Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: **Genetic dissection of Neuropilin1-triggered signaling pathway in Chronic Kindey Disease progression**

Keywords: Neuropilin 1, fibrosis, smooth muscle cells, CKD, vascular permeability, VEGF, Semaphorin.

Joint supervision: YES (name/surname) / Dr Christos Chatziantoniou and Dr Amélie Calmont

Joint PhD (cotutelle): NO

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Institution: INSERM

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

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from Molecular Events to Precision Medicine'
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SORBONNE UNIVERSITÉ

Subject description (2 pages max):

1) Study context

Regardless of the initial insult, human **Chronic Kidney Disease (CKD)** is characterised by progressive destruction of the renal parenchyma and the loss of functional nephrons, the filtering units of a kidney. Renal fibrosis is the common end point of CKD, the hallmark of which is the deposition of pathological matrix by myofibroblasts (1). The worldwide incidence of CKD has reached epidemic proportions and is a major health care burden, with an estimated 10-14% of the adult population known to have CKD (2). These patients display an increased risk of death and cardiovascular morbidity that is proportional to the decline of renal function (3). Moreover, because of its progressive nature, CKD may lead to end-stage renal disease, which requires renal replacement therapy (dialysis and kidney transplantation), substantially altering life quality and expectancy. The only option for reducing the dialysis population or for keeping it stable over the long term is to improve the early detection of CKDs. Stopping the disease from advancing or slow down its progress is an enormous prospect in the field of early detection. Therefore, the discovery of novel **therapeutic targets** of renal fibrosis will very much facilitate the development of preventive therapies.

Neuropilin1 (NRP1) is a membrane-anchored receptor which can non-competitively bind class III Semaphorin proteins and VEGF-A, and trigger corresponding downstream pathways (4). Moreover, NRP1 may act without ligand binding, mainly via regulating signalling pathways. For example, NRP1 can modulate the ligand-receptor binding affinity of PDGF/PDGFRβ and VEGF/VEGFR2 (5). Importantly, NRP1 plays critical deleterious functions in promoting fibrosis in various physiological contexts. Indeed, NRP1 promotes liver fibrosis in mice and humans by enhancing PDGF/TGFβ signalling in hepatic stellate cells (5). In pancreatic ductal adenocarcinoma, characterised by an intense fibrotic reaction responsible in part for its aggressiveness, genetic invalidation of *Nrp1* reduces tumour fibrosis and tumour progression (6). Finally, NRP1 mediates cardiac fibroblast activation and collagen production, contributing to cardiac fibrosis (7).

AIM of the research project: To determine the *in vivo* role of NRP1 in kidney fibrosis progression. We propose to use genetically engineered mice to 1) dissect the tissue-specific functions of NRP1 in CKD progression, 2) investigate the potential mechanisms by which NRP1 could promote CKD progression.

Our project intends to study the disease biology of CKD with the aim to provide an improved mechanistic understanding of the disease. Our work could establish a hierarchy towards using the neuropilin 1-induced signalling pathway as novel therapeutic targets in kidney disease and to open up new directions for the development of novel treatments targeting this disease spectrum.

2) Details of the proposal

2.1) To determine the *in vivo* role of *Nrp1* in fibrogenesis during CKD progression

Clinical studies have shown that the decline of renal function correlates more closely with the severity of **interstitial fibrosis** than with glomerular damage (8). Renal fibrosis is characterised by the deposition of pathological matrix in the potential space between tubules and peritubular capillaries by **myofibroblasts** (1). Interstitial fibrosis interferes with the normal function of tubules to secrete toxins from peritubular capillaries, mediate reabsorption to the capillaries, and receive nutrients from the circulation. In this regard, the understanding of the molecular processes triggering and maintaining myofibroblast deleterious functions is a crucial step towards blocking CKD progression.

Recent comprehensive studies have concluded that resident fibroblasts, which derive from a P0-positive population, are the major source of myofibroblasts during CKD development (9). Our preliminary data showed that NRP1 is expressed in this myofibroblast precursor population while it undergoes pathophysiological transdifferentiation, raising the question of a causative role for NRP1 signalling pathway in this process. We therefore propose to delete *Nrp1* specifically in P0-expressing cells to assay its potential pro-fibrotic function during CKD progression.

Experimental Strategy: *Nrp1* MYO-conditional mutant *P0-Cre;*Rosa^{YFP}:*Nrp1*$^{flox/flox}$ animals will be subjected to two well-characterised models of CKD progression: the UUO (Unilateral Ureteral Obstruction) and the FA (Folic Acid) nephrotoxicity, which are routinely used in our laboratory. We will score renal fibrosis in *Nrp1* conditional mutants versus their wt littermate controls. We will also use primary culture of myofibroblast precursor cells to elucidate NRP1–downstream effectors in cell proliferation, survival, transdifferentiation and collagen production.

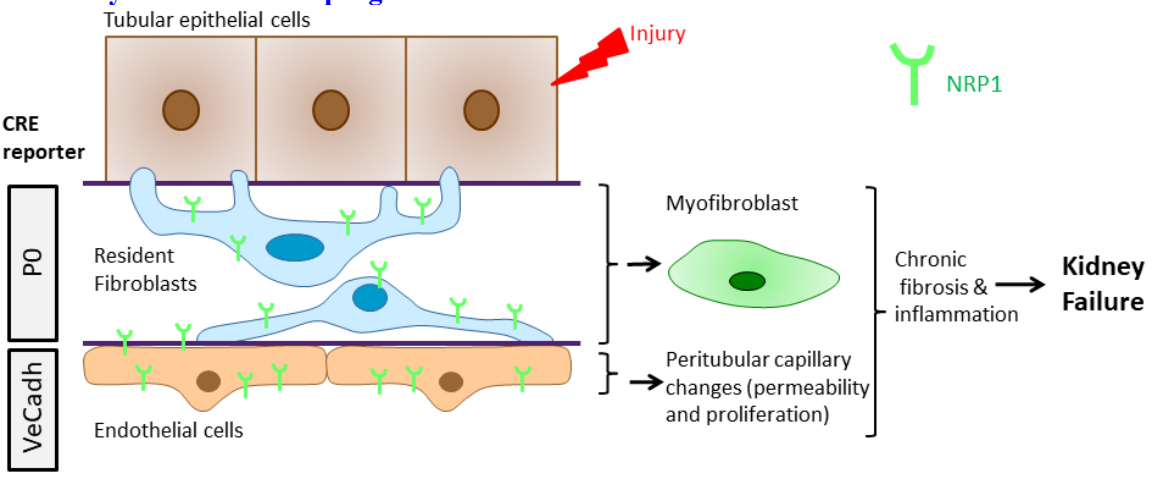
2.2) To determine the *in vivo* role of *Nrp1* in renal microvasculature rearrangement during CKD progression

Another key component of CKD progression and chronic disease progression in general, is **peritubular capillary changes** observed in both animal models and patients (10). Irrespective of the underlying cause, peritubular capillaries undergo substantial ultrastructural and functional changes during progressive renal disease (11-13), pointing toward the relevance of studying microvascular dysfunction and rarefaction as an important piece of CKD

progression. Changes in microvascular permeability, reduction of functional capillaries and decrease in capillary diameter were observed in all models of progressive CKD (11, 14, 15). In this regard, the understanding of signalling pathways altering or preserving the peritubular microvasculature is a crucial step towards blocking CKD progression. Our preliminary data showed that NRP1 is expressed in peritubular capillary endothelial cells during CKD progression. NRP1 functions in vascular remodelling are well-characterised during physiological and pathological angiogenesis, as well as in promoting capillary leakage (16-19). We hypothesise that NRP1 signalling pathway plays deleterious roles in microvasculature rearrangements and that early functional alteration of peritubular capillaries could initiate/participate in fibrosis production. To test this hypothesis, we propose to delete *Nrp1* specifically in endothelial cells to assay its function during CKD progression.

Experimental Strategy: *Nrp1* Endo-conditional mutant *VeCadherin-CreERTM;*Rosa^{YFP}:*Nrp1*$^{flox/flox}$ animals will be subjected to two well-characterised models of CKD progression: the UUO (Unilateral Ureteral Obstruction) and the FA (Folic Acid) nephrotoxicity. We will score renal fibrosis in *Nrp1* conditional mutants versus their wt littermate controls. We will use intravital microscopy to address the endothelial-specific function(s) of NRP1-driven pathways during CKD progression, a technique available in our laboratory (Cai. A et al. data in revision). This technique allows *in vivo* quantification of microvascular flow rates, endothelial cell dysfunction and microvascular permeability. We will also use primary culture of peritubular endothelial cells to elucidate NRP1–downstream functions in cell proliferation, survival, and permeability.

Summary of the research programme:



3) References

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

The applicant should have a very good knowledge of English and French, good communication skills and capacity for a smooth adaptation to a foreign environment.

At a technical level, the candidate should have previous experience and solid knowledge of renal pathophysiology, molecular and cellular biology (PCR, cell cultures, western blotting), histology and immunostaining, and ability to handle and perform animal experiments (mice). A Master 2 degree or its equivalent is mandatory. Scientific publications (original studies or reviewes) will be a plus.

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