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Research Report

The different HMGA1 expression of total population of glioblastoma cell line U251 and glioma stem cells isolated from U251

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ABSTRACT

The high-mobility group A1 (HMGA1) protein is a non-histone architectural nuclear factor and participates in diverse biological processes, including gene transcription, embryogenesis, cell cycle regulation, apoptosis, and even neoplastic transformation. In our study, glioma stem cells (GSCs) expressing the surface marker CD133 from human glioblastoma cell line U251 were isolated using MACS column and were analyzed using immunofluorescence and flow cytometry (FCM). The different expression of HMGA1 was detected using real-time RT–PCR and Western blot at transcriptional and translational levels between U251 and isolated GSCs. The results show that GSCs were successfully isolated from U251 and cultured in serum-free medium (SMF). The percentage of GSCs in U251 was 0.32%±0.07%. HMGA1 expression was significantly higher in GSCs than in glioblastoma cells ($P<0.05$), up to 6.13±0.25-fold and 2.75±0.99-fold at transcriptional and translational levels, respectively. These results indicated HMGA1 is overexpressed in GSCs as compared to glioblastoma cell line U251, which points to the expression of HMGA1 being closely related to malignant proliferation, invasion, and differentiation of tumors from the prospective of tumor stem cells (TSCs). We conclude that HMGA1 may be a potential biomarker and rational therapeutic target for glioblastoma and GSC.

1. Introduction

The high-mobility group A1 (HMGA1) protein is a non-histone architectural nuclear factor and encoded by the gene at chromosomal loci 6p21.3 (Johnson et al., 1989). HMGA1 contains three basic DNA-binding domains termed "AT-hooks," which mediate binding to the AT-rich regions in the narrow minor groove of DNA. Although HMGA1 has no intrinsic transcriptional activity alone, through protein–protein and protein–DNA interactions, it can modulate transcription by altering chromatin architecture (Lovell-Badge, 1995; Reeves and Nissen, 1990; Reeves and Beckerbauer, 2001). It participates in diverse biological processes, including gene transcription, embryogenesis, cell cycle regulation, apoptosis, and even neoplastic transformation (Fedele and Fusco, 2010; Fusco and Fedele, 2007; Sgarra et al., 2004). Although widely expressed during embryonic develop-

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ment, its expression level is negligible or absent in fully differentiated adult tissues including normal brain tissues (Chiappetta et al., 1996; Hirning-Folz et al., 1998; Zhou et al., 1995). Recently published studies showed that HMGA1 was overexpressed in several human benign and malignant tumors (Chiappetta et al., 1995; Chiappetta et al., 1998; Chiappetta et al., 2004; Donato et al., 2004; Fedele et al., 1996; Frasca et al., 2006; Hristov et al., 2010; Kim et al., 1999; Mu et al., 2010).

A small subpopulation of cells with TSCs' properties has been identified in brain tumors that express the neural stem cell (NSC) markers CD133 and nestin protein (Hemmati et al., 2003; Kondo et al., 2004; Singh et al., 2004). These cells have many distinct characteristics of stem cells including limitless proliferation and invasion and were responsible for the genesis, growth, and recurrence of tumors (Dell'Albani, 2008; Qiang et al., 2009; Tang et al., 2007; Yuan et al., 2004). We designed this study to further characterize these cancer stem cells and to exploit their roles in the pathogenesis, prevention, and treatment of tumors. The purposes of this study were to extract GSCs from glioblastoma cell line U251, to investigate the expression of HMGA1 in GSCs and U251, and then to further demonstrate that HMGA1 was correlated with malignant proliferation, invasion, and differentiation of tumors from the prospective of TSCs.

2. Results

2.1. *GSCs in glioblastoma cell line U251*

In this research, the ratio of CD133 positive cells in U251 was analyzed using flow cytometry assay. As shown in Fig. 1, the glioblastoma cell line U251 contained about 0.32%±0.07% CD133 positive cells under the culture condition of 5% CO_2 and 95% air atmosphere. Other research reported that hypoxia induced a reversible up-regulation of CD133 expression in glioma (Griguer et al., 2008).

2.2. *Isolation and culture of GSCs*

GSCs were isolated using MACS and cultured in SMF. The cells were in non-adherent growth and spheres formed after two days (Fig. 2). After ten days of rapid growth, the cell spheres has become so big that cells in the center died due to lack of adequate oxygen and nutrient supply.

2.3. *Immunofluorescence of GSC spheres*

GSC spheres grew quickly in SMF. This study demonstrated that most of the cells of the growing GSC sphere expressed NSC markers CD133 and nestin protein and not the neuro-glial maker glia fibrillary acidic protein (GFAP). In our study, we had added anti-differentiating agents while culturing the cells, but this was not entirely effective and cells at the periphery of the tumors still differentiated. In contrast, U251 cells expressed GFAP but not CD133 and nestin protein (Fig. 3).

2.4. *The HMGA1 expression in GSC and U251 at transcriptional level*

To quantify the relative amounts of HMGA1 mRNA in GSCs and U251, real-time RT-PCR was employed. The RT–PCR data confirmed that the expression of HMGA1 was 6.13±0.25-fold higher in GSCs than that in U251 ($P<0.05$; Fig. 4).

2.5. *The HMGA1 expression in GSC and U251 at translational level*

We performed Western blot analysis and a similar expression difference was observed. The Western blot showed that the protein expression of HMGA1 in GSCs was 2.75±0.99-fold higher than that in U251 ($P<0.05$; Fig. 5).

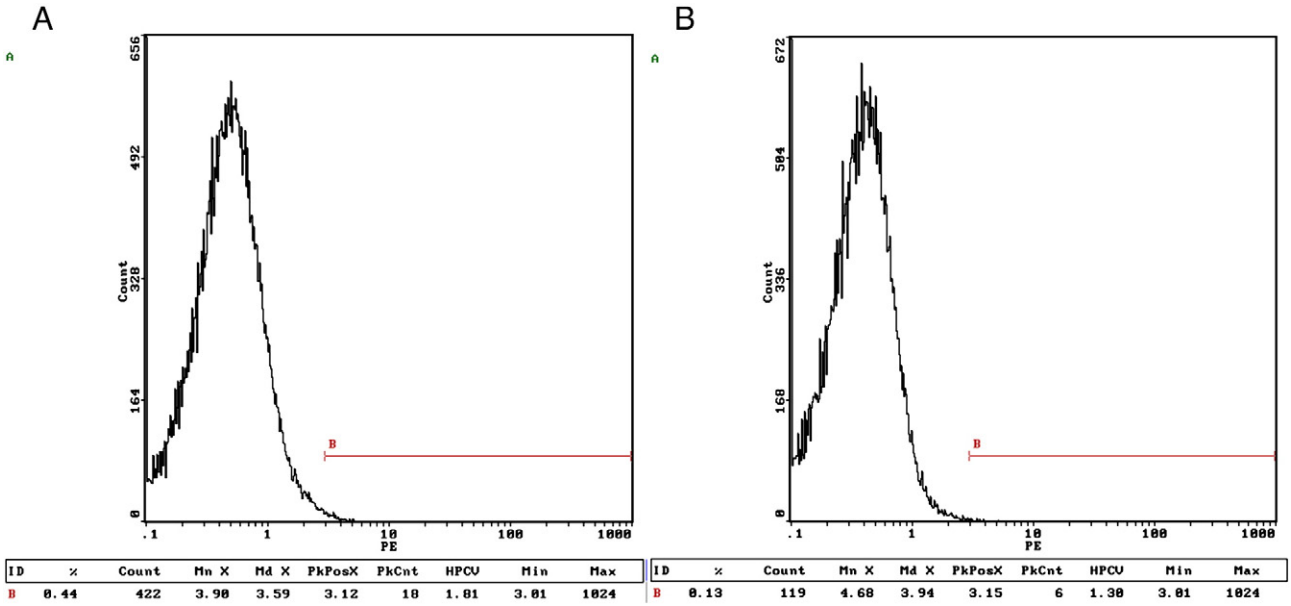

Fig. 1 – The ratio of CD133-positive GSCs in human glioma cell line U251 was determined using flow cytometry (FCM). A, experimental group incubated with CD133/2-PE; B, the reciprocal isotype control group incubated with PBS instead of CD133/2-PE.