Study of the ACKR3/CXCR7 receptor in glioblastoma

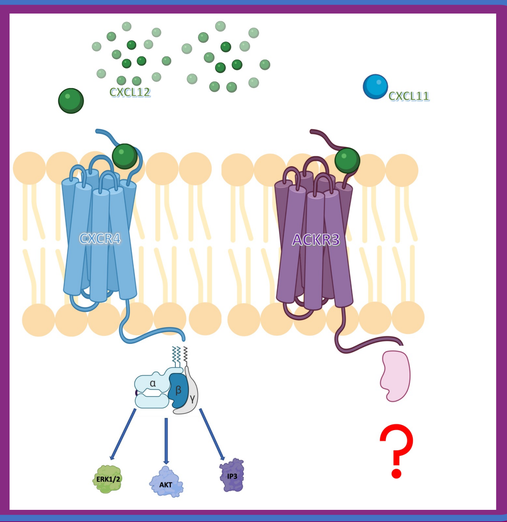
Isci, D.[1]; Da Veiga, M.A.[1]; Cokaiko, J.[1]; Lombard, A[1,2].; Chevigné, A.[3]; Rogister, B.[1,4]; Neirinckx, V.[1]

[1] Laboratory of Nervous System Diseases and Therapy, GIGA Neuroscience, GIGA Institute, University of Liège, Belgium.
[2] University Hospital, Neurosurgery Department, University of Liège, Belgium.
[3] Immuno-Pharmacology and Interactomics, Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg.
[4] University Hospital, Neurology Department, University of Liège, Belgium.

Contact: damla.isci@uliege.be

INTRODUCTION

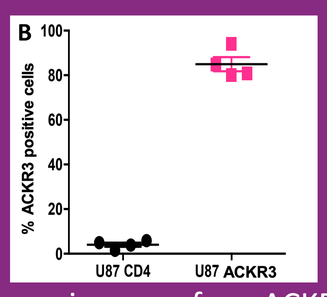
Glioblastoma (GBM) is the most frequent primary malignant tumor of the Central Nervous System, associated with invariably fatal outcome. After current standard treatment combining maximal resection surgery and concomitant radio-chemotherapy, the median survival after diagnosis is approximately 16 months, since the progression of the disease is characterized by systematic relapses. We have recently demonstrated the function of CXCR4 (a receptor for the chemokine CXCL12) in GBM stem-like cells (GSC), especially in their migration towards the subventricular zone (SVZ) and in the CXCL12-mediated protection from radiation therapy. In this project, we aim to investigate ACKR3, the second receptor for CXCL12, which expression in GBM is upregulated and correlated with a poor prognosis. We will study ACKR3 expression in GBM tissue and GBM stem-like cell cultures, unravel ACKR3 function in GBM cell proliferation, invasion and resistance to therapy, and decipher the molecular aspects underlying ACKR3 signaling and interaction with CXCR4 .



RESULTS

1) Impact of ACKR3 overexpression in GBM cells

We started with studying ACKR3 expression and function in the U87 GBM cell line, genetically engineered for expressing ACKR3.



Expression of ACKR3 receptor does not induce any effect on cell proliferation or on the expression of stem cell markers in U87 cells *in vitro*.

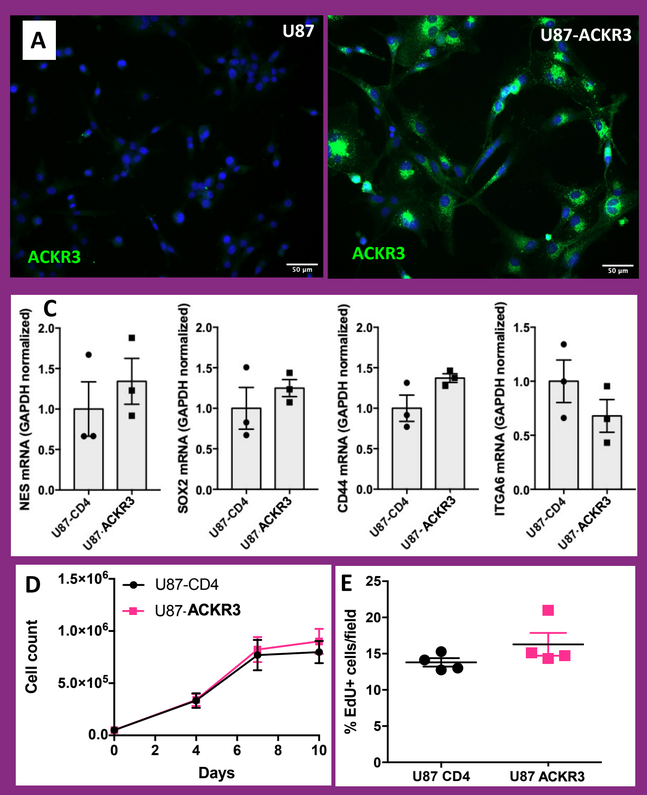


***FIGURE 1*: Study of ACKR3 expression U87 cell lines.** Representative images of Immunofluorescent staining where ACKR3 (green) DAPI (blue) was used to counterstain nuclei. Scale bar = 50 µm (**A**). Flow cytometry analysis of ACKR3 membrane expression in U87 (**B**) quantitative RT-PCR analysis of ACKR3 normalized to GAPDH in U87 (**C**) Cell counting assay in U87 cells (4,7,10 days counting) (**D**). EdU assay in U87 cells (**E**).

2) ACKR3 expression is low in GBM stem-like cells and cell lines

We quantified ACKR3 expression at the membrane of adult patient-derived GBM stem-like cell cultures, maintained as neurospheres. ACKR3 expression appears very low in five tested cultures (<8%). Stimulation with CXCL12 did not induce any change in ACKR3 expression at the membrane.

We also could verify ACKR3 expression in pediatric glioma cell lines, in which we could highlight a higher proporiton of ACKR3 positive cells

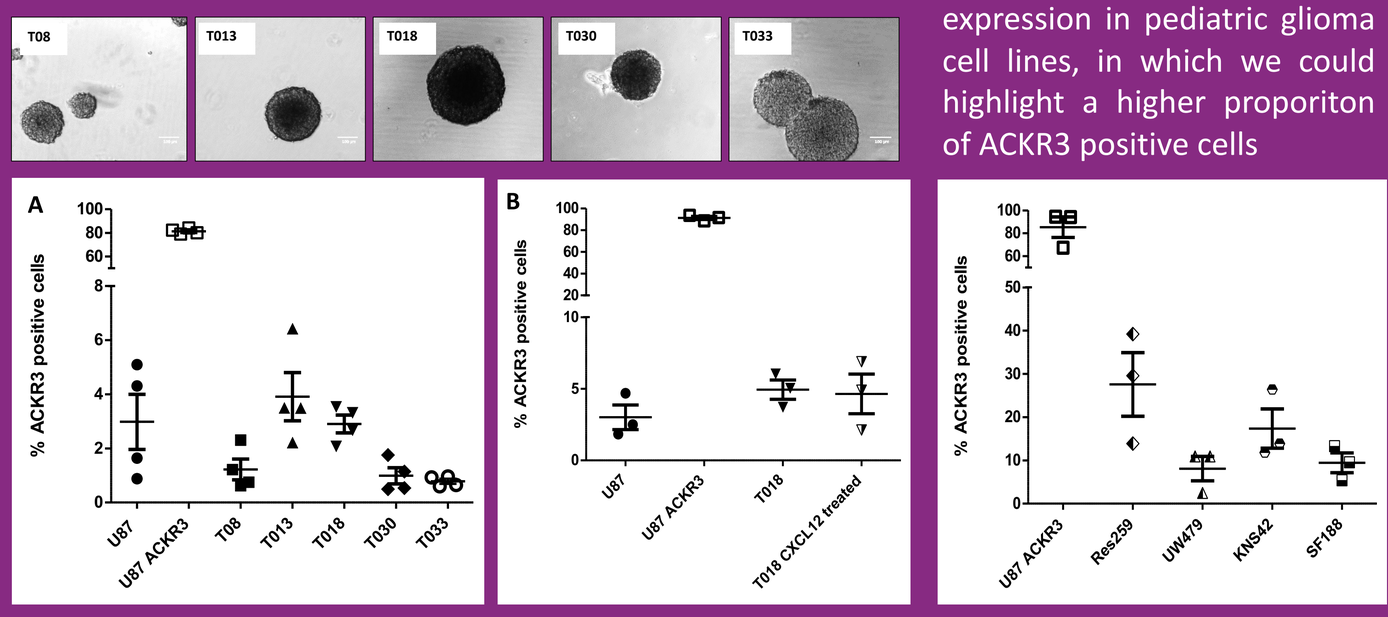


***FIGURE 2*: ACKR3 expression in patient derived GSCs and pediatric cell lines.** Flow cytometry analysis of ACKR3 membrane expression in patient derived GSCs (**A**). Flow cytometry analysis of ACKR3 membrane expression in patient derived GSCs (T018) with CXCL12 treatment (25ng/ml, 48h) (**B**). Flow cytometry analysis of ACKR3 membrane expression in pediatric glioma cell lines (**C**).

3) ACKR3 expression is detected in GBM patient tissue and appears distributed in various cell subtypes within the tumor

Single cell transcriptomic data from patient-derived GBM tissue indicates that ACKR3 is expressed in neoplastic cells, but also in nonvascular cells, myeloid cells, as well as astrocytes. Using immunostaining of patient GBM tissue, we confirmed that ACKR3 is detected in different cell types, which suggests intricate functions for this receptor in modulating tumor growth and behavior.

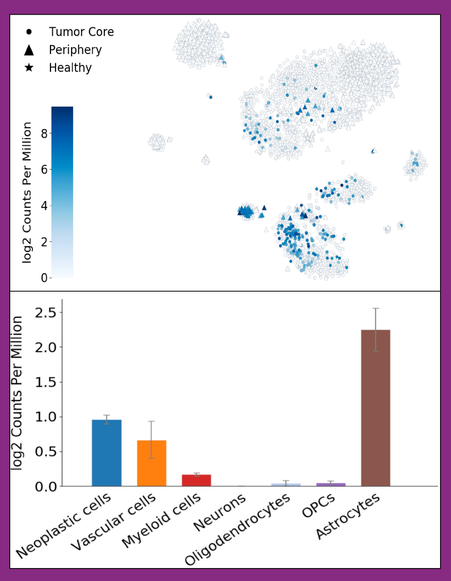


***FIGURE 3*: ACKR3 expression in cell subtypes within GBM tumors** (Single cell RNAseq data from adult GBM, Darmanis et al, 2017 (GBMseq online tool).

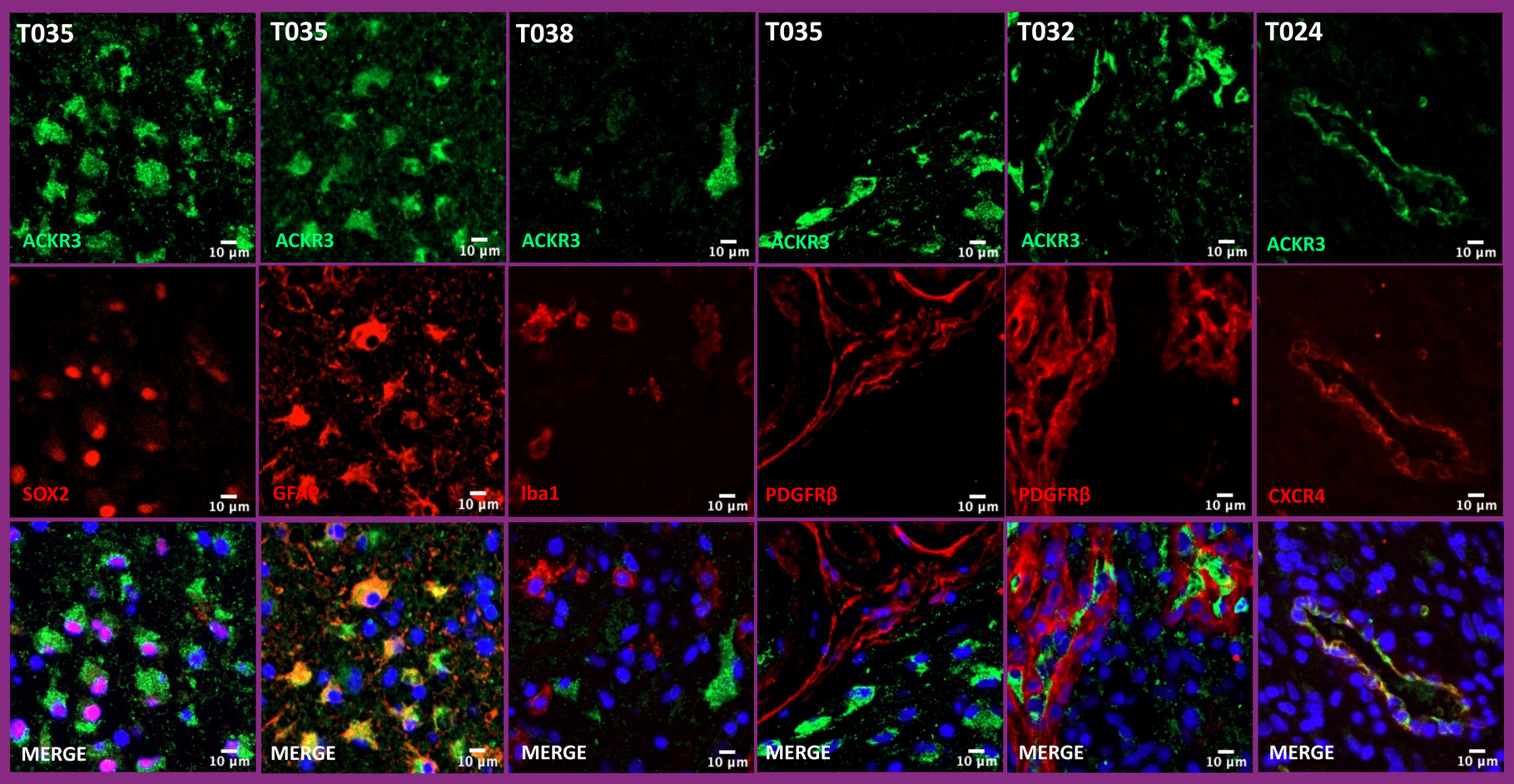


***FIGURE 4*: Characterization of ACKR3 positive cells in glioblastoma FFPE tissue.** Representative images of Immunofluorescent staining where ACKR3 (green) co-labeled with SOX2, GFAP and CXCR4 in GBM patients tissues. Co-labeling of ACKR3 with Iba1 and PDGFRβ has not been reported. DAPI (blue) was used to counterstain nuclei. Scale bar = 10 µm.

CONCLUSION

After validation of ACKR3 staining by using U87-ACKR3 cell line, we showed that ACKR3 expression was very low in patients GBM stem-like cells. However we detect the receptor on GBM patient tissue, where it appears distributed in various cell subtypes. Both public transcriptomic datasets of GBM and results that we have obtained have revealed that ACKR3 is expressed in tumor cells *in situ*, but also in cells from the surrounding tumor microenvironment. Morever, ACKR3 expression pattern varies in different regions of a tumor, and between different patients. The role of ACKR3 in GBM growth might be more subtle than expected, and likely involves malignant GBM cells as well as their microenvironment. The role of CXCR4 together with ACKR3 also deserves deep investigation.