IMP2 TARGETING IN NOTCH-HIGH GLIOBLASTOMA STEM CELLS AS A THERAPEUTIC APPROACH FOR GLIOBLASTOMA MULTIFORME

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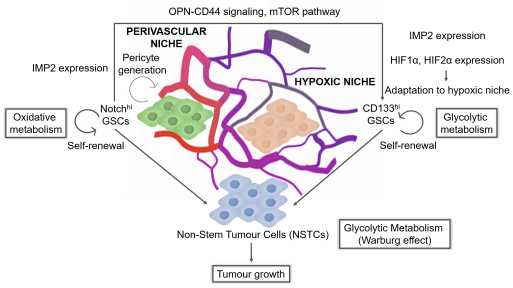
PROJECT BACKGROUND

Glioblastoma multiforme (GBM) is the most prevalent and lethal brain tumour, characterized by a low survival rate and a lack of effective treatment options. As its high intra-tumoural heterogeneity and the presence of glioblastoma stem cells (GSCs) are the cornerstones of therapeutic resistance, novel therapeutic approaches should target the GSC population as a means to avoid tumour relapse, being a potential strategy to attack GSCs metabolism.

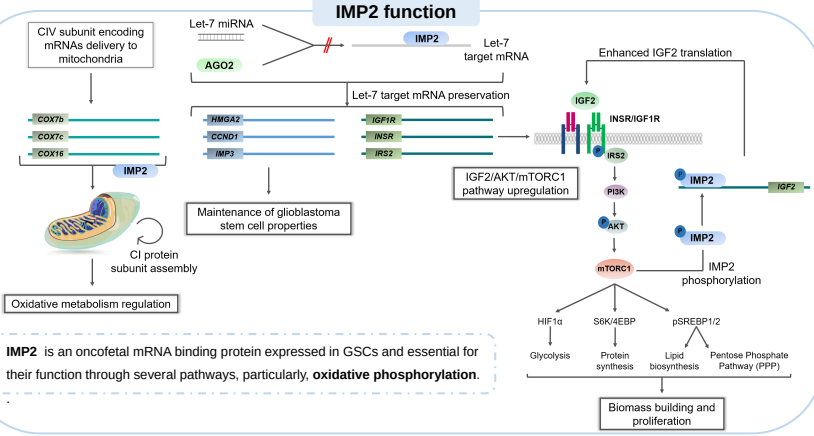
GBM cellular structure



GBM contains different GSCs populations:

- **Notch^{hi} GSCs** rely on oxidative metabolism and show the broadest differentiation potential.
- **CD133^{hi} GSCs** recur to anaerobic glycolysis and present a reduced differentiation potential.

IMP2 function



IMP2 is an oncofetal mRNA binding protein expressed in GSCs and essential for their function through several pathways, particularly, **oxidative phosphorylation**.

HYPOTHESIS: As the Notch^{hi} GSCs present the broadest differentiation potential, we hypothesize that this is the main GBM cell population responsible for tumour relapse. IMP2-targeting could be used to block oxidative metabolism, and consequently, Notch^{hi} GSCs growth, giving rise to a potential adjuvant therapy with ability to improve the clinical outcome.

PROJECT GOALS

To propose an **experimental design** in order to:

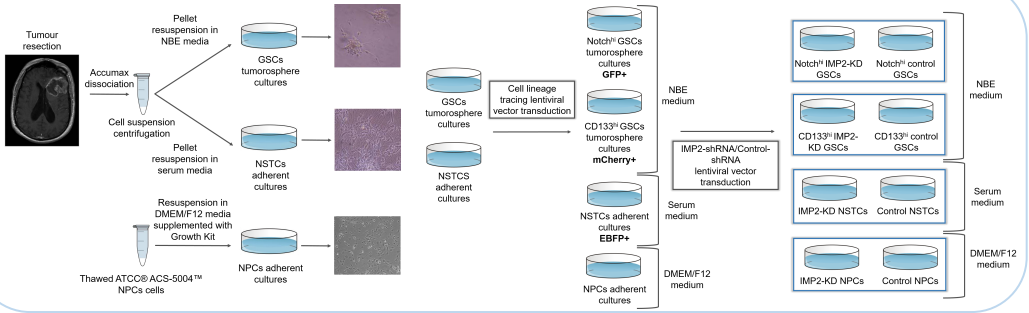
1. Evaluate *in vitro* the effect of IMP2-shRNA knockdown (KD) on the metabolism and survival of the Notch^{hi} GSCs.
2. Elucidate *in vivo* the use of IMP2 KD as a therapeutic approach to avoid tumour relapse.
3. Characterize the impact of IMP2 KD on the CD133^{hi} GSCs, NSTCs and neural progenitor cells (NPCs).

METHODOLOGY

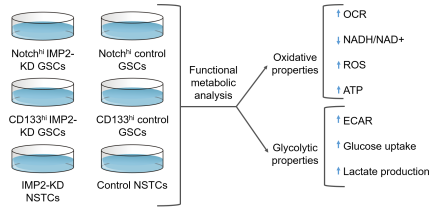
- Bibliographic search in PubMed to identify relevant references.
  - **Keywords**: "Glioblastoma multiforme", "Glioblastoma Stem Cells", "metabolism", "oxidative phosphorylation", "IMP2".
  - **Publication years**: 2004-2018.
- Identification of the materials and methods applicable to the proposed experimental design and protocols reading.

EXPERIMENTAL DESIGN

YEAR 1 – Cell culture establishment

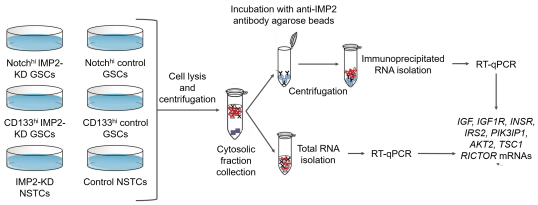


YEAR 2 – *In vitro* functional metabolic analysis



OCR – Oxygen Consumption Rate
ECAR – Extracellular Acidification Rate

YEAR 2 – *In vitro* study of IGF2/AKT/mTOR pathway activation



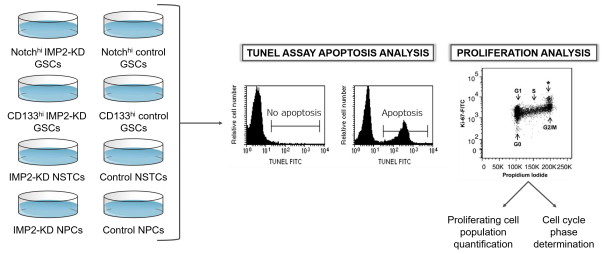
Western Blot

- mTORC1 activation status:
  - P-S6K/S6K
  - P-4EBP/4EBP
  - P-IMP2/IMP2
- Upstream pathway activation:
  - P-INSR/INSR
  - P-IGF1R/IGF1R
  - P-AKT/AKT
  - IGF2

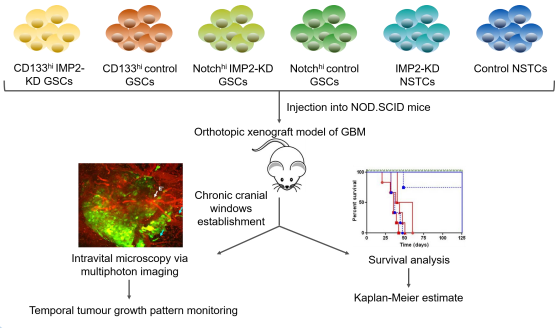
RT-qPCR

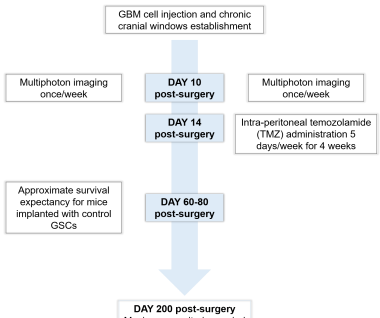
- Downstream metabolic changes:
  - Key glycolysis, PPP and lipid biosynthesis enzymes expression.

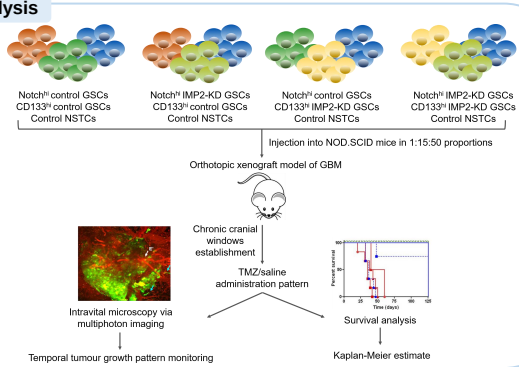
YEAR 2 – *In vitro* apoptosis and proliferation analysis



YEAR 3 – *In vivo* tumour growth and survival analysis







EXPECTED RESULTS

- IMP2 downregulation causes a **decrease in cell viability** and proliferation of the Notch^{hi} GSCs *in vitro* due to impaired oxidative phosphorylation.
- IMP2 downregulation undermines Notch^{hi} GSCs ability to recapitulate a GBM parental tumour phenotype when used for xenograft establishment *in vivo* – **extending the survival rate**.
- IMP2 downregulation **improves the treatment outcome** of TMZ chemotherapy.

DISSEMINATION PLAN

The results will be communicated to the academic and private sector through:

- Publication in high impact journals.
- National and international conferences.
- Seminars.

CONCLUSION

- This research project will advance the knowledge of the pathophysiological mechanisms of GBM – and, particularly, the GSCs – to develop new therapy strategies focused on GSCs metabolism to **avoid tumour relapse**.
- The obtained results could give rise to the development of **IMP2-targeting drugs** to be used in combination therapies both with TMZ chemotherapy and drugs targeting other GBM cell populations.

Bayin NS, Frenster JD, Sen R, Si S, Modrek AS, Galifianakis N et al. Notch signaling regulates metabolic heterogeneity in glioblastoma stem cells. Oncotarget 2017; 8(39): 64932-64953. Janiszewska M, Suvà ML, Riggi N, Houtkooper RH, Auwerx J, Clément-Schatlo V et al. Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. Genes Dev 2012; 26(17): 1926-1944. Vlashi E, Lagadec C, Vergnes L, Matsutani T, Masui K, Poulou M et al. Metabolic state of glioma stem cells and nontumorigenic cells. Proc Natl Acad Sci U S A 2011; 108(38): 16062-16067.