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

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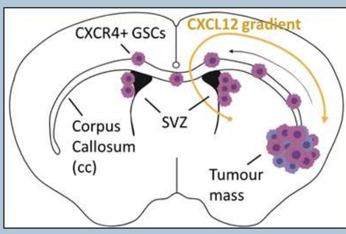
Introduction

Glioblastoma is an aggressive high-grade astrocytoma (WHO, grade 4). Current standard treatments consist of maximal surgical resection followed by chemo-/radio- therapy. Unfortunately, this treatment protocol is impaired by **systematic tumour reformation** promoted by **CXCR4+ glioblastoma stem-like cells (GSCs)**.

Via autocrine and paracrine stimulation, **CXCL12/CXCR4 pathway promotes self-renewal and migration** but also proliferation, tumour angiogenesis and pro-tumoral microenvironment formation.

Thus, GSCs and CXCL12/CXCR4 pathway became targets for new therapies against GBM recurrence and pro-tumoral microenvironment development.

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.



GSCs are able to migrate from the tumour mass (TM) to the sub ventricular zone (SVZ) through the corpus callosum (cc), following a CXCL12 gradient (CXCR4 ligand).

Goffart, N. et al.

Materials and Methods

In vivo experiments.

Orthotopic nude mouse model. 100k GB138 RFP+ cells are stereotaxically engrafted in the right hemisphere of nude mice's brains (Day 0). On day 20, mice are treated with PBS, or oHSV-WT/P2G. On day 50, 3D images of brains are acquired by lightsheet microscopy. Then, TM and cc are modelized and the cells migrating trough the cc are identified. The migration volume ratio is measured as $\frac{Migrating\ tumour\ cells}{Whole\ tumour\ mass}\ volume$.

In vitro experiments.

Stemness markers expression level was assessed on GB138 cells after 48h culture upon infection (or not) by oHSV-WT/P2G. The expression level was evaluated by RT-qPCR relatively to not infected cells.

Tumorospheres formation assay measures the % of cells forming tumorospheres in 4 days upon infection (or not) by oHSV-WT/P2G. % tumorospheres formation is measured as $\frac{Final\ number\ of\ tumorospheres}{Initial\ number\ of\ cells} * 100$.

Spheroid migration assay measures the migration ratio of GSCs plated on a poly-D-lysine coated surface and left to migrate for 24 hours in conditioned media. Migration ratio is measured as $\frac{Aera\ 24h - Area\ 1h}{Area\ 24h} * 100$.

1. Stemness markers expression is impaired upon infection by oHSV-P2G, in a CXCR4-dependent manner

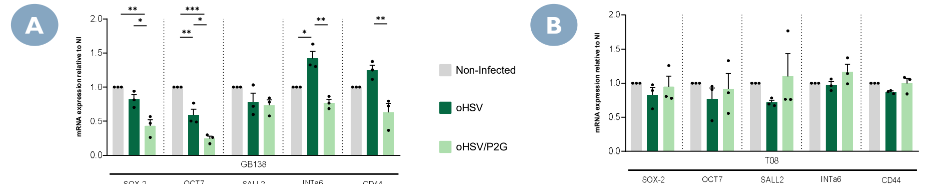


Figure 1. Stemness markers expression was evaluated on CXCR4 high GB138 and CXCR4 low T08 cells upon 48h of infection (or not) by oHSV-WT/P2G, by RT-qPCR relatively to NI. (A, B) Stemness markers expression. Data are mean ± SEM. * p<0.05; ** p<0.01, *** p<0.001.

2. Self-renewal abilities of GSCs are impaired upon infection by oHSV-P2G, in a CXCR4-dependent manner

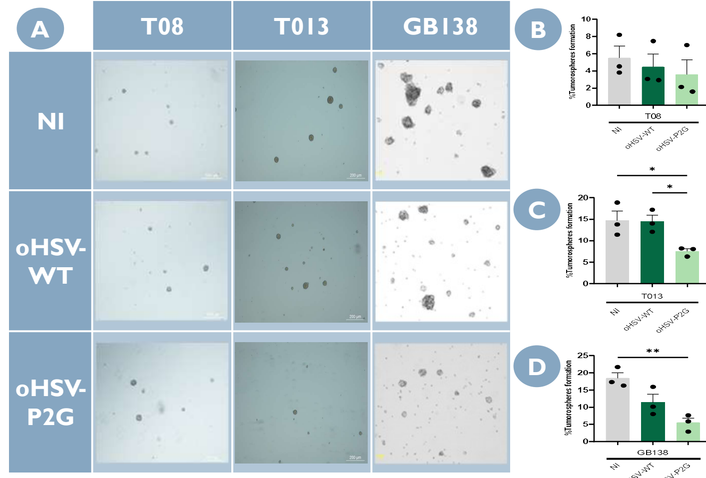


Figure 2. Tumorospheres formation assay performed on GSCs and human glioblastoma primary cells with high (T013, GB138) and low expression of CXCR4 (T08). Cells were left to form tumorospheres in specific media for 4 days upon infection. (A) Representative pictures. (B, C, D) % Tumorospheres formation. Data are mean ± SEM. * p<0.05; ** p<0.01.

3. Migration abilities of GSCs are impaired upon infection by oHSV-P2G, in a CXCR4-dependent manner

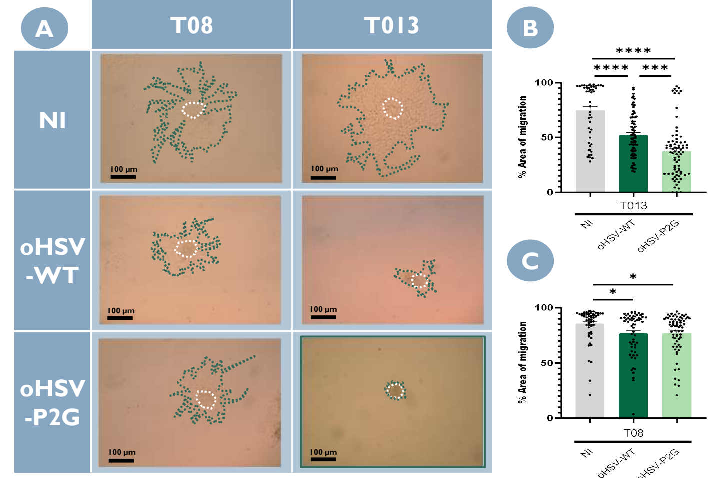


Figure 3. Tumorospheres migration assay performed on GSCs with high and low expression of CXCR4 (T013 and T08, respectively). Cells were left to migrate on a poly-D-lysine coated surface for 24h upon infection. (A) Representative pictures. (B,C) % Area of Migration. Data are mean ± SEM. * p<0.05; ** p<0.01, *** p<0.001, **** p<0.001.

4. *In vivo* migration of human glioblastoma primary cells is inhibited by stereotaxically injected oHSV-P2G

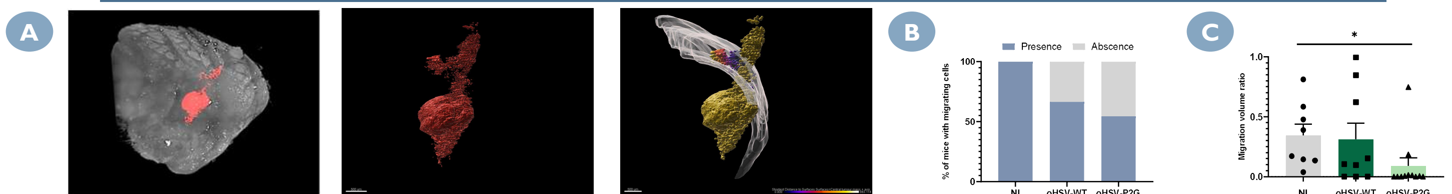


Figure 4. *In vivo* experiment performed on nude mice divided into three treatment groups (PBS, 1x10^6 pfu oHSV-WT, 1x10^6 pfu oHSV-P2G). Treatment were stereotaxically injected 20 days after engraftment of 100k GB138 RFP+ Luc+ cells. On day 50, brains were collected after saline and PAF 4% perfusion and clarified for lightsheet microscopy. (A) Snapshot of a representative tumor and corpus callosum modulizations. 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 ± SEM. . * p<0.05.

Discussion

oHSV-P2G was shown to be able to inhibit CXCR4/CXCL12 pathway (data not shown), to reduce stemness markers expression and hamper with tumor cells self-renewal *in vitro*. Moreover, oHSV-P2G was shown to inhibit *in vitro* and *in vivo* tumor cells migration abilities. One important point that is still to be addressed is the impact of oHSV-P2G in reviving the pro-inflammatory immune response which is known to be impaired by the CXCR4/CXCL12-driven pro-tumoral microenvironment.