Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: **Portal mesenchymal cells in liver fibrosis**

Keywords: Mesenchymal stem cells; Fluorescence-activated cell sorting (FACS); Cell culture; Single-cell RNA sequencing (ScRNAseq); Genetically-engineered animal models; Mouse models of liver injury.

Joint supervision: yes (Axelle Cadoret, PhD and Sara Lemoinne, MD, PhD)

Joint PhD (cotutelle): no

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Institution: Sorbonne Université

Doctoral school (N°+name): ED 394 Physiologie, Physiopathologie et Thérapeutique

Research laboratory: Centre de Recherche Saint-Antoine (CRSA)

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Subject description (2 pages max):

1) Study context

Fibrotic diseases account for up to 45% of deaths worldwide. Liver fibrosis directly causes ~1,500,000 deaths per year worldwide, while the severity of all chronic liver diseases, including the emergence of primary liver cancer, is closely linked to the development of fibrosis. As a consequence of organ injury, mesenchymal cells differentiate into myofibroblasts, the key effectors of fibrosis in all organs. In the liver, fibrosis expands from the portal tract along with a collateral vascular plexus to form cirrhosis, the stage at which lethal complications occur. While the paradigm of hepatic stellate cell (HSC) phenotypic change into myofibroblasts has dominated the focus of research on liver fibrosis over the past 30 years, our group identified a sub-

population of liver myofibroblasts that derive from portal cells, distinct from HSCs, and that we referred to as portal myofibroblasts (PMFs) (1, 2). PMFs are highly proliferative, as opposed to HSCs, and they trigger angiogenesis by producing vascular endothelial growth factor A (VEGFA)-containing microparticles and acting as mural cells for newly formed vessels (1, 2). They express type 15 collagen alpha 1 (COL15A1), a multiplexin that provides stability and resilience to mechanical forces in skeletal/heart muscle and microvessels, and that anchors basement membranes to interstitial collagen. Our working hypothesis is that PMFs derive from a small population of portal perivascular mesenchymal cells with stem cell properties, and promote angiogenesis and fibrosis progression by forming a scaffold for the deposition of interstitial collagen, to a large extent produced by HSC-derived myofibroblasts (HSC-MFs). Thereby PMFs would drive scar progression from portal tracts into the parenchyma, and cause the formation of cirrhosis.

Mesenchymal stem cells (MSCs) were first isolated from the bone marrow on the basis of their self-renewal capacity and their ability to differentiate down multiple lineages (3). Cells with similar properties as bone marrow MSCs have been found in multiple organs with an enrichment in perivascular locations, and have also been referred to as MSCs (4). Among potential markers of these cells, Gli1, a transcription factor of the Hedgehog pathway, marks a network of perivascular mesenchymal stem cells that give rise to myofibroblasts across different tissues including the liver (5). In the liver, $Gli1^+$ cells are located in portal tracts (5, 6), with the same distribution as $COL15A1^+$ cells, around the vasculature and bile ducts (1). In collaboration with Thierry Jaffredo at Sorbonne Université, we have performed single-cell RNA sequencing (scRNAseq) analyses to define the atlas of portal mesenchymal cells in normal mouse liver. These analyses have revealed several clusters of portal mesenchymal cells, including $Col15a1^+$ cells. Trajectory analysis enabled inferring a small cell population further defined by a minimal set of surface markers used to isolate it by FACS. This population consists of portal mesenchymal stem cells (PMSCs) according to their mesenchymal stem cell attributes, *i.e.*, a high clonogenic potential and the ability to undergo trilineage differentiation (chondrogenic, osteogenic and adipogenic), able to generate $Col15a1^+$ myofibroblasts in culture (Lei *et al*., Manuscript to be submitted).

2) Details of the proposal

The aim of the project is to uncover the reprograming of portal mesenchymal cells, notably PMSCs, in liver disease and determine their contribution to liver fibrosis.

To investigate the reprograming of portal mesenchymal cells, notably PMSCs, in liver disease and decode the cellular and molecular basis of fibrosis progression from the portal tract in liver diseases, the PhD student will perform bulk RNAseq and scRNAseq analyses of the bilio-vascular trees, that comprise the portal mesenchyme, from fibrotic livers (murine and human) in comparison with normal liver. We have access to different mouse models of liver fibrosis including of biliary type and of non-alcoholic steatohepatitis (NASH). *Via* the Human HepCell platform (https://www.ican-institute.org/ican-human-hep-cell/), we also have access to human liver tissue samples from patients with biliary diseases or NASH, at different stages of liver fibrosis, *i.e.*, F1-F2 or F3-F4, according to the METAVIR scoring system. Expanding on our preliminary results and publicly available single cell dataset of human liver, we will define *i)* the sub-populations of liver myofibroblasts in mouse and human fibrotic liver, still poorly defined in scRNAseq studies so far; *ii)* key ligand-receptor paracrine interactions between portal mesenchymal cells and derived myofibroblasts with other liver cell types in liver angiogenesis and fibrosis. To refine the identification of the portal mesenchymal cells and their fates in liver fibrosis, normal and pathological single cell mouse and/or human transcriptomes will be used in collaboration with Thierry Jaffredo, who will apply single cell trajectory inference methods and thereby order the cells along the normal-to-pathological pathway. By using Weighted Gene Correlation Network Analysis (WGCNA) (7), optimally suited to identify correlated networks, we will identify gene-regulatory modules, reconstruct gene networks and identify hubs operating in the different cell types in normal and pathological situations.

To determine the contribution of PMSCs to liver fibrosis *in vivo*, the PhD student will perform PMSC genetic lineage tracing and cell depletion experiments in mouse models of liver fibrosis. The preliminary results of our mouse RNAseq analyses indicate that Gli1, is expressed in portal mesenchymal cells, especially PMSCs, with the exception of all other liver cell types. Gli1 expression is fully suppressed during the myofibroblastic differentiation of PMSCs, but the genetic cell-fate tracing of $Gli1^+$ cells has been possible by developing a Gli1-$CreER^{t2}$;tdTomato mouse model, which confirmed that Gli1 demarcates a non-HSC population of fibrogenic cells in the liver (5, 6). We undertook the breeding of Gli1-$CreER^{t2}$;tdTomato mice for cell fate tracing, and of Gli1-$CreER^{t2}$;iDTR and tri-genic Gli1-$CreER^{t2}$;tdTomato;iDTR mice for cell depletion. In cell fate tracing experiments, the combination of Tomato staining with multiplex labeling of PMSC markers identified by RNAseq analyses, using immunolabeling or *in situ* hybridization, will allow us to address the heterogeneity of PMSCs in normal liver and of the population arising from these cells in fibrotic liver. Ultimately, *in vivo* genetic ablation of $Gli1^+$ cells using tri-genic Gli1-$CreER^{t2}$;tdTomato;iDTR mice and the different models of liver fibrosis, will allow us to precisely determine the contribution of $Gli1^+$ cells in liver fibrosis and other aspects of liver tissue repair including angiogenesis and liver regeneration.

Overall, the implication of different mesenchymal cells in liver fibrosis remains largely unknown. Upon completion of this project, we expect to demonstrate that PMSCs are a small population of myofibroblast precursors that largely expand with the progression of liver fibrosis. Targeting new antifibrotic strategies to both a small and critical mesenchymal sub-population in the liver, is a major translational and therapeutic perspective.

3) References

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6. Gupta V, Gupta I, Park J, Bram Y, Schwartz RE. Hedgehog Signaling Demarcates a Niche of Fibrogenic Peribiliary Mesenchymal Cells. Gastroenterology. 2020.
7. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559.

4) Profile of the Applicant (skills/diploma…)

Diploma: Master Degree in Biology and/or Bioinformatics

Skills: Laboratory experience in bioinformatics analysis, molecular and cell biology (Cell culture, RT-qPCR, Western blot, Tissue imaging), and animal experiments (Mouse genotyping, gavage, injection).

Basic knowledge of English (writing and speaking)

Training in Medical or Veterinary schools is welcome.

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