Transformation Foci in Diffuse Low-Grade Gliomas Show STAT3 Pathway Activation and Downregulation of the Phospho-Ethanolamine Catabolism Enzyme ETNPPL Acting as a Negative Regulator of Glioma Cell Growth

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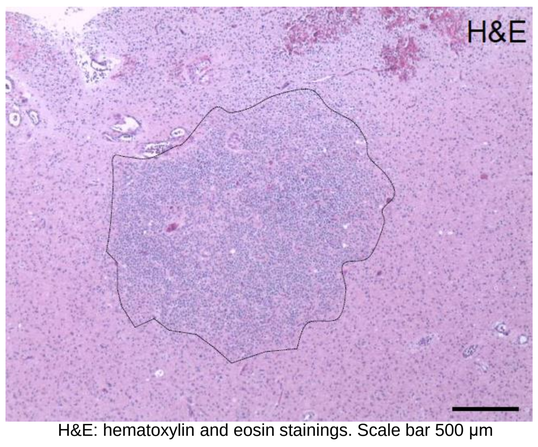
Aims

Diffuse low grade gliomas are slow-growing brain tumors which progress into high-grade gliomas. The early molecular events causing this progression are ill-defined. Previous studies revealed that 20% of these tumors already have transformation foci. These foci offer opportunities to better understand malignant progression. We used immunohistochemistry and high throughput RNA profiling to characterize foci cells.

Figure 1: *Left*: Example of a high cell density foci detected in a diffuse low grade glioma. *Right*: Patients with foci have a reduced overall survival



H&E: hematoxylin and eosin stainings. Scale bar 500 µm

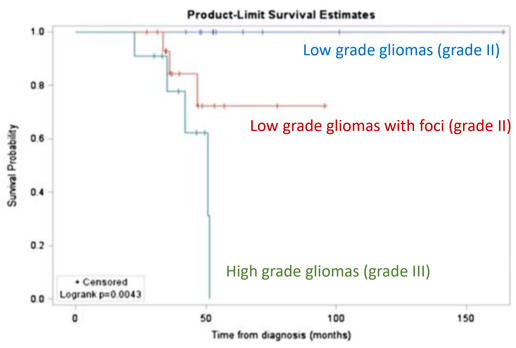


Figure 2: *Left*: Foci cells express a higher % of phosphorylated form of STAT3. *Right*: Co-staining of pSTAT3 with IDH1 R132H and ATRX, 2 mutated proteins found in low grade gliomas, show that pSTAT3 cells are tumoral cells

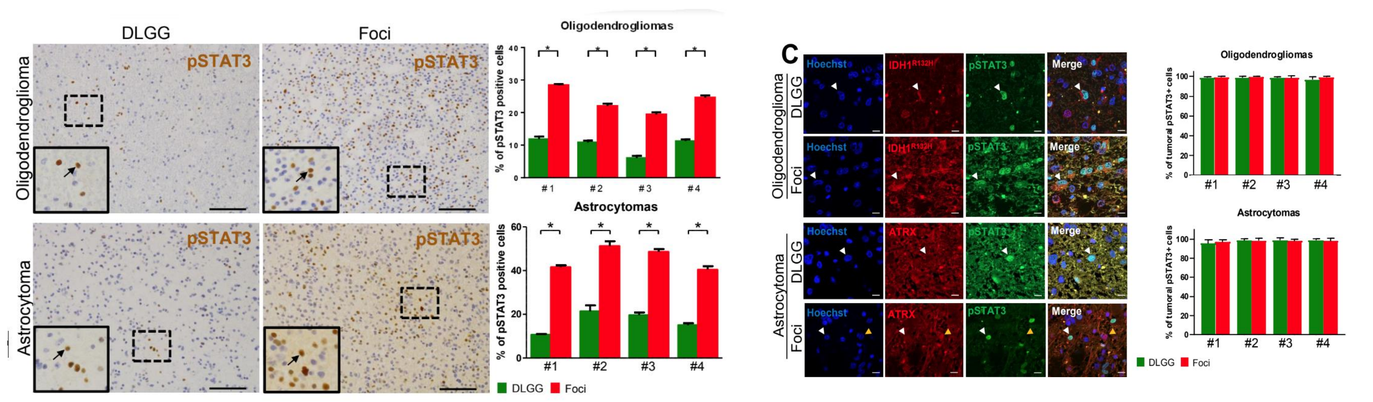


Figure 3: RNA profiling of microdissected foci compared to the rest of the tumor revealed the downregulation of 13 genes involved in Wnt, EGFR signaling and metabolism

Table 1: Identification of dysregulated genes in foci

Gene Symbol	Description	DLGG (log2)	Foci (log2)	Fold Change (DLGG/foci)	p-value
SFRP2	Secreted Frizzled Related Protein 2	6.51	5.5	2	0.0475
CST3	Cystatin C	8.94	8.1	1.79	0.0277
DAAM2	Dishevelled Associated Activator Of Morphogenesis 2	7.2	6.4	1.75	0.0253
ETNPPL	Ethanolamine-Phosphate Phospho-Lyase	6.01	5.22	1.72	0.0096
TMEM47	Transmembrane Protein 47	6.16	5.48	1.61	0.0126
MLC1	Megalencephalic Leukoencephalopathy With Subcortical Cysts 1	6.86	6.23	1.54	0.0028
KCNN3	Potassium Calcium-Activated Channel Subfamily N Member 3	6.42	5.8	1.54	0.0377
ADCYAP1R1	Adenylate Cyclase Activating Polypeptide 1 (Pituitary) Receptor Type I	7.28	6.72	1.47	0.0116
GJA1	Gap Junction Protein Alpha 1	5.79	5.27	1.43	0.0223
EZR	Ezrin	6.98	6.46	1.43	0.0048
ALDOC	Aldolase, Fructose-Bisphosphate C	7.81	7.36	1.37	0.0107
ATP1A2	ATPase Na+/K+ Transporting Subunit Alpha 2	6.88	6.47	1.33	0.0072
SLC1A3	Solute Carrier Family 1 Member 3	5.96	5.57	1.32	0.0273

Figure 4: QPCR validation of downregulated genes (n=7 tumors)

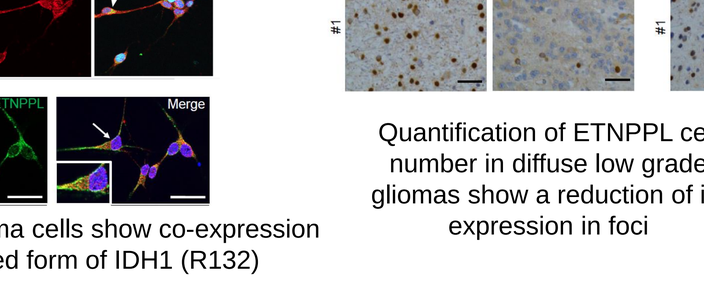
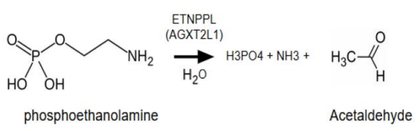
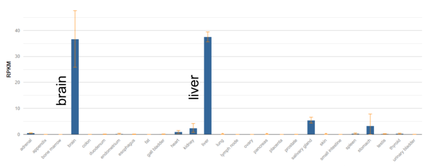


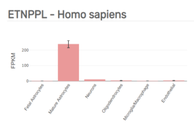
Figure 5: One of the gene dysregulated in foci is ETNPPL coding for Ethanolamine-Phosphate Phospho-Lyase, an enzyme barely studied (13 publication). ETNPPL is only expressed in liver and in brain and is an astrocytic specific enzyme



Breakdown of phosphoethanolamine by ETNPPL

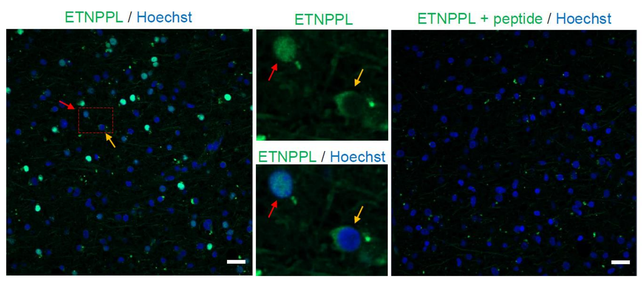


Human tissue expression of ETNPPL (NCBI database)



Brain cell specificity of ETNPPL expression (B Barres's database)

Figure 6: **Left:** Immunofluorescence for ETNPPL in the brain show that this enzyme is present in the cell nucleus but can also be observed in the cytoplasm;
Right: Co-labelling of ETNPPL with astrocyte markers (CHI3L1, Vim, GFAP, Aldh1L1) confirms its preferential expression in astrocytes in the human brain



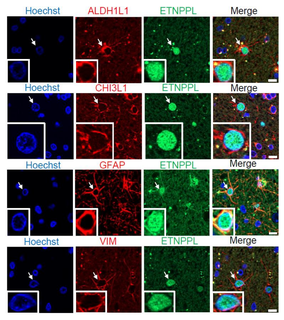
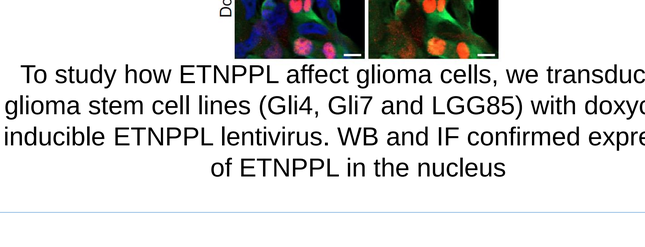
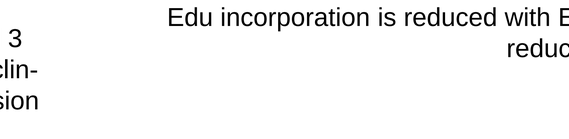


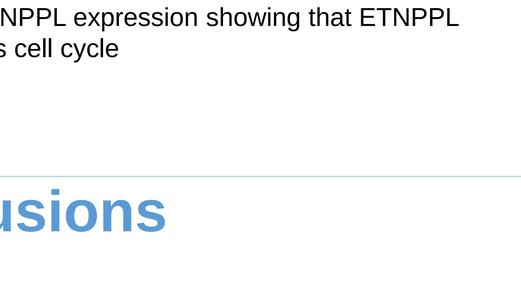
Figure 7: Expression of ETNPPL in gliomas



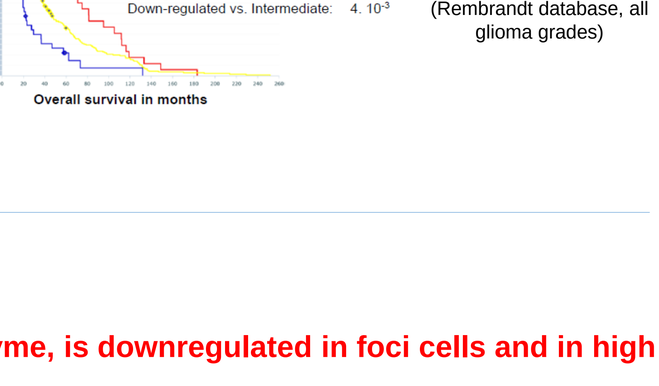
ETNPPL protein is detected in the nucleus or cytoplasm of tumoral cells in diffuse low grade gliomas but not in glioblastomas



WB confirmation of expression of ETNPPL protein in diffuse low grade gliomas but not in glioblastomas



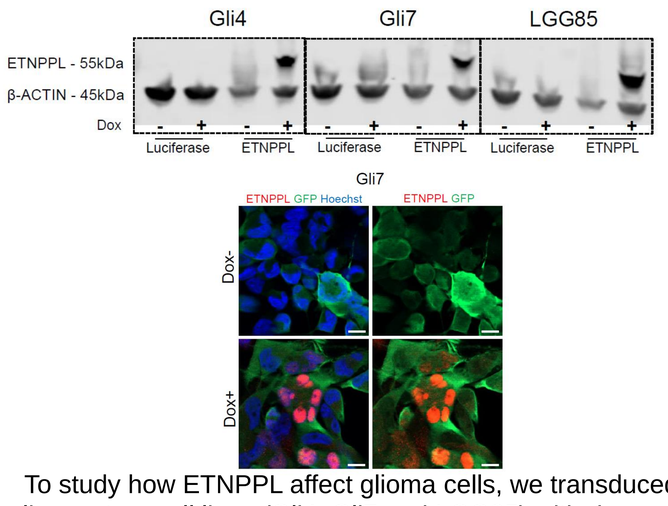
Culture of diffuse low grade glioma cells show co-expression of ETNPPL with the mutated form of IDH1 (R132)



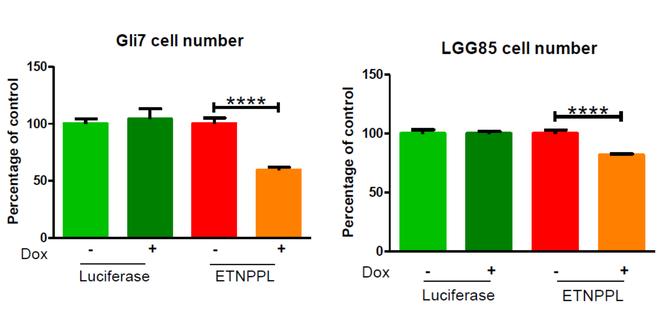
Quantification of ETNPPL cell number in diffuse low grade gliomas show a reduction of its expression in foci

Figure 8: Functional study of ETNPPL in gliomas

Figure 9: Database mining for ETNPPL

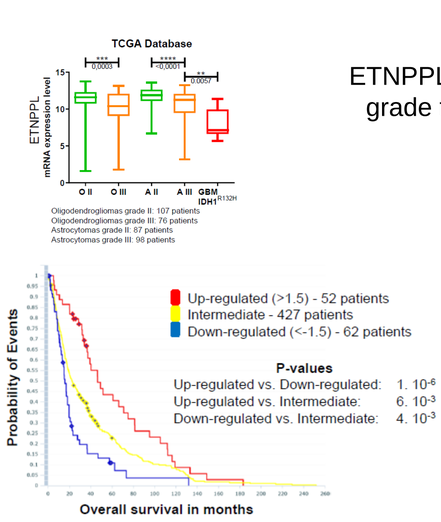


To study how ETNPPL affect glioma cells, we transduced 3 glioma stem cell lines (Gli4, Gli7 and LGG85) with doxycyclin-inducible ETNPPL lentivirus. WB and IF confirmed expression of ETNPPL in the nucleus



Compared to control luciferase virus, ETNPPL expression reduced cell number after 7 days in Gli7 and LGG85 cells. No effect was found in Gli4 cells.

Edu incorporation is reduced with ETNPPL expression showing that ETNPPL reduces cell cycle



ETNPPL RNA is reduced in grade tumors (III and IV)

Patients with low expression of ETNPPL have a reduced overall suvival
(Rembrandt database, all glioma grades)

Conclusions

1-We found that transformation foci cells in diffuse low grade upregulate STAT3 signalling.

2- 13 genes were found downregulated in foci cells

3-ETNPPL, a lipid metabolism enzyme, is downregulated in foci cells and in high grade gliomas

4-Overexpression of ETNPPL reduce growth of glioma stem cells in vitro