Dynamics of microglial activation and expression of cytokines in the site of glioblastoma tumor resection

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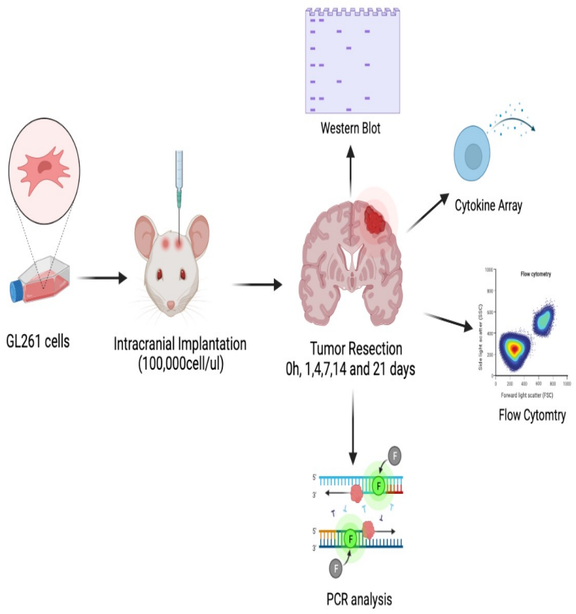
Introduction

Glioblastomas (GBMs) are the most aggressive primary brain tumors, due to their malignant growth and invasion into the brain parenchyma coupled with resistance to therapy, GBMs are among the deadliest of all cancers. GBM cells dynamically respond to their local tissue microenvironment, which, in turn, plays a critical role in tumor invasion and treatment resistance. GBM tumors harbor microglial cells, which can contribute up to 30% of the brain tumor mass[1,2]. Microphage-like M1 microglia releases pro-inflammatory cytokines in contrast to tumor-associated M2 microglia which releases anti- inflammatory cytokines, associated with wound healing, and thus, supporting tumor growth and dispersal[3,4,5].

Surgical resection of brain tumors results in tissue damage, causing microglia activation in the surgical area. These activated microglia are likely to exacerbate the malignant properties of the tumor cells that fail to be eliminated by resection and stimulate the tumor recurrence. Because M1/M2 ratio of microglia varies during the healing after the tumor resection, it is likely to have distinct impacts on tumor re-growth[6,7]. To identify the best strategies targeting microglial-driven glioma recurrence, the type of microglial activation in the site of tumor resection has to be evaluated. The polarization state of microglia in the site of tumor resection, it's cytokines expression profile and the effect on glioma regrowth, remain unclear. The unique tumor microenvironment, established after the tumor resection, must be taken in consideration in treatment protocols[8,9,10].

The goals of this study are to evaluate the role of microglial polarization state in tumor resection area, in tumor relapse and to identify target for therapeutic disruption of glioma-microglia interaction. The outcome of this research will provide insight into understanding how the tumor microenvironment changes after a surgical resection and how discerning the dynamics of microglia can potentially be translated into a new treatment for Glioblastoma patients.

Methods



Results

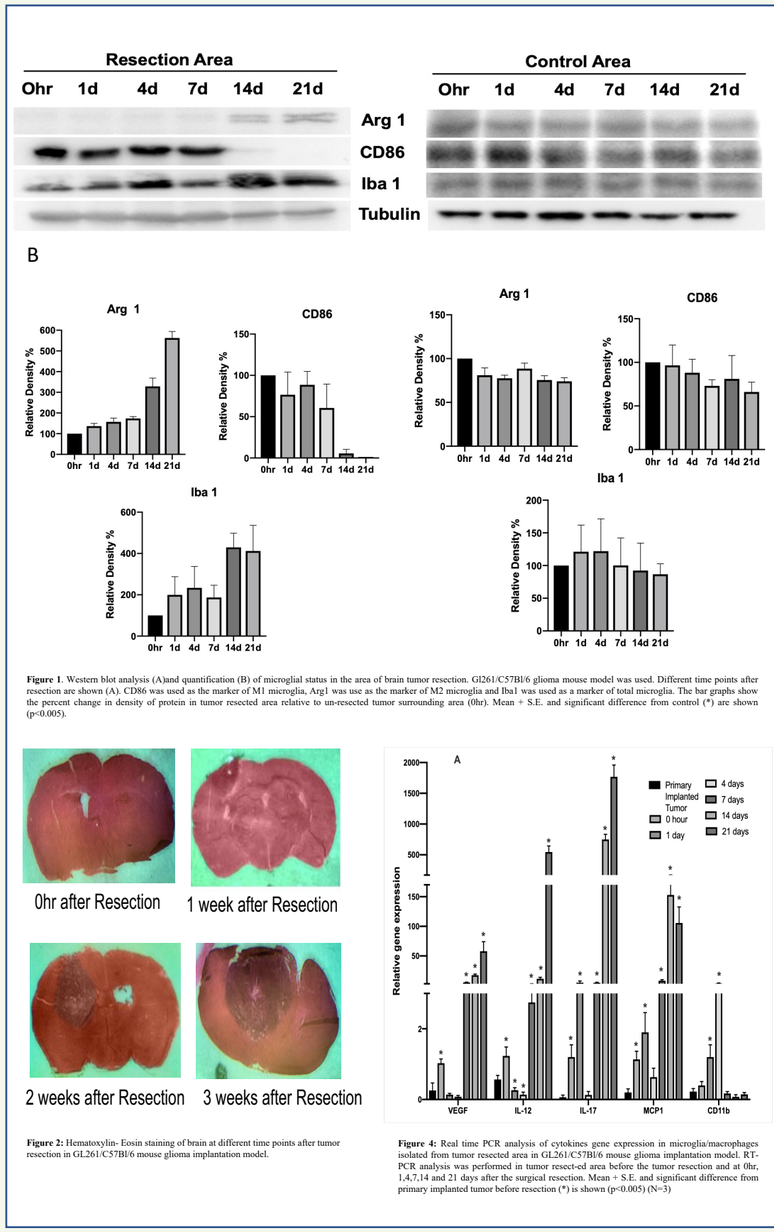


Figure 1. Western blot analysis (A)and quantification (B) of microglial status in the area of brain tumor resection. Gl261/C57Bl/6 glioma mouse model was used. Different time points after resection are shown (A). CD86 was used as the marker of M1 microglia, Arg1 was use as the marker of M2 microglia and Iba1 was used as a marker of total microglia. The bar graphs show the percent change in density of protein in tumor resected area relative to un-resected tumor surrounding area (0hr). Mean + S.E. and significant difference from control (*) are shown (p<0.005).

Figure 2: Hematoxylin- Eosin staining of brain at different time points after tumor resection in GL261/C57Bl/6 mouse glioma implantation model.

Figure 4: Real time PCR analysis of cytokines gene expression in microglia/macrophages isolated from tumor resected area in GL261/C57Bl/6 mouse glioma implantation model. RT-PCR analysis was performed in tumor resect-ed area before the tumor resection and at 0hr, 1,4,7,14 and 21 days after the surgical resection. Mean + S.E. and significant difference from primary implanted tumor before resection (*) is shown (p<0.005) (N=3)

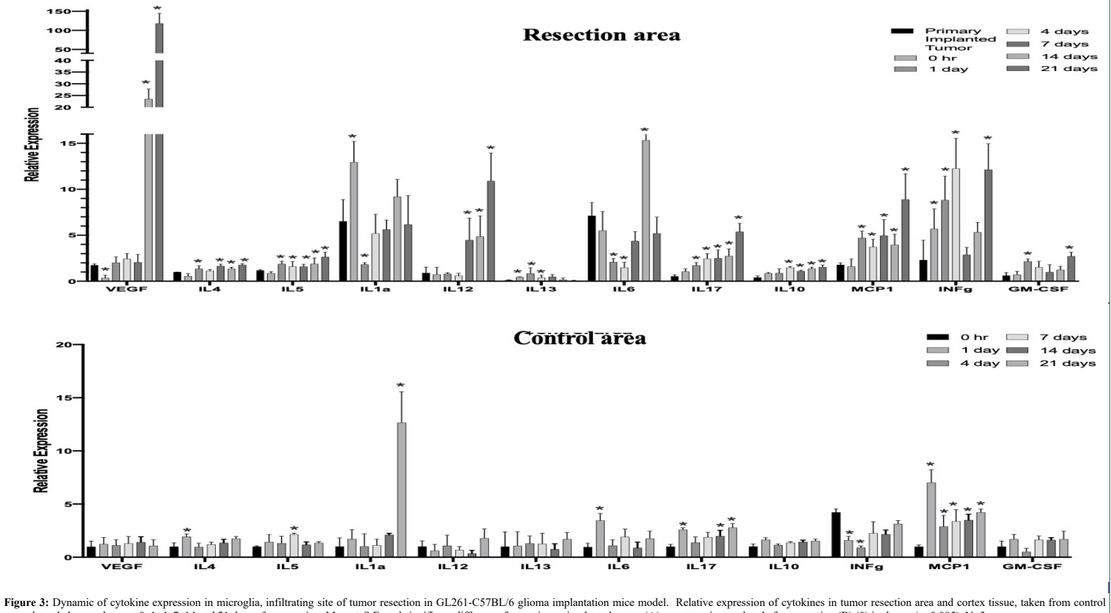


Figure 3: Dynamic of cytokine expression in microglia, infiltrating site of tumor resection in GL261-C57BL/6 glioma implantation mice model. Relative expression of cytokines in tumor resection area and cortex tissue, taken from control contralateral chemosphere, at 0, 1, 4, 7, 14 and 21 days after resection. Mean + S.E. and significant difference from primary implanted tumor (A) or cortex tissue taken before resection (B) (*) is shown (p<0.005). N=3

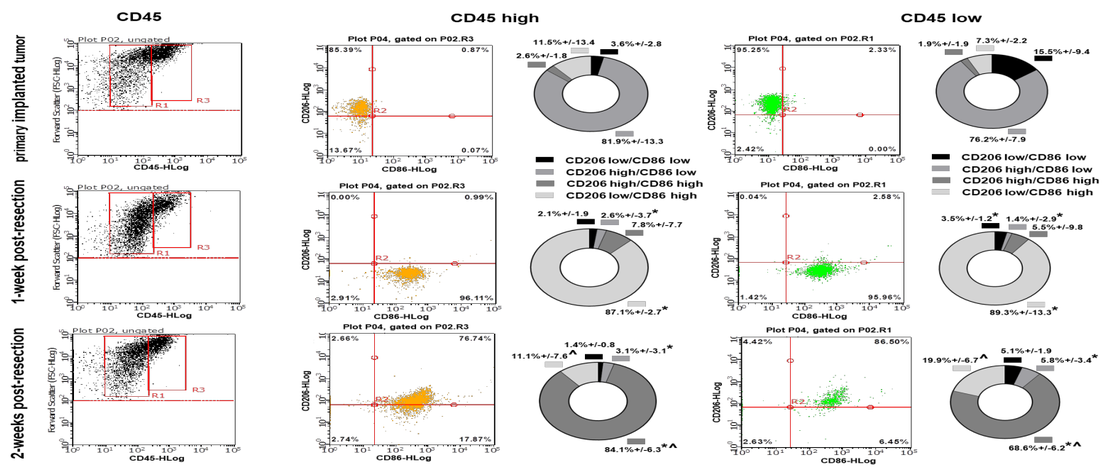


Figure 5: Flow-cytometric analysis of CD86/CD206 expression in tumor-infiltration microglia/macrophages, purified from primary implanted tumors and tumors re-grown during 1 and 2 weeks after resection in GL261-C57BL/6 mouse glioma implantation model. The left graphs represent forward scatter (FSC) vs. CD45 plots demonstrating CD45 populations: low (R1), and high (R3). The middle graphs represent CD86 vs. CD206 events plotted from CD45 high and CD45 low populations. Parts of whole graphical representation of cells expressing CD86 and CD206 markers are shown on the right. Mean + S.E. and significant difference from primary implant-ed tumor (*) and from 1-week post-resected tumors (^) are shown (p<0.005) (N=6).

Conclusions

- The increase of arginase-positive (M2) microglia/macrophages was identified in tumor resection area beginning the 14 days after the tumor resection in Gl261/C57Bl/6 glioma mouse model. This effect was sustained during 21 days after the surgery. Similar dynamics of CD86- positive (M1) microglia/macrophages was identified in tumor resection area, however with a decreased at 14 and 21 days..
- PCR analysis confirmed our cytokines array results, where it demonstrated that cytokines such as VEGF, IL12, IL17, MCP1 at gene level were also higher in the re-grown tumor.
- Microglia/macrophages purified from the re-grown tumor represent significantly higher expression of VEGF, IL12, IL17, MCP1 and GM- CSF, compared to the primary tumor.
- Flow cytometry demosntrated that from primary implanted to 1-week re-grown tumors, there was a shift toward CD86 marker expression shortly after tumor resection and then another shift towards high expression of both CD206 and CD86 in fully 2-weeks re-grown tumors. Suggesting that the activation state of tumor infiltrating myeloid cells undergo significant modulation in re-grown tumors.

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