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Stem Cell Research 2018- Periostin is Secreted by Glioblastoma CD90-positive Stromal Cells and Acts as a Pericyte Chemoattractant- Edona Emini- Lund University`

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Introduction:

Glioblastoma multiforme (GBM) is the most common and aggressive brain tumor in adults. Despite intense research and clinical effort, the median survival time of GBM patients undergoing conventional treatment (ie surgery, radiotherapy and chemotherapy) is only 15 months. This neoplasm is characterized by areas of hypoxia and necrosis and an extensive stromal component supporting neovascularization induced by hypoxia and tumor growth. The tumor stroma is made up of several types of cells, including vascular elements, immune cells, and mesenchymal stromal cells (MSCs). Mesenchymal stromal cells have recently been isolated from GBM and characterized. Less aggressive astroglial tumors in adults, ie grade II astrocytoma and anaplastic astrocytoma, do not contain necrotic areas and have considerably less stroma and angioproliferation. Periostin (POSTN) is a secreted extracellular matrix protein that plays a crucial role in the progression of GBM by promoting invasiveness and angiogenesis and plays a potential role in the clinical response to angiogenic therapy. The intra-tumor level of POSTN is correlated with the increase in glioma malignancy as well as with an expanding stromal component. It is important to note that POSTN levels are correlated with the risk of recurrence and vice versa with the patient's overall survival. Recently, it has been demonstrated that POSTN is involved in the recruitment of tumor-promoting M2 macrophages in GBM. However, the cellular sources of secreted POSTN derived from the glioma and whether POSTN can also act as a chemoattractor for other cellular constituents of the GBM stroma is not known. The aim of the present study is to identify the specific cellular distribution of POSTN in GBM and to explore the possibility that POSTN acts as an attractant for vasculogenic pericytes gliomas.

Material and Methods

Ethical statement

All animal work was approved by the Committee of Animal Ethics in Lund-Malmö, Sweden (permit number: M259-12).

Tumor inoculation Nine female heterozygous rgs5GFP / + males aged 7 to 17 weeks were inoculated with mouse glioma 261 (GL261) tumor model cells. The Rgs5GFP / + mouse line is a C57BL / 6 knockout / knock-in mouse line in which GFP is expressed under the promoter RGS5 specific for pericyte [12]. Before inoculation, the mice were anesthetized with isoflurane (Forene, Abbott, CA, USA) and positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Local anesthesia (0.025 ml of 2.5 mg / ml of bupivacaine containing 5 µg / ml of epinephrine (Marcain), AstraZeneca AB, Södertälje, Sweden) was injected subcutaneously into the head region, a 1 cm long sagittal skin incision was made and a hole was drilled in the skull. Five thousand GL261 tumor cells in 5 µl of R0 medium (RPMI 1640 medium supplemented with 1 mM sodium pyruvate and 10 mM HEPES) were injected using a 10 µl syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) at 1 µl / min in the caudate nucleus. The following coordinates were used: 1.5 mm lateral and 1.0 mm anterior bregma, 2.75 mm ventral to the skull bone. The needle was left in the brain for 5 minutes after the injection before being slowly retracted and the hole in the skull was sealed with bone wax. Immunofluorescence On the 19th day after inoculation of the tumor, the animals were sacrificed by transcardial perfusion with a 0.9% NaCl solution (Merck KGaA, Darmstadt, Germany) followed by 4% of paraformaldehyde (PFA, Electron Microscopy Sciences , Hatfield, PA, USA). The brains were removed from the skull and postfixed in 4% PFA (Electron Microscopy Sciences, Hatfield, PA, USA) at 4 ° C overnight and then transferred to a 30% sucrose solution (Merck KGaA, Darmstadt, Germany) . Coronal

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sections 40 µm thick were cut using a sliding microtome SM200 R (Leica Biosystems Nussloch GmbH, Nussloch, Germany). The coronal sections were stored at -20 ° C in an antifreeze solution (30% ethylene glycol and 30% glycerol (both from VWR International, Radnor, PA, USA) in 0.012 M NaH2 PO4 • H2 O and 0.031 M Na2 HPO4 • 2H2 O (both from Sigma-Aldrich, Stockholm, Sweden) After the washing steps, the free floating sections were first incubated in 10% normal goat / donkey serum (NGS / NDS, Jackson ImmunoResearch Europe Ltd., Suffolk, United Kingdom Immuno Research) and 1% Triton X-100 Solution (Sigma Aldrich, Stockholm, Sweden) in potassium phosphate buffer (KPBS), then incubated for two nights at 4 ° C with anti-POSTN rabbit (1: 400, Abcam, Cambridge, United Kingdom); human anti-integrin β1 mouse (1: 400, Sigma Aldrich, Stockholm, Sweden); anti-CD90 mouse (1: 200 , Santa Cruz Biotechnology Inc, USA); anti-GFP chicken (1: 400, Abcam, Cambridge, UK); mouse antiNestin (diluted 1: 200, Merck Millipore, Billerica, MA, USA) in 3.3% NGS (Sigma A ldrich, Stockholm, Sweden) and 0.3% Triton x-100 in KPBS. were then washed in KPBS and incubated with appropriate secondary antibodies in 3.3% NGS / NDS and 0.3% Triton X-100 (Sigma Aldrich, Stockholm, Sweden) in KPBS for two hours in dark at room temperature. After a final washing step, the sections were mounted on SuperFrost Plus glasses (Thermo Fisher Scientific Inc., Waltham, MA, USA) and covered with polyvinyl alcohol (PVA, Sigma Aldrich, Stockholm, Sweden) -1.4 diazabicyclo [2.2.2] octane (DABCO, Sigma Aldrich, Stockholm, Sweden) supplemented with Hoechst 33342 (1 µl / ml, Sigma Aldrich, Stockholm, Sweden) for nuclear staining.

Cell culture:

The isolation and culture of CD90 + and CD90- cells of the human MSC type from human gliomas were recently reported by our group [3]. In short, the cells were obtained from primary brain tumor surgery at the neurosurgery department of the Skane University Hospital in Lund, Sweden, ethical permit H15 642/2008. Primary cells of passage 2-4, cultured adhesively on plastic, were sorted on a FACS Aria III cell sorter (BD Biosciences, Heidelberg, Germany) based on specific mesenchymal stromal cells defining markers (CD73, CD90 , CD105 and HLA class I) with cytometric flow in a FACS Aria III cell sorter (BD Biosciences, Heidelberg, Germany). Doublets, dead cells and cells expressing negative lineage markers (CD14, CD19, CD34, CD45 and HLA-DR) were used as a cocktail in Lin TO-PRO-1 and excluded. These MSC markers are defined by the International Society for Cell Therapy (ISCT; [3,13]). The cells were then expanded, frozen and kept at -150 ° C until used.

Discussion:

The present study demonstrates that the POSTN expression is largely limited to the perivascular stromal niche of the mouse glioblastoma GL261. In this compartment, the expression POSTN is mainly localized to CDM + CD90 + and pericytes recruited from the tumor. This stromal expression joins previous work on pancreatic cancer showing that the stromal cells are at the origin of POSTN [19]. Findings in the mouse glioma model are further confirmed in human tissue, in particular by CD90 + MSCs acutely isolated from glioblastoma being a source of POSTN

References :

1. Stupp R (2002) Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol 20: 1375-82. [PubMed]

2. Hossain A (2015) Mesenchymal Stem Cells Isolated From Human Gliomas Increase Proliferation and Maintain Stemness of Glioma Stem Cells Through the IL-6/gp130/STAT3 Pathway. Stem Cells 33: 2400-2415. [PubMed]

3. Svensson A (2017) Identification of two distinct mesenchymal stromal cell populations in human malignant glioma. J Neurooncol 131: 245-254. [PubMed]

4. Louis DN (2007) The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114: 97-109. [Pub Med]

5. Wang HY, Wang C, Jiang C (2013) Stromal protein periostin identified as a progression associated and prognostic biomarker in glioma via inducing an invasive and proliferative phenotype. Int J Oncol 42: 1716-1724. [PubMed]

This work is partly presented at 10th World Congress and Expo on Cell & Stem Cell Research March 19-21, 2018 held in New York, USA

6. Mustafa DA (2012) A proteome comparison between physiological angiogenesis and angiogenesis in glioblastoma. Mol Cell Proteomics 11: M111.008466. [PubMed]

7. Park SY (2016) Periostin (POSTN) Regulates Tumor Resistance to Antiangiogenic Therapy in Glioma Models. Mol Cancer Ther 15: 2187-2197. [PubMed]

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