**SUPPLEMENTARY DATA**

**Supplementary Procedures.**

**Neurospheres generation assays**

The related protocol, previously described ([18](#_ENREF_18" \o "Dahan, 2014 #11)), is detailed in the Supplementary Procedures. NS from GIC were dissociated and viable GIC were plated in 96 well-plates (16 wells/condition) at different cellular densities (31 to 500 cells/well) in order to assess their ability to generate primary NS through limiting dilution assays. After 15 days, NS were counted by microscopy in each well. Cells could also be sorted by FACS to select the β8 negative or positive cell population (SRB1 and SRC3 stables cell lines) before running the NS generation assay and plated in 96 well-plates (16 wells/condition) at different cellular densities (1 to 32 cells/well) in order to assess their ability to generate secondary NS through limiting dilution assays. After 15 days, secondary NS were counted by microscopy in each well. Of note, for neurospheres generation assay on SRB1 and SRC3 stables cell lines, a FACS sorting (Beckman MoFlo Astrios) was performed prior to the assay in order to only sort the GIC population GFP+/β8+ for shRNA-CTR or GFP+/β8- for shRNA-B8-1 or-4.

**Western blotting analyses**

The detailed protocols are described in the Supplementary Experimental Procedures. Cells were lysed inRIPA buffer complemented with cocktails of proteases and phosphatase inhibitors (Merck). Proteins (20µg) were then separated by SDS-PAGE, electroblotted onto Nitrocellulose membranes (Amersham). The primary antibodies, incubated overnight, were: mouse anti-Bcl2, rabbit anti-ITGα6, rabbit anti-ITGαV, rabbit anti-ITGβ3, mouse anti-ITGB4, rabbit anti-ITGβ5, rabbit anti-ITGβ8, rabbit anti-OMG (Oligodendrocyte Myelin Glycoprotein), mouse anti-TUJ1 (Neuron-specific Class III β-tubulin) and rabbit anti-Sox2 (Abcam, Cambridge, UK); mouse anti-Actin, rabbit anti-GFAP (astrocytic marker, Glial Fibrillary Acidic Protein), mouse anti-Nestin and rabbit anti-Olig2 (Millipore, Billerica, MA, USA); rabbit anti-Bax, rabbit anti-Caspase 3, rabbit anti-Caspase 7 and rabbit anti-PARP (Cell Signaling, Danvers, MA, USA) and mouse anti-ITGβ1 (Santa Cruz Biotechnology, Dallas, TX, USA). Membranes were then washed three times and incubated with secondary antibodies for 1h before revelation. All blots were representative of at least 3 independent experiments.

**Flow cytometry analyses**

Direct immunofluorescence assay was performed by FACS as previously described ([18](#_ENREF_18)). The antibodies used were A2B5-APC, CD51/ITGαv-FITC and O4-PE (Miltenyi Biotech, Bergisch Gladbach, Germany); ITGαvβ3-PE, ITGαvβ5-APC, ITGβ4-AlexaFluor488, ITGβ8-APC and Sox2-APC (R&D Systems Minneapolis, MN, USA); GFAP-PE (Millipore, Billerica, MA, USA); ITGα6-PE and ITGβ1-FITC (Thermo Fisher Scientific, Waltham, MA, USA) and TUJ1-AlexaFluor 488 (BD Biosciences, San Diego, CA, USA). ITGαvβ8 antibody was a gift from Pr Nishimura ([19](#_ENREF_21)). To evaluate the marker expression, we determined the specific fluorescence index (SFI) using the mean fluorescence intensity (MFI). The SFI was calculated with the following formula SFI = (MFI antibody - MFI isotype control) / MFI isotype control. The gating strategy used in these analyses is based on previously published protocol ([18](#_ENREF_18)).

**Quantitative real-time RT-PCR**

Total RNAs were isolated either from GIC, GDC or conventional cell lines using RNeasy kit (Qiagen, Hilden, Germany). RNA from human neural stem cells (NSC), normal cortex and normal white matter were obtained from Biochain, Origene, Clontech, and Agilent. Total RNAs were then reverse-transcribed using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). Real-time qPCR reactions were carried out using Evagreen dye and ABI-Stepone+ Detection System (Applied Biosystems, Foster City, CA, USA) or the Fluidigm 96.96 dynamic array integrated fluidic circuits and the Biomark HD System according Advanced Development Protocol n°37 (Toulouse GeT Platform, France) ([18](#_ENREF_18" \o "Dahan, 2014 #11)). β2-microglobulin (β2M) was used as endogenous control in the ΔCt analysis. Amplification folds were measured by the 2-ΔΔCt method. The different primers (Eurogentec, Liege, Belgium) used in this study were described in **Supplementary Table 2**.

**Cell death assays**

SubG1 and Annexin V/propidium iodide (AV/PI) staining were performed as previously described ([18](#_ENREF_18)). The related protocols are detailed in the Supplementary Experimental Procedures.

 Dead cells were quantified using FACS by determining the percentage of cells with subG1-DNA content. This subG1 population was analyzed after cell permeabilization and subsequent PI staining, as previously described ([18](#_ENREF_18)). Apoptosis and necrosis were also quantified on non-permeabilized cells by flow cytometry with an Alexa Fluor 488-conjugated AnnexinV (AV) and PI kit, according to the manufacturer's protocol (Invitrogen). SubG1 and AV measurements, previously described ([18](#_ENREF_18)), were conducted on at least 10 000 events, acquired on CellQuest software (BD Biosciences, San Diego, CA, USA) and analyzed with VenturiOne software (Applied Cytometry, Sheffield, UK).

Clonogenic irradiation assays

Stable cell lines SRB1 and SRC3 were dissociated and plated in 96 well-plates (500 cells/wells), irradiated at escalating doses (0, 2, 4, 6, 8 and 10 Gy) with the Gamma-cell Exactor 40, as previously described ([18](#_ENREF_18)). Irradiated cells were subsequently incubated at 37°C for 15 days. Survival fractions were then determined by counting the number of surviving NS clones by microscopy.

Orthotopic xenograft generation

Orthotopic human GB xenografts were established in 4-6 weeks-old female nude mice (Janvier Labs, France) as previously described ([18](#_ENREF_18)). Briefly, mice received a stereotaxically guided injection of 2.5x105 cells (SRB1 and SRC3 stables cell lines) resuspended in 5µl of DMEM-F12. The injection was precisely located into right forebrain (2mm lateral and 1mm anterior to bregma at a 5 mm depth from the skull surface). Survival curves were established and mice were sacrificed at the appearance of neurological signs. Excised brains were collected for subsequent immunohistochemistry analysis.

**Supplemental figure and legends**

**Supplemental Figure 1. Phenotypic characterization of GIC-enriched neurospheres and related GDC cultures**. **(A-D)** GIC-enriched neurosphere cell lines isolated from 4 patient GB (SRA5, SRB1, SRC3, and SB7) were kept in stem cell medium (SCM) or allowed to differentiate as adherent GB cells for at least 15 days in FCS medium. **(A)** Phase contrast photomicrographs of neurospheres or GB differentiated cells. Magnification x10, scale bar 6 µm. **(B)** qPCR analysis of the stem (Olig2 and Sox2) and differentiation (TUJ1 and GFAP) markers in GIC and GDC. Shown are the fold inductions expressed as means ± SEM of at least 3 independent experiments. **(C)** Immunoblots of the stem (Olig2 and Sox2) and differentiation (TUJ1 and GFAP) markers in GIC or GDC. Equal gel loading and transfer efficiency were checked with anti-actin antibody. Blots were quantified using Image J software. Then, the ratios relative to the corresponding Actin loading control were calculated and indicated under each blot.Shown are the ratios expressed as means ± SEM of at least 3 three independent experiments. **(D)** Immunofluorescence FACS analysis of the stem (A2B5 and Sox2) and differentiation (TUJ1 and GFAP) markers in GIC and GDC. Shown SFI were representative of 3 independent experiments. **(B-D)** ∗*p*<0.05, ∗∗*p*<0.01, ∗∗∗*p*<0.001 compared with the related control.

**Supplemental Figure 2. Expression of major integrins in GIC compared to GDC. (A-C)** GIC-enriched neurospheres cell lines isolated from 4 patient GB (SRA5, SRB1, SRC3, and SB7) were maintained in stem cell medium or allowed to differentiate as adherent GB cells for 15 days in FCS-enriched medium. Analysis of the major integrins (ITGA6, ITGAV, ITGB1, ITGB3, ITGB4 and ITGB5) expression in GIC and GDC was performed at the mRNA or protein level either by **(A)** qPCR or **(B)** immunoblots. (**C)** Immunofluorescence FACS analysis was also performed to determine membrane expression of ITGA6, ITGAV, ITGB1, ITGAVB3, ITGB4 and ITGAVB5. **(A, C)** Results are expressed as the means ± SEM of at 3 three independent experiments. *\* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001*.

**Supplemental Figure 3. Correlation of ITGB8 and stem/differentiated markers in GB patients from the TCGA database**. Shown are the statistical correlation graphs between the expression of ITGB8 and **(A)** stem (NESTIN, SOX2, GLI1, NANOG, ITGA6, MSI1, CD44 and SHH) or **(B)** differentiated markers (MAP2, TUJ1, MAL and OMG) based on the TCGA database analysis (n=146 GB patients). Shown are the Spearman’s rank correlation coefficients and related *p*-values.

**Supplemental Figure 4. Immunohistological pattern of ITGβ8 and Sox2 staining.** **(A)** Shown are some representative phase contrast photomicrographs of IHC stainings either with the antibody of interest (positive control) or the related immunoglobulin (negative control) in human lung adenocarcinoma biopsies **(**used as a positive control). These controls were realized on serial paraffin sections. **(B)** Immunohistochemical colocalizations of ITGβ8 (Permanent Red) and Sox2 (DAB) in GB biopsies. Shown are the representative microphotographs of n=10 patient biopsies. Magnification x63, scale bar 50 µm.

**Supplemental Figure 5. siRNA-mediated ITGβ8 knock-down impairs GIC stemness and induces apoptosis.** **(A-G)** SRB1 and SRC3 GIC were transfected with 2 different ITGβ8-targeting siRNA (siB8-5 or -6) or with a negative control siRNA (siCTR). Five days after transfection, ITGβ8 expression levels were then analyzed at the mRNA level by RT-qPCR **(A)** or at the protein level either by Immunoblot **(B)** or FACS **(C)**. Results are expressed as the means ± SEM of at least 3 independent experiments. *\* p ≤ 0.05, \*\*\* p ≤ 0.001* compared with the related control condition (siCTR). **(D-E)** Expression levels of stem (Sox2, Nestin, NG2 and Olig2) and differentiation (TUJ1, OMG and GFAP) markers were analyzed in GIC at the mRNA or protein level either by **(D)** qPCR or **(E)** immunoblots respectively. **(F-G)** In order to analyze the pro-apoptotic process, GIC were subjected either to **(F)** qPCR analysis of cell death markers (BCL2, Survivin) or **(G)** Caspases 8 and 3-7 activity assays. **(A-G)** Results are expressed as the means ± SEM of at least 3 independent experiments. *\* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001* compared with the related siCTR condition.

**Supplemental Tables**

**Supplemental Table 1: Statistical correlation analysis between expression of ITGB8 and stem or differentiated markers.**

Statistical correlation analysis between expression of ITGB8 and stem (NG2, GLI1, MSI1, NANOG, NES, NOTCH1, SOX2, SHH, CD133, ITGA6, OLIG2 and CD44) or differentiated markers (GFAP, MAP2, NeuN, TUJ1, MAL and OMG) from TCGA database (n=146 GB patients). Shown are the Spearman’s rank correlation coefficients (Rho) and related *p*-values.**.**

**Supplemental Table 2: qPCR and fluidigm primers.**

|  |  |  |
| --- | --- | --- |
| **GENE** | **PRIMER FORWARD (5’ – 3’)** | **PRIMER REVERSE (3’ – 5’)** |
| β2M |

|  |  |
| --- | --- |
|  acccccactgaaaaagatga | atcttcaaacctccatgatg |

 |  atcttcaaacctccatgatg |
| BAX | CGAGAGGTCTTTTTCCGAGTG | TAGAAAAGGGCGACAACCCG |
| BCL2 | TGCGGCCTCTGTTTGATTTC | GGGCCAAACTGAGCAGAGTCT |
| GFAP | GGCAAAAGCACCAAAGACGG | GGCGGCGTTCCATTTACAAT |
| ITGA6 | TGATCGAAATTCCTACCCTGATG | TAATCACAGGCCGGGATCTG |
| ITGAV | GGAGCAATTCGACGAGCACT | TTCATCCCGCAGATACGCTA |
| ITGB1 | CAAAGGAACAGCAGAGAAGC | ATTGAGTAAGACAGGTCCATAAGG |
| ITGB3 | GTGGTAGAAGAGCCAGAGTGTCC | CGTGGATGGTGATGAGGAGTTTC |
| ITGB4 | CTGTACCCGTATTGCGACT | AGGCCATAGCAGACCTCGTA |
| ITGB5 | AAGTGCCACCTCATGTGAAGA | CCACAGCCATTTTTGACAAGG |
| ITGB8 | ACCAGGAGAAGTGTCTATCCAG | CCAAGACGAAAGTCACGGGA |
| MAL | CGCTGCCCTCTTTTACCTCAG | GAAGCCGTCTTGCATCGTGAT |
| NESTIN | ATCGCTCAGGTCCTGGAAGG | AAGCTGAGGGAAGTCTTGGAG |
| Olig2 | CAGAAGCGCTGATGGTCATA | TCGGCAGTTTTGGGTTATTC |
| Survivin | CGAGGCTGGCTTCATCCA | AGAAGAAACACTGGGCCAAGTC |
| Sox2 | GCACATGAACGGCTGGAGCAACG | TGCTGCGAGTAGGACATGCTGTAGG |
| TUJ1 | GCTCAGGGGCCTTTGGACATCTCTT | TTTTCACACTCCTTCCGCACCACATC |