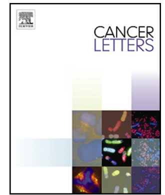
Cancer Letters ■■ (2015) ■■–■■

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet



Original Articles

Bone marrow derived myeloid cells orchestrate antiangiogenic resistance in glioblastoma through coordinated molecular networks

B.R. Achyut [a], Adarsh Shankar [a], A.S.M. Iskander [a], Roxan Ara [a], Kartik Angara [a], Peng Zeng [a], Robert A. Knight [b], Alfonso G. Scicli [c], Ali S. Arbab [a,*]

[a] *Tumor Angiogenesis Laboratory, Biochemistry and Molecular Biology, Cancer Center, Georgia Regents University, Augusta, GA, USA*
[b] *NMR Center, Henry Ford Health System, Detroit, MI, USA*
[c] *Cellular and Molecular Imaging Laboratory, Henry Ford Health System, Detroit, MI, USA*

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 29 August 2015
Accepted 9 September 2015

Keywords:
Glioblastoma
Resistance
Tumor angiogenesis
Bone marrow
VEGF
Tumor microenvironment

ABSTRACT

Glioblastoma (GBM) is a hypervascular and malignant form of brain tumors. Anti-angiogenic therapies (AAT) were used as an adjuvant against VEGF–VEGFR pathway to normalize blood vessels in clinical and preclinical studies, which resulted into marked hypoxia and recruited bone marrow derived cells (BMDCs) to the tumor microenvironment (TME). *In vivo* animal models to track BMDCs and investigate molecular mechanisms in AAT resistance are rare. We exploited recently established chimeric mouse to develop orthotopic U251 tumor, which uses as low as 5×10^6 GFP+ BM cells in athymic nude mice and engrafted >70% GFP+ cells within 14 days. Our unpublished data and published studies have indicated the involvement of immunosuppressive myeloid cells in therapeutic resistance in glioma. Similarly, in the present study, vatalanib significantly increased CD68+ myeloid cells, and CD133+, CD34+ and Tie2+ endothelial cell signatures. Therefore, we tested inhibition of CSF1R+ myeloid cells using GW2580 that reduced tumor growth by decreasing myeloid (Gr1+ CD11b+ and F4/80+) and angiogenic (CD202b+ and VEGFR2+) cell signatures in TME. CSF1R blockade significantly decreased inflammatory, proangiogenic and immunosuppressive molecular signatures compared to vehicle, vatalanib or combination. TCK1 or CXCL7, a potent chemoattractant and activator of neutrophils, was observed as most significantly decreased cytokine in CSF1R blockade. ERK MAPK pathway was involved in cytokine network regulation. In conclusion, present study confirmed the contribution of myeloid cells in GBM development and therapeutic resistance using chimeric mouse model. We identified novel molecular networks including CXCL7 chemokine as a promising target for future studies. Nonetheless, survival studies are required to assess the beneficial effect of CSF1R blockade.

© 2015 Elsevier Ireland Ltd. All rights reserved.

Introduction

Glioblastoma (GBM), a grade IV glioma classified by World Health Organization (WHO), is considered highly malignant, vascular and invasive subtype [1]. GBM is most lethal during first year after initial diagnosis despite surgical resection, radiotherapy and/or chemotherapy [1,2]. Median survival of patients diagnosed with GBM is only 12–15 months [1,2]. Hypoxia and neovascularization are histopathologic features of GBM [3]. Anti-angiogenic therapies (AAT) were used as adjuvants mainly against VEGF–VEGFR pathway to normalize tumor vasculatures. However, all of them provided minimal to none effect with no change in overall survival [4,5]. Therefore, current challenge is to investigate mechanisms of undesirable outcomes in GBM clinical trials. Preclinical studies involving AATs have shown marked hypoxia, increased homing of bone marrow derived cells (BMDCs) to the tumor and activation of alternative pathways of neovascularization [6,7].

BMDCs play a pivotal role in tumor development [8] and endothelial progenitor cells (EPCs) from BM pool are recruited to tumor microenvironment (TME) [9–12]. BMDCs associated therapeutic resistance falls under evasive or adaptive resistance, where tumor itself after an initial response phase acquires evasive properties against therapeutic blockade by inducing alternate mechanisms that enable neovascularization, leading to renewed tumor growth and progression [13]. Distinct potential mechanism of resistance might be at cellular level [14] mediated through up-regulation of HIF1-α followed by induction of SDF1α, secretion of pro-angiogenic factors and recruitment of CXCR4+ BMDCs to the tumor [9–11,15]. These recruited cells were characterized as pro-angiogenic CD45+VEGFR2+ EPCs, or CD45+Tie2+ monocytes [16,17]. Interestingly, lin-ckit+Sca-1+ and their derived cells demonstrated recruitment to tumor but do not functionally contribute to tumor neovascularization [18]. BMDCs derived MMP9 modulates neovessel remodeling, thereby playing role in tumor growth [15,19].

* Corresponding author. Tel.: +1 706 721 8909; fax: +1 706 434 6406.
E-mail address: aarbab@gru.edu (A.S. Arbab).

http://dx.doi.org/10.1016/j.canlet.2015.09.004
0304-3835/© 2015 Elsevier Ireland Ltd. All rights reserved.

Please cite this article in press as: B.R. Achyut, Adarsh Shankar, A.S.M. Iskander, Roxan Ara, Kartik Angara, Peng Zeng, Robert A. Knight, Alfonso G. Scicli, Ali S. Arbab, Bone marrow derived myeloid cells orchestrate antiangiogenic resistance in glioblastoma through coordinated molecular networks, Cancer Letters (2015), doi: 10.1016/j.canlet.2015.09.004

Studies have indicated that resistance to AAT has profound involvement of immune system [20–26]. Role of myeloid cells in tumor angiogenesis is an established phenomenon as shown by other authors [27–31] and supported by our previous study (unpublished), which showed that majority of bone marrow derived GFP+ cells acquire both myeloid signature (CD68) and endothelial signatures (CD202b and CD34) in the TME under vatalanib (VEGFR tyrosine kinase inhibitor) treatment. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors [32], whereas, SDF-1 played an important role in the invasiveness of brain tumor and infiltration of macrophages from bone marrow [33]. AATs in glioma were associated with increased myeloid cell infiltration and stem cell accumulation [34]. However, investigations whether those phenotypes have bone marrow component, were lacking. In other study, authors noticed infiltration in myeloid populations in the tumor bulk and in the infiltrative regions after AAT [23]. Together, studies suggest that immune suppressive myeloid cells, especially myeloid derived suppressor cells (MDSCs) and tumor associated macrophages (TAM) [35–37], may participate in escape from AATs and represent a potential biomarker of resistance with potential therapeutic target in GBM [23,38].

Several chemokines such as macrophage colony-stimulating factor-1 (MCSF/CSF1) and monocyte chemotactic protein-1 (MCP1/CCL2) are known to contribute in the recruitment of TAMs to the tumor [39,40]. CSF1R expression has been reported on immunosuppressive myeloid cells and dendritic cells [41–43]. CSF1-CSF1R signaling regulates survival, differentiation, and proliferation of monocytes and macrophages [44,45], and have critical role in angiogenesis and tumor progression [46,47]. Therefore, the goals of the present study are to (1) investigate the effect of CSF1R blockade on orthotopic glioma development in a recently established preclinical chimeric mouse model, (2) to evaluate whether CSF1R blockade alone or in combination with VEGFR2 blockade could inhibit the homing of myeloid BMDCs to the glioma, (3) to identify signature immune cell populations following CSF1R inhibition that could have profound role in glioma growth and (4) to investigate key secreted molecular signatures in GBM TME following CSF1R inhibition.

Materials and methods

All animal related experimental procedures were approved by the Institutional Animal Care and Use Committee and Institutional Review Board of Georgia Regents University (animal protocol #2014-0625). All efforts were made to ameliorate suffering of animals. CO2 with secondary method was used to euthanize animals for tissue collection at the end of the study.

Establishing chimeric mouse

Chimeric mouse for orthotopic U251 glioma was established with IACUC approved protocol and published method [48]. Transgenic mice with universally expressing GFP under the human ubiquitin C promoter (C57BL/6-tg(UBC-GFP)30Scha) were used as donors (Jackson Laboratory, Maine, USA). Athymic nude mice (NCr-nu/nu) were used as recipients (Charles River, Frederick, MD, USA), and were whole body irradiated with sub-lethal dose of 6Gy (using Cs-137 source). After 24 hours, recipient mice were injected intravenous (n = 3) and intraperitoneal (n = 3) routes with BM cells (5×10^6 cells) collected from donor transgenic mice. All mononuclear cells were separated from red blood cells using lymphocyte cell separation media (Corning, Cellgro, USA), counted and 5×10^6 cells/100 µl were injected into each mouse. Ten microliter of blood (from orbital sinus) were collected from each mouse on days 7 to 56 (n = 3 each time point) following transplantation of BM to determine BM engraftment efficiency (GFP positivity) in peripheral blood using flow cytometer. Cells from athymic mice without irradiation and GFP+ cell transplantation were used as control for flow cytometry.

Animal model of human glioma

Precisely, animals were anesthetized with 100 mg/kg ketamine and 15 mg/kg xylazine i.p. The surgical zone was swabbed with betadine solution, the eyes coated with Lacri-lube and the animals were immobilized in a small animal stereotactic device (Kopf, Cayunga, CA). After draping, a 1-cm incision was made 2 mm to the right of the midline 1 mm retro-orbitally; the skull exposed with cotton-tip applicators and a 23G needle tip was used to drill a hole 2 mm to the right of the bregma, taking care not to penetrate the dura. A 10 µl Hamilton syringe with a 26G-needle containing tumor cells (2.4×10^5) in 3 µl was lowered to a depth of 2.5 mm, and then raised to a depth of 2 mm. During and after the injection, careful note was made of any reflux from the injection site. After completing the injection, we waited 2–3 minutes before withdrawing in a stepwise manner. The surgical hole was sealed with bone wax. Finally, the skull was swabbed with betadine before suturing the skin over the injection site.

In vivo *multispectral optical imaging*

Multispectral optical images were acquired using excitation profiles of 460–480 nm range and emission of 535 nm to monitor the GFP positivity on days 7, 14 and 21 after tumor cell implantation. RFP positivity was determined at excitation 587 nm and emission 610 nm. All optical imaging data were acquired by Spectral AMI (Spectral Instruments Imaging, LLC) machines and analyzed by AMI view software.

Drug treatments

Orthotopically implanted chimeric mice with U251 tumor cells were allowed to grow for 7 days and then started oral treatments of either vehicle or receptor tyrosine kinase inhibitors (vatalanib (50 mg/kg/day) [49], GW2580 (160 mg/kg/day) [43] and combination of both GW2580+ vatalanib, daily for two weeks. Seven days waiting period was followed after tumor implantation to mimic clinical scenario, where treatment is being done following detection of tumor.

In vivo *magnetic resonance imaging (MRI)*

All MRI experiments were conducted using a 7 Tesla 12 cm (clear bore) magnet interfaced to a varian console with actively shielded gradients of 49 gauss/cm and 100 µs rise times or a horizontal 7 Tesla BioSpec MRI spectrometer (Brucker Instruments, Billerica, MA) equipped with a 12 cm self-shielded gradient set (45 gauss/cm max). Detailed MRI procedure was adopted from our several previous publications [50–54]. An appropriate state of anesthesia was obtained with isoflurane (2.5% for induction, 0.7% to 1.5% for maintenance in a 2:1 mixture of N_2:O_2). After positioning using a triplanar FLASH sequence, MR studies were performed using pre-contrast T1, T2-weighted and post contrast T1-weighted MRI scans with following parameters (1) Standard T1-weighted multislice sequence (TR/TE = 500/10 ms, 256 × 256 matrix, 13–15 slices, 1 mm thick slice, 32 mm field of view (FOV), # of averages = 4). (2) T2-mapping sequence (2D multi-slice, multi-echo (MSME) sequence, TE = 10, 20, 30, 40, 50, 60 ms, TR = 3000 ms, 256 × 256 matrix, 13–15 slices, 1 mm thick slice, 32 mm field of view (FOV), # of averages = 2). Post contrast T1WI was used to determine volume of tumors in vehicle and drug treated mice by drawing irregular ROI to encircle whole tumor in each image section containing tumor using ImageJ software, and area was then multiplied by thickness of image slice to determine volume (cm^3). Two investigators blinded to the animal groups determined tumor volume.

Collection of GFP+ cells and determination of immune cell populations

Freshly isolated brain samples were separated into left and right (tumor bearing) hemispheres from each group and were homogenized to pass through 40 µm cell strainer to make single cell. Similarly, cells were collected from spleen and BM. Cells were labeled with antibodies (BioLegend) such as CD45, CD4, CD8, Gr1, CD11b, F4/80, CD68, CD133, CD31, CD34, CD202b (Tie2), and CD309 (VEGFR2) (other than FITC) to identify BM recruited cell types (GFP+) in the tumor as well as phenotypes of GFP+ cells in spleen and bone marrow. Flow cytometry data were acquired using Accuri C6 machine (BD Biosciences) and analyzed by BD Accuri C6 software.

Immunofluorescence study

After MRI at day 22, animals were euthanized and brains were collected for frozen tissue sections and later stained for immunofluorescence study to determine expression of myeloid cell signature markers CD11b (Abcam), F4/80 (Santa Cruz Biotechnology), p-ERK (Cell Signaling), Ki67 (DAKO), CXCL7 (Millipore) and CD31 (Abcam) at the site of tumor. Migration and incorporation pattern of GFP+ BMDCs was determined in different regions of the tumor.

Western blot analysis

Tumors were collected and processed for protein isolation using T-PER, tissue protein extraction reagent for tissue and Pierce RIPA buffer for tumor cells (Thermo Scientific, USA). Protein concentrations were estimated with Pierce, BCA protein assay kit (Thermo Scientific, USA) and separated by standard Tris/Glysine/SDS gel electrophoresis. Membranes were incubated with primary antibodies against IDO (1:1000, Santa Cruz Biotechnology), p-ERK and ERK (1:1000, Cell Signaling), and α-Tubulin (1:5000, Abcam) followed by horseradish peroxidase-conjugated secondary antibody (1:5000, Biorad). The blots were developed using a Pierce SuperSignal West Pico Chemiluminescent substrate kit (Thermo Scientific, USA). Western blot images were acquired by Las-3000 imaging machine (Fuji Film, Japan).

Please cite this article in press as: B.R. Achyut, Adarsh Shankar, A.S.M. Iskander, Roxan Ara, Kartik Angara, Peng Zeng, Robert A. Knight, Alfonso G. Scicli, Ali S. Arbab, Bone marrow derived myeloid cells orchestrate antiangiogenic resistance in glioblastoma through coordinated molecular networks, Cancer Letters (2015), doi: 10.1016/j.canlet.2015.09.004

Download English Version:

https://daneshyari.com/en/article/10899492

Download Persian Version:

https://daneshyari.com/article/10899492

Daneshyari.com