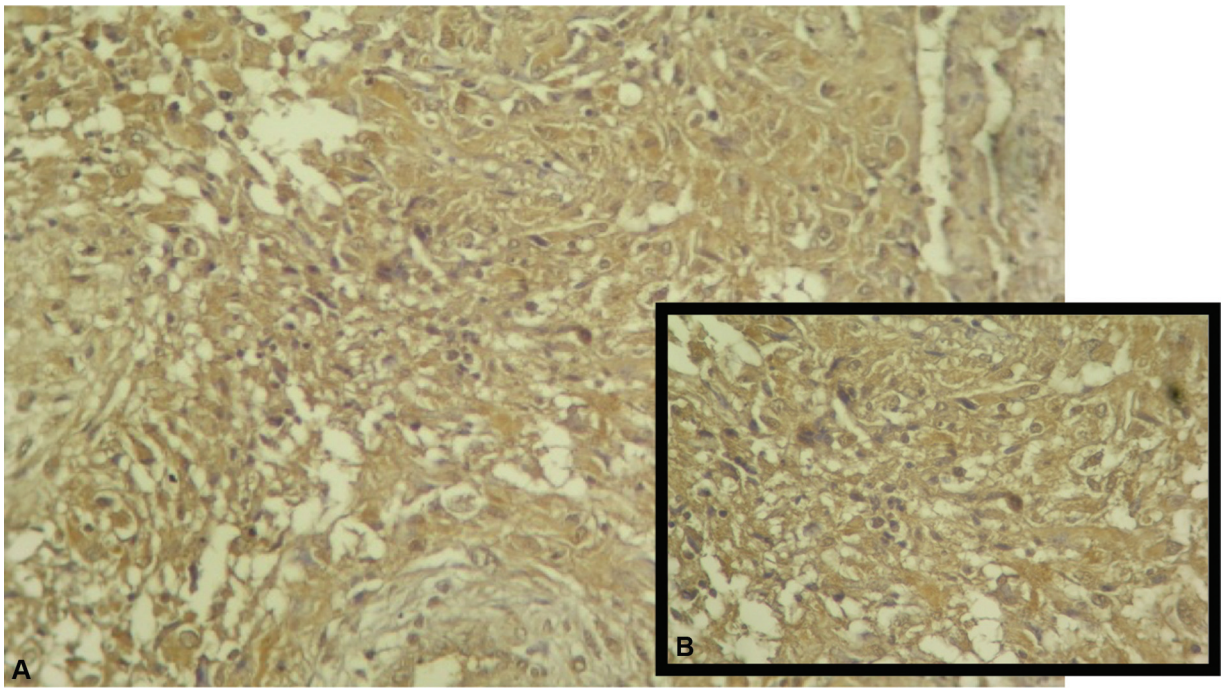


Fig. 2 Transforming growth factor beta 1 immunoreactivity in tumor (GBM) tissue. (**A**) x200, (**B**) x400.

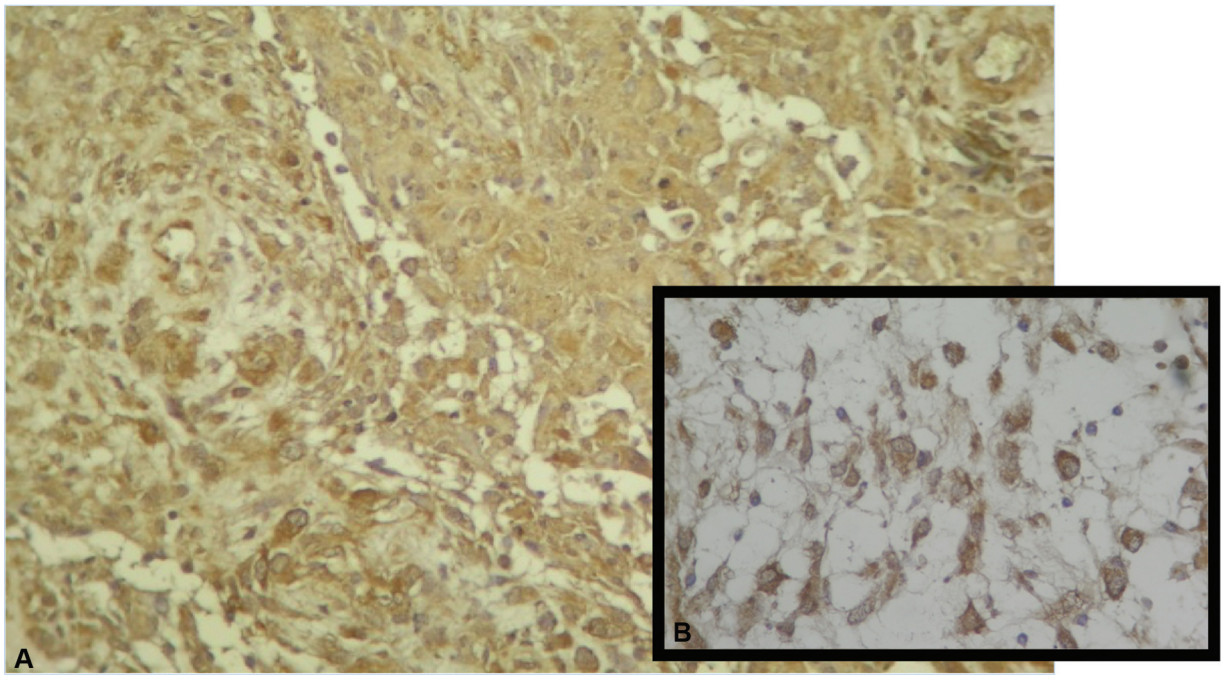


Fig. 3 Transforming growth factor beta 2immunoreactivity in tumor (GBM) tissue. (**A**) x200, (**B**) x400.

apart from the Smads, the signal transduction mechanism of these pathways at the molecular level is not fully known.

Transforming growth factor beta superfamily members act by activating transmembranic receptors. It, then, initiates the flow of regulatory SMAD proteins located from the cytoplasm to the nucleus, acting as transcriptional regulators.

Transforming growth factor beta receptors in glycoprotein structure of TGF-β superfamily members are classified as type I (TßR-I; 55kDa), type II (TßR-II; 70–100 kDa), and type III (TßR-III; 200–400 kDa). Types I and II are responsible for signal transmission. Seven type-I receptors (ALKs 1–7) and 5 type-II receptors (Act-IIA, Act-IIB, BMPR-II, AMHR-II, TBR-II) were detected in the human genome.

Transforming growth factor beta and activins bind directly to type-II receptors first, and then these are activated. Next, the phosphorylation of SMAD proteins, which provides

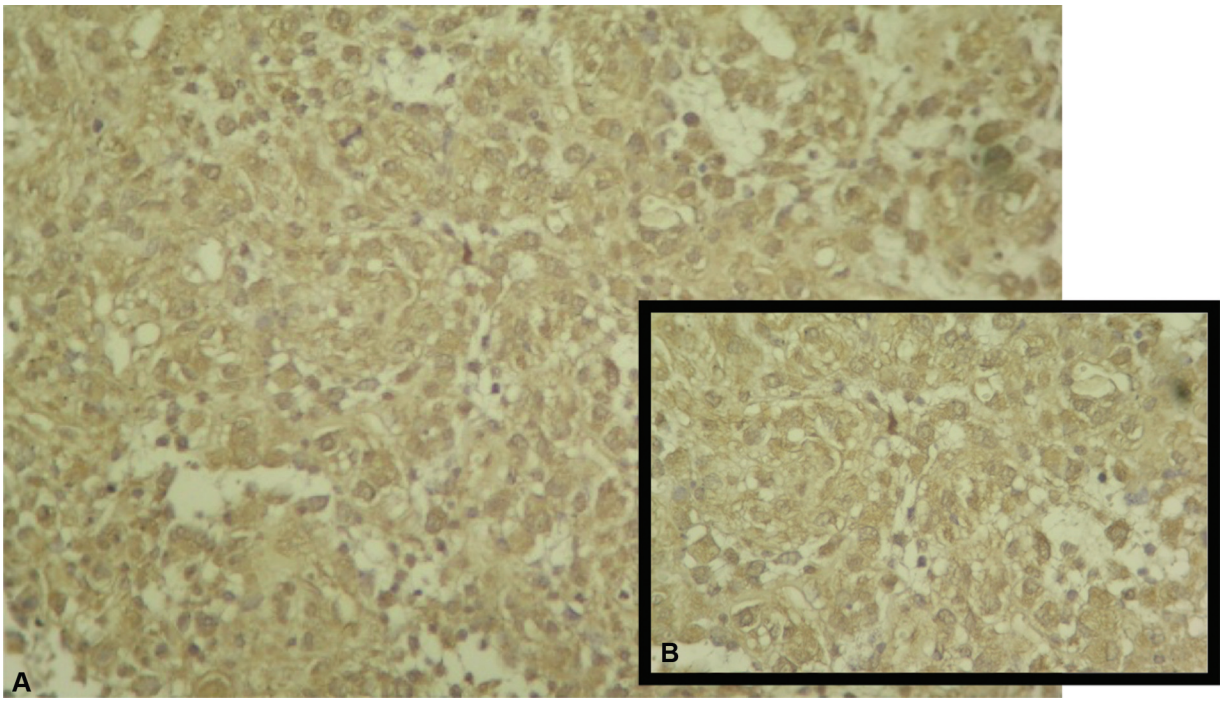


Fig. 4 Transforming growth factor beta 3immunoreactivity in tumor (GBM) tissue. (**A**) x200, (**B**) x400.

Table 2 Statistical immunoreactivity values of the transforming growth factors beta family and Smad receptors

RECEPTOR COMPARISON	*P-value*
TGF-β1 - TGF-β2	$P < 0.001^*$
TGF-β1 - TGF-β3	$P > 0.05$
TGF-β2 - TGF-β3	$P < 0.001$-
Smad 1/2/3 - Smad 6	$P > 0.05$
Smad 1/2/3 - Smad 7	$P > 0.05$
Smad 6 - Smad 7	$P < 0.001^*$

**p-value* < 0.001 is statistically significant.

the signal flow from cytoplasm to the nucleus, is achieved through type-I receptors.

Another receptor of the TGF-β family that mediates signal transduction is the type-III receptor. Type-III receptors meet extracellular ligands to reach signal receptors (types I and II).

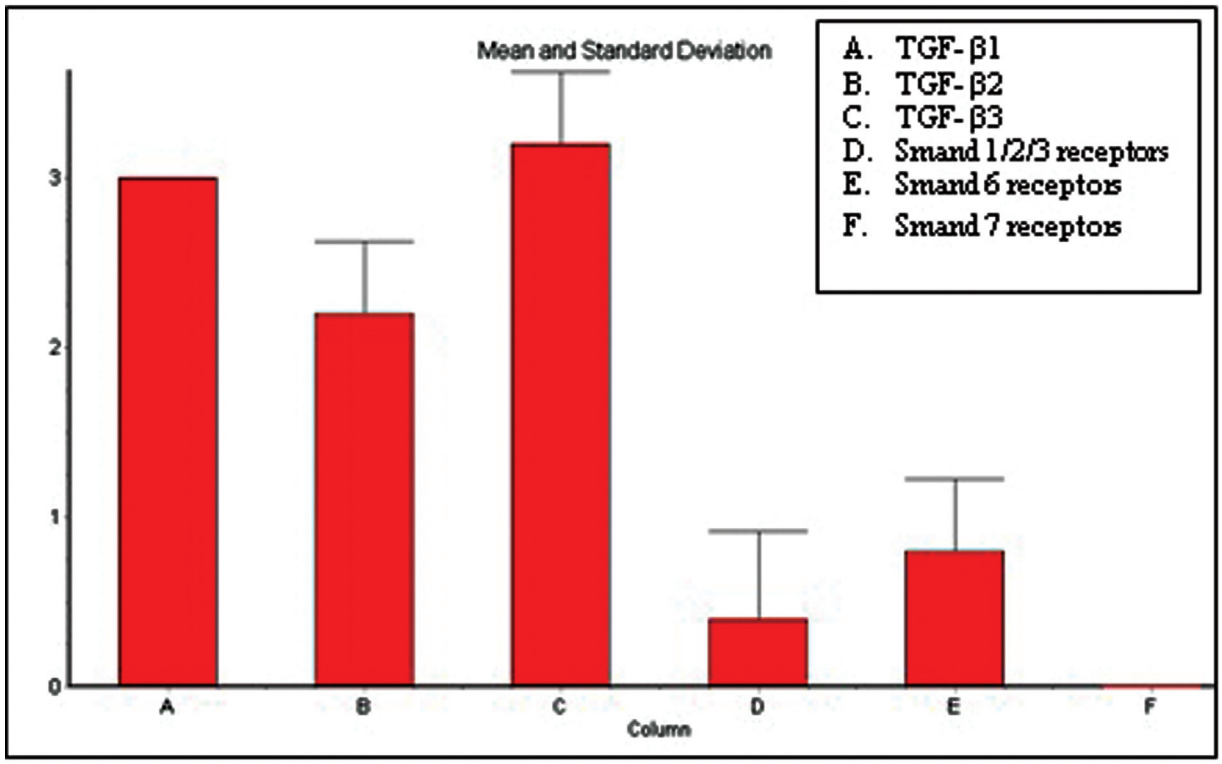
The Smad family proteins were first identified as substrates for type-I receptor kinases. They play a central role in transmitting the receptor signal to target genes in the nucleus. Approximately 10 Smad proteins have been identified to date. The Smad family is classified into three subgroups according to its structural and functional characteristics. The first group, which includes Smads 1, 2, 3, 5, 8 members, is called the "receptor-regulator Smad" (R-Smad). Smads 1, 5, and 8 interact specifically with bone morphogenetic protein (BMP) type-I receptors. Smads 2 and 3 interact with the receptors of activin and TGF-β. The second subgroup is the "common agent-Smad" (common-Smad = co-Smad). Co-Smads found in vertebrae are only Smad 4.

Co-Smads form heterooligomers with R-Smads and activate signal transduction to the nucleus. In vertebrae, the third group is the "inhibitory smad" proteins, including Smads 6 and 7. The only known function of Smads in this group is that R-Smads inhibit the signal activity. Smad 6 inhibits BMP signaling. Smad 7 inhibits both TGF-β and BMP signaling. It was observed that the levels of Smad 6 and 7 proteins act as negative feedback control of the function of Smads in response to BMP, activin, or TGF-β increase.[9]

Sasaki et al. showed active and latent forms of both TGF-β1 and TGF-β2 in malignant glioma cell culture. However, furthermore, the possible antitumoral activity of the TGF-β signal in gliomas has not been demonstrated[10]. On the contrary, evidence has been found that TGF-β expression in malignant brain tumors causes tumor cells to proliferate, migrate and invasion in the survival, while providing an advantage to tumor cells by causing angiogenesis and immune suppression.

Microvascular proliferation and necrosis are found in these aggressive and highly malignant tumors. Glial fibrillary acidic protein (GFAP) is undoubtedly the most important marker in neoplastic astrocytes, and many cells, including most aggressive GBMs, are stained with GFAP staining. Vimentin and fibronectin expressions are also frequent, but less specific.[11]

The K_i-67 nuclear antigen is a proliferation marker that is secreted only from proliferating cells. It has been shown that cell proliferation is particularly closely related to the S (synthesis) phase.[12,13] Quantitative K_i-67 measurements provide accurate information about the proliferation index of tumors. As a result of the studies on this subject, it has been revealed that it is closely related with cancer prognosis.[14]



Graphic 1 Average and standard deviation values of immunoreactivities of TGF-βs family and Smad receptors.

Epithelial-mesenchymal change is seen in the management of morphogenesis in multicellular organisms. This process also plays a role in the development of fibrosis and carcinoma. Well-preserved pathways play a role on this. As the epithelial cells lose their polarity, embryonic mesenchymal cell formation is observed with the loss of cell connections, deterioration of the cytoskeleton, and change of organelles. The loss of vimentin and e-cadherin is held responsible for this situation. Epithelial-mesenchymal transition development is followed by pathways associated with the tyrosine kinase surface receptor, TGF-β signaling pathways, influence of Smad proteins, and GTPase activity. In

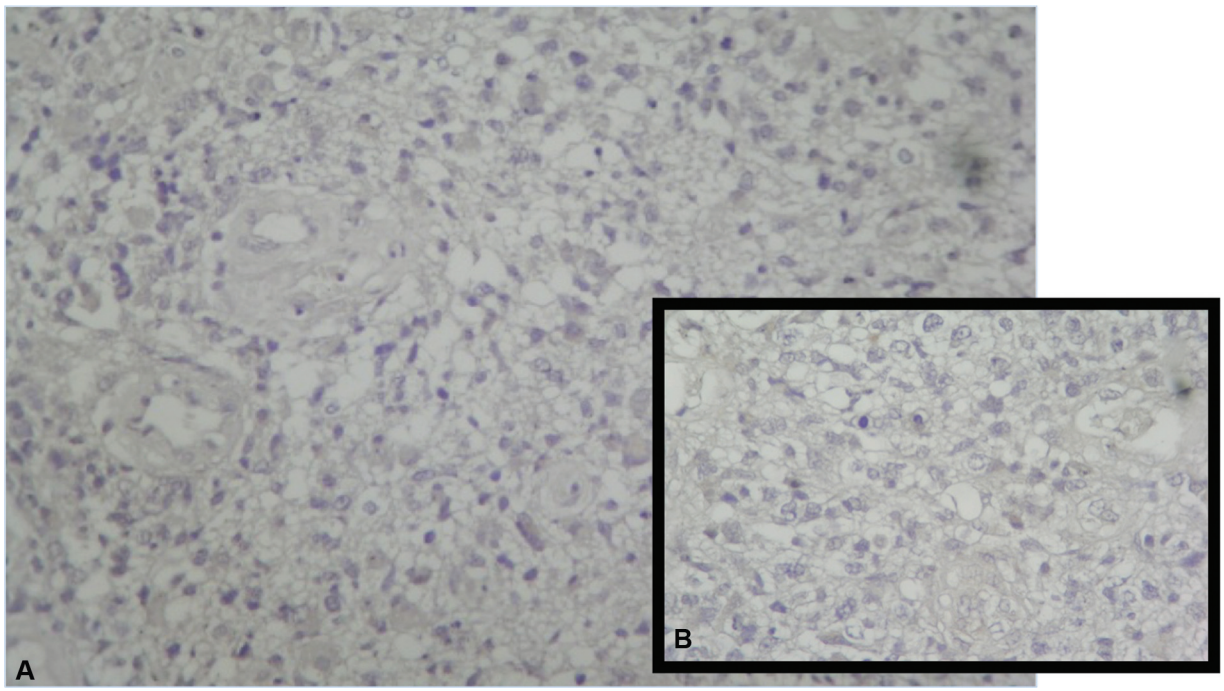


Fig. 5 Smad1/2/3 immunoreactivity in tumor (GBM) tissue (**A**) × 200, (**B**) × 400.

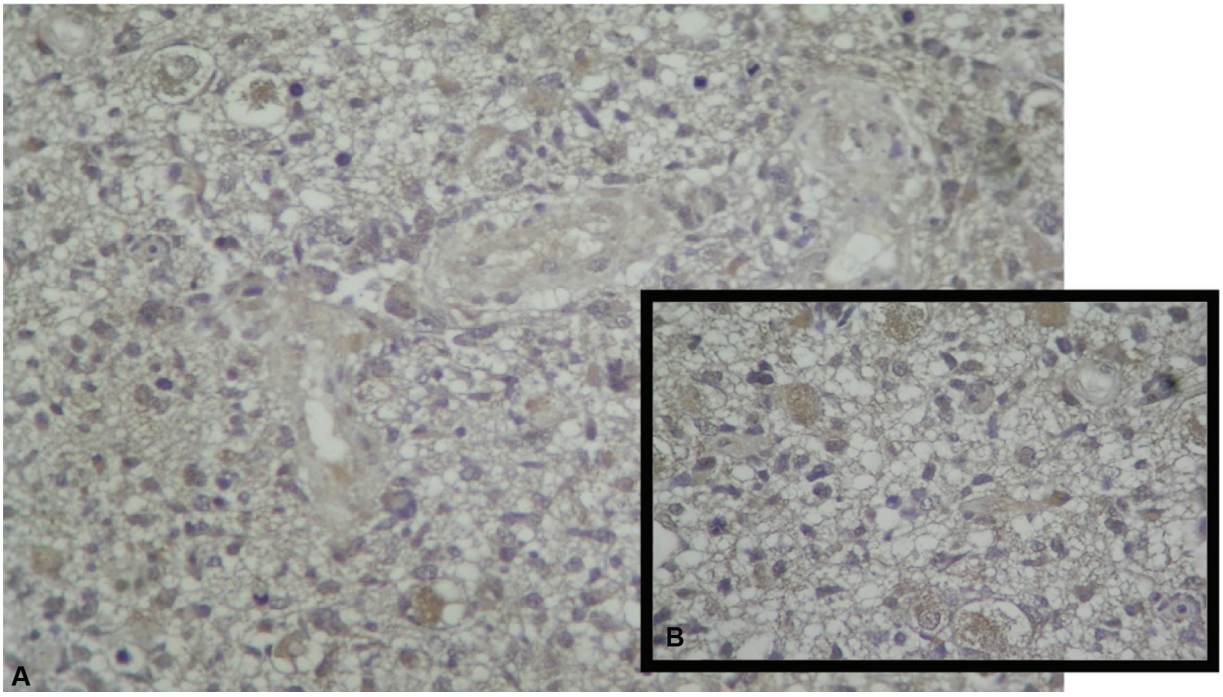


Fig. 6 Smad 6 immunoreactivity in tumor (GBM) tissue. (**A**) × 200, (**B**) × 400.

particular, TGF activity triggers the transcription of intracellular Smad family members by acting on type-I and type-II receptors. Epithelial cell plasticity and dedifferentiation are important steps in carcinoma development for invasive and metastatic tumor morphogenesis. Studies on the TGF family and activation on their receptors are particularly limited. Transforming growth factor family members are known to be important in cell proliferation, differentiation, and migration. Since it is thought that TGF family members may have roles in epithelial tumors—both primary and metastatic tumors—it is thought that knowing which receptors they activate, and which other pathways are activated after the activation of these receptors in the cell will be important in personalized treatments and effective tumor treatment. Since the roles of the TGF family and receptors in the EMT in metastatic cancer cells are also known, it will be important

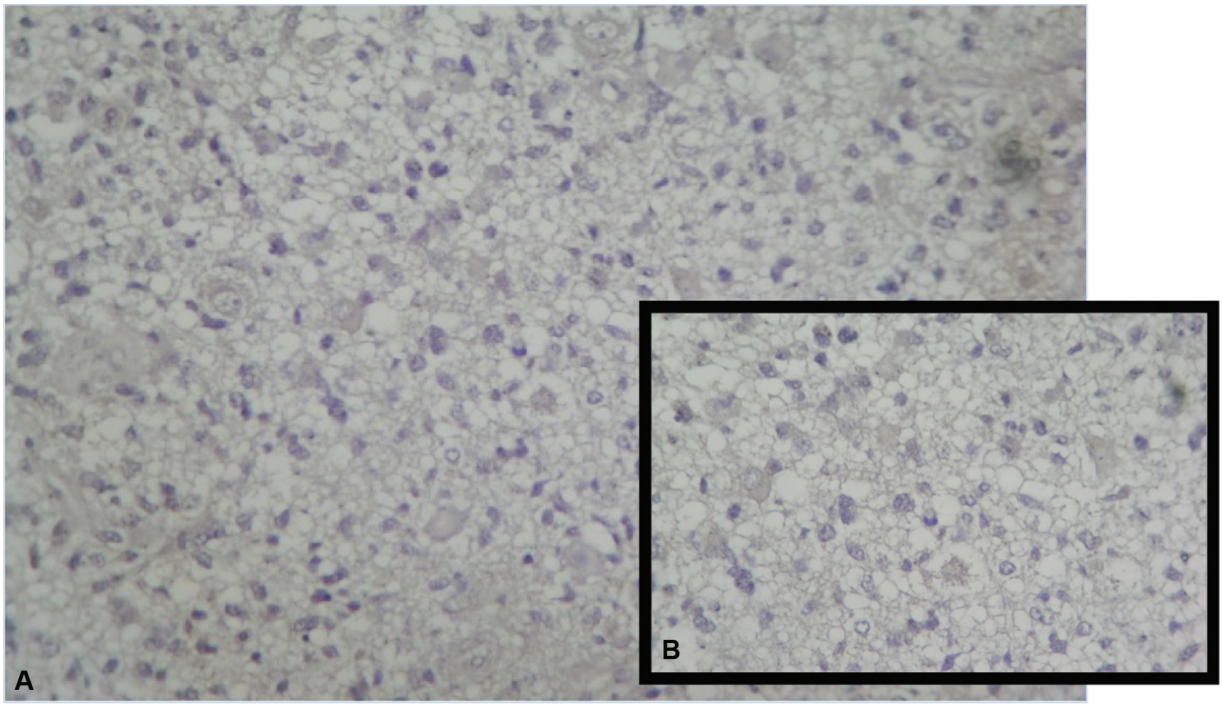


Fig. 7 Smad 7 immunoreactivity in tumor (GBM) tissue (**A**) × 200, (**B**) × 400.

to evaluate such patient groups in terms of the control mechanisms of the primary tumor and the tendency to metastatic tumor development. Although each of these pathways is the target of detailed studies, studies have been performed on its effect on prognosis and resistance to chemotherapy.[15]

High-grade gliomas tend to show severe invasion. Tumor relapse occurs frequently ($>90\%$) at the tumor margins, even after total tumor resection and many surgical procedures. This invasive phenotypic feature of GBM is thought to be due to the activation of some surface receptors, including receptor tyrosine kinase (RTKs), G-protein-coupled receptor (GPCRs), integrins, tumor necrosis factor (TNF), cytokine receptors, protein tyrosine phosphatase receptors, and the TGF-β family. On the other hand, locally released TGF is thought to play an active role in metastasis formation with phosphorylated Smad 2.

In the basal part, Smads are found as homooligomers. Type-I receptor kinases phosphorylate specific Smads after ligand activation of the receptor complex. The phosphorylated Smads then combine with Smad 4 and are transported to the cell nucleus. These complexes can be found alone or in conjunction with the DNA-binding subunit in the core and activate target genes by binding to specific promoter elements.

Nuclear translocations of Smads activated by the receptor occur through the phosphorylation induced by agonists and the binding kinetics with Smad 4. Smad 4 translocates into the cell nucleus in response to TGF-β and BMP, and translocation occurs in the presence of Smad 1 and Smad 2. Receptor-activating Smads bind to Smad 4 in the cytoplasm and transport it into the cell nucleus.

Various forms of negative regulation of cell proliferation, such as arresting the cell at the G_1 stage, increasing terminal differentiation or activation of cell death mechanisms, are the effects of TGF-β on target cells. It is thought that susceptibility to cancer or cancer may occur due to disruption of TGF-β signaling.

The fact that especially TGF-β1 and TGF-β3 immunoreactivities are high in the patient samples, in GBM cases, the tumor cells release these two TGF proteins, and as the receptor, Smad 6, which is the inhibitor receptor, is secreted more than Smad 7, suggesting that it has a protective effect in the tissue. Smad 6 and Smad 7 are inhibitors of signal transduction with R-Smads. In the absence of expression, Smad 6 inhibits BMP and partially TGF-β signal transduction. Smad 7 can also inhibit TGF-β and BMP signal transduction. On the other hand, Smad 1, 2, and 3 proteins phosphorylated by type-I receptor were found to be positive in these samples, and they were phosphorylated in some patient groups and initiated intracellular signaling; however, it results in some patient groups not being activated much.

Smads, the most important mediators of the TGF-β family of signal transduction, are exposed to different types of regulation mechanisms to complete and adapt the signal transduction according to the state of the cell. In cancer, the TGF-β signal transduction network is damaged by Smad 2, and Smad4/DPC4 mutations. Smad4/DPC4 has been identified as a candidate tumor suppressor gene on chromosome 18q21. Since TGF family members are activated with type-I and type-II surface receptors other than Smad receptors, the observation of positively stained TGFs, as well as the fact that Smad family members are less stained, TGF-β family members can trigger other receptors in addition to Smad protein in these patient samples and its effect suggests that it can show it that way. Precilinic data and experimental studies show that treatments targeting TGF-β are of great value in the treatment of gliomas.[1]

The observation of TGF-β2 immunoreactivity in both tumor cells and brain connective tissue showed that it is not only tumor-specific but also secreted from the cortex, suggesting that it is less important to evaluate it clinically in this patient group compared with TGF-β1 and TGF-β3.

Our study has potential limitations. One limitation is its retrospective design, and the other limitation is that this is a single-center study.

Conclusion

As a result of the histochemical and immunohistochemical staining of patients with high grade primary brain tumor (glioblastoma multiforme), it is observed that the expression of TGF-β1 and TGF-β3 proteins increases. Among the inhibitory receptors, Smad 6 is secreted more than Smad 7, suggesting that Smad 6 has a protective role in the tissue. Smad 1, 2, and 3 receptors were also partially increased in some patients and not at all in others. The intensive immunohistochemical staining of TGF-β family proteins activated by type-I and type-II receptors and the partial staining of the Smad family suggest that the TGF-β family can also act via a receptor mechanism other than Smad receptors.

Therefore, in addition to the investigation of Smad receptors, receptors such as Shc-GBR2, SARA, and Ras-Erk1/2 should also be examined, as they are important to regulate patient-specific chemotherapy regimens and immunotherapies for treatment and prognosis.

Conflict of Interests

The authors have no conflict of interests to delcare.

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