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The expression of BST2 in human and experimental mouse brain tumors

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Abstract

Glioblastoma multiforme (grade IV astrocytoma) is a highly malignant brain tumor with poor treatment options and an average lifespan of 15 months after diagnosis. Previous work has demonstrated that BST2 (bone marrow stromal cell antigen 2; also known as PDCA-1, CD137 and HM1.24) is expressed by multiple myeloma, endometrial cancer and primary lung cancer cells. BST2 is expressed on the plasma membrane, which makes it an ideal target for immunotherapy. Accordingly, several groups have shown BST2 mAb to be effective for targeting tumor cells. In this report, we hypothesized that BST2 is expressed in human and mouse brain tumors and plays a critical role in brain tumor progression. We show that BST2 mRNA expression is increased in mouse brain IC-injected with GL261 cells, when compared to mouse brain IC-injected with saline at 3 weeks post-operative ($p < 0.05$). To test the relevance of BST2, we utilized the intracranially (IC)-injected GL261 cell-based malignant brain tumor mouse model. We show that BST2 mRNA expression is increased in mouse brain IC-injected GL261 cells, when compared to mouse brain IC-injected saline at 3 weeks post-operative ($p < 0.05$). Furthermore, BST2 immunofluorescence predominantly localized to mouse brain tumor cells. Finally, mice IC-injected with GL261 cells transduced with shRNA for BST2 ± pre-incubation with BST2 mAb show no difference in overall lifespan when compared to mice IC-injected with GL261 cells transduced with a scrambled shRNA ± pre-incubation with BST2 mAb. Collectively, these data show that while BST2 expression increases during brain tumor progression in both human and mouse brain tumors, it has no apparent consequences to overall lifespan in an orthotopic mouse brain tumor model.

Keywords

BST2; glioma; glioblastoma; immunotherapy; gene therapy

Introduction

Glioma is a primary brain tumor that arises from a cell in the central nervous system with glial lineage and is a categorical designation for multiple types of glial tumors, including ependymomas, astrocytomas and oligodendrogliomas. Notably, astrocytoma (grade IV), otherwise referred to as glioblastoma multiforme (GBM), is a highly malignant tumor where most patients undergo debulking surgery resulting in an average lifespan of 12.1 months when also treated with radiotherapy, which can be extended to 14.6 months if treated with

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both radiotherapy and the chemotherapeutic drug, temozolomide (Stupp et al., 2005). Given the lack of effective therapies for patients with GBM, our laboratory continues to explore targets that may be amenable for future gene-and immuno-therapeutic approaches. BST2 (bone marrow stromal cell antigen 2; also referred to as PDCA-1, CD137 and HM1.24) has been identified as a cell surface membrane protein with increased expression on multiple myeloma (Ohtomo et al., 1999), endometrial cancer (Wong et al., 2007) and primary lung cancer cells (Wang et al., 2009). Given the convenient cell surface location of BST2, several groups have recently attempted various immunotherapeutic approaches using anti-BST2 antibodies in a mouse model of lung cancer (Wang et al., 2009), as well as in adeno-associated virus-gene loaded dendritic cells which generated cytotoxic T lymphocyte responses against multiple myeloma cells which expressed BST2 (Chiriva-Internati et al., 2003).

BST2 was originally identified while searching for a specific marker of terminally differentiated B cells (Goto et al., 1994). Co-incidentally, BST2 is expressed both by normal and neoplastic B cells. Furthermore, BST2 is a 30- to 36-kDa type II transmembrane protein, consisting of 180 amino acids (Ishikawa et al., 1995). Recently, Neil et al. (2008) discovered that BST2 acts as a tetherin, binding human immunodeficiency virus virions to the cell surface or in $BST2^{+}$ compartments, intracellularly. Additionally, BST2 has been shown to be expressed by plasmacytoid dendritic cells (pDCs), which secrete large amounts of type I interferons after sensing nucleic acids through toll-like receptor (TLR) 7 and/or 9. When BST2 binds to the orphan receptor, immunoglobulin-like transcript 7, the production of interferons and pro-inflammatory cytokines by pDCs is inhibited (Cao et al., 2009). Collectively, the tethering and cytokine regulating functions of BST2 suggest that this molecule may be a target for future investigation.

In this report, we investigated the expression of BST2 at the mRNA and protein level in both human and experimental mouse models that recapitulate brain tumors. Our data indicate that BST2 is upregulated during brain tumor progression in humans at both the mRNA and protein levels. To study BST2 further, we used the orthotopic mouse model of intracranially (IC)-injected GL261 cells which recapitulates malignant brain tumors in mice. We show that the mouse glioma cell line, GL261, normally expresses BST2 *in vitro*. Furthermore, we demonstrate that BST2 mRNA is upregulated in mouse brain with GL261-cell based brain tumors when compared to normal mouse brain. We also show that BST2 immunofluorescence predominantly localizes to mouse brain tumor cells. Finally, we determined that BST2 does not play a role in brain tumor progression since neither BST2 knock-down nor immunotherapeutic targeting using BST2 mAb resulted in a significant increase in overall lifespan. These data suggest that although BST2 is highly expressed in both human and mouse brain tumors, BST2 alone is not an amenable therapeutic target for brain tumor clearance.

Materials and methods

Animals

Male C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME), maintained in our laboratory and used at ages between 6–9 weeks. All mice were provided autoclaved food pellets and water ad libitum. All surgical procedures were completed in accordance with an approved protocol by the University of Chicago IACUC.

Mouse glioma cell line

GL261 cells carry a point mutation in the K-ras and p53 genes, do not express major histocompatibility complex II and have been extensively characterized (Szatmári et al.,

2006). GL261 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10 % fetal calf serum as well as streptomycin (100 mg/ml) and penicillin (100 U/ml) at 37 °C in a humidified atmosphere of 95 % air / 5 % CO_2. All cell culture products were purchased from Gibco Invitrogen (Carlsbad, CA).

Mouse intracranial-injection model

Mice were anesthetized with an intraperitoneal injection of 0.1 ml of a stock solution containing ketamine HCl (25 mg/ml), xylazine (2.5 mg/ml), and 14.25 % ethyl alcohol (diluted 1 : 3 in 0.9 % NaCl). For the stereotactic intracranial injection, the surgical site was shaved and prepared with 70 % ethyl alcohol. A midline incision was made, and a 1 mm diameter right parietal burr hole, centered 2 mm posterior to the coronal suture and 2 mm lateral to the sagittal suture, was drilled. Mice were placed in a stereotactic frame and 2.5 μL saline or 4×10^5 GL261 cells in 2.5 μL saline were injected intracranially with a 26-gauge needle at a depth of 3 mm. The needle was removed and the skin was sutured with 4-0 nylon thread.

Immunofluorescence

Brains were taken from mice with GL261-cell based brain tumors at 3 weeks post-operative (WPO) and were flash frozen [in 62.5 % n-Butyl Bromide (Fisher Scientific) + 37.5 % 2-methylbutane (Fisher Scientific) surrounded by crushed dry ice] as previously described by Wainwright et al. (2010). Briefly, frozen tissue was immersed into Tissue Teck O.C.T. Compound (Sakura Finetek) and sectioned at a temperature of −24 °C at 8 μm intervals and thaw-mounted onto pre-cleaned SuperFrost slides (Fisher Scientific). Sections were post-fixed with 4 % paraformaldehyde, blocked for endogenous biotin for 5 min (1 % H_2O_2 in PBS), and blocked for non-specific staining with 10 % bovine serum albumin (Sigma-Aldrich) in PBS for 1 hr. Sections of mouse tissue were incubated with anti-BST2-alexa fluor 647 (eBio927, 1:500; Ebioscience; San Diego, CA) and anti-CD11c-alexa fluor 488 (N418, 1:50; Ebioscience) in PBS at 4 °C overnight. Following extensive washing in PBS, sections were covered with Ultra Cruz mounting media with DAPI (Santa Cruz). Images of antibody-stained sections were captured using either the Sp5 Tandem Scanner 2-photon confocal microscope (Leica Microsystems; Bannockburn, IL) running LAS AF software (Leica Microsystems) using the argon, and red NeHe laser lines or the Macroscope (MVX10; Olympus; Center Valley, PA) using cellSens digital imaging software (Olympus). Confocal fluorescent images were captured using the 20× or 63× objectives.

Patient samples

Resected specimens from patients who underwent operations in the Section of Neurosurgery at the University of Chicago Medical Center between 2009 and 2010 were evaluated in this study. According to the WHO classification, samples included normal brain, grade II, III or IV astrocytoma. Normal brain control tissue consisted of nonmalignant tissue obtained during resection from a patient with a brain tumor. Histological confirmation of the diagnosis for tumors was obtained in all cases by an attending neuropathologist. The tissue was collected in accordance with a protocol approved by the Institutional Review Board (IRB) at the University of Chicago.

RNA isolation, semi-quantitative PCR and real-time PCR

Total cellular RNA from normal WT mouse brain, mouse brain IC-injected saline at 3 WPO, mouse brain IC-injected with GL261 cells at 3 WPO or normal (non-malignant) human brain and human astrocytoma grades 2 – 4 were isolated using the RNeasy Mini kit (Qiagen; Valencia, CA) according to the manufacturer's protocol. Equivalent amounts of RNA were reverse-transcribed with the iScript cDNA Synthesis Kit (Bio-rad Laboratories; Hercules,

CA). Semi-quantitative PCR was performed using 5 uL Taq PCR Master Mix, 2.5 μM forward primer, 2.5 μM reverse primer, 2 uL RNase-free H_2O and 1 ng input cDNA per reaction. Amplification was performed using the iCycler Thermal Cycler (Bio-Rad Laboratories) under the following conditions: 3 min at 95 °C, followed by 30 cycles of 30 sec at 95 °C, 30 sec at 60 °C and 30 sec at 72 °C, followed by 5 min at 72 °C. PCR products combined with 2 uL 6X DNA Loading Dye (Fermentas Life Sciences; Glen Burnie, MD) were ran on a 2 % agarose gel with the GeneRuler 100 bp DNA ladder. The gel was visualized in the ChemiDoc XRS Universal Hood II (Bio-rad Laboratories). Real-time PCR was performed using 5 uL SYBR GreenER qPCR Supermix Universal (Invitrogen; Carlsbad, CA), 0.5 μM forward primer, 0.5 μM reverse primer, 3 uL RNase-free H_2O and 1 ng input cDNA per reaction. Amplification and detection were performed using the Opticon 2 Real-Time PCR Detector (Bio-rad Laboratories) using Opticon Monitor software (version 3.1.32; Bio-rad Laboratories) under the following conditions: 15 min hot start at 95 °C, 15 sec denaturation at 95 °C, 20 sec annealing of primers at 60 °C, and 15 sec elongation at 72 °C, for 35 cycles. Triplicate reactions were performed for all cDNA samples. For mouse and human samples, percent change in mRNA levels was calculated using the formula ((Experimental brain group / normal brain group) × 100)) – 100 %. Mouse and human primer sequences used for PCR reactions are found in Table 1.

Human brain tumor array

The multiple brain cancer and normal adjacent tissue array (US Biomax; GL1001t) was deparaffinized in xylene and rehydrated through a descending strength ethanol series before being rinsed in tap water. The slide was heated in a sodium citrate buffer (10 mM sodium citrate, 0.05 % Tween-20, pH 6.0) at 97 °C for 40 min and cooled at room temp for 20 min. Sections were rinsed in PBS, serum- and biotin-blocked as described above and incubated with anti-BST2-alexa fluor 488 (26F8, 1:250; Ebioscience) overnight. Following extensive washing in PBS, sections were covered with Ultra Cruz mounting media (Santa Cruz). Photomicrographs of all antibody-stained tissue specimens were taken using the Macroscope (MVX10; Olympus; Center Valley, PA) using cellSens digital imaging software (Olympus). For each representative antibody-stained section, the mean fluorescent intensity was calculated using image J based on standardized arbitrary units. The fluorescent intensities represent averages of individual astrocytoma patient samples that were provided on the array in triplicate.

Flow cytometry

GL261 cells were adjusted to 1×10^6 and stained in PBS + 1 % bovine serum albumin (Sigma-Aldrich) for 30 min on ice and stained with: anti-BST2-PerCP-eFluor 710 (eBio927; Ebioscience) or IgG_{2B}-PerCP-eFluor 710 (Ebioscience). Cells were washed extensively in PBS and the cellular frequency and geometric mean fluorescence intensity were determined by the LSR II flow cytometer (BD; Franklin Lakes, NJ) and Flowjo analysis software (TreeStar; Cupertino, CA). At least ten-thousand events were collected for each sample.

BST2 knockdown and anti-BST2 pre-incubation with GL261 cells

BST2 shRNA lentiviral particles containing 3 target-specific constructs or shRNA constructs encoding a scrambled sequence were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). To knock down BST2 gene expression, GL261 mouse glioma cells were transduced with control or BST2 shRNA lentiviral particles at a ratio of 5 infectious units of virus per cell in the presence of 8 μg/mL polybrene for 6 hours. The next day, fresh media containing puromycin at 2 μg/mL was added to the media for the selection of cells that had stably incorporated shRNA. Selected cells were expanded and transduced with control or BST2 shRNA lentiviral particles and the protocol described above was repeated. BST2 gene silencing in these cells was confirmed at the protein level by flow cytometry. Finally, cells

were stained with anti-BST2-PerCP-eFluor 710 (eBio927; Ebioscience) and sorted on the FACSAria II (BD) isolating the BST2-expressing and BST2-deficient cells from GL261 cells transduced with scrambled and BST2 shRNA, respectively. The cells were then maintained as previously described. For BST2 pre-incubation, 5×10^6 GL261 cells were stained with 50 ug/mL purified anti-BST2 (eBio927; Ebioscience) in PBS + 1 % bovine serum albumin (Sigma-Aldrich) for 30 min on ice and washed in PBS extensively. The cells were then intracranially-injected 1 – 2 hours after pre-incubation.

Statistical analysis

Survival curves were calculated according to the method of Kaplan–Meier. Overall survival is defined as the time from injection of GL261 cells to the last day of the time course. The *p*-value was obtained by logrank statistical analysis and was considered significant when $p < 0.05$. For non-survival curves, data are presented as ± SEM and were analyzed by one-way ANOVA or the Student's *t* test and a $p < 0.05$ was considered significant. All analyses were performed using GraphPad Prism version 4.00 (GraphPad Software, Inc.).

Results

BST2 expression increases in high grade astrocytoma

To investigate whether BST2 expression is affected by the grade of brain tumor, BST2 expression was analyzed at the mRNA and protein level in various grades of human astrocytoma. BST2 mRNA expression was detectable in both low (grade II)- and higher (grade III – IV)-human astrocytoma (Fig. 1A). The percent change in BST2 mRNA levels was 32% higher in low grade (II) astrocytoma (n = 3) when compared to non-malignant human brain (Fig. 1B). Moreover, BST2 mRNA levels increased to 355% in higher grade (III – IV) astrocytoma (n = 3) when compared to non-malignant human brain. To determine if BST2 protein expression levels increased during astrocytoma tumor progression co-incident with what was observed at the mRNA level, a human brain tumor tissue microarray was stained with anti-BST2 conjugated to alexa fluor 488 and the mean fluorescence intensity (MFI) was determined between low- and higher-grade astrocytoma. The BST2 MFI in low grade astrocytoma was 8.59 ± 2.87 (n = 6), which increased to 45 ± 12 (n = 10) in higher grades of astrocytoma ($p = 0.0017$). Thus, BST2 expression increases in human brain tumors at both the mRNA and protein level during tumor progression.

BST2 expression is increased in an orthotopic mouse brain tumor model

To test the hypothesis that BST2 plays a role in brain tumor progression, we first had to verify that BST2 expression is increased in a mouse model that recapitulates malignant brain tumors. We therefore utilized the mouse model whereby murine GL261 (glioma) cells are intracranially (IC)-injected orthotopically. *In vitro* cultured GL261 cells expressed BST2 at the protein level when compared to an IgG_{2B} isotype control antibody (Fig. 2A). Furthermore, mRNA for BST2 expression was detectable from *in vitro* cultured GL261 cells, as well as in normal mouse brain, or mouse brain that was IC-injected with saline or GL261 cells, at 3 weeks post-operative (WPO) (Fig. 2B). However, to accurately quantify the level of change in mRNA expression levels, quantitative PCR was performed. Compared to normal mouse brain, BST2 mRNA expression levels decreased to −47 ± 18% in mouse brain IC-injected with saline at 3 WPO, when compared to normal mouse brain (n = 3, respectively). In contrast, BST2 mRNA expression levels increased to 1979 ± 553% ($p = 0.025$) in mouse brain IC-injected with GL261 cells, when compared to normal mouse brain at 3 WPO (n = 3, respectively). Collectively, the data indicate that BST2 expression increases due to the presence of brain tumor, rather than simply increasing due to the surgical injury resulting from the intracranial injection.

BST2 is expressed by tumor cells in an orthotopic mouse brain tumor model

Although we established that BST2 is expressed by GL261 cells *in vitro* and is increased in mouse brains with tumor, it remains to be established if the brain tumor cells or other cell types express BST2 *in vivo*. To address this question, mice were intracranially-injected with GL261 cells orthotopically and analyzed for BST2 localization *in situ*. Immunofluorescence for BST2 expression predominantly co-localized with the tightly packed DAPI-staining brain tumor in the mouse brain at 3 weeks post-operative (WPO) (Fig. 3A). However to rule out the possibility that BST2 immunofluorescence is simply due to the recruitment of plasmacytoid dendritic (pDCs), $CD11c^+$ antigen presenting cells that express high levels of BST2 (Blasius et al., 2006) and have been shown to play a negative role in tumor progression, mouse brains with tumor were co-stained with anti-CD11c conjugated to alexa fluor 488 and anti-BST2 conjugated to alexa fluor 647 (Fig. 3 B,C). Although $CD11c^+$ cells were present in mouse brain tumors at 3 WPO and did co-localize, in part, with BST2, $CD11c^+BST2^+$ pDCs did not account for the vast majority of BST2 immunofluorescence (Fig. 3 B). Thus, the data suggest that the majority of BST2 expression predominantly localizes to tumor cells in mouse brain.

BST2 does not affect lifespan in an orthotopic mouse brain tumor model

Recent studies have demonstrated that BST2 may be a ligand that is amenable to immunotherapeutic targeting *in vivo* with the potential to decrease tumor size (Wang et al., 2009; Ozaki et al., 1997), consequently increasing the lifespan in mouse models of multiple myeloma (Ozaki et al., 1997). To address whether BST2 affects the lifespan of mice with brain tumors, we modified GL261 cells using non-replicating lentiviral particles transduced with shRNA for scrambled sequences or selective for BST2 (Fig. 4A). 96 ± 0.87 % of GL261 cells transduced with scrambled shRNA were positive for BST2 expression, which was decreased to 5.6 ± 0.35 % ($p < 0.001$) of GL261 cells transduced with shRNA for BST2 (Fig. 4B). Accordingly, the MFI of BST2 of GL261 cells transduced with scrambled shRNA was 8341 ± 234%, which decreased to 153 ± 54% ($p < 0.001$) of GL261 cells transduced with shRNA for BST2 (Fig. 4C). To determine if the expression of BST2 affected the lifespan of mice with brain tumors, mice were intracranially (IC)-injected with GL261 cells transduced with shRNA for scrambled sequences or selective for BST2. As an additional control, cells were pre-incubated with or without BST2 mAb to further test whether BST2 was an amenable target for immunotherapy (Fig. 4D). The median survival of mice post-intracranial GL261 cell implantation transduced with shRNA for scrambled sequences (n = 3) or selective for BST2 (n = 3) was 35- and 34-days, respectively. Similarly, the median survival of mice post-intracranial GL261 cell implantation transduced with shRNA for scrambled sequences (n = 4) or selective for BST2 (n = 4) that were pre-incubated with anti-BST2 mAb was 41- and 42.5-days, respectively. There were no statistically significant differences between any of the groups. Collectively, the data suggest that neither the presence of BST2, nor the immunotherapeutic targeting of BST2, affects the lifespan in a mouse brain tumor model.

Discussion

Glioblastoma multiforme (GBM) is an incurable brain cancer with poor treatment options. Finding new promising therapeutic targets for GBM, which are over-expressed in malignant tissue, but expressed at low levels or not at all on normal tissue, has been challenging. This study investigated the expression of BST2 in malignant human and experimental mouse brain tumors. Although we were able to demonstrate that BST2 expression is increased in higher grades of human astrocytoma, as well as expressed in a mouse model that recapitulates malignant brain tumors, we were unable to detect any effect of BST2 expression on the overall lifespan in a mouse brain tumor model.

Recent studies have suggested that BST2 is an effective therapeutic target in renal cell mouse carcinoma xenograft models (Kawai et al., 2008), lung cancer models (Wang et al., 2009) and multiple myeloma models (Ozaki et al., 1997). Combined with the increased expression of BST2 during mouse and human brain tumor progression, we hypothesized that BST2 would be pro-tumorigenic and therefore negatively affect overall lifespan. However, we were unable to see any effect on mouse lifespan in a malignant mouse brain tumor model when BST2 expression was suppressed with small hairpin RNA. When BST2 silencing proved ineffective, we revised our hypothesis to include the possibility that BST2 does not serve a role in tumorigenesis, but as a bystander ligand, may serve to allow for immunotherapeutic targeting. However, mouse lifespan was not affected in mice intracranially-implanted with GL261 cells that had been pre-incubated with BST2 mAb. Thus, the GL261 cell-based mouse brain tumor model is not amenable to BST2 immunotherapy, in contrast to the mouse tumor models described above. This may be due to the very different circumstances in which the tumors present themselves. The renal-, lung- and myeloma mouse tumor models are present within the peripheral nervous system, which is constantly surveilled by the innate and adaptive immune systems. In contrast, the mouse brain tumor model is present within the central nervous system, which is protected by normal immune surveillance via the blood-brain-barrier, lack of lymphatic drainage and low level of MHC II expression, which may be contributing factors for the lack of therapeutic efficacy.

While this study did not determine why BST2 is over expressed in brain tumors, it is possible that BST2 is a downstream target and/or positively regulated ligand which is dispensible for brain tumor progression. Alternatively, BST2 may be upregulated as one of many factors that are compensated for by other related and/or functionally redundant pathways. Furthermore, we cannot conclude that immunotherapeutic targeting of BST2 is a scientifically dead route for future immunotherapy, since the antibody used in this study was not specifically designed as a neutralizing and/or depleting antibody. Also, given the unique environment of the central nervous system, it is possible that the pro-oncogenic role of BST2 in peripheral tumors is negated and/or compensated for by brain-resident cells such as neurons, astrocytes, microglia and/or oligodendrocytes. As such, future studies aim to determine if GL261 cells expressing- and deficient for-BST2 show different rates of growth in a peripheral flank tumor model.

Acknowledgments

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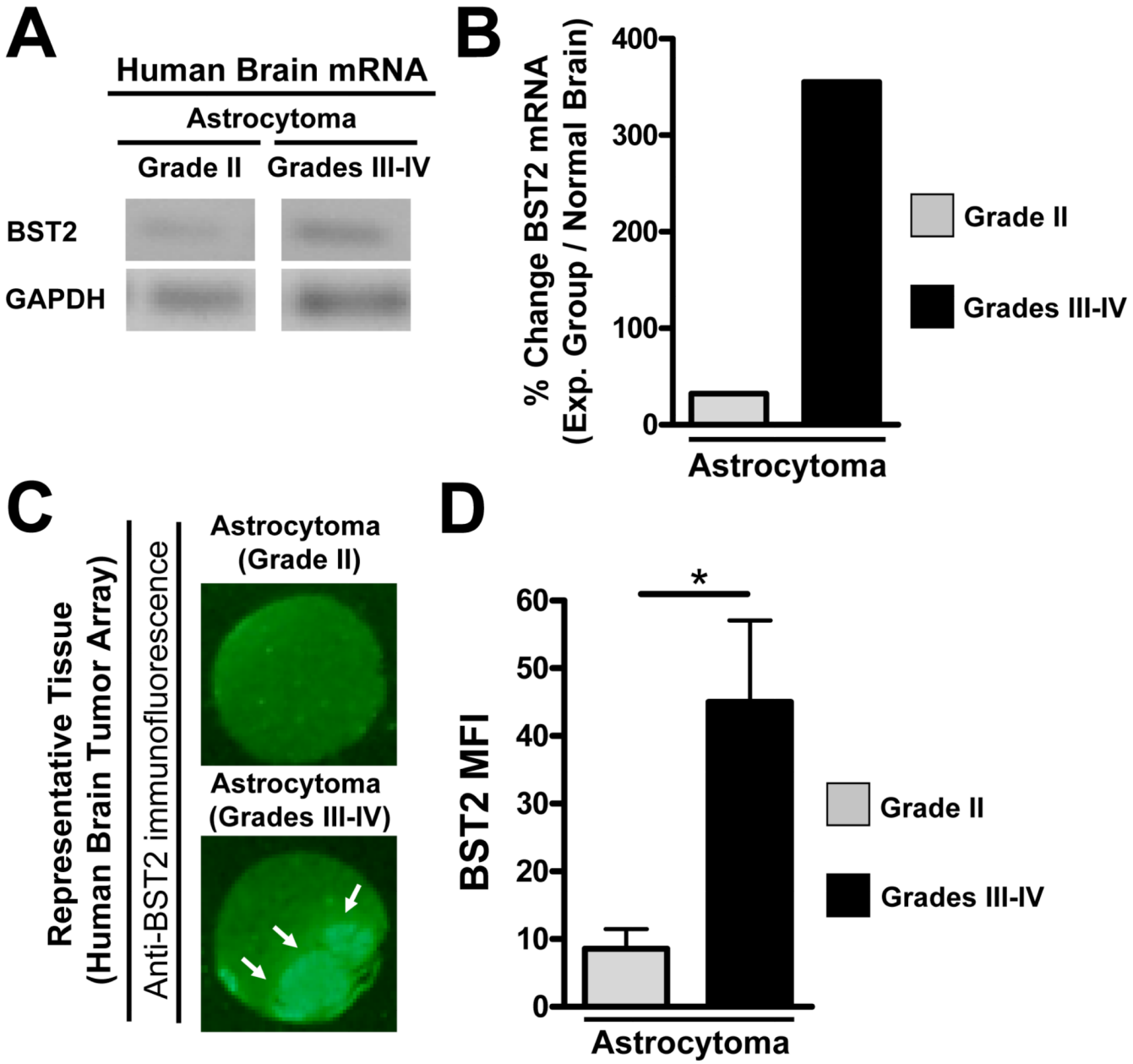

Fig. 1.

Human BST2 mRNA and protein expression levels in normal (non-malignant) brain and grades II – IV astrocytoma. A) Representative PCR products run on a 1% polyacrylamide gel for BST2 and GAPDH in astrocytoma grades II – IV. B) BST2 mRNA levels are displayed as the percent change between the different grades of astrocytoma (Exp. Group) and the normal brain. C) Representative BST2 immunofluorescence in samples of grade II astrocytoma or grades III – IV astrocytoma on the human brain tumor array. D) BST2 mean fluorescence intensity (MFI) for grade II astrocytoma and grades III – IV astrocytoma, respectively. Bar heights represent means (± SEM). * denotes significant differences at $p < 0.05$.

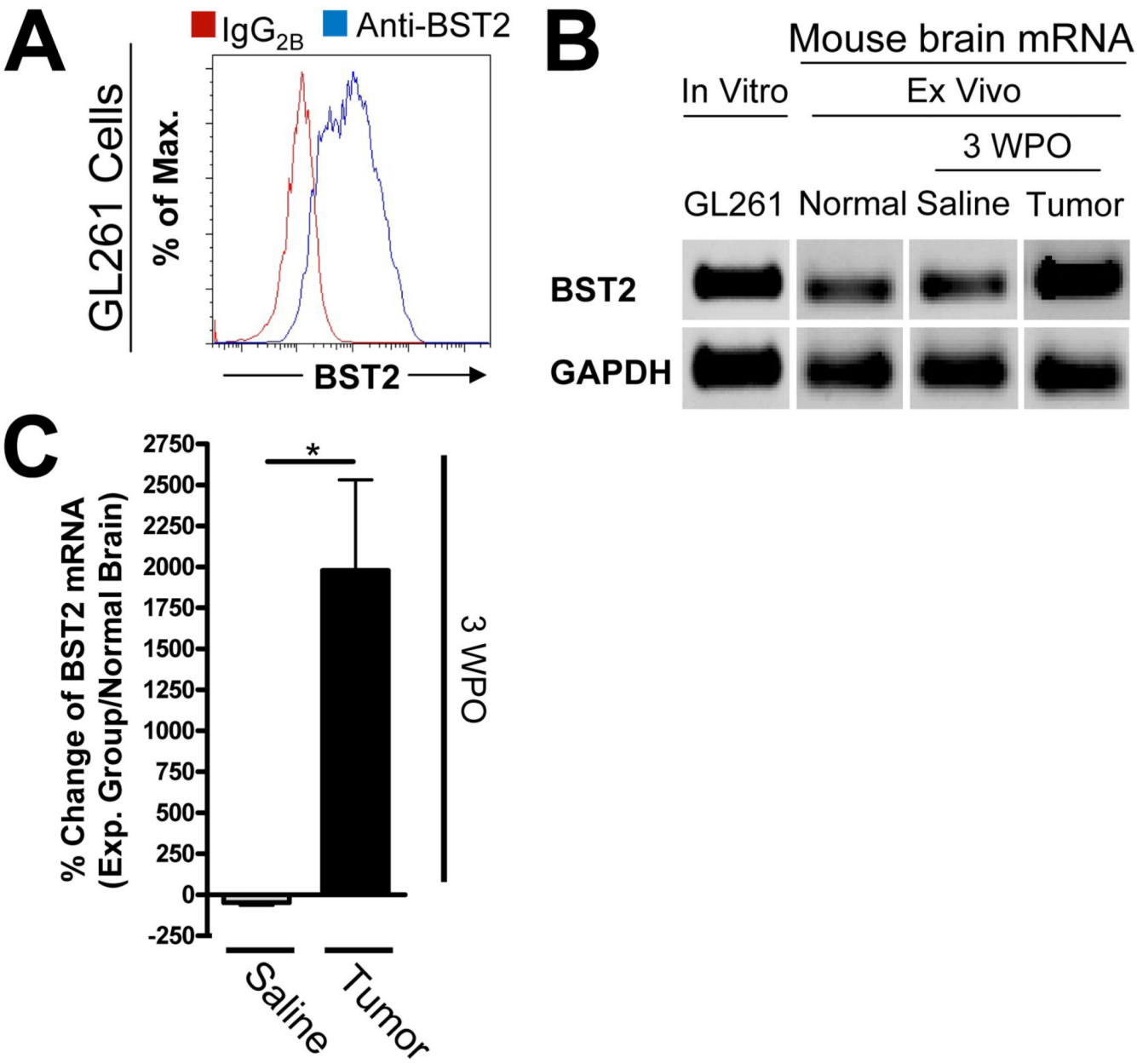

Fig. 2.

BST2 expression in GL261 cells and normal-, 3 week post-operative (WPO) saline-injected- and 3 WPO GL261-cell injected- mouse brain. A) Flow cytometric histograms of *in vitro*-cultured GL261 cells stained with IgG_{2B} (red line) and anti-BST2 (blue line). B) Representative PCR products run on a 1% polyacrylamide gel for BST2 and GAPDH in GL261 cells, normal- or 3 WPO saline-injected- and GL261 cell-injected (Tumor)- mouse brain. C) BST2 mRNA levels are displayed as the percent change between the experimental brain group (Exp. Group; i.e. intracranially-injected with saline or GL261 cells, respectively) and the normal brain group. Bar heights represent means (± SEM). * denotes significant differences at $p < 0.05$.

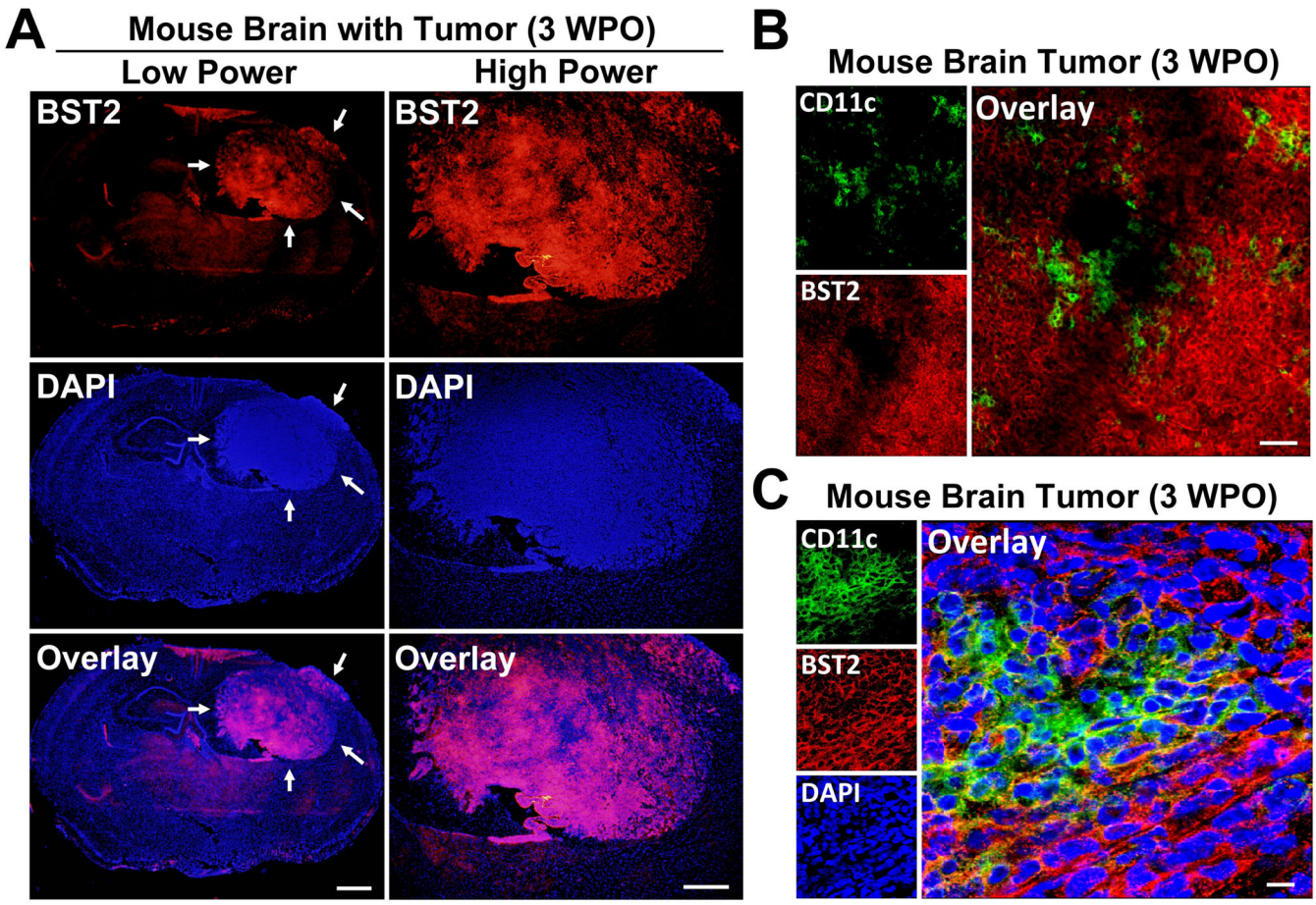

Fig. 3.

Immunofluorescence for BST2 in mouse brain intracranially-implanted with GL261 cells at 3 weeks post-operative (WPO). A) Low-power (left-column) and high-power (right column) immunofluorescence photomicrographs of mouse brain with a GL261 cell-based tumor immunoreactive for BST2 (red) and nuclear counterstain with DAPI (blue), as well as the overlay demonstrating co-localization (purple). Bars, 1 mm. B,C) Immunofluorescence photomicrographs of mouse brain tumor immunoreactive for CD11c (green), BST2 (red) and nuclear-counterstained with DAPI [blue; shown only in (C)], as well as the overlay. Bars, 100 μm (B) and 10 μm (C).

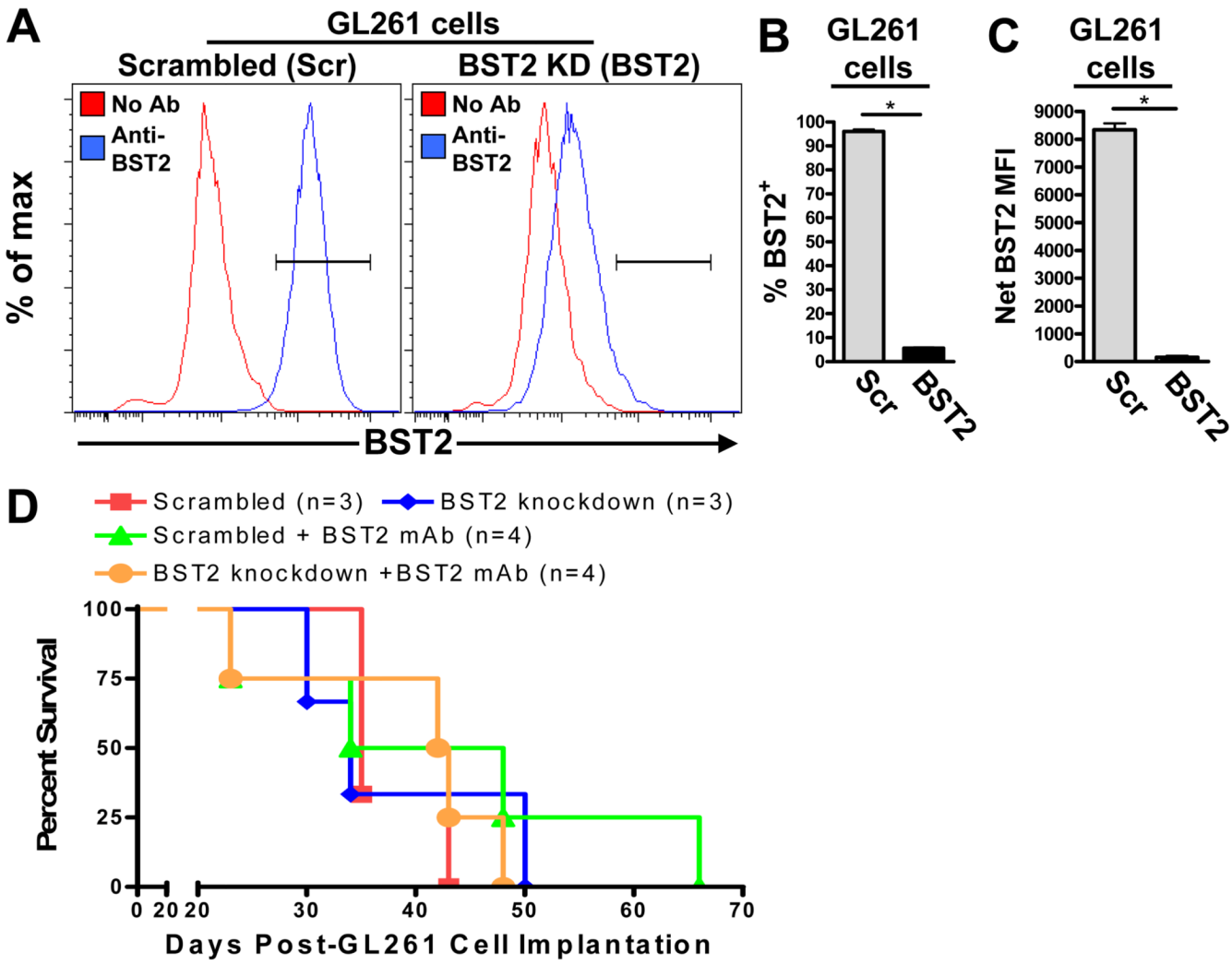

Fig. 4.

BST2 expression in GL261 cells transduced with scrambled- or BST2-shRNA (BST2 KD) and impact on lifespan in the GL261 cell-based mouse brain tumor model. A) Representative flow cytometric histograms of unstained GL261 cells (red line) or incubated with anti-BST2. B) The percentage of GL261 cells that are positive for BST2 expression [as gated in (A)]. C) The net mean fluorescence intensity (MFI) of GL261 cells. The net MFI was calculated by substracting the MFI of GL261 cells stained with anti-BST2 from the MFI of unstained cells. D) A Kaplan-Meier curve representing mouse survival times over 67 days. Bar heights represent means (± SEM). * denotes significant differences at $p < 0.001$.

Table 1

Mouse (m) and human (h) primer sequences. The gene symbols used above indicate: BST2 = bone marrow stromal cell antigen 2; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

Gene symbol	GenBank accession	Forward primer 5'->3'	Reverse primer 5'->3'	Amplicon size(bp)
mBST2	NM_198095.2	ACATGGCGCCCTCTTTCTATCACT	TGACGGCGAAGTAGATTGTCAGGA	156
mGAPDH	NM_008084.2	TCAACAGCAACTCCCACTCTTCCA	ACCCTGTTGCTGTAGCCGTATTCA	115
hBST2	NM_004335.2	TGATCTCATCAGTTCTGAGCGGGT	AGACCTGCTCCAGAGGCCCTTCTC	89
hGAPDH	NM_002046.3	TCTCCTCTGACTTCAACAGCGACA	GACAAAGTGGTCGTTGAGGGCAAT	80