SRP Diabetes Postdoctoral Fellowship Program 2025

Project Title:

UNDERSTANDING THE PATHOPHYSIOLOGY OF SYSTEMIC MASLD

Background

Metabolic dysfunction associated steatotic liver disease (MASLD) is a growing burden comprising liver fat accumulation and progressing to inflammation, namely steatohepatitis (MASH), fibrosis, and ultimately life-threatening conditions, namely cirrhosis and cancer. Although MASLD early stages can be partially treated by lifestyle changes [1], late stages of the disease may be only treated by liver transplantation. Recently, the U.S. food and drug administration (FDA) approved Resmetirom and Semaglutide is soon to be approved for the treatment of MASH with moderate to advanced liver fibrosis. However, 2/3 individuals did not respond to the treatment, indicating the *need for a better understanding of the disease and for more effective therapeutic options.*

MASLD frequently coexists with diabetes and poses an elevated risk of cardiovascular events [2]. However, its progression to diabetes and cardiometabolic complications varies, suggesting the presence of distinct MASLD subtypes with unique clinical trajectories. Through human genetic studies, we recently identified two distinct types of MASLD: the first, named *systemic*, is associated with liver disease and a significantly heightened risk of type-2 diabetes and cardiovascular disease. The second, referred to as *liver-specific*, involves liver disease but carries minimal risk of type-2 diabetes and offers protection against cardiovascular disease [3].

While the pathophysiological mechanisms of the liver-specific MASLD have been partially elucidated, *the mechanisms of systemic MASLD and the relationship with diabetes are unknown.*

Objectives

The overall aim of this grant is to understand the pathophysiology of the newly identified systemic MASLD and its association with diabetes. To achieve this, the Postdoctoral Fellow (Postdoc) will analyze bulk and single-nuclei (sn) RNA sequencing data from paired liver and visceral adipose tissue (VAT) samples obtained from individuals with obesity [4]. *This analysis aims to identify transcriptomic signatures and specific cell subtypes in the liver and VAT that contribute to diabetes in individuals with systemic MASLD.*

Methodology and Work Plan

PLAN: Systemic and liver-specific MASLD will be defined based on the partitioned polygenic risk score (pPRS), calculated as previously described [3].

***To identify the transcriptomic signature* of systemic MASLD**, the Postdoc will examine bulk transcriptomic data from paired liver and VAT from individuals living with obesity, stratified based on the quantiles of the 2 pPRS (75^{th} vs 25^{th}) and identify the most up- or down-regulated tissue-specific genes. Then, the Postdoc will identify the metabolic pathways responsible for systemic MASLD by Gene Ontology enRIchment anaLysis and visuaLizAtion tool (GOrilla) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) software.

To identify cell subtypes causing systemic MASLD, the Postdoc will analyze snRNA-sequencing data from both liver and VAT, stratified based on the quantiles of the 2 pPRS (75^{th} vs 25^{th}, n=16 per group, matched by age, sex, BMI and statin use). To ensure clean clustering, the Postdoc will utilize the DIEM tool to remove debris-contaminated droplets [7]. For each person, cell type, and gene, the Postdoc will combine the unique molecular index (UMI) counts to construct cell-type-level gene UMI counts. These gene counts will be then normalized by the total cell-type gene UMI counts and log transformed for downstream analyses. The cell-type marker genes and expression data will be used to correct for cell-type heterogeneity and identify cell-type specific effects.

Validation: results will be validated in independent European cohorts.

Statistics: Differential gene expression analysis will be performed using the DESeq2 R package (2_1.6.3). P-values will be adjusted by Benjamini and Hochberg's to account for False Discovery Rate and for age, gender, and RNA Integrity Number (RIN). For snRNAseq data, after clustering nuclei based on expression using Seurat, cell-type marker genes will be identified by using Wilcoxon Rank Sum test using Bonferroni correction. A gene will be considered as a cell-type marker if it has an adjusted $p<0.05$ and a log fold change >0.25.

Power: For bulk transcriptomic study, a simulation-based power analysis using R package RnaSeqSampleSize [8] estimated a power=0.95. For snRNA-seq data, formal power calculation is not feasible due to the high heterogeneity in cell composition.

Relevance of project for Diabetes

In systemic MASLD, the liver is a key player in the crosstalk among metabolic organs. In this type of MASLD, dysfunctional visceral adipose tissue (VAT) releases excess energy substrates, which are metabolized by the liver, leading to liver steatosis. This, in turn, may exacerbate hepatic insulin resistance, creating a vicious cycle that heightens the risk of diabetes. Understanding the pathophysiology of systemic MASLD will enable the identification of effective drug targets specifically tailored to this disease subtype, with the potential to simultaneously improve both liver disease and diabetes.

Timeline for Postdoc

Year 1: Analyze bulk transcriptomic data. Presenting data at National meetings.
Year 2: Analyze snRNA-sequencing data and validate both bulk and snRNA-sequencing results. Presenting data at an international meeting. Writing paper.

References cited:

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8 Zhao S, Li CI, Guo Y, Sheng Q, Shyr Y. RnaSeqSampleSize: real data based sample size estimation for RNA sequencing. BMC Bioinformatics 2018;**19**:191.

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