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Increased expression of stress inducible protein 1 in glioma-associated microglia/macrophages

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ABSTRACT

Factors released by glioma-associated microglia/macrophages (GAMs) play an important role in the growth and infiltration of tumors. We have previously demonstrated that the co-chaperone stress-inducible protein 1 (STI1) secreted by microglia promotes proliferation and migration of human glioblastoma (GBM) cell lines *in vitro*. In the present study, in order to investigate the role of STI1 in a physiological context, we used a glioma model to evaluate STI1 expression *in vivo*. Here, we demonstrate that STI1 expression in both the tumor and in the infiltrating GAMs and lymphocytes significantly increased with tumor progression. Interestingly, high expression of STI1 was observed in macrophages and lymphocytes that infiltrated brain tumors, whereas STI1 expression in the circulating blood monocytes and lymphocytes remained unchanged. Our results correlate, for the first time, the expression of STI1 and glioma progression, and suggest that STI1 expression in GAMs and infiltrating lymphocytes is modulated by the brain tumor microenvironment.

1. Introduction

The prognosis of patients with glioblastoma (GBM), the most common primary brain tumor, remains dismal despite aggressive treatment. The high proliferative rate, intense invasiveness, and presence of the blood–brain barrier (BBB), which limits the penetration of large molecules into the central nervous system (CNS), all contribute to poor GBM response to conventional therapies (Lima et al., 2012). Besides, the inflammatory function of the active mediators of the innate immune response, namely microglia (MG) and macrophages (MP), seems to be suppressed in gliomas and these cells may even promote tumor progression (Charles et al., 2011; Fonseca and Badie, in press). High infiltration of MG and MP (here referred to as glioma-associated microglia/macrophages or GAMs) into malignant gliomas (Badie and Schartner, 2000; Graeber et al., 2002), however, suggests that targeted therapies that modulate GAMs function may be an effective immunotherapeutic approach for GBM.

The co-chaperone stress inducible protein 1 (STI1) has been described as a ligand of the cellular prion protein (PrP^C), which has several cellular functions and is highly expressed in the brain (Martins et al., 1997; Prusiner, 1998; Zanata et al., 2002; Linden et al., 2008). The interaction between these proteins promotes neuroprotection (Chiarini et al., 2002; Zanata et al., 2002; Lopes et al., 2005), neuronal survival (Lima et al., 2007), neuritogenesis (Lopes et al., 2005; Lima et al., 2007), memory formation and consolidation (Coitinho et al., 2007), astrocytic survival and differentiation (Arantes et al., 2009; Hartmann et al., 2013). On the other hand, STI1 modulates the proliferation of both wild-type and PrP^C-null astrocytes (Arantes et al., 2009), and induces GBM proliferation in a PrP^C-independent manner (Fonseca et al., 2012). In fact, we were the first to demonstrate a tumor promoting role of STI1 (Erlich et al., 2007).

Recently, we demonstrated that STI1 released by microglia promotes tumor proliferation, modulates MMP-9 activity and favors the migration of human GBM cell lines *in vitro* (Fonseca et al., 2012). These observations raised the question of the role of STI1 in a physiological context. In order to investigate this, we used a glioma model to evaluate the expression of STI1 *in vivo*. Here, we show that STI1 expression increases with tumor progression, and is also upregulated in GAMs and infiltrating lymphocytes. In contrast, STI1 expression did not significantly change in circulating leukocytes, and even decreased in leukocytes that infiltrated tumors propagated in the subcutaneous tissue. These observations suggest that STI1 expression is modulated by brain tumor microenvironment.

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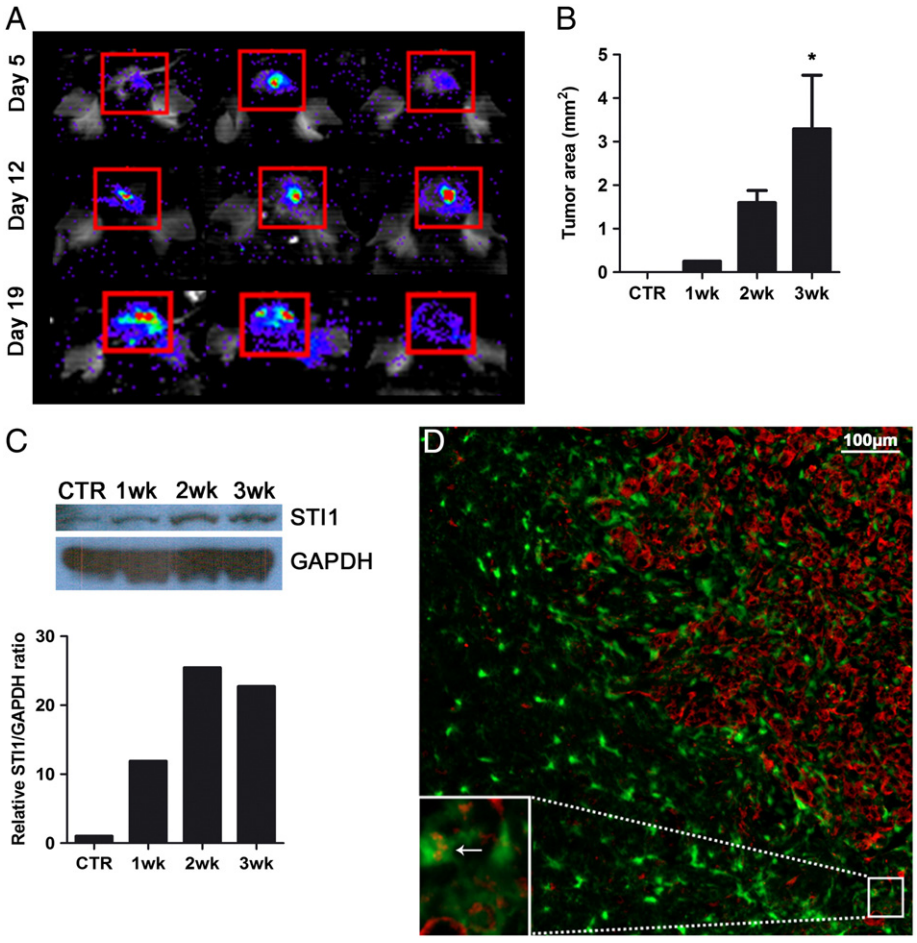

Fig. 1. STI1 expression increases with tumor progression. (A) Representative Xenogen images of three mice 5, 12 and 19 days after tumor implantation. (B) Graph illustrating the tumor size of samples used for STI1 analysis; n = 4 animals per group; *p < 0.05. (C) Western blot of normal brain (CTR) and brain tumors 1, 2 and 3 weeks after intracranial injection. All samples probed with anti-STI1 antibody showed a band at the expected molecular weight (66 kDa). (D) A two-week old GL261 glioma in a CX3CR1^{GFP} mouse was immunolabeled with anti-STI1 antibody (red) demonstrating the expression of STI1 by tumor and GAMs (inset, arrow); nuclei were stained with DAPI (blue). Bar, 100 µm. Data is representative of two separate experiments.

2. Materials and methods

2.1. Cell culture

Luciferase-expressing GL261 murine glioma cell line (GL261-ffluc) was generated as described before (Zhang et al., 2011) and was cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 µg/mL) at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Tumor implantation

All animals were housed and handled in accordance with the guidelines of City of Hope Institutional Animal Care and Use Committee (IACUC, California, USA). Intracranial tumor implantation was performed as described previously (Zhang et al., 2009). GL261-ffluc cells were harvested by trypsinization, counted, and resuspended in PBS. Female C57BL/6 or CX3CR1^{GFP} mice that express eGFP under control of the endogenous Cx3cr1 locus (Jackson Laboratory) weighing 15–25 g were anesthetized by intraperitoneal (i.p.) administration of ketamine (132 mg/kg) and xylazine (8.8 mg/kg), and immobilized in a stereotactic head frame. Through a small burr hole, 3 µl of PBS containing 1×10^5 tumor cells was injected unilaterally as described before (Zhang et al., 2009). Subcutaneous tumors were generated by injecting 100 µl of PBS containing 1×10^6 tumor cells (Fan et al., 2012). Tumor growth was assessed by a Xenogen IVIS *In Vivo* Imaging System (Xenogen) as previously described (Zhao et al., 2011).

2.3. Flow cytometry analysis

Brain tumors, subcutaneous tumors and blood samples were harvested and examined by flow cytometry as described previously (Zhang et al., 2011). Cell suspensions from brain and subcutaneous tissue were forced through a 40-µm filter. Blood samples were incubated in Gey's buffer (pH 7.2) for 10 min. For extracellular staining of immune markers, freshly prepared samples were resuspended in 0.1 mol/L PBS containing 1% fetal bovine serum and 2 mmol/L EDTA and incubated with Purified Rat Anti-Mouse CD16/CD32 (BD Pharmingen; #553141) to prevent nonspecific binding. Samples were then stained with CD11b (1:100 dilution; eBioscience; #17-0112-82) and CD45 (1:100 dilution; BD Pharmingen; #557235) antibodies for 15 min at 4 °C. For intracellular staining, cells were fixed in 4% paraformaldehyde and permeabilized in BD Cytofix/Cytoperm Buffer before incubation with STI1 antibody (1:50 dilution; Abgent; #AP2817b) for 45 min at 4 °C. Samples were washed with PBS containing 1% bovine serum albumin three times for 5 min and incubated with secondary antibody (1:100 dilution; Santa Cruz; #sc-2012). Fluorescence data were collected on a CyAn fluorescence cell sorter (BDIS). Inflammatory cells were gated based on forward vs. side-scatter analysis and their staining characteristics. FlowJo 9.0 software (Tree Star, Inc.) was used for data analysis. Glioma macrophages (MPs) were gated as CD11b$^+$/CD45^{high}, microglia