DOCTORAT BIOLOGIE BRETAGNE SANTE LOIRE

Year 2021/2022

# THESIS TOPIC

| **Subject N° (to be completed by the ED):** | **FUNDING:** ☒ **Requested** ☐ **Acquired** | **Funding origin:** |
|---|---|---|
| Thesis title:<br>**Characterization of genetic fusions in glioblastoma and study of their therapeutic targeting by RNAi.** | | 3 keywords:<br>Glioblastoma ; Fusion genes targeting ; RNAi |
| Unit / team: **CRCINA, INSERM UMR 1232, Team 17 GLIAD** | | |
| Supervisor's names:<br>**Emmanuel GARCION / Audrey ROUSSEAU (Supervisor / Co-supevisor)** | | Phone number: 02 44 68 85 43 (EG)<br>02 41 35 52 73 (AR)<br>Email address: emmanuel.garcion@univ-angers.fr et audreygrousseau@gmail.com |

Socio-economic and scientific context (approximately 10 lines):

Glioblastomas (GB) are singularly aggressive primary tumors of the central nervous system (CNS). With an increasing incidence, reaching up to 7 new cases per year per 100,000 inhabitants, their usual therapeutic approach did not changed a lot in thirty years and remains palliative. Conventional treatment consists of surgical excision when possible followed by external radiotherapy combined with Temozolomide. Despite improvements in neurosurgery and molecular typing techniques, the prognosis for GB remains very poor with a median survival of 15 months. Illustrating their genomic instability, various major chromosomal rearrangements can lead in GB to the juxtaposition of coding sequences of two genes resulting in the formation of fusion genes potentially oncogenic and contributing to resistance to treatment as well as allowing recurrence. The identification of these fusions and the validation of the gene products (mRNA, fusion protein) as targets (intrinsic or capable of controlling the tumor ecosystem) open up new areas of research and therapeutic innovation. In this context, the use of RNA interference (RNAi) associated with the development of new locoregional technologies to control the disease (while avoiding the problem of the blood-brain barrier and reducing systemic toxicity), represents a technological challenge. and offers possibilities for the application of precision medicine with potentially significant socio-economic impact.

Working hypothesis and aims (approximately 8 lines):
While certain chromosomal rearrangements in GB can lead to the loss of tumor suppressor genes and the amplification of oncogenes, then the juxtaposition of coding sequences of two genes can also lead to the formation of chimeric genes with oncogenic potential. The identification of the FGFR3-TACC3 fusion has made it possible in the GB to develop therapies against the constitutively active domain of tyrosine kinase (European phase II clinical trial TARGET, AstraZeneca, Jansen). Studies carried out within our team in conjunction with the Department of Pathology of Angers University Hospital have very recently made it possible to select new candidate fusion genes through a prospective analysis of detection by SNP array carried out on 205 patients. The refinement of the analysis of the results by Sanger sequencing and RTqPCR allowed us to start this project by choosing relevant original fusion targets (eg. EGFR-SEPT14,…) and by developing cell models suitable for understanding the role of these. We are also developing new tools to improve their targeting. As such an innovative protein vectorization of RNAi for locoregional application was also implemented. The objective of this thesis work will be to finalize the "Molecular and functional characterization of these new genetic fusions in GB and the study of their therapeutic targeting through protein vectorization of RNAi".

Main milestones of the thesis (approximately 12 lines):
1) Functional constitutive role and gain of function resulting from the expression of proteins of interest within the tumor cell and healthy cells. GB cells and astrocytes have been modified to express the fusions of interest, their functionality (proliferation, adhesion, migration, radiation resistance) will be studied by standard biochemistry, cell biology and molecular techniques. The cellular bio-distribution of fusion proteins and the modified / amplified signals they cause will be particularly investigated.
2) In vitro impact of exogenous modulation on the expression of these fusion proteins. The modulation of these fusions by siRNAs designed to specifically target them will be tested through commercially available systems (RNAiMax, NTER) and siRNA / Argonaute-2 (AGO2) nanoparticles currently being developed in the laboratory. The link with existing chemical molecules can be made.
3) In vivo impact through a locoregional implant with prolonged release of RNAi targeting these fusions. One or two models will be developed in vivo for therapeutic investigations at the preclinical level using the identified genetic fusions and for their targeting through the sustained release system of siRNA characterized to be the most relevant. The gain of function at the level of healthy astrocytes will also be tested for tumorigenicity with regard to the number of cells injected (link with 1).

Scientific and technical skills required by the candidate (2 lines):
Molecular and cell biology, Bio-engineering, *In vitro* and *in vivo* experimental models, Team work necessary !
Motivation, Autonomy, Oral and editorial quality.

3 publications de l'équipe d'accueil relatives au domaine (5 dernières années) :

1. Ah-Pine F, Casas D, Menei P, Boisselier B, Garcion E, Rousseau A. RNA-sequencing of IDH-wild-type glioblastoma with chromothripsis identifies novel gene fusions with potential oncogenic properties. Translational Oncology, 4 (2021) 100884. (IF: 3.558)
2. Anthiya S., Griveau A., Loussouarn C., Baril P., Garnett M., Issartel JP., Garcion E.. MicroRNA-based drugs for brain tumors. *Trends in Cancer*, 4 (2) (2018) 37-53. (IF: 8.884)
3. Séhédic D., Chourpa I., Tétaud C., Griveau A., Loussouarn C., Avril S., Legendre C., Lepareur N., Wion D., Hindré F., Davodeau F., Garcion E. Locoregional confinement and major clinical benefit of $^{188}$Re-loaded CXCR4-targeted nanocarriers in an orthotopic human to mouse model of glioblastoma. *Theranostics*, 7 (18) (2017) 74517-4536. (IF: 8.537)


Collaborations nationales et internationales : Patrick Baril, CBM, Orléans ; Moreno GALLENI, CIP, Liège ; Benjamin Lemasson, GIN, Grenoble ; Consortium GLIOSILK Euronanomed III ; LABEX IRON ; CGO.