# Sorbonne Université/China Scholarship Council program 2021

## Thesis proposal

Title of the research project: **MITOCHONDRIAL ACTIVITY IN HEPATIC FIBROGENESIS**

Keywords: **mitochondria, metabolism, hepatic stellate cells, liver, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steato-hepatitis (NASH), fibrosis.**

Joint supervision: **yes**

Joint PhD (cotutelle): **no**

Thesis supervisor: **Dr Fabienne FOUFELLE**

Email address of the thesis supervisor: **fabienne.foufelle@crc.jussieu.fr**

Institution: **Centre de Recherche des Cordeliers (Inserm, Sorbonne Université, Université de Paris)**

Doctoral school (N°+name): **ED 394 Physiology, Pathophysiology and Therapeutics**

Research laboratory: **Metabolic diseases, diabetes and comorbidities**

http://www.crc.jussieu.fr/fabienne_foufelle2019.html

Address of the laboratory: **Centre de Recherche des Cordeliers, 15 rue de l'Ecole de Médecine, 75006 Paris**

Name of the laboratory director: **Dr Fabienne FOUFELLE**

Email address of the laboratory director: fabienne.foufelle@crc.jussieu.fr

## Subject description (2 pages max):

### 1) Study context

The non-alcoholic fatty liver disease (NAFLD) is rapidly expanding worldwide as its incidence is correlated with the obesity pandemic. Today, NAFLD prevalence is about 20 to 30% in the general population and reaches about 80% among obese people. NAFLD encompasses a spectrum of pathologies ranging from simple steatosis characterized by triglyceride (TG) accumulation in hepatocytes, to non-alcoholic steatohepatitis (NASH) that can progress in some cases to fibrosis, cirrhosis and hepato-cellular carcinoma (HCC). NAFLD is also associated with extra-hepatic complications such as cardiovascular diseases that are the leading cause of mortality in NASH and fibrotic patients (1). In addition to TG accumulation in hepatocytes, NASH is also characterized by hepatic inflammation and fibrogenesis. The later corresponds to extracellular matrix (ECM)

production by myofibroblasts, originated mainly from hepatic stellate cells (HSC) (2). In a healthy liver, quiescent HSC are characterized by the presence of lipid droplets that contain TG and retinyl esters (vitamin A) (3, 4). In response to liver injury, HSC activate and undergo trans-differentiation into myofibroblasts (5), capable of proliferating and migrating within the liver. This trans-differentiation is characterized by the loss of the lipid droplets and the synthesis of ECM components such as type I and III collagen fibers (3, 6). Repetitive injuries to the liver, such as those that occur during prolonged steatosis, engage chronic and excessive ECM production by myofibroblasts, leading to the development of liver fibrosis, cirrhosis and, ultimately HCC. The onset of a sustained fibrogenic response, such as appearing in early NASH, is recognized as a tipping point during the course of NAFLD progression and, although HSC trans-differentiation constitutes the cornerstone of the fibrogenic response, the underlying cellular and molecular mechanisms have not yet been fully elucidated. This project therefore seeks to define cellular and molecular mechanisms governing HSC trans-differentiation and subsequent hepatic fibrogenesis.

## 2) Details of the proposal

During the trans-differentiation process, HSC undergo an important metabolic transition, shifting from energy storage to energy production, in order to meet the costly bioenergetic demands of their newly acquired functions, which include proliferation, migration and ECM production. Given the emblematic lipid droplet loss undergone by HSC upon activation, it is not surprising that the catabolism of lipids has been implicated in the trans-differentiation process of these cells (7, 8). On the contrary, the maintenance of the lipid droplet has been shown to promote persistence in the quiescent state (9). However, the lipid catabolic pathway, initiated at the lipophagy/lipolysis level, will drive energy production provided that it is effectively coupled to an increased mitochondrial fatty acid oxidation and subsequent oxidative phosphorylation, both terms together being referred to as mitochondrial activity. Given the changes of cellular lipid content observed upon HSC trans-differentiation and due to mounting evidence of the involvement of mitochondrial function in cellular stemness and differentiation in the literature (10) **we hypothesize that the mitochondrial activity plays a key role in HSC trans-differentiation by sustaining the metabolic transition encountered by the cells.**

Based on the hypotheses formulated above, the main goals of this PhD project will be the followings:

**2.1. To assess the changes in mitochondrial activity that occur during trans-differentiation of quiescent HSC into myofibroblasts.**
To this end, mitochondrial content and activity in different independent models of trans-differentiation will be carefully assessed. In order to obtain HSC in the quiescent and myofibroblastic stages, HSC will be isolated from the liver of mice after they have been subjected to fibrogenic or control regimen. In addition, in order to follow HSC at different stages of their trans-differentiation, primary HSC from healthy mice will be isolated and their activation kinetics will be modulated and monitored *ex vivo*, for instance following treatments by pro- *versus* anti-fibrogenic compounds. To determine if intrinsic modulation of mitochondrial metabolism occurs during HSCs trans-differentiation, oxygen consumption, mitochondrial potential, content and morphology will be measured on the aforementioned models, in parallel with the expression levels of fibrogenic markers.**2.2. To study the impact of the modulation of HSC mitochondrial activity on NAFLD progression *in vivo***

We have at our disposal several rodent models with HSC-specific invalidation of genes (système loxP-Cre) that have been identified as key players for mitochondrial activity, either by influencing mitochondrial biogenesis, morphology or intrinsic activity. Depending on the results obtained in the first work package, *i.e.* after having defined which facet of mitochondrial activity is the most altered during HSC trans-differentiation, we will select the most relevant of these models. Primary HSC will be isolated from this model and the trans-differentiation kinetics of the knockout (KO) *versus* control HSC will be compared in culture and correlated to their mitochondrial content, morphology and activity. HSC-specific KO mice for the gene of interest and their control littermates will most importantly be subjected to different procedures inducing NASH or hepatic fibrosis. Total livers from these animals will be then analyzed by immunohistochemistry and gene expression profiling, in order to determine whether the progression of the fibrogenic response can be slowed down by the inhibition of some specific mitochondria-related gene expression in HSC.

**2.3. To identify the mitochondria-related genes that regulate HSC trans-differentiation and fibrogenesis progression.**

To gain further insights into the mitochondrial genes required for the metabolic transition and trans-differentiation of HSC, we will perform a siRNA screening in a human HSC line. To this end, we will use a library which contains siRNAs directed against all known and predicted human mitochondrial genes (>1500) in a format compatible with high content screening and fluorescence microscopy. Using this approach, we will individually knockdown known and putative mitochondrial genes in a HSC line and determine the effects on trans-differentiation by assessing the expression of established markers of fibrogenesis. We thereby expect to identify all the mitochondria-related genes whose silencing is able to suppress or accelerate trans-differentiation in human HSCs.

## 3) References


*1. Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. J Hepatol 2019;70:531-544.*

*2. Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, Pradere JP, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun 2013;4:2823.*

*3. Blaner WS, O'Byrne SM, Wongsiriroj N, Kluwe J, D'Ambrosio DM, Jiang H, Schwabe RF, et al. Hepatic stellate cell lipid droplets: a specialized lipid droplet for retinoid storage. Biochim Biophys Acta 2009;1791:467-473.*

*4. Mormone E, George J, Nieto N. Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. Chem Biol Interact 2011;193:225-231.*

*5. Puche JE, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. Compr Physiol 2013;3:1473-1492.*

*6. Mormone E, George J, Nieto N. Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. Chem Biol Interact 2011;193:225-231.*

*7. Taschler U, Schreiber R, Chitraju C, Grabner GF, Romauch M, Wolinski H, Haemmerle G, et al. Adipose triglyceride lipase is involved in the mobilization of triglyceride and retinoid stores of hepatic stellate cells. Biochim Biophys Acta 2015;1851:937-945.*

*8. Yu F, Su L, Ji S, Zhang S, Yu P, Zheng Y, Zhang Q. Inhibition of hepatic stellate cell activation and liver fibrosis by fat-specific protein 27. Mol Cell Biochem 2012;369:35-43.*

*9. Tsuchida T, Friedman SL. Mechanisms of hepatic stellate cell activation. Nat Rev Gastroenterol Hepatol 2017;14:397-411.*

*10. Papa L, Djedaini M, Hoffman R. Mitochondrial Role in Stemness and Differentiation of Hematopoietic Stem Cells. Stem Cells Int 2019;2019:4067162.*

SORBONNE UNIVERSITÉ

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

We are looking for an enthusiastic, dedicated and collaborative PhD student with a Master's degree in Biological Sciences (or equivalent diploma) with at least 6 months of lab experience.

The applicant should have good English oral proficiency and writing skills.

Previous experience in animal physiology and/or primary cell culture, and/or imaging would be an asset.

## Contacts:


### Thesis supervisor

Dr Fabienne FOUFELLE

Email address of the thesis supervisor: fabienne.foufelle@crc.jussieu.fr