Investigating metabolic disease in human induced pluripotent stem cells : apidocyte size, insulin signaling and hepatic lipids

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Summary

Metabolic disease has become pandemic in the developed world, and an incredible burden on the global healthcare system. Given our lack of understanding of its molecular pathology, we are often unable to diagnose patients before they reach an irreversible state of diabetes or cardiovascular disease. Much research has been done on the role of insulin signaling in metabolic disease, as well as the resultant disturbed lipid homeostasis present in cardiovascular disease and atherosclerosis. Here we add to the existing body of work by developing new tools and sketching out the pathology of dysregulated adipose insulin signaling.

Chapter 2 of this thesis discusses lipodystrophy, a lack of adipose tissue, and its effect on metabolism. FPLD2 (Familial Partial Lipodystrophy Type 2) iPSC-derived adipocytes displayed aberrant lipid metabolism, a deficiency in insulin signaling, and perturbed mitochondrial metabolism phenotypes. The FPLD2 adipocytes showed decreased propensity to differentiate into adipocytes, and increased autophagy markers. Altogether these phenotypes hint at the mechanism causing lipodystrophy patients to present with reduced adipose tissue mass.
In chapter 3 we dig deeper into the direct effects of adipose insulin signaling. Through murine adipose-specific deletion of the insulin receptor (*INSR*, CD220) we show dramatic deleterious effects. We observe reduced insulin sensitivity and glucose clearance, as well as disrupted liver metabolism, which led to hepatomegaly and hepatosteatosis and an overall significantly decreased lifespan. We also find AIRKO mice are protected from diet-induced obesity due to the near-complete absence of adipose tissue.
Chapter 4 establishes a relationship between obesity and inflammation. In obese human adipocytes *IRF1* (Interferon Regulatory Factor 1) is upregulated. When ectopically expressed in adipocytes differentiated *in vitro*, *IRF1* alters insulin signaling, and regulates lipid metabolism generating more unilocular and saturated lipid droplets. In mice these adipocytes also attracted more inflammatory macrophages.

In chapter 5 we show the development of a robust differentiation and purification protocol for hepatocytes. We isolate cells based on the mature hepatocyte-specific cell-surface marker ASGR1 (Asialoglycoprotein Receptor 1). These purified cells display increased functional capabilities and were much more similar to human primary hepatocytes. Regardless of iPSC line-to-line variation in hepatocyte differentiation capacity, the purified cells unanimously display a hepatocyte gene expression signature.
Lastly, Chapter 6 utilizes the tool developed in the previous chapter to demonstrate a proof-of-concept study modeling a GWAS-identified SNP *in vitro*. Leveraging a cohort of 68 iPSC lines differentiated into adipocytes and hepatocytes we are able to recapitulate the effect of a SNP on the liver-specific expression of *SORT1* (Sortilin). We replicate the phenotype as found *in vivo* and are able to perform additional studies, such as metabolomics, with the abundance of hepatocyte cellular material provided by the differentiated iPSCs.

The results of all experimental efforts are described in chapter 7, along with a discussion of the implication and future perspectives of the results.