SRP Diabetes Postdoctoral Fellowship Program 2024

Project title: Spatially resolving the response to anti-hyperglycemic drugs in adipose tissue

Background

Insulin resistance, defined as an attenuated response to normal insulin concentrations in peripheral tissues such as the liver, skeletal muscle, and white adipose tissue (WAT), is a major determinant of type 2 diabetes and cardiovascular disease.[1] Despite this, there is still limited understanding of the cellular aspects that underlie and resolve insulin resistance. The current model is based on physiological readouts and posits that insulin resistance is a homogenous feature of all parenchymal cells within peripheral tissues.[2] As displayed in **Figure 1**, we and others have challenged this concept and instead suggest that cellular heterogeneity in different peripheral organs is a key determinant of insulin sensitivity.[3,4] Thus, strategies to identify, reconstitute, target, or maintain specific cell identities may provide new treatment avenues in the era of precision medicine.

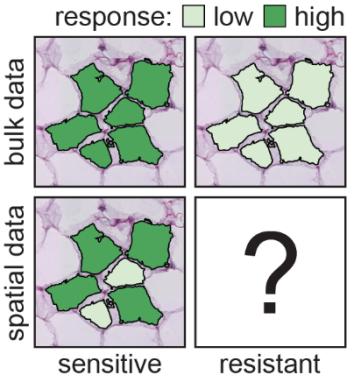

Figure 1. New *vs*. old model of insulin resistance comparing bulk and spatial mapping data.

Adipocytes are formed *de novo* from progenitor cells and have previously been considered to constitute a homogenous cell population. We recently applied spatial transcriptomics to human WAT and identified three distinct adipocyte subtypes.[3,5] Through follow-up experiments on WAT biopsies before and two hours following hyperinsulinemic euglycemic clamps, we found that only one subpopulation, termed Adipo^{PLIN}, responds to insulin in vivo. Comparing donors, we observed marked variations in the proportions and insulin response of Adipo^{PLIN}, suggesting that this subpopulation is an important determinant of insulin sensitivity. However, as our discovery was based on healthy, insulin sensitive individuals, the disease-relevance of our findings is presently unclear. Herein, we therefore focus on how the formation and function of different adipocyte subtypes are regulated by treatments aimed at improving insulin sensitivity.

Objectives

We hypothesize that interindividual variations in the formation of and/or function in distinct adipocyte subtypes determine insulin sensitivity and outcomes following interventions with anti-hyperglycemic drugs. We will test this by spatially resolving treatment responses in human WAT based on samples from a clinical trial called DIASPAX (NCT05501483).

Methodology

DIASPAX is a randomized open-label clinical trial where we compare the effects on insulin sensitivity of pioglitazone, oral semaglutide and empagliflozin in people with type 2 diabetes treated with metformin. Research participants undergo WAT biopsies before and after hyperinsulinemic euglycemic clamps at baseline and six-month follow-up. We chose the three drugs because they are cardioprotective and improve insulin sensitivity via different mechanisms.[6–8] Our initiative is the first randomized trial to study the effects of anti-hyperglycemic drugs with proven cardiovascular benefit on peripheral tissues. We started the study in February 2023 and aim to include a total of 60 men and women (we currently have n=10 participants enrolled). Comparing clinical measures obtained at baseline and six months follow-up, will allow us to subdivide study participants into responders *vs*. non-responders based on improvements in HbA1c, insulin sensitivity, body weight and body fat distribution. As reported elsewhere[9], we expect that approximately one third of the randomized participants will be categorized as high responders to each treatment. The WAT biopsy material will be analyzed using a set of established, e.g., single-nucleus RNA sequencing (snSeq) and spatial transcriptomics, as well as beyond-state-of-the-art techniques, including spatial assay for transposase-accessible chromatin (spatial ATAC), which we recently have developed[10].

Workplan

Our published and preliminary data demonstrate that by combining spatial and single-cell approaches we can define **i)** cell composition, **ii)** cytoarchitecture and **iii)** insulin response of WAT down to the single-cell level. Herein, we will relate these aspects to treatment effects. To achieve this, the recruited postdoctoral fellow will work on two well-defined subaims:

Spatially resolve transcriptomic and epigenomic WAT profiles (months 1-12): Transcription is regulated at multiple levels involving both gene regulatory networks and epigenetic mechanisms. To obtain insights into these aspects and enhance the precision of our cellular annotations, the fellow will integrate spatial transcriptomics[11] with our recently developed spatial ATAC method[10]. The latter employs capture probes to enable profiling of open chromatin in tissue sections. We will apply this cutting-edge combination of spatial methods on consecutive tissue sections to study fresh frozen WAT samples from individuals who have undergone hyperinsulinemic euglycemic clamps before and six months following drug treatment. More specifically, the recruited postdoc will cut 16 µm thick WAT sections, stain them with hematoxylin/eosin and create barcoded libraries by fixing and permeabilizing the sections and capturing the RNA. These pooled samples will be sequenced using Illumina's Nextseq 2000 instrument and raw reads preprocessed using 10X Genomics' CellRanger pipelines. As the spatial approaches are spot based, the postdoc will combine them with snSeq to deconvolve the information obtained. For this, commonly available (*SoupX*[12], *doubletfinder*[13] and *SpotClean*[14]) and in-house developed scripts (*semla*[15]) will be applied.

Relate treatment responses to spatial data (months 13-24): Following data integration, the postdoc will determine whether the proportion, position and/or insulin sensitivity of adipocyte subtypes differs comparing drug responders *vs*. non-responders. Proportion and localization will be determined by calculating the percentages and positions of spots that are annotated as a specific fat cell subtype *vs*. other cell types, respectively. Insulin sensitivity will be calculated by measuring the effects of insulin on known insulin target genes. Together, these results will provide information on whether there are inter-individual variations in the response to the different drug treatments, which in turn are linked to spatial biology of WAT. To validate our findings, the fellow will immunostain WAT sections using established marker proteins for subtypes and insulin targets.

Suitability as a postdoctoral project

The project builds upon an established collaboration between experts in WAT (Mejhert) and spatial (Ståhl) biology. Thus, the postdoc will obtain unique insights into beyond-state-of-the-art transcriptomic/epigenomic platforms and be involved in both data generation and analyses, including integration between clinical and spatial data. The feasibility is supported by the fact that the approaches are established, and preliminary data have been generated.

Relevance of project for diabetes

Our discovery that human WAT contains adipocyte subtypes with qualitatively distinct functions constitutes a breakthrough in understanding WAT function and its link to metabolic disease.[3] In this proposal, we will for the first time define how differences in the proportion and function of distinct fat cell populations might explain variations in the clinical response to anti-hyperglycemic drugs. Results from this project may therefore enable a precision medicine approach for the treatment of insulin resistance and type 2 diabetes.

References

1. James, D. E., Stöckli, J. & Birnbaum, M. J. Nat Rev Mol Cell Biol 22, 751–771 (2021). **2.** Klein, S., Gastaldelli, A., Yki-Järvinen, H. & Scherer, P. E. Cell Metab 34, 11–20 (2022). **3.** Bäckdahl, J. et al. Cell Metab 33, 1869-1882.e6 (2021). **4.** Rubio-Navarro, A. et al. Nat Cell Biol 25, 565–578 (2023). **5.** Massier, L. et al. Nat Commun 14, 1438 (2023). **6.** B, Z. et al. N Engl J Med 373, 17–18 (2015). **7.** Dormandy, J. A. et al. Lancet 366, 1279–1289 (2005). **8.** Marso, S. P. et al. N Engl J Med 375, 1834–1844 (2016). **9.** Shields, B. M. et al. Nat Med 29, 376–383 (2023). **10.** Llorens-Bobadilla, E. et al. Nat Biotechnol (2023) **11.** Ståhl, P. L. et al. Science 353, 78–82 (2016). **12.** Young, M. D. & Behjati, S. Gigascience 9, 1–10 (2020). **13.** McGinnis, C. S., Murrow, L. M. & Gartner, Z. J. Cell Syst 8, 329-337.e4 (2019). **14.** Ni, Z. et al. Nat Commun 13, (2022). **15.** Larsson, L., Franzen, L., Ståhl, P. L. & Lundeberg, J. Bioinformatics 39, (2023).

Contact details:

Main supervisor:
Name: Niklas Mejhert
Academic affiliation: Department of Medicine Huddinge, Karolinska Institutet
E-mail: niklas.mejhert@ki.se
Telephone: +46 70 491 85 41
Website: https://ki.se/en/medh/ryden-mejhert-group

Co-supervisor:
Name: Patrik Ståhl
Academic affiliation: Division of Gene Technology, SciLifeLab
E-mail: patrik.stahl@scilifelab.se
Telephone: +46 70 363 02 63
Website: https://www.kth.se/profile/steel?l=en