SRP Diabetes Postdoctoral Fellowship Program 2024

Project title: Understanding how long non-coding RNAs promote metabolic flexibility through controlling adipocyte insulin sensitivity.

Background

The white adipocyte is a highly specialised cell with a remarkable capacity to store or breakdown lipid in response to the nutritional state or hormonal cues of the organism. In obesity, adipocytes are the first cells to display insulin resistance (IR)[1]. Adipocyte IR leads to increased spontaneous lipolysis and sustained elevations in circulating free fatty acids (FFA). Consequently, the circulating FFAs propel systemic hyperglycemia by perturbing insulin-stimulated glucose uptake in skeletal muscle which drives hepatic glucose production (HGP) and progression to type 2 diabetes. Restoring adipocyte sensitivity to insulin, and thereby restoring the antilipolytic response, is necessary to dampen HGP and ameliorate systemic hyperglycemia[2]. Thus, re-sensitizing the adipocyte to insulin presents a promising approach to prevent type 2 diabetes (T2D).

Long non-coding RNAs (lncRNAs) (defined as any RNA molecule >200 nucleotides in size with no protein coding ability) are important regulators of chromatin stability, transcription, translation, and protein-protein interactions. Recently, our laboratory[3] and others[4] have shown that cytoplasmic lncRNAs interact with important enzymes and scaffold proteins to alter adipocyte lipid metabolism. However, whether specific lncRNAs regulate the adipocyte's response to insulin in health and disease is not understood. The proposed project will integrate clinically relevant human tissue samples and novel, bespoke molecular approaches to delineate how lncRNAs enable the adipocyte to rapidly respond to insulin, and how this may go awry to drive cardiometabolic disease pathogenesis.

Objectives and methodology

1. **Identify lncRNAs that respond to insulin in human white adipose tissue and are perturbed in states of insulin resistance.**

The postdoctoral fellow will identify candidate lncRNAs using a bioinformatic pipeline which combines multiple lines of clinical evidence to filter for adipocyte-enriched lncRNAs associated with insulin action and resistance. A unique clinical cohort will be used to define the lncRNAs that directly respond to insulin and that are perturbed under states of insulin resistance. The cohort consists of 30 subjects who have underwent a euglycemic hyperinsulinemic clamp alongside paired white adipose tissue biopsies whereby transcriptomics has been performed. The subjects have been extensively phenotyped in relation to insulin sensitivity and lncRNAs found to be altered in states of insulin resistance will be further prioritized by examining their association with measures of insulin action.

2. **Define the lncRNAs that regulate the human adipocyte response to insulin.**

In the laboratory we have developed a novel cassette system capable of expressing the CRISPRi or CRISPRa, Cas9-conjugated repression/expression systems, alongside a gRNA targeting the promoter of identified lncRNAs. The system can be used at scale and is easily modified for silencing or overexpression, allowing lncRNAs to be screened for multiple effects on the insulin response. The postdoctoral fellow will identify the lncRNAs that regulate the responsiveness of the adipocytes to insulin. The broader impact on adipocyte metabolism will be uncovered using ^{13}C isotopic labelled tracers followed by gas chromatography-mass spectrometry and RNA-sequencing. The combined omics

information gained will inform on how the lncRNA affects specific pathway activity or enzyme function in the adipocyte and guide future mechanistic studies.

3. To determine the molecular mechanism of action for insulin response-regulating lncRNAs.
Our laboratory is the inventor of a state-of-the-art method, termed TROOPS, which identifies the specific binding partners of of a lncRNA in mature adipocytes[3]. For lncRNAs found to regulate the insulin response, TROOPS will be performed across basal and insulin-stimulated conditions to assess dynamic changes in the lncRNA interactome. All interactions will be validated using size exclusion chromatography and advanced light and electron microscopy. The identified interacting protein(s) of the lncRNA will be fluorescently-tagged and the effect of the lncRNA on the interacting protein can be directly visualised. In all, the postdoctoral fellow project will springboard from unique clinical cohort data to uncover how the identified lncRNAs regulate the insulin response at a molecular resolution. This project will add new understanding to insulin signalling and may discover novel targets that can be approached therapeutically.

Relevance of project for diabetes

The proposed research will examine how lncRNAs regulate adipocyte insulin signalling and contribute to adipose insulin resistance. The adipose tissue is the first tissue to become unresponsive to insulin in obesity[1]. This inability of insulin to supress lipolysis leads to increased systemic free fatty acids, ectopic lipid deposition in metabolic organs and type-2 diabetes pathogenesis[2]. The lncRNAs examined here may provide a novel targeting strategy to restore the responsiveness of the adipocyte to the effects of insulin.

References

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2. Perry, R. J. *et al.* Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell* **160**, 745–758 (2015).
3. Kerr, A. G. *et al.* The long noncoding RNA ADIPINT regulates human adipocyte metabolism via pyruvate carboxylase. *Nat Commun* **13**, (2022).
4. Tran, K. Van *et al.* Human thermogenic adipocyte regulation by the long noncoding RNA LINC00473. *Nat Metab* **2**, 397–412 (2020).

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