### Thesis Title
Biomarkers and new therapeutic approaches targeting the metabolism of cancer stem cells in hepatocellular carcinoma

### Disciplinary Fields
Autres et Autres

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#### Thesis Title
Biomarkers and new therapeutic approaches targeting the metabolism of cancer stem cells in hepatocellular carcinoma

#### 3 keywords
hepatocellular carcinoma / cancer stem cells / metabolic reprogramming

#### Unit/Team of supervising
Nutrition, Métabolismes et Cancer (NuMeCan). UMR 1241, Rennes / Team EXPRES

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### Socio-economic and scientific context
Hepatocellular carcinoma (HCC) is one of the most frequent cancers in adults (500,000 cases per year worldwide, including 5,000 in France). The identity of the cells causing HCC is controversial; however, the existence of stem/progenitor cells (SCC) in some mixed tumors (hepatocarcinoma/cholangiocarcinoma) has been reported. These tumors have a very aggressive phenotype and are associated with a poor prognosis. It has been suggested that CHCs escape conventional therapies and are responsible for the recurrence of HCCs. Improved biological knowledge of CHCs is essential to identify biomarkers that can be used to target CHCs. Preclinical validation of candidate molecules on HCC cell lines and on organoids obtained from patient samples is a key step in developing CSC-targeting therapies and complementary to validated chemotherapies.

#### Assumptions and questions
The ability of tumor hepatocytes to retrodifferentiate into CSCs in an inflammatory environment contributes to HCC cellular heterogeneity and therapeutic escape, as conventional chemotherapies preferentially target proliferating cells. The metabolism of SCCs, which is different from that of differentiated tumor cells, may provide an opportunity to target and destroy these cells. Our work on different hepatoma lines shows that hepatic SCCs have a reduced energy metabolism, corresponding to a state of metabolic dormancy. Our hypothesis is that the reactivation of oxidative phosphorylation could be deleterious for these cells due to the production of reactive oxygen species that the cell could not detoxify and which induce apoptosis. The proposed work aims to test the possibility of resensitizing CSCs to conventional chemotherapies by combining them with drugs that promote tricarboxylic acid (TCA) cycling and oxidative phosphorylation in CSCs.

#### The main steps of the thesis and demarche
1. 1st year: The main objective will be the characterization of ANGPTL4/PDK4/PPARγ cells present in hepatocellular carcinomas. Our results indicate that tumor cells can retrodifferentiate under the influence of inflammatory signals. This process is accompanied by metabolic reprogramming characterized in particular by overexpression of ANGPTL4 and PDK4 genes. The first year of the thesis will aim to better characterize ANGPTL4/PDK4/PPARγ cells in patient samples, particularly in fibrous nests, by evaluating the co-expression of stem cell markers and the colocalization of immune cells (multiplex immunohistochemistry), and by performing RNA sequencing of these cells.

2. 2nd year: This 2nd stage of the thesis will consist in evaluating on hepatoma lines (HepaRG, BC2, Huh7, HepG2) the anti-tumor efficacy of combination associating conventional chemotherapies and inhibitors of metabolic regulators that we assume play a major role in metabolic reprogramming, especially PDK4 and PPARγ. Through a collaboration with a Spanish team, we will study the interplay between Insulin receptor substrate 2 (IRS2) and epithelial-mesenchymal transition.

3. 3rd year: the last part of the thesis will focus on validating these results on a model of hepatic organoids. After the validation phase of the protocols which make it possible to obtain organoids from liver stem cells, we will characterize these organoids by multiplex IHC and we will evaluate the effect of the drug candidates selected in step 2.

#### Methodological and technical approaches considered
- Cell culture: implementation of the protocol for obtaining organoids from hepatic cancer stem cells (patient samples will be provided by the CRB Santé of the Rennes University Hospital).
- Multiplex immunocytochemistry: detection of six biomarkers on a single tissue slide - High Precision Histopathology platform - SFR UMS CNRS 3480 - INSERM 018
- RNA sequencing - Environmental and Human Genomics platform in Rennes (https://geh.univ-rennes1.fr/)
- Immunolabelling coupled with image analysis to study the functionality of mitochondria - ImPACCell platform
- Gas chromatography coupled with mass spectrometry (GS-MS)

#### Scientific and technical skills required by the candidate
The candidate must have validated graduate training in the field of cell and molecular biology. He/she must have a technical background in cell culture, immunohistochemistry and data analysis (RNA sequencing).