

## Control of fibrotic response in cardiovascular diseases

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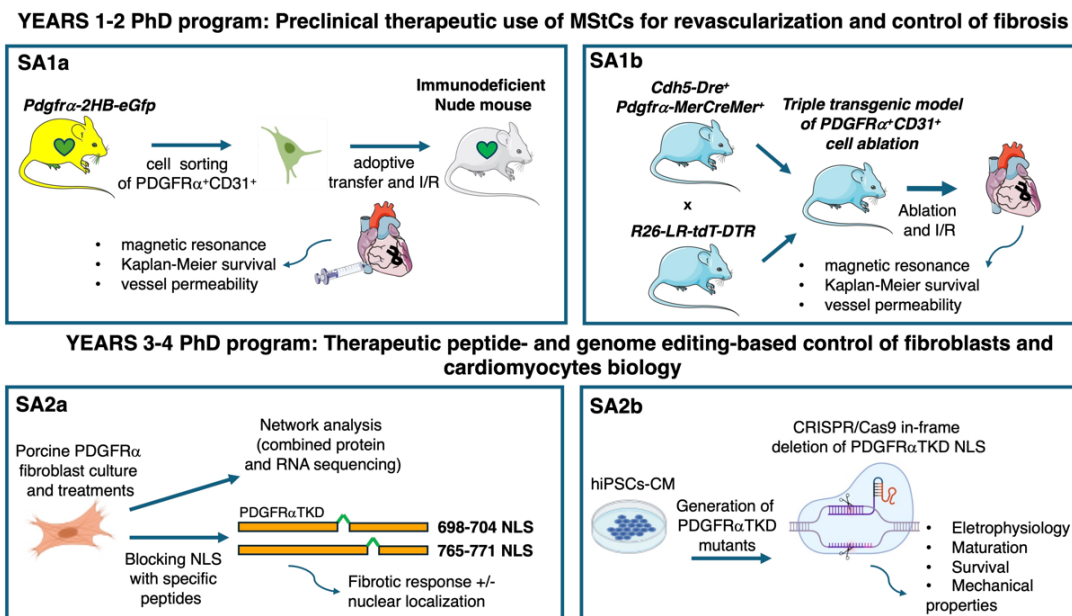
**Lab: Translational and Regenerative Medicine, Human Physiology Laboratories, Department of Molecular Medicine**

**Background and Specific Aims:** In this laboratory, we investigate the mechanisms and therapeutic approaches for inhibiting multiorgan fibrosis in diseases and aging, with a special emphasis on the cardiovascular system and skeletal muscle. Platelet-derived growth factor receptor alpha (PDGFR $\alpha$ ) signaling is associated with cardiac fibro-adipogenic pathologies due to the differentiation of PDGFR $\alpha$  mesenchymal stromal cells (i.e., PDGFR $\alpha$  MStCs) into myofibroblasts and adipocytes in response to tissue damage<sup>1</sup>. Building upon our discoveries in regenerating organs<sup>2</sup>, we preliminarily observed novel features of cardiac PDGFR $\alpha$  biology, which challenge the prevailing notions on its signaling, localization and cellular heterogeneity. Briefly, we discovered that **(1)** PDGFR $\alpha$  is not a unique marker of fibroblasts but it is expressed in several cardiac populations, indicating that the heart contains an extended cellular domain associated with PDGFR $\alpha$  expression; **(2)** nuclear shuttling of PDGFR $\alpha$  tyrosine kinase domain (TKD) is present in cardiomyocytes and to a lower extent in fibroblasts in response to ischemia, suggesting that spatiotemporal control of PDGFR $\alpha$  define the functional 'status' rather than the type of cardiac cells in response to an injury. Based on these data, we generated the **general hypothesis** that distinct populations within the PDGFR $\alpha$  cellular domain exist in the heart, which may modulate fibrosis and myocardial integrity through spatiotemporal distribution of PDGFR $\alpha$  tyrosine kinase activity. We will test this hypothesis in the following Specific Aims (SA) during the PhD program:

**SA1: Define the spatiotemporal regenerative functions of specific cardiac PDGFR $\alpha$  MStCs using novel orthogonal transgenic technologies.** This Aim will be developed during Years 1-2 of the PhD program and include SA1a and b (Figure 1). Here, we will characterize the therapeutic properties of specific cardiac PDGFR $\alpha$  MStCs, focusing our attention on the endothelial-like population (CD31<sup>+</sup>) to have insightful analyses on the revascularization properties of these cells. In SA1a, we will employ gain of function analyses by adoptive transfer after myocardial ischemia/reperfusion (I/R) of PDGFR $\alpha$ +GFP<sup>+</sup> MStCs harvested from *Pdgfra*H2B-eGfp mice and sorted according to the co-positive marker CD31. In SA1b, we will further undertake loss of function experiments using transgenic mice that have been genetically modified to ablate specifically and exclusively PDGFR $\alpha$ +CD31<sup>+</sup> cells using a novel yet validated orthogonal *Cdh5*Dre<sup>+</sup>/*Mer*Cre*Mer**Pdgfra*<sup>+</sup>-LR-DTR mouse model<sup>3</sup>. Main measurements for this Aim include survival, vessel permeability and magnetic resonance analyses of left ventricular function.

**SA2: Identify the biological significance of PDGFR $\alpha$  TKD nuclear localization signaling using systems biology and mutagenesis analyses.** This Aim will be developed in Years 3-4 of the PhD program and include SA2a and b (Figure 1). Here, we will investigate whether modulation of PDGFR $\alpha$  TKD nuclear localization using peptide- and genome editing-based techniques affects fibroblasts and cardiomyocytes biology. In SA2a, we will investigate through integrated network analyses the combined proteomic and transcriptomic profile of ex vivo isolated cardiac porcine fibroblasts in response to

conditions that favor or pharmaceutically inhibit PDGFR $\alpha$  TKD nuclear transport and fibrotic response. We will further verify the relevance of putative nuclear localization sequences (NLS) located on PDGFR $\alpha$  TKD molecule in regulating nuclear dynamics and fibrosis using peptide-targeted sequence blockage. In SA2b, we will use human induced pluripotent stem cells-derived cardiomyocytes (hiPSC-CM) from human fibroblasts to generate inducible mutants of PDGFR $\alpha$  TKD with in-frame deletion of NLS by



**Figure 1: Timeline of PhD program.**

CRISPR/Cas9 mediated targeted mutagenesis. Mutants will be analyzed for differentiation potential, electrophysiological maturation and response to biomechanical forces as present in the infarcted heart.

**Impact:** This study integrates methodologies including mutagenesis, network analyses, CRISPR/Cas9 mediated mutagenesis and translational technologies to investigate the therapeutic properties of PDGFR $\alpha$  spatiotemporal distribution in the heart. We believe that this analysis will accelerate the discovery of novel pharmacological and clinical therapies to treat cardiovascular fibrosis and restore cardiac function.

**Summary of relevant techniques:** Network analyses, electrophysiology, new orthogonal transgenic technologies, culture and differentiation of hiPSCs, CRISPR/Cas9 methodologies, biomechanical studies. The analyses will be developed within the Department of Molecular Medicine in collaboration with the cardiology and structural biology laboratories. There is the possibility to travel to Utrecht, Netherlands, and to New York City, USA, to the laboratories of my collaborators to learn iPSCs and CRISPR/Cas9 manipulations as well as biomechanical tensile analyses.

**Literature:**

1. Chong JJ, Reinecke H, Iwata M, Torok-Storb B, Stempien-Otero A, Murry CE. Progenitor cells identified by PDGFR-alpha expression in the developing and diseased human heart. *Stem cells and development*. 2013;22:1932-1943. doi: 10.1089/scd.2012.0542
2. Santini MP, Malide D, Hoffman G, Pandey G, D'Escamard V, Nomura-Kitabayashi A, Rovira I, Kataoka H, Ochando J, Harvey RP, et al. Tissue-Resident PDGFRalpha(+) Progenitor Cells Contribute to Fibrosis versus Healing in a Context- and Spatiotemporally Dependent Manner. *Cell Rep*. 2020;30:555-570 e557. doi: 10.1016/j.celrep.2019.12.045
3. Wang H, He L, Li Y, Pu W, Zhang S, Han X, Lui KO, Zhou B. Dual Cre and Dre recombinases mediate synchronized lineage tracing and cell subset ablation in vivo. *J Biol Chem*. 2022;298:101965. doi: 10.1016/j.jbc.2022.101965