A biobank of IDH1-mutant astrocytoma cell lines for studying tumour heterogeneity, progression and target identification

L.Garcia[1,2], D.Pineau[1,2], S.Hideg[1], M.Augustus[1], F.Cavalli[4], M.Salma[5], E.Soler[5], F.Boussin[6], E.Huillard[7], M.Sanson[7], V.Rigau[1,3], H.Duffau[1,3], J-P. Hugnot[1,2]

Contact: leonor.garcia@igf.cnrs.fr; jean-philippe.hugnot@igf.cnrs.fr

[1]Institut de Génomique Fonctionnelle, Montpellier (IGF), [2]Université de Montpellier, [3]CHU Hôpital Gui de Chauliac , Montpellier, [4]Institut Curie, Paris, [5] Institut de Génétique Moléculaire, Montpellier (IGMM), [6]CEA, Paris, [7]Institut du Cerveau, Paris (ICM)

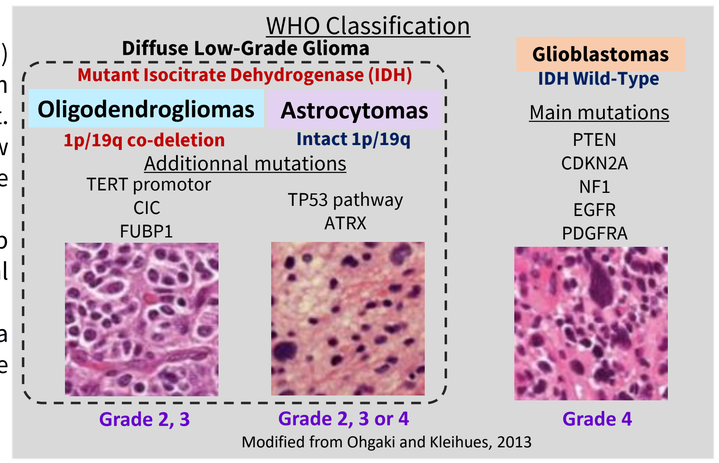
Introduction

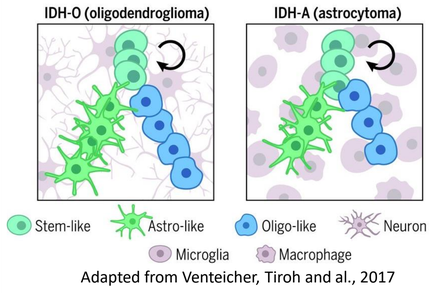
➢ Context

Diffuse Low-Grade Gliomas (DLGG, Grade 2) are primary rare brain cancers arising from glial cells preferentially in young adult. Unlike glioblastoma, LGG grow at a slow rate, but often degenerate into high grade gliomas (grade 3 or 4).

DLGG constitute an heterogenous group classified by WHO according to histological and genetic criteria.

In 70% of cases, these tumours carry a missense mutation on isocitrate dehydrogenase 1 (IDH1) gene.





➢ Challenges

- Previous studies show a high cellular heterogeneity in DLGG. They are composed by three cell types; stem-like cells, astrocyte-like and oligodendrocyte-like cells. This heterogeneity probably defeats current therapies, causing a poor overall survival of patients.
- There are few cellular tools to study *in vitro* these low grade gliomas .

➢ Aim

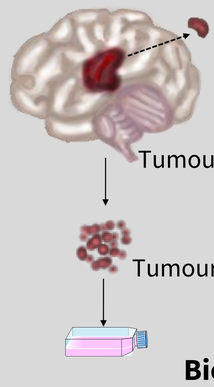
Our aim was to isolate and characterise IDH1-mutant astrocytoma cell lines and show they are relevant *in vitro* models to study low grade gliomas

➢ Strategy

We developed a method to derive cells lines from patients tumoral resections to constitute a biobank of astrocytoma cell lines. Then, we use a multi-omic approach to compare each cell line and validate the model.

Methods

Constitution of a biobank



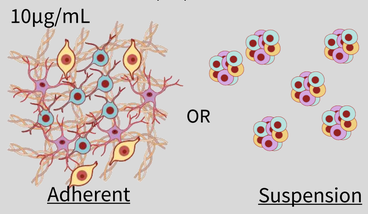
Patients informations

Cell lines	Sex	Age	Diagnostic	Clinical characteristics	Localisation	Treatment
LGG275	Female	40	Astrocytoma Grade 2	IDH1-mutant R132H ATRX lost	-	Surgery
LGG336	Female	40	Astrocytoma Grade 2	IDH1-mutant R132H, ATRX kept, p53 20%	-	Surgery
LGG85	Male	38	Astrocytoma Grade 4	IDH1-mutant R132H, p53 100%, c-met negative, ATRX kept	Left fronto insular	Surgery and chemotherapy (TMZ)
LGG349	Female	57	Astrocytoma Grade 4	IDH1-mutant R132H, ATRX kept, p53 20%	-	Surgery, chemotherapy (PCV), radiotherapy

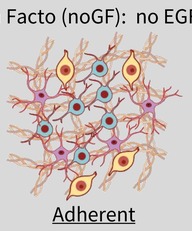
Biobank characterisation and validation as a relevant *in vitro* model

1) Culture conditions

Growth Factors (GF): EGF and FGF2 10µg/mL

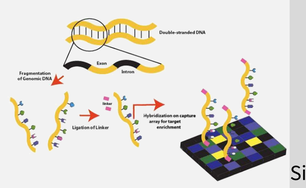


No Growth Facto (noGF): no EGF and no FGF2



2) Mutational profil

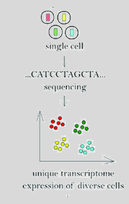
Whole Exome Sequencing



3) Transcriptomics

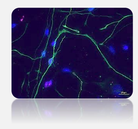
RNA sequencing

Single cell RNA sequencing



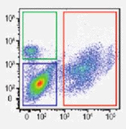
4) Proteomics

Immunofluorescence



Western Blot

Flow cytometry



I - Grade 4 astrocytoma cell lines are less adherent and grow faster than Grade 2 astrocytoma cell lines in adherent conditions

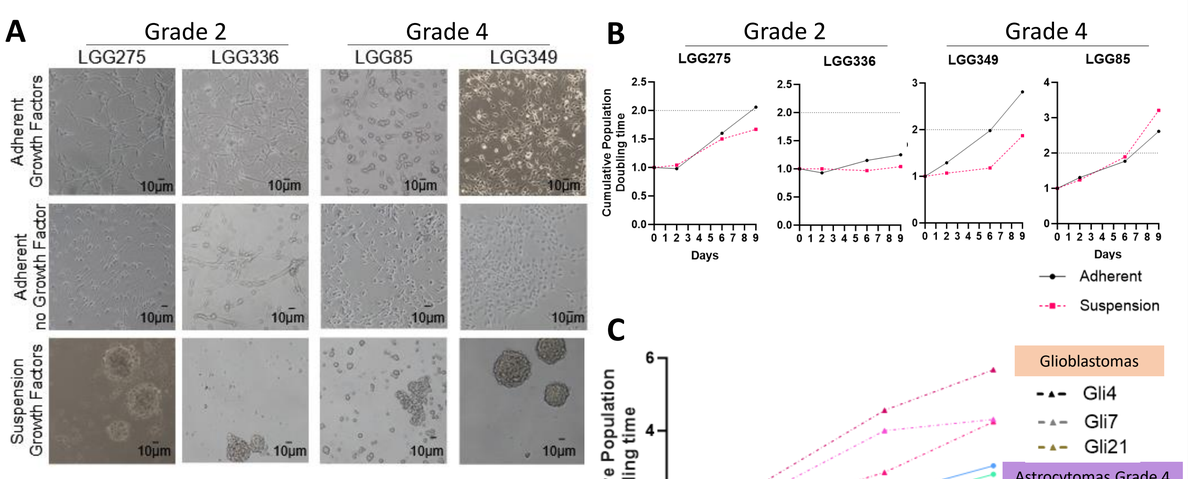
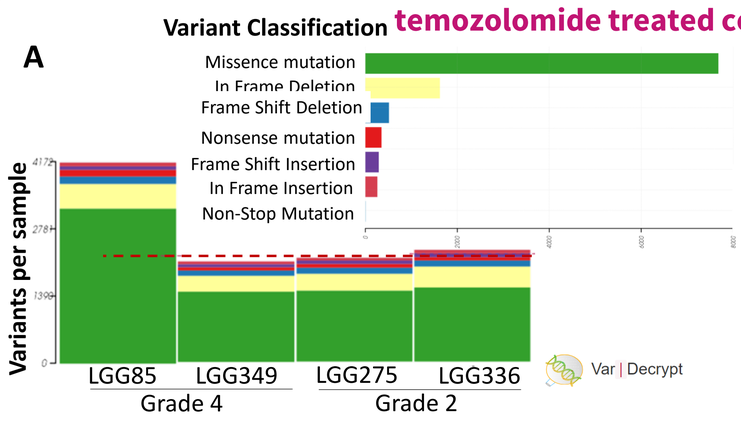


Figure 1: A) Cell morphology in adherent or suspension conditions, in presence or absence of growth factor (EGF, FGF2). B) Population doubling time in adherent or suspension condition. C) Population doubling time of each cell line in adherent conditions

II – Maintenance of IDH1 mutation in cell lines and hypertmutation in one temozolomide treated cell line



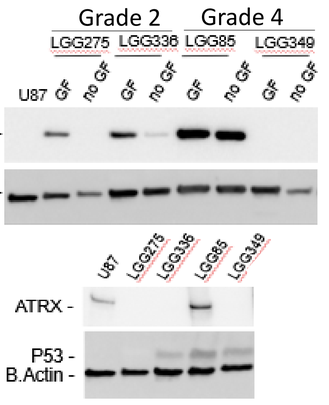
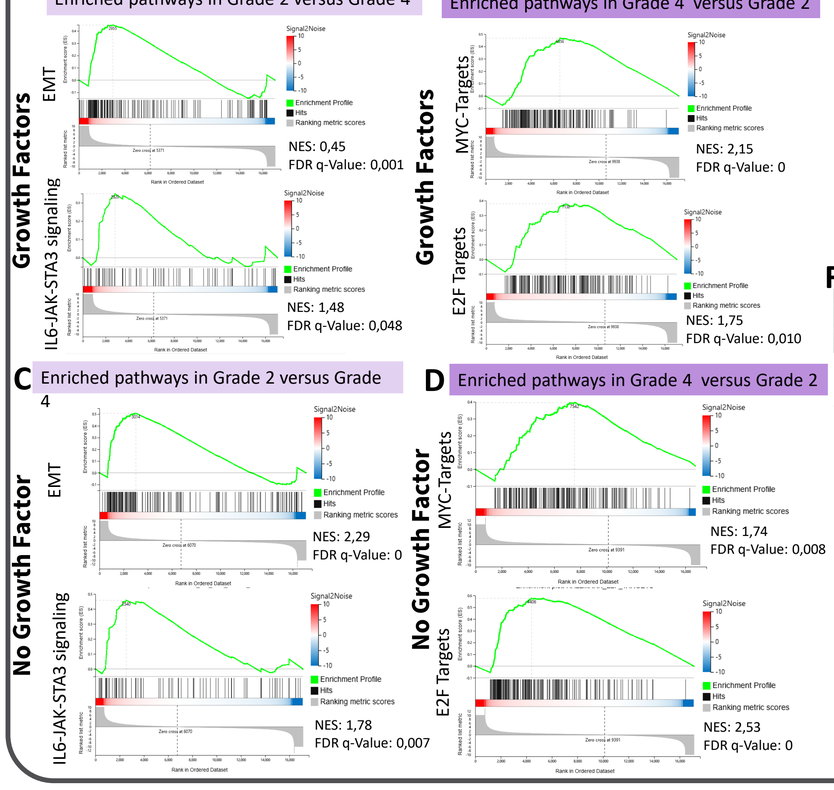


Figure 2: A) Variants number and type in grade 2 and grade 4 astrocytoma cell lines B) Mutation profils of *IDH1, ATRX, TP53* and *CIC* genes in cell lines using Var/Decrypt. C) Expression (WB) of IDH1-mutant, ATRX and P53 by western blot in presence (GF) or absence of growth factors (no GF)

III - EMT and STAT3 pathways are enriched in Grade 2 astrocytomas and could be involved in migration



E Enriched genes in EMT pathway

Gene Symbol	Rank metric score
COL6A3	8,89
MFAP5	7,39
LOXL1	6,12
COL5A3	6,00
ABI3BP	5,37
SPP1	4,88
COL11A1	4,87
ECM2	4,79
BASP1	4,65
ANPEP	4,64

F

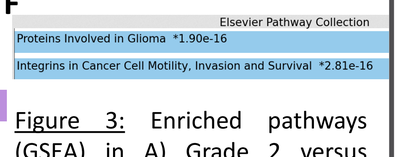
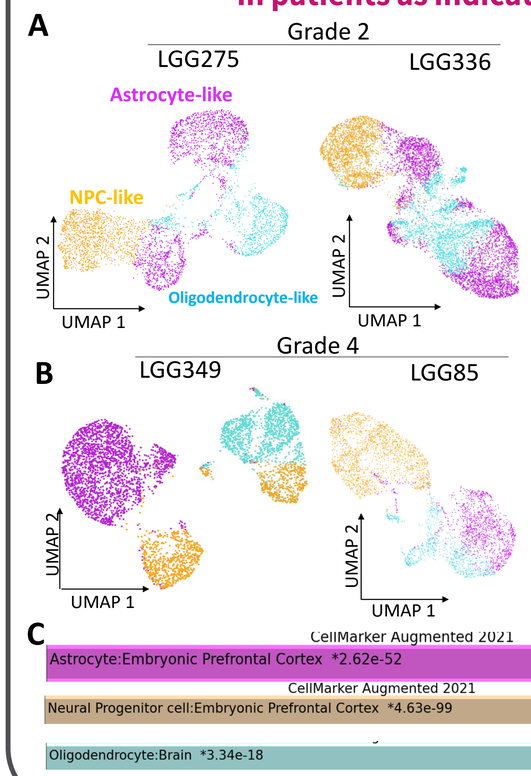


Figure 3: Enriched pathways (GSEA) in A) Grade 2 versus Grade 4 and B) Grade 4 versus Grade 2 astrocytomas in presence of growth factors. Enriched pathways in C) Grade 2 versus Grade and D) Grade 4 versus Grade 2 astrocytomas without growth factor. E) Top 10 most enriched genes in EMT pathways and F) their implication in biologicals process.

IV - Astrocytoma cell lines show cellular heterogeneity similar to the one found in patients as indicated by single-cell RNA sequencing



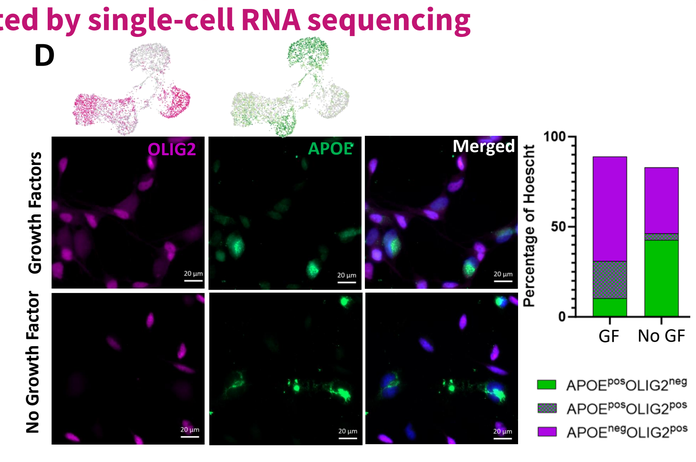


Figure 4: UMAP Of A) Grade 2 and B) Grade 4 astrocytomas showing three clusters of cells. C) Pathways analalysis of TOP 200 most differentially expressed genes in each cluster showing three cellular identities: Astrocyte-like, Oligodendrocyte-like and NPC-like. D) Transcipt and protein co-expression (immunofluorescence) of OLIG2, enriched in the oligodendrocyte cluster and APOE, enriched in the astrocyte cluster in presence or absence of growth factors.

Conclusion

- We constituted a richly-annotated biobank derived from 4 astrocytomas.
- Some of these cell lines maintain mutations found in tumours (IDH1, P53, ATRX)
- Cell lines derived from grade 4 astrocytomas grow faster than grade 2-derived cell lines.
- Single cell RNA seq show that these cell lines contain cell subpopulations (oligodendrocyte-like, astrocytes like and stem cell-like cells) akin to those found in tumors
- These cell lines represent useful models to 1/ understand the formation of cellular heterogeneity in low grade gliomas 2/the molecular mechanisms underlying progression from grade 2 to grade 4, 3/ identify new targets and treatment

References

- Ohgaki H, Kleihues P**. The definition of primary and secondary glioblastoma.** Clin Cancer Res. 2013 Feb 15;19(4):764-72.
- Venteicher AS, Tirosh I, Hebert C, Yizhak K, Neftel C, Filbin MG, Hovestadt V, Escalante LE, Shaw ML, Rodman C, Gillespie SM, Dionne D, Luo CC, Ravichandran H, Mylvaganam R, Mount C, Onozato ML, Nahed BV, Wakimoto H, Curry WT, Iafrate AJ, Rivera MN, Frosch MP, Golub TR, Brastianos PK, Getz G, Patel AP, Monje M, Cahill DP, Rozenblatt-Rosen O, Louis DN, Bernstein BE, Regev A, Suvà ML. **Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq.** Science. 2017 Mar 31;355(6332):eaai8478.
- Augustus M, Pineau D, Aimond F, Azar S, Lecca D, Scamps F, Muxel S, Darlix A, Ritchie W, Gozé C, Rigau V, Duffau H, Hugnot JP. **Identification of CRYAB⁺ KCNN3⁺ SOX9⁺ Astrocyte-Like and EGFR⁺ PDGFRA⁺ OLIG1⁺ Oligodendrocyte-Like Tumoral Cells in Diffuse IDH1-Mutant Gliomas and Implication of NOTCH1 Signalling in Their Genesis.** Cancers (Basel). 2021 Apr 27;13(9):2107.