Decoding development: in search of key drivers of human mesenchymal progenitors and pancreatic mesenchyme formation *in vitro*

Have you ever wondered how our organs form and grow during embryonic development? It is a fascinating process called organogenesis, involving intricate interactions between different cell types. Take the pancreas, for instance – it's made up of endocrine and exocrine cells (the epithelial part), surrounded by blood vessels, immune cells, nerve cells, and a multitude of mesenchymal cells. These mesenchymal cells are indispensable for proper pancreas development.

Research from our lab and others suggests that the pancreatic mesenchyme originates in part from mesothelial cells, which surround pancreas and other visceral organs and are marked by a specific transcription factor called Wilms Tumor 1 (WT1). During embryonic development, these mesothelial cells emerge from a broad, heterogeneous tissue called the splanchnic mesoderm, which contributes to the formation of the pancreas and other organs in digestive and respiratory systems, as well as heart.

However, we still have much to learn about how mesothelial cells differentiate into the mesenchymal cells residing within organs, and whether there are pancreas-specific mesenchyme subtypes. Understanding this process is crucial because mesenchyme plays a vital role in proper human organogenesis, and ultimately, in the development of pancreatic beta cells – the insulin-producing cells that are compromised in diabetes, a widespread civilization disease affecting millions worldwide.

Through this project, I aim to answer two key questions: 1) Are there previously unknown factors involved in development of mesothelial cells from splanchnic mesoderm? 2) Does the embryonic pancreas possess its own specific mesenchyme progenitor subpopulation that can be derived in the lab?

In my research, I have taken human pluripotent stem cells (hPSCs) and guided them to become mesothelial cells using small chemical molecules or proteins – a process called directed differentiation. I then performed single-cell RNA sequencing (scRNA-seq) on these cells at various stages of differentiation to uncover the molecular changes driving this process. scRNA-seq is a powerful technique that allows researchers to analyze the gene expression patterns of individual cells within a complex tissue or organ, revealing the intricate cellular diversity and interactions that drive development. My initial bioinformatics analysis has already uncovered potential novel genes driving mesothelial development. Moreover, by analysis of publicly available scRNA-Seq data of human mesenchyme from developing pancreas and other organs, I identified differential signaling pathway activities within the pancreatic mesenchymal cells.

Moving forward, my research aims are two-fold: 1) Define the molecular blueprint of splanchnic mesoderm and mesothelial lineages by testing the roles of chosen candidate genes by manipulating activity of encoded proteins. 2) Derive human early pancreatic mesenchyme in the lab by manipulating identified signaling pathways. For both aims, I will use hPSC *in vitro* differentiation as the research model, while flow cytometry and confocal microscopy technologies to evaluate results.

The outcomes of this project will fill gaps in our understanding of human mesenchymal lineage differentiation, particularly in the field of pancreatic biology. My data could serve as a valuable resource for generating multilineage organoids – 3D in vitro models composed of multiple differentiated cell types that faithfully reconstruct organ development and disease processes, including cancer formation. Ultimately, this research paves the way for developing cell-based therapies for diabetes, offering hope for better management and potential cures for this widespread condition.