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Research Report

PAX3 is overexpressed in human glioblastomas and critically regulates the tumorigenicity of glioma cells

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ABSTRACT

Paired box 3 (PAX3) is overexpressed in glioma tissues compared to normal brain tissues, however, the pathogenic role of PAX3 in human glioma cells remains to be elucidated. In this study, we selected the human glioma cell lines U251, U87, SHG-44, and the normal human astrocytes, 1800, which have differential PAX3 expression depending upon the person. SiRNA targeting PAX3 and PAX3 overexpression vectors were transfected into U87 and SHG-44 glioma cell lines, and cell proliferation, invasion, apoptosis, and differentiation were examined by CCK-8 assays, transwell chamber assays, tunnel staining, Annexin V/PI analysis, and Western blotting, respectively. In addition, we used subcutaneous tumor models to study the effect of PAX3 on the growth of glioma cells in vivo. We found that PAX3 was upregulated in the three glioma cell lines. PAX3 knockdown inhibited cell proliferation and invasion, and induced apoptosis in the U87MG glioblastoma cell line, whereas PAX3 upregulation promoted proliferation, inhibited apoptosis, and increased invasion in the SHG-44 glioma cell line. Moreover, we found that targeting PAX3 expression in glioma cell lines together with chemotherapeutic treatment could increase glioma cell susceptibility to the drug. In subcutaneous tumor models in nude mice using glioma cell lines U-87MG and SHG-44, inhibition of PAX3 expression in glioblastoma U-87MG cells suppressed tumorigenicity, and upregulation of PAX3 expression in glioma SHG-44 cells promoted tumor formation in vivo. These results indicate that PAX3 in glioma is essential for gliomagenesis; thus, targeting PAX3 or its downstream targets may lead to novel therapies for this disease.

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1. Introduction

Malignant gliomas, the most common type of primary brain tumors (Komotar et al., 2008), are one of the most deadly cancers. Glioma cells are rapidly proliferative, invasive, and resistant to surgical resection, chemotherapy, and radiation (Soffietti et al., 2007; Stupp et al., 2007). To date, factors governing glioma tumor progression and invasion are not completely understood.

PAX3 protein (paired box 3) is a critical transcription factor for neuronal development, as its expression in the neural crest and neural tube is required for the migration and differentiation of neural crest cells (Buckingham and Relaix, 2007). PAX3 has also been found to play an important role in oncogenesis (Wang et al., 2008). For example, the hyperactivity of PAX3 has been reported in melanomas and rhabdomyosarcoma (Frascella et al., 1998; Plummer et al., 2008). Moreover, separate studies have reported that PAX3 is upregulated and highly expressed in other malignancies, such as Ewing sarcoma, and breast and small cell lung cancers (Muratovska et al., 2003; Parker et al., 2004; Schulte et al., 1997).

In our previous study (Chen et al., 2012), we found that PAX3 expression was upregulated in tissue specimens from high-grade gliomas compared to low-grade gliomas and normal brain tissues, and increased with ascending tumor World Health Organization (WHO) grade. These results suggested that PAX3 might be an intrinsic regulator of progression in glioma cells. Furthermore, we also showed that PAX3 negatively regulated glial fibrillary acidic protein (GFAP, astrocyte maturation marker) expression during astrocyte differentiation in vitro (Liu et al., 2011). However, the oncogenic role of PAX3 in human gliomas has not been well examined. In this study, we attempted to investigate the expression and pathological roles of PAX3 in human glioma, and to determine whether PAX3 could serve as a potential chemotherapy target for glioma patients.

2. Results

2.1. PAX3 is overexpressed in glioma cells

We examined the expression of PAX3 in the established human glioma cell lines, U87, U251, SHG44, and the normal astroglial cell line, 1800. Using RT-PCR and Western blot analysis, we found that PAX3 mRNA and protein were highly expressed in glioma cells (U87, U251, SHG44), whereas low expression was observed in the normal astroglial cell line (1800). Moreover, PAX3 was more highly expressed in U87 than U251 and SHG44 cells (Fig. 1, $P<0.05$).

2.2. PAX3 promotes cell proliferation in glioma cells

To determine if glioma cell oncogenicity is dependent upon PAX3, we reduced PAX3 expression using PAX3 siRNA to specifically knockdown PAX3 mRNA, and enhanced PAX3 expression using the Flag-PAX3 plasmid. PAX3 expression was highest in U87 cells and lowest in SHG-44 cells (Fig. 1). PAX3 siRNA was transfected into U87 cells to downregulate PAX3 expression, and the Flag-PAX3 plasmid was transfected into SHG-44 cells to enhance PAX3 expression. We initially tested four different siRNA sequences, siPAX3 (1–4), and determined by RT-PCR that one construct, siPAX3-2, was most effective in reducing PAX3 mRNA levels. As shown in Fig. 2A and C, siPAX3-2 knocked down PAX3 mRNA levels in U87 cell lines by approximately 80% within two days post-

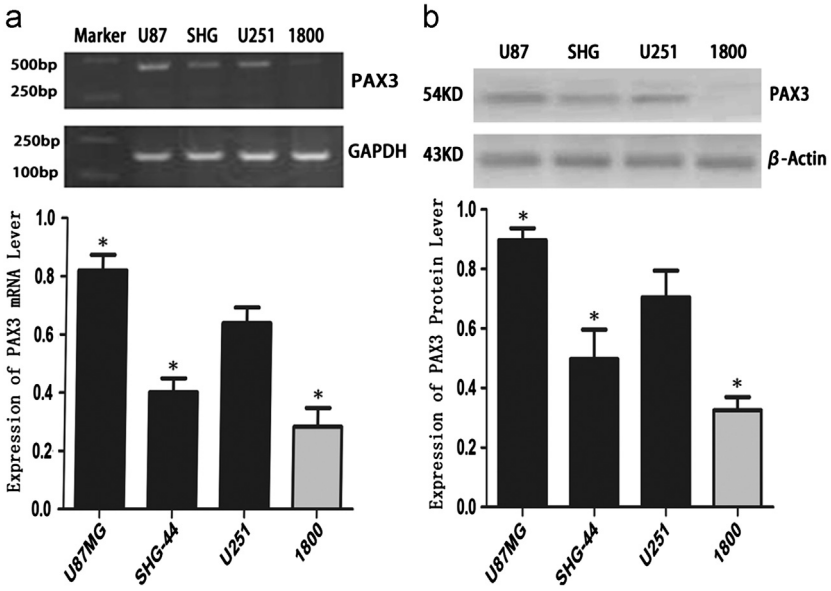

Fig. 1 – Expression of PAX3 mRNA (a) and protein (b) in three human glioma cell lines (U87, U251, SHG44) and the astroglial cell 1800. (A) Representative agarose gel of RT-PCR data. The bar graph shows GAPDH-normalized PAX3 mRNA expression in those cell lines. (B) The expression of PAX3 protein in all cell lines was measured by Western blotting. The anti-Pax3 antibody recognized a band around 56 kDa and this band was blocked by the addition of immunizing peptides. The bar graph shows β-actin-normalized PAX3 protein expression in those cell lines. The intensities of the results were quantified by densitometry and ImageQuant software (molecular dynamics, $^{*}p<0.05$).