

Oncolytic Herpes Simplex Virus type 1 armed with CXCL12-antagonist "P2G" to disrupt CXCR4 pathway in Glioblastoma

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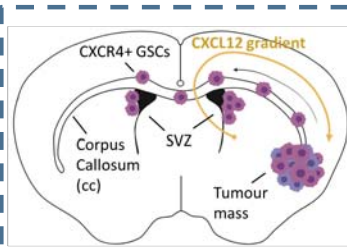


Introduction

Glioblastoma is an aggressive high-grade astrocytoma (WHO, grade 4). Current standard treatments consist of maximal surgical resection followed by chemo-/radio-therapy.

Unfortunately, new therapies are needed as this protocol is impaired by **Glioblastoma Stem-like Cells (GSCs)** which promote tumour reformation and pro-tumoral microenvironment development through CXCL12/CXCR4 pathway.

In this context, stereotaxic injection of **oncolytic HSV-1 (oHSV)** expressing a specific CXCL12/CXCR4 pathway inhibitors (oHSV-P2G) seems to be a potent therapeutic strategy.



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CXCR4+ GSCs are able to migrate to the SVZ, escape resection, resist to standard therapies, self-renew, leading to tumour recurrence. In the meantime, **CXCR4 pathway inhibit the immune response and promote angiogenesis.**

Materials and Methods

Migration - Orthotopic nude mouse model

100k GB138 RFP+ cells are stereotactically engrafted in the right hemisphere of the brain (Day 0). On day 20, mice are treated with PBS, oHSV or oHSV/P2G. On day 50, 3D images are acquired by lightsheet microscopy, then TM and cc are modeled. The migration volume ratio is measured as $\frac{\text{Migrating tumour cells}}{\text{Whole tumour mass}} \text{ volume}$.

Tumour microenvironment - Orthotopic C57BL/6J mouse model

100k GL261N4 cells are stereotactically engrafted in the right hemisphere of the brain (Day 0). On day 7 & 14, mice are treated with PBS, oHSV or oHSV/P2G. On day 21, Cd45+ leucocytes are isolated from 12 brains per treatment and 10x genomics single-cell protocol was followed. Analysis are performed on R with Seurat.

1. GSCs : oHSV-P2G inhibits *in vivo* migration of human glioblastoma primary cells

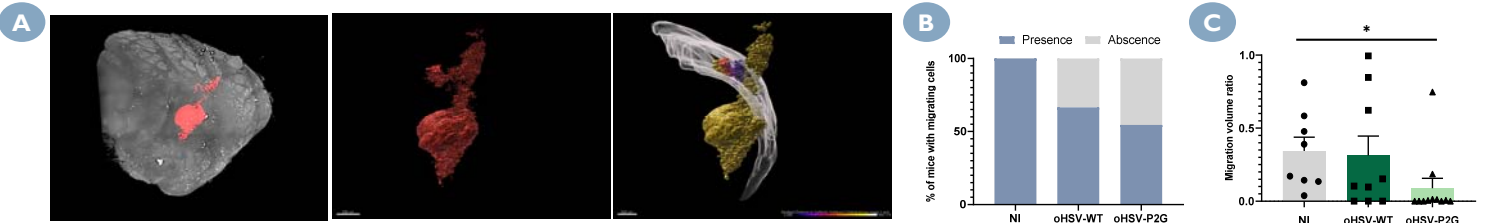


Figure 1. *In vivo* experiment performed on nude mice divided into three treatment groups (PBS, 1×10^6 pfu oHSV-WT, 1×10^6 pfu oHSV-P2G). Treatment were stereotaxically injected 20 days after engraftment of 100k GB138 RFP+ Luc+ cells. On day 50, brains were collected after saline and PAF 4% perfusion and clarified for lightsheet microscopy. (A) Snapshot of a representative tumor and corpus callosum modulations. Central tumor mass and axis (corresponding to injection path) are represented in yellow and cells migrating in the cc are statistically colored relatively to element distance from central axis. (B) % of mice with migrating cells in the cc (C) Migration volume ratio. Data are mean \pm SEM. * $p < 0.05$.

2. Lymphocytes : oHSV/P2G induces T and B cells recruitment & inverts lymphoid/myeloid cells ratio

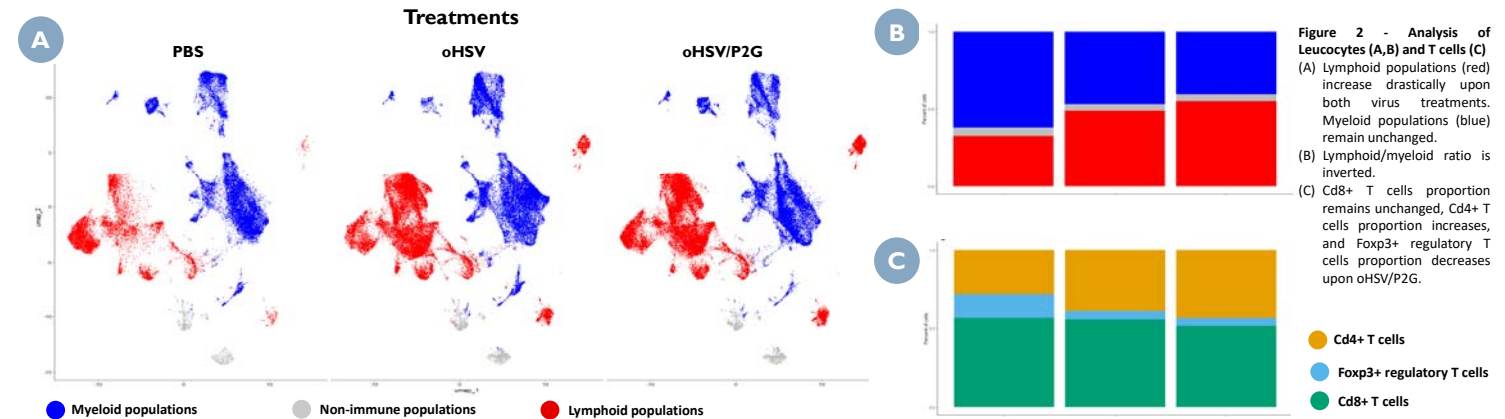


Figure 2 - Analysis of Leucocytes (A,B) and T cells (C)
 (A) Lymphoid populations (red) increase drastically upon both virus treatments. Myeloid populations (blue) remain unchanged.
 (B) Lymphoid/myeloid ratio is inverted.
 (C) Cd8+ T cells proportion remains unchanged, Cd4+ T cells proportion increases, and Foxp3+ regulatory T cells proportion decreases upon oHSV/P2G.

3. Macrophages : oHSV/P2G induces pro-tumoral & pro-angiogenic population decrease and pro-inflammatory population increase

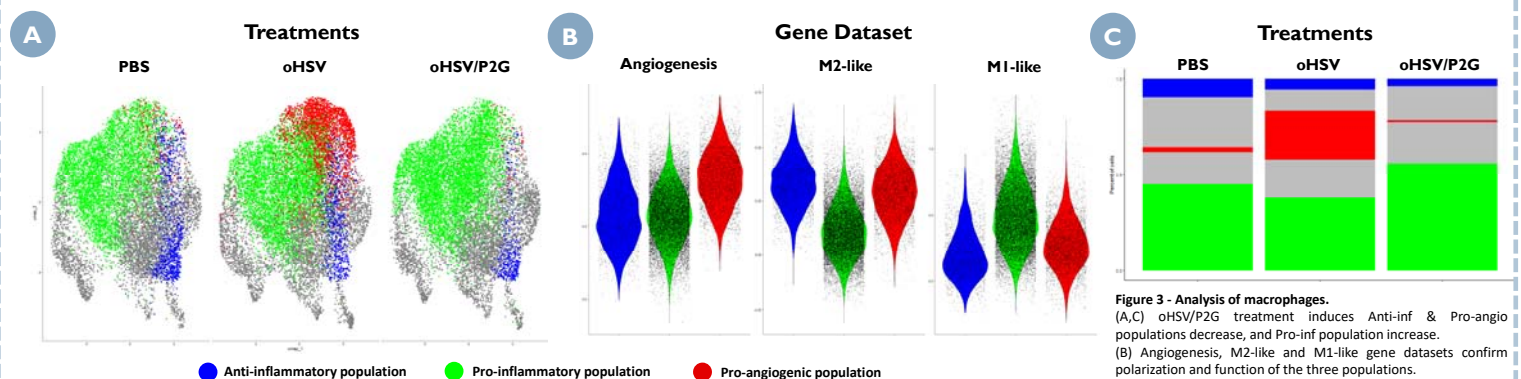


Figure 3 - Analysis of macrophages.
 (A,C) oHSV/P2G treatment induces Anti-inf & Pro-angio populations decrease, and Pro-inf population increase.
 (B) Angiogenesis, M2-like and M1-like gene datasets confirm polarization and function of the three populations.

Discussion

Our previous works show that oHSV-P2G inhibits CXCR4/CXCL12 pathway, hampers tumor cells self-renewal and migration abilities. The above results points towards an activation of adaptative lymphoid immune response, as well as a polarization of macrophages towards a pro-inflammatory and anti-angiogenic phenotype compared to PBS and oHSV. These encouraging data confirm the beneficial impact of oHSV/P2G on the tumor microenvironment.