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Supplemental Information

Microglia Induce PDGFRB Expression in Glioma Cells to Enhance Their Migratory Capacity

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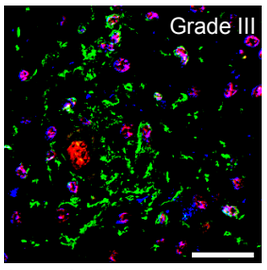
Figure S1



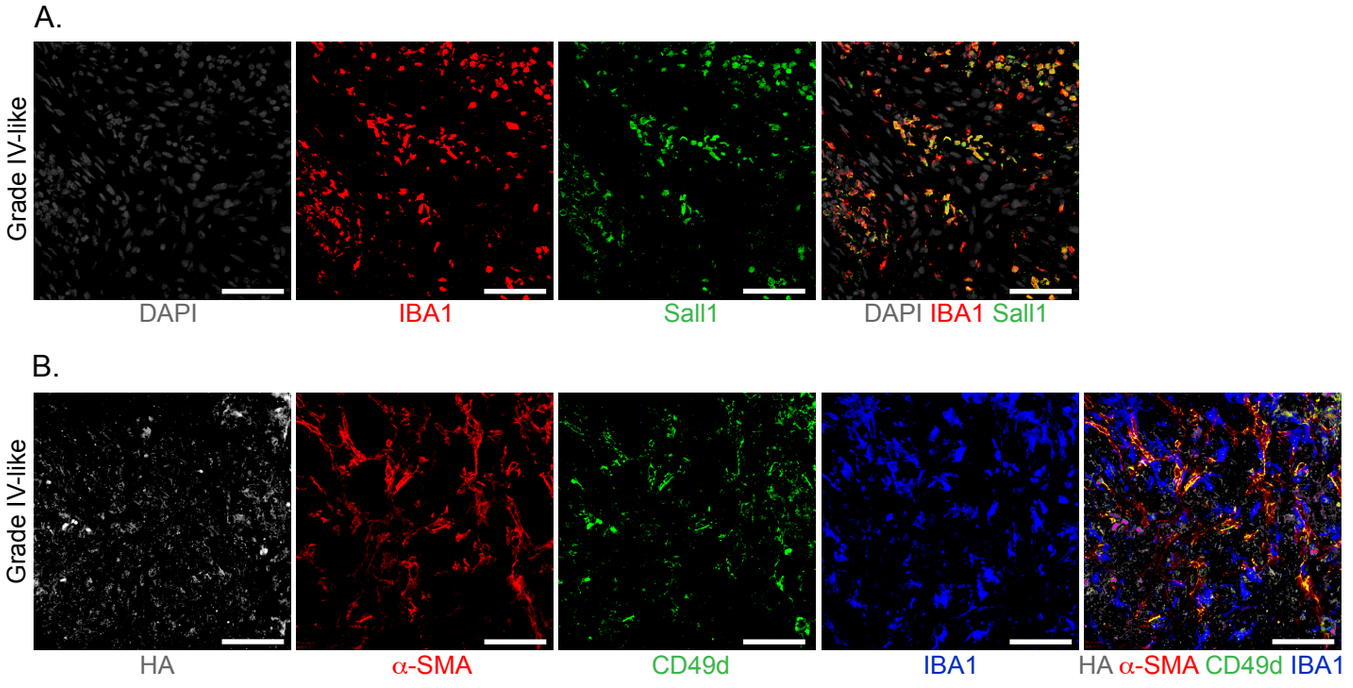
IDH1-R132H α-SMA IBA1

SUPPLEMENTAL FIGURE LEGEND

Figure S1. [α-SMA$^+$ glioma cells are in close proximity to IBA1$^+$ TAMMs], Related to Figure 4.

Human Grade III astrocytoma sections were immunostained for IDH1-R132H (blue), α-SMA (red) and IBA1 (green). Bars 50 μm.

Figure S2


A.
Grade IV-like
DAPI
IBA1
Sall1
DAPI IBA1 Sall1
B.
Grade IV-like
HA
α-SMA
CD49d
IBA1
HA α-SMA CD49d IBA1

SUPPLEMENTAL FIGURE LEGEND

Figure S2. [IBA1^{+} TAMMs express Sall1], Related to Figure 5.

(A) Mouse Grade IV-like glioma sections were immunostained for DAPI (grey), IBA1 (red) and Sall1 (green). Bars 50 μm.

(B) Mouse Grade IV-like glioma sections were immunostained for HA (grey), α-SMA (red), CD49d (green) and IBA1 (blue). Bars 50 μm.

TABLE S1. [Quantitative PCR Probes], Related to Figure 6.

TaqMan probes (Life Technologies)

α-sma	Mm00725412_s1
pdgfrb	Mm00435546_m1
β-actin	Mm00607939_s1
cxcl9	Mm00434946_m1
cxcl10	Mm00445235_m1
cxcl11	Mm00444662_m1
il-6	Mm00446190_m1
il-1b	Mm01336189_m1
arginase1	Mm00475988_m1
mrc1	Mm01329362_m1
ym1	Mm00657889_mH

SYBR green probes (Sigma)

pdgfra	FW 5'-ATATTTGAGACATTGCTGGC RW 5'-CTAGTTCCTGCATCCATTTTG
egfr	FW 5'-CTGTCGCAAAGTTTGTAATG RW 5'-GAATTTCTAGTTCTCGTGGG
fgfr	FW 5'-TATGTCCAGATCCTGAAGAC RW 5'-GAGAGTCCGATAGAGTTACC
hgfr	FW 5'-CGACAAATACGTTGAAATGC RW 5'-GATCTACATAGGAGAATGCAC
igf-1r	FW 5'-AGAACCGAATCATCATAACG RW 5'-TTTTAAATGGTGCCTCCTTG
cnp	FW 5'-CTTCGACACTTTATTTCTGGAG RW 5'-AATTTGGTTGTACAGTGCAG
gfap	FW 5'-GGAAGATCTATGAGGAGGAAG RW 5'-CTGCAAACTTAGACCGATAC

hes1	FW 5'-AAGCCTATCATGGAGAAGAG RW 5'-GGAGCTATCTTTCTTAAGTGC
ng2	FW 5'-GGCAGCACTGCCTCCTGGAC RW 5'-CCCTGGCCCCACTGCAACTG
β-actin	FW 5'-GATGTATGAAGGCTTTGGTC RW 5'-TGTGCACTTTTATTGGTCTC

SYBR green probes (QIAGEN)

nestin	QT00316799
olig2	QT01041089
sox2	QT02249347

TRANSPARENT METHODS

Mouse tumor models

We used a PDGF-B-driven replication-competent leukosis virus splice acceptor (RCAS)/*tv-a* mouse glioma model in which transgenic expression of the avian tv-a receptor expression is under the nestin (NES) promoter (Holland and Varmus, 1998) in a homozygous p19Arf-deficient ($Arf^{-/-}$) background and denoted *N/tv-a;*$Arf^{-/-}$ mice (Tchougounova et al., 2007). In order to induce glioma, 4×10^5 RCAS virus-encoding PDGF-B-producing DF-1 chicken fibroblasts were transplanted through intracerebral injections into neonatal mice. RCAS is replication incompetent in mammalian cells and infection of nestin-expressing target cells will occur during the first days after injection. Within three months injected mice will develop gliomas with different degree of malignancies (Karrlander et al., 2009; Tchougounova et al., 2007). Experiments were conducted in accordance with the local Animal Ethics Committee decision C300/10.

Patient glioma material

Paraffin-embedded human astrocytoma samples were obtained from the Research Biobank for intracranial neoplasms at Bergen University, Bergen, Norway. Surgical specimens were collected during surgical resections at the Department of Neurosurgery, Haukeland Univeristy Hospital, Bergen, Norway. All material in the Research Biobank was collected with written informed consent from patients and the Biobank is approved by the regional ethical committee (013.09, 2013/720 and 2018/1520).

Histological analysis

Tumor samples embedded in paraffin or in OCT were cut in 6µm or 10µm thick sections, and stained as previously described (Wallerius et al., 2016). Tumor

sections were stained with hematoxylen harris and eosin (HistoLab) or treated for antigen retrival with sodium citrate buffer (Thermo Scientific Fisher and Biocare Medical) and immunostained with appropriate antibodies: anti-CD31 (Santa Cruz), Podocalyxin (R&D systems), α-SMA (Sigma), PDGFRB (Cell Signalling and Abcam), CD44 (Santa Cruz), Sox2, GFAP, Olig2 and NG2 (Millipore), Ki67 (Abcam), MRC1 (CD206; AbD), Iba 1 (Wako chemicals and Abcam), HA (Acris and Abcam), GFAP (Abcam), IDH-R132H (Dianova); streptavidin and all secondary antibodies were conjugated with AlexaFluor 405, AlexaFluor 488, AlexaFluor 546, AlexaFluor 555 or AlexaFluor 647 fluorochromes (Molecular Probes). Cell nuclei were labeled with DAPI (Invitrogen Corp). Tumor hypoxia was detected by intraperitoneal injection of 60mg/kg pimonidazole hydrochloride (HypoxiProbe) into tumor-bearing mice. Vessel functionality was assessed by intravenous injection of 1mg of FITC-conjugated lectin (Molecular Probes) into tumor-bearing mice (this compound binds to the inner lumen of vessels) (Criscuoli et al., 2005; Dreher et al., 2006). Ten minutes later mice were sacrificed and their brains were fixed in 4% formalin and embedded in paraffin or in OCT embedding material and frozen in isobutanol on dry ice. At least 6 independent fields from sections from each tumor were analyzed by using LSM 510 META, LSM700 META and LSM T-PMT Zeiss confocal microscopes and quantified using ImageJ software.

Primary mouse glioma cells

The cultured mouse glioma stem cells were derived from RCAS-PDGF-B-induced grade IV gliomas in *N/tv-a;Arf*$^{-/-}$ mice (Jiang et al., 2017) and cultured under stem cell conditions as previously described (Jiang et al., 2011). These cells have all functional characteristics of GBM stem cells and will be denoted GSCs.

Isolation of microglia

6-to-8-week-old naïve mice were deeply anesthetized and perfused with ice-cold PBS (Sigma). Brains were removed without meninges, enzymatically digested and resuspended in 0.5 mg/ml DNaseI in HBSS. Cell pellets were resuspended in DMEM/F12 complete (complemented with 10% FBS, 1% Penicillin Streptomycin (P/S, 100 units/ml) and 1% Glutamine (100µg/ml, Sigma) supplemented with Macrophage Colony Stimulating Factor (M-CSF) (20ng/ml, Miltenyi) and cultured at 37°C and 5% CO_2. The primary cells were cultured until 80% confluence. Microglia were isolated with CD11b magnetic microbeads (Miltenyi Biotec 130-049-601) according to the manufacturer's instructions and seeded in DMEM/F12 medium complete supplemented with 50ng/ml M-CSF (Zhang et al., 2014).

Isolation of BMDMs

Bone marrow precursors were acquired by flushing the bone marrow from the femurs and tibias of 6-to-8-week-old naïve BALB/c mice. Erythrocytes were lysed with red blood cell lysis buffer (RCB, Sigma) and cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 1% P/S (Gibco), 1% Glutamine (Gibco) and 50ng/ml M-CSF (Miltenyi) for 6-to-7 days (Wallerius et al., 2016).

M1- or M2- polarized microglia and BMDMs

Microglia or BMDMs were polarized to either an M1-phenotype (100 ng/ml LPS (Sigma) and 200 units/ml IFNγ (Peprotech)) or an M2-phenotype (20 ng/ml IL-4 (Peprotech), 10 ng/ml TGF-β (Peprotech), 20 ng/ml IL-10 (R&D)) for 24 hours.

Co-culture experiments

M1- and M2-polarized microglia or M1- and M2-polarized BMDMs were washed two times with PBS before N/tv-a PDGF-B GFP^+GCSs were added and co-cultured with or without M1- or M2-polarized microglia or M1- or M2-polarized BMDMs for 24h at

37°C. Control medium (M0) or conditioned medium from M1- or M2-polarized microglia was added to N/tv-a PDGF-B GFP$^+$GCSs for 24 h at 37°C. GFP$^+$GCSs were then flow-sorted and subjected to RNA isolation and cDNA synthesis.

RNA Extraction, cDNA Synthesis and quantitative RT-PCR

Total RNA was extracted from N/tv-a PDGF-B GFP$^+$GCSs, microglia or BMDMs. Cells were collected in RLT buffer and RNA was extracted and cDNA synthesized from collected glioma cells, microglia or BMDMs as previously described. Gene expression was analyzed by performing quantitative RT-PCR. TaqMan and SyBr green probes are listed in Supplemental table 1.

Migration Assay

The mouse glioma cells were co-cultured with M1- or M2-polarized microglia for 8h before they were seeded on laminin (5 μg/ml)-coated transwell membranes (VWR) +/- 10 μg/ml PDGFRB neutralizing antibody (ThermoFisher) for 24h at 37°C. Cells in the upper part of the membrane were removed. The membranes were fixed in 4% paraformaldehyde and immunostained with an Olig2 antibody (Millipore) and the appropriate secondary antibody conjugated with AlexaFluor 488 (Molecular Probes). Cell nuclei were labeled with DAPI. Cells were counted using a LSM T-PMT Zeiss confocal microscope and quantified using ImageJ software.

Statistical analysis

In all studies, values are expressed as Standard Deviation (SD) and mean. Statistical analyses were performed using One-way ANOVA. Stars and hash tags in the graphs indicate significance as depicted in figure legends. Differences were considered statistically significant at $p<0.05$.