

Genomic and therapeutic hallmarks of mesenchymal stromal cells in muscle senescence and diseases

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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. Aging causes various changes in mammalian skeletal muscles at the structural, molecular, and functional levels, with loss of stem cell clonality being the characteristic for excessive extracellular matrix accumulation and senescence-associated secretory response. Skeletal muscle mesenchymal stem/stromal cells expressing the platelet-derived growth factor receptor alpha, here referred to as PDGFR α MStCs, have been shown to be associated with skeletal muscle pathologies, such as fibro-fatty infiltration in human Duchenne Muscular Dystrophy (DMD)¹. Contrary to this notion, **we discovered** that PDGFR α MStCs may have dual functions, in which they may induce relevant aging-like processes but also promote therapeutic control of the pathological mechanisms leading to senescence^{2,3}. We observed that the regenerative efficiency of young PDGFR α MStCs is associated to regulation of transcripts involved in resolution of inflammation (*Cxcl9*) and increased mesodermal (*Mest1*) and proliferative signaling (*Runx1*)³. Based on these data, we generated the **hypothesis** that young PDGFR α MStCs have an intrinsic regenerative potential that is lost during senescence through modulation of pathways involved in resolution of inflammation and re-establishment of a mesodermal niche. To test this hypothesis, we will develop in the timeline of the PhD program two Specific Aims (SA) as follows:

SA1. Genomic and functional analyses of PDGFR α MStCs during aging. This Aim will be developed during Years 1-2 of the PhD program and include SA1a and b (Figure 1). In SA1a, we will generate for the first time an atlas of the aging PDGFR α MStCs transcriptomic profile using combined single cell RNA sequencing (scRNA-seq) and lineage tracing at different stages after postnatal development. Cells will be harvested from mice expressing conditional yellow fluorescent protein under the PDGFR α promoter (*MCM⁺/eYfp⁺*). In SA1b, to study the importance of the discovered regenerative molecules in controlling relevant senescence properties of PDGFR α MStCs, we will generate conditional knockout mice under the PDGFR α -promoter of *Mest1* (*MCM⁺/Mest^{fllox/+}*), *Runx1* (*MCM⁺/Runx1^{fllox/+}*) and *Cxcl9* (*MCM⁺/Cxcl9^{fllox/+}*). We will measure vessel stability through colorimetric assay³, muscle integrity through microscopy, and fibro-adipogenic response through picrosirius red and oil red O staining at different stages after postnatal development.

SA2. Therapeutic control of muscle regeneration and rejuvenation by adoptive transfer of PDGFR α MStCs. This Aim will be developed in

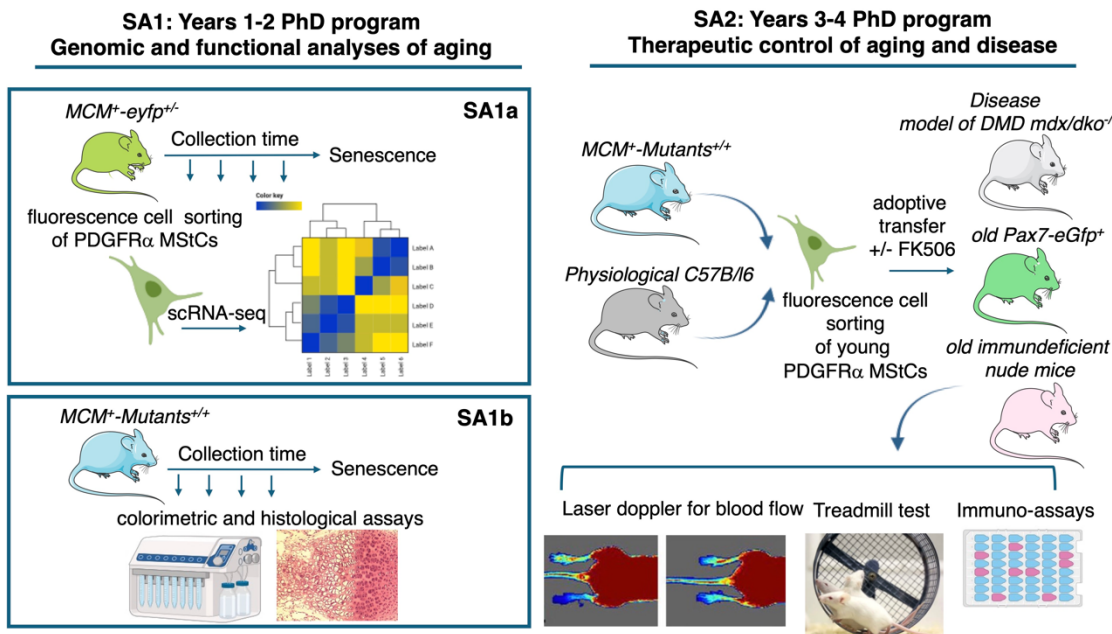


Figure 1: Timeline of PhD program

type and size using immuno-histochemistry analyses. To analyze how satellite cells are modulated by adoptive transfer of juvenile MStCs, we will lineage trace them with GFP expressed under pax7 promoter (i.e., *pax7-egfp*). Furthermore, mitigation or exacerbation of senescence associated secretory phenotype (SASP) will be profiled in tissue and serum by a custom-made xMAP bead-based immunoassay. Whether necessary, allotransplantation will be performed in immunodeficient mice or in mice treated with the immunosuppressant FK506.

Impact: The success of this project would have profound biological and preclinical implications, which entail the opportunity to discover key genomic drivers of mesenchymal stromal cell functions during aging and the possible therapeutic mechanisms modulating their regenerative properties during pathologies and rejuvenation processes.

Summary of relevant techniques: scRNA-seq, flow cytometry, transgenic technologies, isolation and adoptive transfer of muscle mesenchymal stromal cells, functional blood flow and muscle strength measurements. The analyses will be developed within the Department of Molecular Medicine in collaboration with the Human Physiology's laboratory.

Literature:

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