Research Article

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SOX7 inhibits tumor progression of glioblastoma and is regulated by miRNA-24

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Abstract: Objective: Sex-determining region Y-box 7 (SOX7) is a putative tumor suppressor in various types of human cancers. In the present study, the expression and function of SOX7 was investigated in human glioblastoma (GBM) cells. Methods: Real-time PCR and western blot were carried out to reveal the expression of SOX7 in GBM specimens and cultured cell lines. A short interfering RNA (siRNA) targeting SOX7 was synthesized and transfected into U87 cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to evaluate the cell proliferation ability in U87 cells. Bioinformatics analysis further predicted its regulation by microRNA-24 (miR-24). Luciferase reporter assay was performed to prove this regulation. Results: SOX7 was downregulated in GBM specimens and cell lines. Inhibition of SOX7 in cultured U87 cells resulted in a slower growth rate. Mechanically, SOX7 was a target of miR-24, demonstrated by reporter assay. Conclusion: SOX7 was a strong tumor suppressor regulated by miR-24 in human GBM cells.

Keywords: SOX7, glioma, miR-24, proliferation, invasion

1 Introduction

SOX proteins are a family of transcription factors, which have high-mobility-group DNA-binding domain (HMG box), and they have been reported to play vital roles in embryogenesis [1-3]. Some members of the SOX family are negative regulators of the WNT-β-catenin-TCF signaling pathway [4]. SOX7, a member of subgroup F along with SOX17 and SOX18, has been reported to regulate hematopoiesis and cardiogenesis originally [5-7]. Recently, accompanied with the increasing evidence, SOX7 in particular has also been revealed to be a tumor suppressor in a number of human cancers. In detail, for example, its downregulation was observed in tumors from the ovarian [8], gastric [9] and lung [10]. Functionally, studies have proved that overexpression of SOX7 could inhibit hepatocellular carcinoma cell growth, with G1 to S phase arrest [11]. ShRNA-mediated SOX7 silencing in non-tumorigenic breast cells increased proliferation, migration, and invasion. Conversely, ectopic SOX7 expression inhibits proliferation, migration, and invasion of breast cancer cells in vitro and tumor growth in vivo [12]. Although its role is finely interpreted in multiple human cancers, the expression, function and regulation have not been addressed in GBM yet.

Glioma constitutes more than 70% of all primary neoplasms that develop in the CNS [13]. GBM, the highest-grade glioma, is the most common and aggressive type of primary brain tumor in human [14,15]. Despite improved understanding of the molecular and physiological features of GBM, there are no effective treatments for this type of brain cancer. The average prognosis is still uniformly poor, and the median life expectancy after diagnosis is 15 months [16]. Hence, there is an urgent need for novel targets, concepts, and approaches to treat this disease.

Recent studies suggest that SOX7 acts as a key tumor suppressor in many cancers, but its actual expression and regulation in GBM remain unclear. In the present study, we demonstrated that SOX7 targeted by miR-24 was significantly decreased in GBM specimens and cell lines, and it could suppress the proliferation ability of U87 cells.
2 Materials and methods

2.1 Cell Lines and Tissues

Human astrocytes were obtained from Gibco (Life Technologies). Human glioblastoma cell lines U251, U343, U87, LN229 and TJ905 were obtained from the Cell Bank of the Chinese Academy of Sciences or ATCC. Cells were cultured in DMEM medium with 10% fetal bovine serum. All cells were maintained in a humidified incubator at 37°C and 5% CO₂.

Tumor tissues with the corresponding paired normal tissues were obtained from glioblastoma patients at the Tianjin Nankai hospital between 2013 and 2015. Informed written consent was obtained from all patients.

2.2 qRT-PCR

Expression levels of total mRNAs were quantified by two-step quantitative real-time PCR. All qRT-PCR reactions were performed in triplicate on ABI PRISM 7900HT Real-Time PCR System. Data were analysed with the RQ Manager 1.2.1 software, using the 2−ΔΔCt method with a relative quantification RQmin/RQmax confidence set at 95%.

2.3 Cell viability assay

Cells were plated in 96-well plates at 2×10³ per well 24 h post-transfection. MTT (20µl, 5mg/ml) was added to each well and cells were incubated for another 4 h at 37°C. The reaction was stopped by addition of 150µl DMSO and optical density at 590nm was determined on a microplate reader.

2.4 Western blot analysis

Total proteins were extracted using RIPA buffer (Beyotime, Jiangsu, China). Then total proteins were separated by SDS-PAGE gel and transferred to nitrocellulose membranes. The membranes were probed with anti-SOX7 and anti-GAPDH antibodies (Santa Cruz). Proteins were visualized by a HRP-conjugated secondary antibody.

2.5 Vector Construction and Transfection

The 3′-UTR of SOX7 containing the putative miR-24 binding site was amplified and cloned into the pGL3basic vector (Promega, Madison, WI). Transfection was performed with Lipofectamine™ 2000 (Invitrogen) according to the manufacturer’s protocol.

2.6 Luciferase activity assay

Luciferase assays were carried out as described below. Briefly, cells were co-transfected with luciferase reporter constructs, miRNA and Renila using Lipofectamine™2000 (Invitrogen), and Firefly and Renilla luciferase activities measured after 48 h with a Dual-LuciferaseReporter Assay System (Promega) using a luminometer.

2.7 MicroRNA Mimics, inhibitors and SOX7 siRNAs

The miR-24 mimics and inhibitors and SOX7 siRNAs were purchased by GenePharma (Shanghai, China). The cells were transfected with mimics or inhibitors using Lipofectamine™ 2000.

2.8 Statistical analysis

All results were expressed as mean ± S.D. Data analysis was performed by SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was analyzed using Student’s t-test. Differences with P < 0.05 were considered statistically significant.

3 Results

3.1 SOX7 was downregulated in primary GBM tumor samples and cell lines

To determine its expression level in GBM, we firstly evaluated the mRNA levels in primary GBM tumor tissues by using quantitative real-time PCR (qRT-PCR). We found that SOX7 was downregulated in more than half of GBM tissues compared with the matched non-tumor brain tissues (Figure 1A). We next examined its expression in five GBM cell lines (U251, U343, U87, LN229, TJ905) compared with
that of in normal human astrocytes. The results showed that the SOX7 mRNA was frequently downregulated in GBM cell lines, especially in U251 and U343 cells (Figure 1B). These findings were in line with previous studies characterizing decreased SOX7 expression in other malignancies [8-12], which further indicated a tumor suppressive role of SOX7 in GBM carcinogenesis.

3.2 Silencing of intrinsic SOX7 inhibited cell growth rate of GBM cell line U87

To address the functional effects of downregulated SOX7 in GBM cells, we transfected U87 cells with small interfering RNAs (siRNAs) that targeted 3'-UTR region of SOX7 gene. The knockdown efficiency was validated by qRT-PCR and western blot (Figure 2A and 2B). To test the ability of SOX7 silencing in inhibiting cell proliferation, MTT assay was performed and as the result a significant elevation of cell growth rate after SOX7 silencing was observed (Figure 2C). These findings strongly supported a tumor suppressive function of SOX7 in GBM, which had not been reported ever since.

3.3 SOX7 was a putative target of microRNA-24 in GBM

Previous studies have pointed out that SOX7 might be a target of microRNA-24 (miR-24) in hepatocellular carcinoma (HCC), in which forced expression of SOX7 substantially attenuated the oncogenic effects of miR-24 [17]. To justify a direct interaction between miR-24 and the 3'-untranslated region (3'-UTR) of SOX7, which contains the putative miR-24 recognition sites, we constructed a renilla luciferase reporter gene in the pGL3 vector by inserting the 3'-UTR region of human SOX7 and flanking sequences. The mimics and inhibitor of miR-24, as well as the negative control oligonucleotides were transfected into U87 cells along with the reporter gene. As shown in Figure 3A, the luciferase activity was significantly decreased in miR-24-mimics-transfected cells (compare lane 2 with lane 1, \( P<0.01 \)), whereas the miR-24-inhibitor-transfected U87 cells exhibited no obvious change of SOX7 transcription (compare lane 3 with lane 1), which might be explained by the low endogenous expression of SOX7 in U87 cells. In addition, we further confirmed the protein expression of SOX7 upon miR-24 mimics or inhibitor transfection, which was also direct evidence, that SOX7 is regulated by miR-24 (Figure 3B). Finally, qRT-PCR was carried out to detect the miR-24 expression in GBM cell lines. The results shown in Figure 3C revealed its upregulation in GBM cell lines, which was another evidence to support the miR-24/SOX7 axis since the negative correction of these two molecules.

4 Discussion

In the present study, we observed the downregulation of SOX7 in primary GBM tumor specimens as well as cell lines. We also discovered that knockdown of SOX7 in GBM U87 cells resulted in cell growth acceleration. Mechanically, we proved that SOX7 was a potential target of miR-24 by base pairing within its 3'-UTR.

Accumulating evidence shows that miRNAs participate in the development and progression of various human cancers, including GBM [18,19]. Several reports have found that miR-24 was increased and appeared as an oncogene in some tumors, including GBM [20,21]. In this study, we also showed that miR-24 was indeed upregulated in our selected GBM cell lines, and targeting of SOX7
Wang Zhen et al provided another explanation of the oncogenic activity of miR-24 in GBM, through negatively regulating SOX7 transcription. Since this targeting had been reported in another paper discussed in HCC [17], we did not pay more attention in this regulation aspect. The result also supported the tumor suppressive role of SOX7 in GBM cells, by inhibiting cell proliferation. However, since it was known that SOX7 could also impact on cancer cell apoptosis, migration and invasion processes [22,23], further evidence demonstrating its role in these mentioned biological processes should be studied.

Data from luciferase reporter assays showed that miR-24 could directly binds to the 3′-UTR of SOX7 mRNA and repressed its expression at translation levels. Yu
and his colleagues reported that in colorectal cancer cells, decreased expression of SOX7 was partially due to the aberrant DNA methylation of the gene [24], in which SOX7 promoter was frequently hypermethylated in colorectal cancers. This finding provided important information about the regulation of intrinsic SOX7 expression. In future studies, we should also consider this epigenetic regulation mechanism governing low SOX7 expression.

Collectively, we demonstrated in this study that SOX7 mRNA was frequently downregulated in primary GBM tumor samples and established cell lines, which was at least partially due to the oncogenic miR-24 targeting. In detail, SOX7 could inhibit GBM cell proliferation. Data presented in this report represent the attempt to disclose the role of SOX7 in suppressing GBM, which may be useful in the development of new therapeutic strategies for GBM.

**Conflict of interest statement:** Authors state no conflict of interest.

**References**

[1] Kamachi Y, Kondoh H. Sox proteins: regulators of cell fate specification and differentiation. Development. 2013 Oct;140(20):4129-4144

[2] Chew LJ, Gallo V. The Yin and Yang of Sox proteins: Activation and repression in development and disease. J Neurosci Res. 2009 Nov 15;87(15):3277-3287

[3] Kamachi Y, Uchikawa M, Kondoh H. Pairing SOX off: with partners in the regulation of embryonic development. Trends Genet. 2000 Apr;16(4):182-187

[4] Morini MF, Dejana E. Transcriptional regulation of arterial differentiation via Wnt, Sox and Notch. Curr Opin Hematol. 2014 May;21(3):229-234

[5] Ceremeta S, Moleri S, Cimbro S, Corti P, Del Giacco L, Amodeo R, Dejana E, Koopman P, Cotelli F, Beltrame M. Sox18 and Sox7 play redundant roles in vascular development. Blood. 2008 Mar 1;111(5):2657-2666

[6] Pendeville H, Winandy M, Manford I, Nivelles O, Motte P, Pasque V, Peers B, Struman I, Martial JA, Voz ML. Zebrafish Sox7 and Sox18 function together to control arterial-venous identity. Dev Biol. 2008 May 15;317(2):405-416

[7] Zhang C, Basta T, Klymkowsky MW. SOX7 and SOX18 are essential for cardiogenesis in Xenopus. Dev Dyn. 2005 Dec;234(4):878-891

[8] Liu H, Yan QZ, Li B, Yin SY, Sun Q, Kou JJ, Ye D, Ferns K, Liu HY, Liu SL. Reduced expression of SOX7 in ovarian cancer: a novel tumor suppressor through the Wnt/β-catenin signaling pathway. J Ovarian Res. 2016 Sep 5;7:7

[9] Cui J, Xi H, Cai A, Bian S, Wei B, Chen L. Decreased expression of Sox7 correlates with the upregulation of the Wnt/β-catenin signaling pathway and the poor survival of gastric cancer patients. Int J Mol Med. 2014 Jul;34(1):197-204

[10] Hayano T, Garg M, Yin D, Sudo M, Kawamata N, Shi S, Chien W, Ding LW, Leong G, Mori S, Xie D, Tan P, Koeffler HP. SOX7 is down-regulated in lung cancer. J Exp Clin Cancer Res. 2013 Apr 4;32:17

[11] Wang C, Guo Y, Wang J, Min Z. The suppressive role of SOX7 in hepatocarcinogenesis. PLoS One. 2014 May 9;9(5):e97433

[12] Stovall DB, Wan M, Miller LD, Cao P, Maglic D, Zhang Q, Stamper MR, Liu W, Xu J, Sui G. The regulation of SOX7 and its tumor suppressive role in breast cancer. Am J Pathol. 2013 Nov;183(5):1645-1653

[13] Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. Acta Neuropathol. 2005 Jan;109(1):93-108

[14] Tzadok S, Beery E, Israeli M, Uziel O, Lahav M, Fenig E, Gil-Ad I, Weizman A, Nordenberg J. In vitro novel combinations of psychotropics and anti-cancer modalities in U87 human glioblastoma cells. Int J Oncol. 2010 Oct;37(4):1043-1051

[15] Fiorenzo P, Mongiardi MP, Dimitri D, Cozzolino M, Ferri A, Montano N, Trevisi G, Maira G, Battistini L, Falchetti ML, Levi A, Pallini R. HIF1-positive and HIF1-negative glioblastoma cells compete in vitro but cooperate in tumor growth in vivo. Int J Oncol. 2010 Apr;36(4):785-791

[16] Wilson TA, Karajannis MA, Harter DH. Glioblastoma multiforme: State of the art and future therapies. Surg Neurol Int. 2014 May 8;5:64

[17] Ma Y, She XG, Ming YZ, Wan QQ. miR-24 promotes the proliferation and invasion of HCC cells by targeting SOX7. Tumour Biol. 2014 Nov;35(11):10731-10736

[18] Luo JW, Wang X, Yang Y, Mao Q. Role of micro-RNA (miRNA) in pathogenesis of glioblastoma. Eur Rev Med Pharmacol Sci. 2015 May;19(9):1630-1639

[19] Costa PM, Cardoso AL, Mano M, de Lima MC. MicroRNAs in glioblastoma: role in pathogenesis and opportunities for targeted therapies. CNS Neurol Disord Drug Targets. 2015;14(2):222-238

[20] Zhao G, Liu L, Zhao T, Jin S, Jiang S, Cao S, Han J, Xin Y, Dong Q, Liu X, Cui J. Upregulation of miR-24 promotes cell proliferation by targeting NAIF1 in non-small cell lung cancer. Tumour Biol. 2015 May;36(5):3693-3701

[21] Chen L, Zhang A, Li Y, Zhang K, Han L, Du W, Yan W, Li R, Wang Y, Wang K, Pu P, Jiang T, Jiang C, Kang C. MiR-24 regulates the proliferation and invasion of glioma by ST7L via β-catenin/Tcf-4 signaling. Cancer Lett. 2013 Feb 8;329(2):174-180

[22] Wang C, Guo Y, Wang J, Min Z. The suppressive role of SOX7 in hepatocarcinogenesis. PLoS One. 2014 May 9;9(5):e97433

[23] Stovall DB, Cao P, Sui G. SOX7: from a developmental regulator to an emerging tumor suppressor. Histol Histopathol. 2014 Apr;29(4):439-445

[24] Zhang Y, Huang S, Dong W, Li L, Feng Y, Pan L, Han Z, Wang X, Ren G, Su D, Huang B, Lu J. SOX7, down-regulated in colorectal cancer, induces apoptosis and inhibits proliferation of colorectal cancer cells. Cancer Lett. 2009 May 8;277(1):29-37