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Repression of Dok7 expression mediated by DNMT1 promotes glioma cells proliferation

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ABSTRACT

Malignant glioma is one of the most common primary human tumors in the central nervous system. The molecular mechanisms of the progression and development of glioma have been largely unexplored. In this study, we illustrated that the expression of Dok7 was downregulation in human glioma tissues. Dok7 overexpression significantly inhibits proliferation and colony formation in vitro, and the xenograft tumor formation in vivo. In addition, 5-Aza-2′-deoxycytidine (5-Aza), a DNA methylation inhibitor, preventing the loss of Dok7 expression by decreasing aberrant hypermethylation of Dok7 promoter in glioma cells. More importantly, DNMT1 knockdown induced the demethylation of Dok7 promoter, and enhanced the expression of Dok7 in gliomas. These results suggest that epigenetic silencing of Dok7 may provide a novel glioma treatment strategy.

1. Introduction

Glioma is one of the most common and aggressive type of human primary malignant tumor with a poor outcome in the brain [1]. Currently, the commonest therapeutic strategy for gliomas is surgical excision and the adjuvant therapy consists of radiotherapy and chemotherapy. Temozolomide is the most commonly used glioma chemotherapy drug, but gliomas are highly resistant to temozolomide. Although many promotions in treatments have been made in recent decades, the prognosis of patients with glioma, especially with high-grade malignant glioma, remains poor and the 5-year survival rate is almost lower than 5% [2–4].It is estimated that there should be 3000–5000 molecular targets that can be used as drugs in human genes, but only about 500 currently mature drug targets. In spite of various cellular and molecular mechanisms are available on malignant process, there is an urgent need to identify a novel target that can be used to suppress the development and progression of human glioma.

Since the tyrosine-phosphorylated and rasGAP-associated 62-kDa protein Dok1 was identified as a major substrate of many PTKs, the Dok-family are already seven members, Dok1 to Dok7, which share structural similarities characterized by the N-terminal pleckstrin homology (PH) and phosphotyrosine-binding (PTB) domains, followed by the src-homology2 (SH2) target motifs in its C-terminal moiety, suggesting an adaptor function [5,6]. Unlike Dok1 to Dok6, Dok7 is preferentially expressed in muscle tissues, and further immunohistochemical studies certify that Dok7 is colocalized with AChRs at the postsynaptic area of NMJ in skeletal muscle [7]. In addition, Dok7 also plays a role in the pathologic process of breast cancer [8]. However, to our knowledge, the role of Dok7 in human glioma has not been reported.

In the current study, we showed that Dok7 expression was downregulated in clinical tissues, whereas overexpression of Dok7 inhibited the proliferation of glioma cells *in vitro and in vivo*. Additionally, the inhibition of DNMT1 by 5-Aza treatment or DNMT1 knockdown induced demethylation of Dok7 and reversed the loss of Dok7 in glioma cells. These results suggest that the repression of Dok7 due to methylation of Dok7 promotes the growth of glioma cells. Therefore, Dok7 may serve as a potential novel target for human gliomas.

2. Materials and methods

2.1. Patients and tissue samples

We collected 48 glioma tissues and 9 normal brain tissues from the Department of Neurosurgery of The Second Affiliated Hospital of Anhui Medical University (Hefei, China) after obtaining all the patient's or

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Table 1
The clinicopathological features of glioma patients.

Parameters	Total
Age(years)	
< 50	11
> 50	37
Gender	
Male	23
Female	25
Clinical grade	
Low grade I-II	19
High grade III-IV	29
KPS	
> 80	36
< 80	12

their client's informed consent. All Samples had confirmed pathological diagnosis according to the WHO criteria and were preserved in -80 °C until use. 48 glioma tissues were used for this study divided into low grade (WHO I/II, n = 29) and high grade(WHO III/IV, n = 19), and all of these patients had similar age and sex with their negative controls (Table 1). This study was approved by the Research Ethics Committee of The Second Affiliated Hospital of Anhui Medical University.

2.2. Cell culture

The human glioma cell lines (U251 and U87) were obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). These cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) /high glucose supplemented with 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA), 100 U/mlpenicillin and 100 U/ml streptomycin. The cultured environment of cells kept the temperature of 37 °C and atmosphere of humidification with 5% CO_2.

2.3. 5-Aza-2′-deoxycytidine treatment

For treatment, U87 and U251 cells were seeded overnight in culture dishes and then treated with 5-Aza (Sigma-Aldrich, St. Louis, MO) and refreshed every 24 h three times. The medium containing PBS only was regarded as a negative control.

2.4. Cell transfection

Cell transfection in glioma cells were performed in cultured glioma cells (2×10^5 cells per 200 mm^2 dish) which were cultured in penicillin/streptomycin-free DMEM with 10% FBS for 12 h. Dok7 expression vector (pcDNA3.1-Dok7) was constructed by sub-cloning the full-length Dok7 coding sequence into pcDNA3.1(+)and confirmed by sequencing. All plasmid overexpression were selected by culturing cells in the presence of puromycin (1 g/mL) 72 h after transfection. Finally, the transfection efficiency was evaluated. Short-hairpin RNA plasmid directed DNMT1 and was indicated as sh-DNMT1 (DNMT1 shRNA: 5′-GGGACUGUGUCUCUGUUAUTT-3′.) and (negative control shRNA: 5′-UUCUCCGAACGUGUCACGUTT-3′.) Cell transfections were

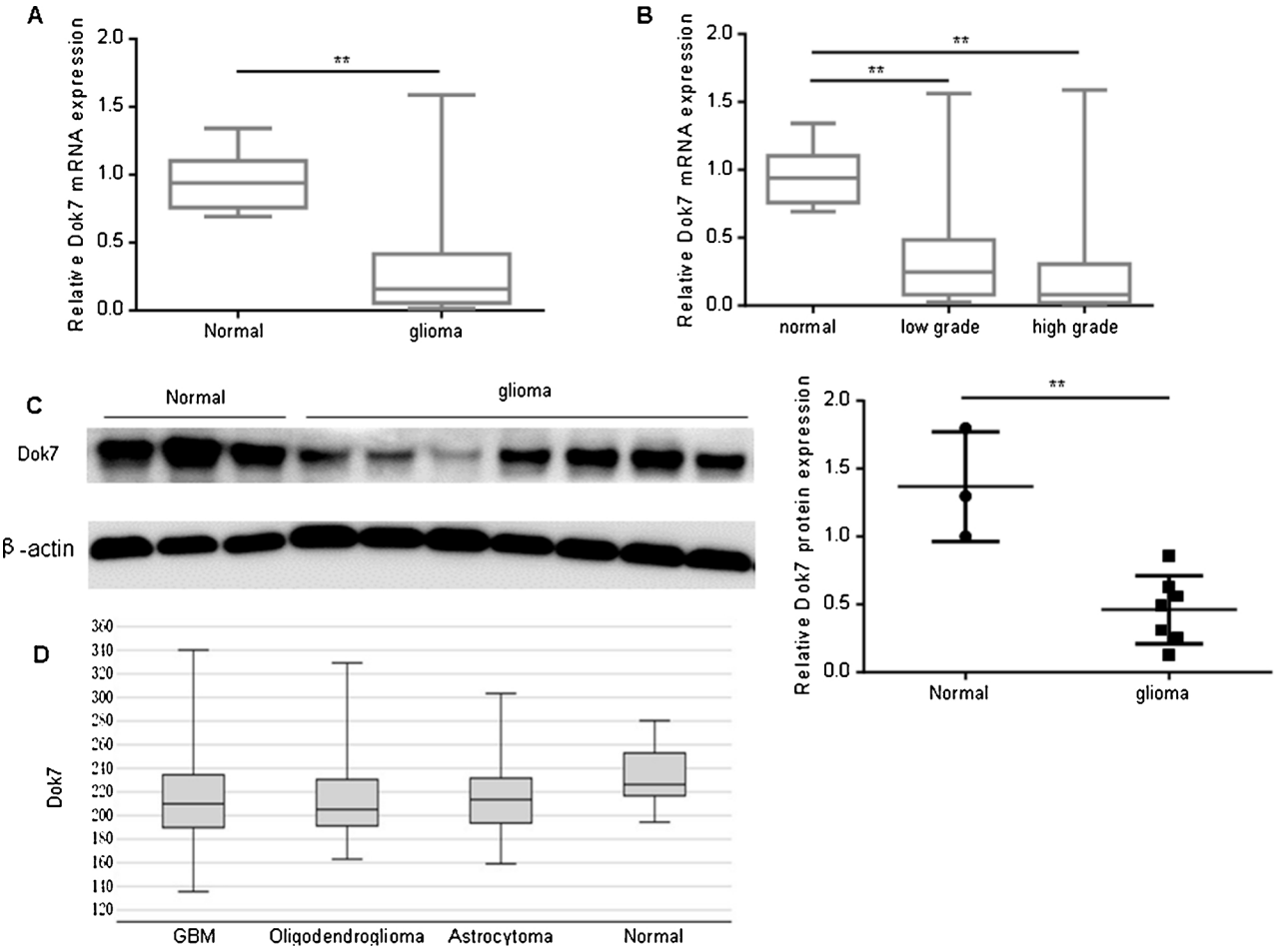

Fig. 1. Dok7 expression was downregulation in gliomas. (A) Dok7 mRNA expression was decreased in glioma samples. (B) Increased malignancy of glioma was correlated with decreased Dok7 mRNA expression. (C) The protein level of Dok7 in glioma and normal brain tissues. (D)Expression of Dok7 glioma and normal brain tissues at REMBRANDT. Each bar represents the mean ± SD of three independent experiments performed in duplicate. **P < 0.01 vs. normal control.