RESEARCH ARTICLE

Editorial Process: Submission:11/29/2017 Acceptance:09/21/2018

Gene Silencing of *TGFβRII* Can Inhibit Glioblastoma Cell Growth

Farzane Ordoni Aval[1], Shaghayegh Askarian Amiri[1], Abbas Azadmehr[1]*, Morteza Oladnabi[2,3], Payam Saadat[4], Hadi Ebrahimi[4], Behzad Baradaran[5], Behzad Mansoori[2,3], Fereshteh Pourabdolhossein[6], Pedram Torabian[2], Mahmoud Hajiahmadi[7]

Abstract

Objective: Glioblastoma (GBM) is the most malignant and aggressive type of glioma, associated with a high rate of mortality. The transforming growth factor-β receptor II (TGFβ RII) is involved in glioma initiation and progression. On the other hand, TGFβ RII silencing is critical to the inhibition of GBM. Therefore, we aimed to determine the effects of specific TGFβ RII siRNA on the survival of U-373MG cells. **Methods:** TGFβ RII siRNA was transfected, and qRT-PCR was performed to examine TGFβ RII mRNA expression. Cell survival was determined using colorimetric MTT assay, and platelet-derived growth factor-BB (PDGF-BB) level was measured in the culture supernatant using ELISA assay. **Result:** Our findings indicated that specific siRNAs could dose-dependently suppress TGFβ RII mRNA expression after 48 hours. In addition, treatment with TGFβ RII siRNA significantly reduced tumor cell survival and decreased the amount of PDGF-BB protein in the cell culture supernatant. **Conclusion:** Our results suggest that TGFβ RII silencing can be a promising complementary treatment for glioma.

Keywords: TGF-β RII- siRNA- U-373 MG cell line- PDGF-BB- Glioblastoma

Asian Pac J Cancer Prev, **19 (9)**, 2681-2686

Introduction

Gliomas or malignant astrocytomas are the most frequent type of primary cancer, arising from the brain. The most malignant and aggressive type of glioma is glioblastoma multiforme (GBM), also known as grade-IV astrocytoma. This disease is recognized as a common and fatal brain tumor in adults, associated with high rates of morbidity and mortality. It accounts for about 15% of all intracranial tumors in 40- to 75-year-old adults (Holland, 2001; Iacob and Dinca, 2009) . The incidence of glioma in the United States is about 5 in 100,000 people per year (Ostrom et al., 2013).

Despite major progress in chemotherapy, radiation, surgery, and complementary medicine in the treatment of tumors, prognosis remains extremely poor (Hofer and Herrmann, 2001; Stupp et al., 2005; Amirghofran et al., 2007; Yamanaka and Saya, 2009; Zare Shahneh et al., 2013). On the other hand, anatomical localization of the brain and suppression of antitumor immune responses in glioblastoma contribute to its aggressiveness (Stupp et al., 2005). Overall, long-term survival of patients with glioma is limited. Therefore, it is necessary to recognize the basic molecular pathways, leading to glioma formation in order to develop new therapeutic strategies (Nakano et al., 1995).

Among different pathways, transforming growth factor β (TGF-β) seems to contribute to glioma initiation and progression owing to its major influence on cell proliferation (Alexandrow and Moses, 1995), tumor invasion (Wesolowska et al., 2008), angiogenesis (Ueki et al., 1992), immunosuppression (Platten et al., 2001) and maintenance of stemness of glioma stem cells (GSCs) (Ikushima et al., 2009). TGF-β cytokine in glioma, due to the increased expression of matrix metalloproteinase and decreased level of tissue inhibitors of metalloproteinase, can improve invasion in cells (Nakano et al., 1995; Platten et al., 2001).

Moreover, type I receptors (TbRI) are phosphorylated and activated through TGF-β binding to type-II receptors (TbRII). Subsequently, activated TbRI initiates cytoplasmic signaling pathways, which phosphorylate

*[1]Cellular and Molecular Biology Research Center, Health Research Institute, [4]Mobility Impairment Research Center, Health Research Institute, [6]Department of Physiology, [7]Department of Biostatistics and Epidemiology, Babol University of Medical Sciences, Babol, [2]Ischemic Disorders Research Center, [3]Gorgan Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, [5]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*For Correspondence: azadmehr2010@gmail.com*

Smad proteins, Smad2 and Smad3, pairing with Smad4 (not phosphorylated by TbRI). In addition, adaptor proteins are recruited by the receptor complex, and some signaling proteins, transmitting biological information, are activated through posttranslational changes (Moustakas and Heldin, 2009; Akhurst and Hata, 2012).

Immunohistochemical studies have shown significantly higher TGFβ RI and TGFβ RII expression in advanced malignant glioma tissues in comparison with nontumorous gliosis (Yamada et al., 1995). In addition, glioma cell proliferation is promoted by TGF-β through inducing the expression of platelet-derived growth factor-BB (PDGF-BB) in a Smad2/3-dependent pathway (Ikushima et al., 2008). RNA interference is a sequence-specific process, which is known as short/small interfering RNA or silencing RNA, operating by double-stranded RNA (Zamore et al., 2000). Synthetic RNAs, which interfere with the target RNA, eventually cause degradation of target mRNA after transcription in mammalian cells (Elbashir et al., 2001).

In RNAi-based treatments, cancer is recognized as one of the most important targets. RNAi-based therapy is currently cost-effective, but shows high specificity and no side effects in comparison with other cancer treatments including chemotherapy (Bora et al., 2012; Ramachandran and Ignacimuthu, 2012). Moreover, another advantage of RNAi in cancer treatment is promotion of the effects of standard cancer drugs (Nieth et al., 2003; Karami et al., 2013; Li et al., 2016). Therefore, targeting TGFβ-mediated signaling with siRNA method may be an appropriate anti-GBM approach. Accordingly, we evaluated the effect of siRNA on the inhibition of TGF-β RII expression in the U-373 MG cell line.

Materials and Methods

Cell line and cell culture

The National Cell Bank of Iran (Tehran, Iran) supplied the human high-grade glioma cell line. The cells were added to RPMI-1640 medium, containing 1% penicillin-streptomycin and 10% fetal bovine serum (Gibco, USA). The U-373MG cells were cultured (5% CO2) at a temperature of 37°C and then used in the logarithmic phase of the experiments.

Transfection of SiRNA

In this study, TGFβ RII siRNA, scrambled control (nonspecific siRNA as the negative control), and transfection reagent were supplied by Santa Cruz Biotechnology Inc. The U-373MG cells were cultured in a 6-well culture plate (2×10^5 each well) in serum and antibiotic-free RPMI1640 medium. On the other hand, untreated cells were considered as the controls. Transfection was carried out in line with the manufacturer's guidelines.

The siRNA transfection medium (Santa Cruz Biotechnology, USA) was used to separately dilute the transfection reagent and TGFβ RII siRNA. After mixing the solution, it was incubated at room temperature for 30 minutes. After rinsing the cells with PBS, the mixture was added to each well of transfection medium (800 µL). Following incubation at 37°C for 6 hours inside a CO_2 incubator, RPMI-1640 medium consisting of 20% fetal bovine serum was added. Under the mentioned conditions, the cells were incubated. Finally, cell harvest was carried out at 48 hours following transfection for further analysis.

RNA isolation and qRT- PCR

Using YTA Total RNA Purification Mini Kit (Yekta Tajhiz Azma, Tehran, Iran), total RNA isolation from U-373MG cells was carried out, based on the manufacturer's guidelines. Afterwards, cDNA from total RNA (1 µg) was synthesized, using a random hexamer primer (1 µL), Moloney Murine Leukemia Virus Reverse Transcriptase (1 µL), 5X reaction buffer (4 µL), dNTPs (1µL; 10 mM each), and RNasin (0.6 µL; 40 U/µL) in line with the established guidelines. Using SYBR Green I Dye, quantitative real-time PCR (qRT-PCR) was carried out in a Light Cycler® 96 System (Roche Life Science, Germany). The dye binds to double-stranded (ds) DNA, producing a fluorescent signal, which can indicate the quantity of dsDNA product in PCR. The products were examined via melting curve analysis to confirm their specificity.

The PCR reaction included cDNA template (1 µL), primers (0.4 µL), SYBR green reagent (10 µL), 50X Passive Reference Dye (0.4 µL), and distilled water (nuclease-free; 7.8 µL). The primer sequences of TGFβ RII included: forward, 5'CACCTCCATCTGTGAGAAGCC-3'; and reverse, 5'GGAACACATGAAGAAAGTCTCACC 3'. On the other hand, the primer sequences of β-actin included: forward, 5'-TCCCTGGAGAAGAGCTACG-3'; and reverse, 5'GTAGTTTCGTGGATGCCACA-3'. The cycling conditions were as follows: denaturation for 60 seconds at a temperature of 95°C, succeeded by 40 cycles for 30 seconds at 95°C, for 30 seconds at 59°C, and for 30 seconds at 72°C. The size of the amplified TGFβ RII product was 193 bp. Finally, the relative expression levels in various groups were calculated using $2^{-(\Delta\Delta Ct)}$ method by normalizing the mRNA expression level in β-actin as the reference gene. All PCR reactions were performed in triplicate.

Cell cytotoxicity assay using MTT

The cytotoxic effect of TGFβ RII siRNA on glioma cell line, U-373MG, was determined using the MTT assay. In brief, 96-well plates were used for culturing the cells (2×10^4 cells per well). The study groups included untreated cells, as well as cells treated with scrambled siRNA (negative control), pure TGFβ RII siRNA, transfection reagent, and different doses of TGFβ RII siRNA (40-80 pmol). Following 2 days of incubation, MTT cytotoxicity kit (Sigma-Aldrich, MO, USA) was used to determine cytotoxicity of treatments, based on the manufacturer's guidelines. The optical density (OD) was calculated in each well at 570 nm (reference, 650 nm), using a sunrise™ System microplate reader (Tecan Life Sciences, Austria)

ELISA

The U-373MG cells were cultured and incubated in 5% CO_2 at 37°C. Afterwards, the cells were divided into four groups including siRNA TGFβ RII treated, control transfected, transfection reagent and non-transfected.

The concentration of PDGF-BB was measured in the supernatant with an ELISA kit (R and D System).

Statistical analysis

To compare the groups, ANOVA test was applied in GraphPad Prism. The findings are expressed as mean±SD, and the significance level was 0.05.

Results

SiRNA down regulated TGF-βRII mRNA expression in U-373 MG cell line

The effect of siRNA on the expression of TGFβ RII genes was examined in U-373 MG glioblastoma cells, according to Figures 1 and 2. In the RT-PCR analysis, the quantitative data from each sample were normalized against β-actin and reported as relative percent knockdown of mRNA levels, compared to the control group (untreated cells; 100%). The present results indicated that treatment with specific siRNA could dose- and time-dependently reduce TGFβ RII mRNA level (P< 0.05; Figures 1 and 2).

Our findings showed that relative expression levels of TGFβ RII were 34.36%, 20.67%, and 60.09% at 24, 48, and 72 hours after transfection, respectively (Figure 1). The optimal knockdown time was considered as 48 hours. The relative expression of different TGFβ RII siRNA concentrations (40, 60 and 80 pmol) was 29.04%, 19.23%, and 10.61%, respectively (P< 0.05; Figure 2).

The optimal knockdown concentration was 80 pmol. In addition, β-actin mRNA expression (internal control for qRT-PCR) was similar among the groups. Based on the findings, treatment with NC siRNA had no major effects on mRNA level in comparison with the controls. In addition, the scrambled control was a sequence, which did not cause specific degradation of cellular mRNA. These findings demonstrated that TGFβ RII specific siRNA could effectively suppress TGFβ RII mRNA expression in U-373 MG glioblastoma cells, without any nonspecific effects on the expression of β-actin mRNA.

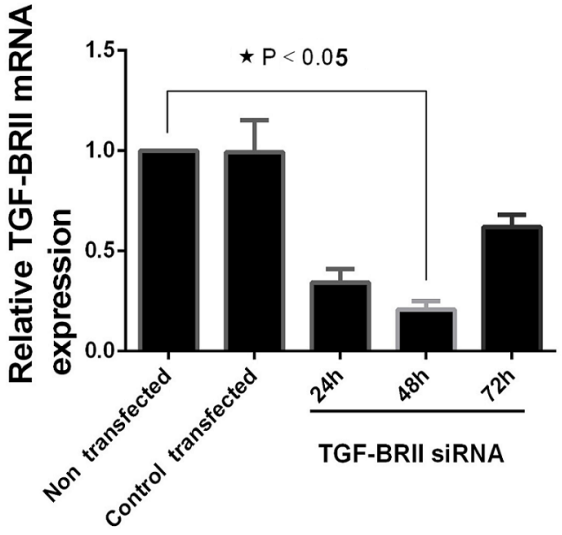

Figure 1. The U-373 MG Tumor Cells Transfected with TGFβ RII siRNA. Total RNA was extracted and mRNA was analyzed via qRT-PCR assay at 24, 48, and 72 hours following transfection. The relative mRNA expression was quantified by 2-(ΔΔCT) formula (β-actin, internal control) (data presented as mean±SD; *P< 0.05 vs. controls).

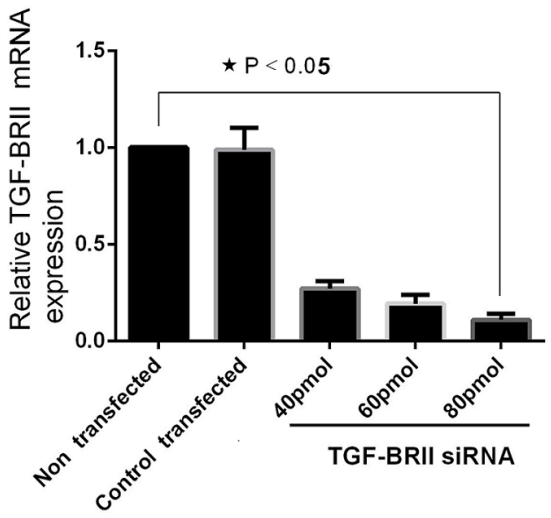

Figure 2. The U-373 MG Tumor Cells Transfected with siRNA (40, 60, and 80 pmol). Total RNA was extracted and mRNA was analyzed via qRT-PCR at 48 hours after transfection. The relative expression of mRNA was determined with 2-(ΔΔCT) formula (β-actin, internal control) (data presented as mean±SD; *P< 0.05 vs. controls).

TGF-βRII suppression caused cytotoxic effect on U-373 MG cells

In this study, the effect of TGFβ RII downregulation was examined in U-373 MG cells. As presented in Figure 3, TGFβ RII specific siRNA treatment, in a dose-dependent manner, could cause cytotoxicity. According to the MTT results, TGFβ RII siRNA (60 and

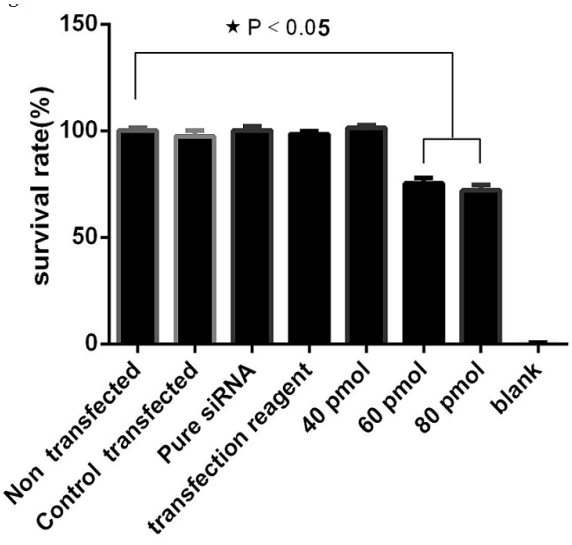

Figure 3. The U-373 MG Tumor Cells Transfected with TGFβ RII siRNA (40, 60, and 80 pmol). Cytotoxicity was examined using MTT assay after 48 hours (data presented as mean±SD; *P< 0.05 vs. controls).

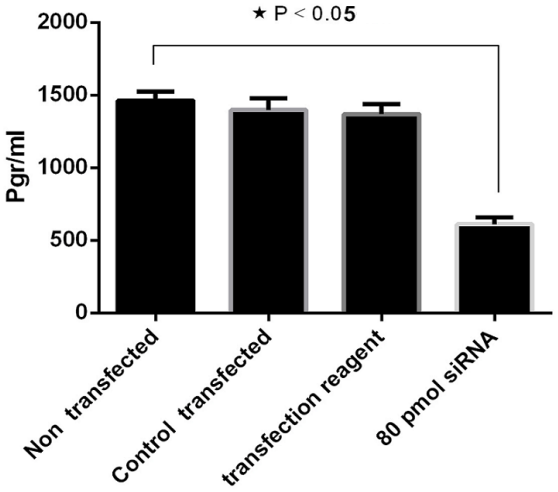

Figure 4. The U-373 MG Tumor Cells Treated with TGFβ RII siRNA (80 pmol). PDGF-BB protein released into the cell culture supernatant was analyzed by ELISA (data presented as mean±SD; *P< 0.05 vs. controls).

80 pmol), in comparison with the negative siRNA control, could significantly decrease cell survival (P< 0.05).

TGF-βRII siRNA decreased the production of PDGF-BB protein in U373-MG cells

GBM by increasing TGF/SMAD signaling pathway induces PDGF-BB expression and leads to proliferation of glioma cells. Therefore, we performed quantitative PDGF-BB ELISA assay, using the cell culture supernatant from the cells. In this study, U-373 MG glioma cells were treated with specific siRNA TGFβ RII. As shown in Figure 4, our results indicated that knockdown of TGFβ RII expression by siRNA in U-373MG cell line significantly reduced the PDGF-BB protein content (P< 0.05).

Discussion

In this study, the suppressive effect of siRNA TGFβ RII in the U-373 MG glioblastoma cell line was examined. GBM is among the most malignant and aggressive primary brain tumors in humans (Furnari et al., 2007; Dunn et al., 2012; Omuro and DeAngelis, 2013). At the present, standard methods for GBM therapy included surgery, radiation, and chemotherapy. Although these treatment methods decrease the number of proliferative glioblastoma cells, recurrence of this tumor is common (Xie et al., 2014).

As shown in the present study, evaluation of molecular mechanisms and genomic characterization could significantly improve our understanding of GBM proliferation and invasion; therefore, it is necessary to find novel methods to control and treat this cancer (Xie et al., 2014). Moreover, siRNA, as a molecular targeted cancer treatment, has been shown to be effective in the knockdown of cancer genes (Elbashir et al., 2001; Karami et al., 2013). Therefore, the suppressive effect of siRNA TGFβ RII on GBM (U-373 MG cell line) was investigated in our in vitro study.

TGF-β is a key molecule, which seems to be involved in the malignancy of numerous cancers (Bierie and Moses, 2006). TGF-β has a dual function in the Suppression or progression of different cancers relative to the condition of tumor microenvironment. On the other hand, the cells secrete significant amounts of TGF-β in the later stages of GBM for cell proliferation (Alexandrow and Moses, 1995), tumor invasion (Wesolowska et al., 2008), angiogenesis (Ueki et al., 1992), immunosuppression (Platten et al., 2001), preservation of GSC stemness (Ikushima et al., 2009), and stimulation of epithelial-mesenchymal transition, allowing migration and metastatic dissemination (Huber et al., 2005).

Activated TGF-β is capable of binding to TGF-β receptors, including type I and II receptor subtypes. Type II receptors phosphorylate and activate type I receptor kinases with a signaling cascade after binding (Massague, 2012). Based on previous studies, TGF-β mediates glioma initiation and progression. It is also overexpressed in malignant glioma tissues, but undetectable in normal brain tissues. These studies suggest that TGF-β (particularly upregulated TGF-β2) contributes to glioma development (Samuels et al., 1989; Maxwell et al., 1992; Yamada et al., 1995).

Moreover, malignant gliomas show major expression of TGF-β and its receptors, while it is associated with lost growth inhibitory responses. These tumors show increased TbIR and TbIIR expression in comparison with normal tissues (often upregulated by TGF-β) (Yamada et al., 1995; DaCosta Byfield et al., 2004; Hjelmeland et al., 2004). TGFβ RII, as a transmembrane protein, seems to mitigate TGF-β signaling pathway in TGF-β binding. Since TGF-β signaling contributes to GBM progression, TGFβ RII mRNA knockdown was investigated in our in vitro study to determine the antitumoral effects of TGFβ RII with specific siRNAs.

We evaluated the expression of mRNA TGFβ RII via real-time PCR assay. The results confirmed that TGFβ RII siRNA significantly downregulated TGFβ RII mRNA in U-373MG cells. Wesolowska et al. showed that silencing of human TGFβ RII with shRNA in glioblastoma T98G cells eliminated glioma invasion and provided a novel tool for the development of glioma gene therapy (Wesolowska et al., 2008).

In addition, other researchers have indicated that TGFβ RI silencing with siRNA could majorly inhibit TGFβ-induced signal transduction and diminish the aggressiveness of bladder cancer cells (Li et al., 2010). Additionally, in a study by Ye et al., (2012), knockdown of TGFβ RII expression in glioma stem-like cells (GSLCs) significantly decreased the invasiveness of GSLCs (Ye et al., 2012). Our findings are in accordance with previous reports, showing that TGFβ RII siRNA significantly downregulated TGFβ RII mRNA in U-373MG glioblastoma cells; however, further studies on U-373MG glioma invasion are suggested.

According to the MTT assay, TGFβ RII downregulation significantly reduced the survival of glioblastoma cells. On the contrary, no major changes were found in cellular events or gene expression due to treatment with negative control siRNA. The findings showed the specific effects of TGFβ RII siRNA. These findings are in line with the

results reported by XU et al., (2011), who demonstrated that TGF-β signaling is essential to the progression of lung adenocarcinoma. Moreover, TGFβ RII knockdown reduced cancer cell survival and proliferation in A549 lung carcinoma cell line.

In the current research, TGFβ RII knockdown could negatively influence proliferation in U-373 MG cell line. However, further investigations regarding the effects of siRNA TGFβ RII on molecular agents, associated with glioblastoma proliferation and survival, are required. On the other hand, previous findings have demonstrated that TGF-β signaling is involved in tumorigenicity and stemness of GSCs (Ikushima et al., 2009). Moreover, through Smad-related induction of leukemia inhibitory factor (LIF), it could induce self-renewal capacity and stimulate JAK-STAT signaling pathway (Penuelas et al., 2009; Hardee et al., 2012).

Moreover, other studies reported that Sox family of proteins could promote GSC survival. They showed that TGF-β could directly trigger the expression of Sox4, which can promote the expression of Sox2, as an essential factor for survival of GSCs (Ikushima et al., 2009).

Our results indicated that TGFβ RII siRNA could suppress TGFβ RII expression in glioblastoma cells. In addition, after treatment with TGFβ RII siRNA, survival of glioblastoma cells significantly reduced. Our findings may be associated with survival maintenance factors, such as Sox2, Sox4, and JAK-STAT signaling pathway. However, future studies are recommended to identify the main mechanism.

PDGFs are identified as growth factors, which are structurally or functionally related to each other. This family consists of 5 dimeric proteins, including PDGF-AA, AB, BB, CC, and DD (Andrae et al., 2008). According to the literature, PDGF-BB influences gliomagenesis (Dai et al., 2001). In a previous study, Bruna et al., (2007) showed that TGF-β induces glioma cell proliferation by PDGF-BB induction in tumors. They indicated that methylation of PDGF-BB gene determines the ability of TGF-β to induce PDGF-BB. This event is an oncogenic response in human glioma, which causes tumor proliferation through hyperactivity of Smad pathway (Bruna et al., 2007).

According to ELISA findings, treatment of U-373 MG cell line with siRNA TGFβ RII significantly decreased PDGF-BB protein in the cell culture supernatant, compared with the negative control. In agreement with previous studies, reduction of PDGF-BB protein after treatment with siRNA TGFβ RII could be associated with TGF-β in the Smad pathway; nonetheless, future studies are suggested in this area.

According to the present findings, the proposed method is a potential therapeutic strategy, which can suppress TGFβ RII expression and control cell proliferation in GBM. Accordingly, blocking the signaling pathway through TGFβ RII downregulation with siRNA can be a potential treatment for GBM.

Acknowledgments

We would like to thank the Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R. Iran for financial support of this project. Moreover, we would like to extend our thanks to Miss, Mahjoobeh Jafari for assisting in the partly of laboratory stage in this study. This manuscript is related to Miss, Ordoni thesis work for receiving of medical master of sciences degree in Medical Immunology. In addition, we used of the experienced scientific peoples in this field and research laboratory facilities of other university especially of Dr. Baradaran, Dr. Mansouri and Ms, Shajari from Tabriz University of Medical Sciences. Hereby, we would like to thank of Dr. Alireza Rafiei, Mr, Kardan and also Dr. Reza Norian, at Cellular and Molecular Biology Research Center from Mazandaran (Sari) University of Medical Sciences and also Qazvin Vice President of Reference Laboratory of food & feed Quality Control of Veterinary Organization for their assistances for performing of partly in laboratory stage of this study, respectively.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Akhurst RJ, Hata A (2012). Targeting the TGFbeta signalling pathway in disease. *Nat Rev Drug Discov*, **11**, 790-811.

Alexandrow MG, Moses HL (1995). Transforming growth factor beta and cell cycle regulation. *Cancer Res*, **55**, 1452-7.

Amirghofran Z, Bahmani M, Azadmehr A, et al (2007). Antitumor activity and apoptosis induction in human cancer cell lines by Dionysia termeana. *Cancer Invest*, **25**, 550-4.

Andrae J, Gallini R, Betsholtz C (2008). Role of platelet-derived growth factors in physiology and medicine. *Genes Dev*, **22**, 1276-312.

Bierie B, Moses HL (2006). Tumour microenvironment: TGFbeta: the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer*, **6**, 506-20.

Bora RS, Gupta D, Mukkur TK, et al (2012). RNA interference therapeutics for cancer: challenges and opportunities (review). *Mol Med Rep*, **6**, 9-15.

Bruna A, Darken RS, Rojo F, et al (2007). High TGFbeta-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. *Cancer Cell*, **11**, 147-60.

DaCosta Byfield S, Major C, Laping NJ, et al (2004). SB-505124 is a selective inhibitor of transforming growth factor-beta type I receptors ALK4, ALK5, and ALK7. *Mol Pharmacol*, **65**, 744-52.

Dai C, Celestino JC, Okada Y, et al (2001). PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes Dev*, **15**, 1913-25.

Dunn GP, Rinne ML, Wykosky J, et al (2012). Emerging insights into the molecular and cellular basis of glioblastoma. *Genes Dev*, **26**, 756-84.

Elbashir SM, Harborth J, Lendeckel W, et al (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, **411**, 494-8.

Furnari FB, Fenton T, Bachoo RM, et al (2007). Malignant astrocytic glioma: genetics, biology, and paths to treatment. *Genes Dev*, **21**, 2683-710.

Hardee ME, Marciscano AE, Medina-Ramirez CM, et al (2012). Resistance of glioblastoma-initiating cells to radiation mediated by the tumor microenvironment can be abolished

by inhibiting transforming growth factor-beta. *Cancer Res*, **72**, 4119-29.
Hjelmeland MD, Hjelmeland AB, Sathornsumetee S, et al (2004). SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. *Mol Cancer Ther*, **3**, 737-45.
Hofer S, Herrmann R (2001). Chemotherapy for malignant brain tumors of astrocytic and oligodendroglial lineage. *J Cancer Res Clin Oncol*, **127**, 91-5.
Holland EC (2001). Gliomagenesis: genetic alterations and mouse models. *Nat Rev Genet*, **2**, 120-9.
Huber MA, Kraut N, Beug H (2005). Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr Opin Cell Biol*, **17**, 548-58.
Iacob G, Dinca EB (2009). Current data and strategy in glioblastoma multiforme. *J Med Life*, **2**, 386-93.
Ikushima H, Komuro A, Isogaya K, et al (2008). An Id-like molecule, HHM, is a synexpression group-restricted regulator of TGF-beta signalling. *EMBO J*, **27**, 2955-65.
Ikushima H, Todo T, Ino Y, et al (2009). Autocrine TGF-beta signaling maintains tumorigenicity of glioma-initiating cells through Sry-related HMG-box factors. *Cell Stem Cell*, **5**, 504-14.
Karami H, Baradaran B, Esfahani A, et al (2013). siRNA-mediated silencing of survivin inhibits proliferation and enhances etoposide chemosensitivity in acute myeloid leukemia cells. *Asian Pac J Cancer Prev*, **14**, 7719-24.
Li W, Jiang P, Sun X, et al (2016). Suppressing H19 modulates tumorigenicity and stemness in U251 and U87MG Glioma cells. *Cell Mol Neurobiol*, **36**, 1219-27.
Li Y, Yang K, Mao Q, et al (2010). Inhibition of TGF-beta receptor I by siRNA suppresses the motility and invasiveness of T24 bladder cancer cells via modulation of integrins and matrix metalloproteinase. *Int Urol Nephrol*, **42**, 315-23.
Massague J (2012). TGFbeta signalling in context. *Nat Rev Mol Cell Biol*, **13**, 616-30.
Maxwell M, Galanopoulos T, Neville-Golden J, et al (1992). Effect of the expression of transforming growth factor-beta 2 in primary human glioblastomas on immunosuppression and loss of immune surveillance. *J Neurosurg*, **76**, 799-804.
Moustakas A, Heldin CH (2009). The regulation of TGFbeta signal transduction. *Development*, **136**, 3699-714.
Nakano A, Tani E, Miyazaki K, et al (1995). Matrix metalloproteinases and tissue inhibitors of metalloproteinases in human gliomas. *J Neurosurg*, **83**, 298-307.
Nieth C, Priebsch A, Stege A, et al (2003). Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). *FEBS Lett*, **545**, 144-50.
Omuro A, DeAngelis LM (2013). Glioblastoma and other malignant gliomas: a clinical review. *JAMA*, **310**, 1842-50.
Ostrom QT, Gittleman H, Farah P, et al (2013). CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006-2010. *Neuro Oncol*, **15**, 1-56.
Penuelas S, Anido J, Prieto-Sanchez RM, et al (2009). TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. *Cancer Cell*, **15**, 315-27.
Platten M, Wick W, Weller M (2001). Malignant glioma biology: role for TGF-beta in growth, motility, angiogenesis, and immune escape. *Microsc Res Tech*, **52**, 401-10.
Ramachandran PV, Ignacimuthu S (2012). RNA interference as a plausible anticancer therapeutic tool. *Asian Pac J Cancer Prev*, **13**, 2445-52.
Samuels V, Barrett JM, Bockman S, et al (1989). Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. *Am J Pathol*, **134**, 894-902.
Stupp R, Mason WP, van den Bent MJ, et al (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*, **352**, 987-96.
Ueki N, Nakazato M, Ohkawa T, et al (1992). Excessive production of transforming growth-factor beta 1 can play an important role in the development of tumorigenesis by its action for angiogenesis: validity of neutralizing antibodies to block tumor growth. *Biochim Biophys Acta*, **1137**, 189-96.
Wesolowska A, Kwiatkowska A, Slomnicki L, et al (2008). Microglia-derived TGF-beta as an important regulator of glioblastoma invasion--an inhibition of TGF-beta-dependent effects by shRNA against human TGF-beta type II receptor. *Oncogene*, **27**, 918-30.
Xie Q, Mittal S, Berens ME (2014). Targeting adaptive glioblastoma: an overview of proliferation and invasion. *Neuro Oncol*, **16**, 1575-84.
Xu CC, Wu LM, Sun W, et al (2011). Effects of TGF-beta signaling blockade on human A549 lung adenocarcinoma cell lines. *Mol Med Rep*, **4**, 1007-15.
Yamada N, Kato M, Yamashita H, et al (1995). Enhanced expression of transforming growth factor-beta and its type-I and type-II receptors in human glioblastoma. *Int J Cancer*, **62**, 386-92.
Yamanaka R, Saya H (2009). Molecularly targeted therapies for glioma. *Ann Neurol*, **66**, 717-29.
Ye XZ, Xu SL, Xin YH, et al (2012). Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-beta1 signaling pathway. *J Immunol*, **189**, 444-53.
Zamore PD, Tuschl T, Sharp PA, et al (2000). RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell*, **101**, 25-33.
Zare Shahneh F, Valiyari S, Baradaran B, et al (2013). Inhibitory and cytotoxic activities of salvia officinalis L. Extract on human lymphoma and leukemia cells by induction of apoptosis. *Adv Pharm Bull*, **3**, 51-5.