PhD Title

DECIPHERING METABOLIC INTRATUMORAL HETEROGENEITY IN IDH1 MUTANT GLIOMAS TO PROPOSE NEW THERAPEUTIC AVENUES

Research unit/Location **IRCM - Institut de Recherche en Cancérologie de Montpellier**

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Keywords

Metabolism, Tumoral heterogneity, IDH mutation, Microenvironnement, Epigenetic, Epitranscriptomic, Cancer

Project description

Gliomas are the most common primary brain tumors affecting approximately 4,000 new patients annually in France. While half of all gliomas are diagnosed as advanced glioblastoma (grade IV), 15% of tumors are detected as grade II diffuse low-grade gliomas (dLGG). These dLGGs mainly affect young adults and the overall survival is 5 to 15 years after diagnosis. Despite surgery, their diffuse nature and their presence in sensitive areas imply the presence of residual cells that will inexorably progress to a very aggressive grade III or IV. **It is therefore essential to better characterize dLGGs in order to facilitate the development of new therapies.**

At the cellular level, dLGGs are divided into two major types: astrocytoma and oligodendroglioma. At the molecular level, while astrocytomas and oligodendrogliomas are mutated on isocitrate dehydrogenase (IDH), astrocytomas are characterized by the p53 mutation and ATRX loss-of-function mutations, while oligodendroglioma has a 1p/19q codeletion. It is important to note that despite this classification, dLGGs, astrocytomas and oligodendrogliomas, show significant cellular heterogeneity with the presence of different subpopulations within the same tumor: differentiated nonproliferative astrocytic-like cells, differentiated nonproliferative oligodendrocytic-like cells, and undifferentiated proliferative cells that resemble neural stem/progenitor cells. **To date, the functional characteristics of these subpopulations remain unexplored.**

In addition, IDH is a key enzyme in metabolism and catalyzes the conversion of isocitrate to α-ketoglutarate (α-KG), producing NADPH. When mutated, this enzyme will result in a gain of function with production of 2-hydroxyglutarate (2-HG) from α-KG and NADPH. The IDH mutation, via the production of 2-HG, leads to the inhibition of α-KG-dependent dioxygenases and thus to a hypermethylation of DNA and histones affecting notably cell differentiation. In addition, more and more studies show the major impact of this mutation on the metabolic reprogramming of cancer cells, different according to the tissue of origin. Thus, while the IDH mutation leads to an increase in mitochondrial activity in acute myeloid leukemia (AML), it leads to a decrease in the concentration of Krebs cycle metabolites, ATP production and mitochondrial respiration in gliomas. However, in most of glioma studies, the models used are glioblastoma lines engineered to carry the IDH mutation, not reflecting the cellular and molecular heterogeneity of dLGGs.

The objective of our project is to determine the metabolic specificities of the different subpopulations present in dLGGs to better understand how molecular, cellular and metabolic heterogeneity are orchestrated. As 2-HG leads to important epigenetic and epitranscriptomic

consequences, we will also focus on the link between the observed metabolic dysregulations and the epigenome and epitranscriptome. To answer these questions, the student will have access to tumors from patients with dLLG extensively characterized in clinic as well as unique patient-derived dLLG cell lines, some of which will be xenografted into mouse models. The student will use mass cytometry techniques (CyTOF) to measure at the single cell level the expression of about 40 proteins in a single sample. These proteins will include phenotypic markers characteristic of the different dLLG subpopulations but also the expression of proteins associated with different metabolic pathways (Krebs cycle, glycolysis, pentose pathway, fatty acid oxidation, amino acid utilization), signaling pathways and epigenetic markers to determine the specificities of these different subpopulations. The spatial module (Hyperion) will also allow mass cytometry imaging to determine the expression of these different proteins on tumor sections and identify areas characterized by the expression of specific metabolic or epigenetic markers. It will also be possible to determine if these tumor areas are closer to some neighboring healthy cells expressing specifically some of these markers, which could highlight metabolic dialogues between dLLG cells and those of the microenvironment (immune and stromal cells in particular). Metabolomics and Chip-seq analyses will confirm the observed metabolic and epigenetic specificities respectively, while mass spectrometry analyses of RNA modifications will determine the associated epitranscriptomic changes.

Thematic

Glioma metabolism and intratumoral heterogeneity

Objectives

The objectives of this project are:

- 1/ To determine the metabolic specificities of the three subpopulations of malignant cells present in dLLG as nonproliferating cells differentiated along the astrocytic and oligodendrocytic lineages, and proliferative undifferentiated cells that resemble neural stem/progenitor cells using tissues from patients' samples collected at resection.

- 2/ To evaluate the importance of epigenetic/epitranscriptomic changes related to the metabolic specificities in each of these subpopulations.

- 3/ To identify the metabolic interplays between these subpopulations and their ecosystem.

- 4/ To evaluate if targeting either the metabolic characteristics, epigenetic/epitranscriptomic changes or specific dialogue with the ecosystem can efficiently target these subpopulations *in vivo* in murine models.

Context

Metabolic flexibility, intra and intertumoral heterogeneity are key factors in resistance to treatments in cancer, including gliomas. Therefore, a better understanding of the molecular mechanisms regulating metabolic flexibility and a more detailed description of the different subpopulations and their respective niches are of major importance.

While most studies of cancer metabolism rely on metabolic features measured in bulk cell populations, overlooking cellular heterogeneity, this project will apply cutting-edge technologies to query the metabolic states of dLLG subpopulations. This innovative strategy should improve our understanding of the distinct adaptive cellular responses that we believe is influenced by the organization of their microenvironment. This study will also decipher the poorly understood links between 2-HG, the epigenome/epitranscriptome and their impact on metabolic plasticity. Identifying these metabolic dependencies and regulators will help design new combination therapies for patients diagnosed with dLLG.

Method

As detailed in the project description, the PhD student will utilize complementary approaches, in particular mass cytometry and imaging mass cytometry, both available at IRCM and for which metabolic, signaling

and epigenetic panels have been developed by Dr Laurent LeCam team. The PhD student will complement these panels with markers specific to the different dLLG subpopulations already described by the team of Pr Jean-Philippe Hugnot (co-director of this PhD project). Metabolomic and isotopic profiling experiments will also be performed to confirm the metabolic specificities and dialogue with the microenvironment. MS-based detection of histone/RNA modifications and ChipSeq will help determine the interplays between metabolic plasticity and epigenetic/epitranscriptomic in dLLG. The student will have unlimited access to several core facilities at the IRCM, Montpellier Biocampus and external collaborators. Validation of some of the findings will be performed *in vivo* using mice models xenografted with primary samples or cell lines derived from dLGG patients.

Expected results

Our project aims at characterizing the metabolic intratumoral heterogeneity in dLLG, in particular to determine the metabolic specificities of the different subpopulations and the metabolic dialogue with their niche. Moreover, the project will address how metabolic rewiring in dLLG contribute to histone/RNAs modifications that modulate gene expression. This project should improve our understanding of the complex interplay between metabolism, the epigenome and the epitranscriptome and the dialogue with the niche and provides potential targets for patients diagnosed with IDH mutant gliomas.

Bibliographic citations

From the labs/researchers and important for the project:

Stuani L*., Sabatier M., Saland E., Cognet G., [*et al*.], Linares LK., Récher C., Portais J-C., Sarry J-E*. ***Mitochondrial metabolism supports resistance to IDH mutant inhibitors in acute myeloid leukemia.*** *J. Exp. Med.* 2021 May;218(5):e20200924. doi: 10.1084/jem.20200924.

Relier S., Amalric A., Attina A., [*et al*.], Hugnot J.P., Duffau H., Bauchet L, Hirtz C.*, Rivals E.*, David A*. ***Multivariate Analysis of RNA Chemistry Marks Uncovers Epitranscriptomics-Based Biomarker Signature for Adult Diffuse Glioma Diagnostics.*** *Anal. Chem.* 2022, 94, 35, 11967–11972. doi: 10.1021/acs.analchem.2c01526.

Augustus M., Pineau D., Aimond F., Azar S., [*et al*.], Gozé C., Rigau V., Duffau H., Hugnot J.P. **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*. 2021 Apr 27;13(9):2107. doi: 10.3390/cancers13092107.

Azar S., Leventoux N., Ripoll C., Rigau V., [*et al*.], Duffau H., Guichet P.O., Rothhut B., Hugnot J.P. **Cellular and molecular characterization of IDH1-mutated diffuse low grade gliomas reveals tumor heterogeneity and absence of EGFR/PDGFRα activation.** *Glia*. 2018 Feb;66(2):239-255. doi: 10.1002/glia.23240.

From the literature - important for the project:

Karimi E., Yu M.W., Maritan S.M., Perus L.J., [*et al*.], Guiot M.C., Siegel P.M., Quail D.F., Walsh L.A. **Single-cell spatial immune landscapes of primary and metastatic brain tumours**. *Nature*. 2023 Feb;614(7948):555-563. doi: 10.1038/s41586-022-05680-3.

Ravi V.M., Will P., Kueckelhaus J., Sun N., [*et al*.], Walch A.K., Delev D., Schnell O., Heiland D.H. **Spatially resolved multi-omics deciphers bidirectional tumor-host interdependence in glioblastoma**. *Cancer Cell*. 2022 Jun 13;40(6):639-655.e13. doi: 10.1016/j.ccell.2022.05.009.

Venteicher A. S., Tirosh I., Hebert C., Yizhak K., [*et al*.], Louis D.N, Bernstein B.E., Regev A., Suvà M.L. **Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq.** *Science*. 2017 Mar 31;355(6332):eaai8478. doi: 10.1126/science.aai8478.

Tirosh I., Venteicher A.S., Hebert C., Escalante L.E., [*et al*.], Bernstein B.E., Louis D.N, Regev A., Suvà M.L. **Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma.** *Nature*. 2016 Nov 10;539(7628):309-313. doi: 10.1038/nature20123.

Details on the thesis supervision

The student will be mostly based at IRCM in Dr Laurent Le Cam team, where she/he will be mentored by Dr Lucille Stuani, a senior scientist with expertise in metabolism and IDH mutation (CPJ INSERM) and also trained by experienced engineers in the team with pluridisciplinary skills. The thesis will be co-directed by Pr Jean-Philippe Hugnot, a recognized expert in diffuse low-grade gliomas who co-leads a team at IGF (Montpellier) with Pr Hugues Duffau, director of the Neuro-oncology department at

Montpellier Hospital Gui de Chauliac; therefore, the student will interact regularly with the different members of the team to benefit from their strong expertise in glioma biology and translational research.

Supervision will be done through daily interactions with team members and regular meetings with both thesis supervisor as well as during weekly labmeetings (L. Le Cam team) and monthly labmeetings (J.P. Hugnot/H. Duffau team) during which the student will be asked to present her/his work every 3 months. She/he will also participate to Institute/department meetings at IRCM once a year and as well as student symposium organized both internally at IRCM and by the doctoral school CBS2. Mandatory PhD committees will be organized once a year. Technical training may also involve training sessions organized by core-facilities (histology, mouse training…) affiliated to Montpellier-Biocampus.

Profile and skills required

We are looking for a highly motivated student who will be involved in a new research program of the laboratory aiming at characterizing metabolic heterogeneity in IDH mutated gliomas. He/she will have to show a strong team spirit to integrate a cohesive and enthousiastic team (3 seniors, 3 post-docs, 2 PhD students, M1/M2, 3 engineers...) with transverse competences, as well as develop a close communication with his/her co-director, Pr JP Hugnot and his team at the IGF. The candidate will benefit from the technical and scientific support of the other members of the two teams in a stimulating environment ideal for developing his/her scientific mind. A first experience, or if not, a particular interest in the study of metabolism and/or brain tumors would be appreciated.