Adipocyte heterogeneity - a novel determinant of insulin resistance and type 2 diabetes

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Project outline

Background

While it is established that disturbances in adipocyte function are linked to type 2 diabetes, the mechanisms promoting these alterations remain unclear. This is partly explained by the fact that fat cells are notoriously difficult to study *ex vivo* (*e.g.* they are short-lived and fragile). Therefore, focus has been on the non-adipocyte cells residing in white adipose tissue (WAT), including adipocyte progenitors. Single cell analyses have shown a pronounced heterogeneity in these cell types, which in turn has been hypothesized to lead to distinct mature fat cell populations that control different aspects of WAT function. However, if specific adipocyte subtypes exist and whether they are linked to clinically relevant phenotypes is not known.

We have together with Dr. Patrik Ståhl (Royal Institute of Technology) recently finalized a project where we for the first time have applied spatial transcriptomics to human WAT. This allowed us to define WAT transcriptional profiles down to the single cell level in subjects with different body weights and insulin sensitivity. By obtaining data before and after hyperinsulinemic euglycemic clamps, we have identified distinct adipocyte subpopulations, which exhibit specific spatial arrangements, unique gene and protein expression and respond qualitatively differently to insulin. This suggests that human white adipocytes are much more heterogenous than hitherto realized. However, as these assessments are based on descriptive analyses of gene expression profiles and immunocytochemistry, functional characterizations are presently lacking.

Objectives

Based on our data, we hypothesize that distinct fat cell subpopulations contribute to WAT homeostasis by regulating specific functions of the organ. Thus, we propose that some adipocytes regulate energy turnover, while others control energy intake. The overarching aim of this project is to functionally characterize the adipocyte subtypes described above and to determine if they are affected in insulin resistant and type 2 diabetic subjects. To accomplish these ambitious goals, we will apply spatial transcriptomics to unique clinical materials and functionally validate our findings in mature adipocytes *ex vivo*. The following two aims are set up for the two-year post-doc period:

Aim 1: Determine the link between adipocyte heterogeneity and insulin resistance/type 2 diabetes in well-phenotyped subjects before/after hyperinsulinemic euglycemic clamps

Aim 2: Isolate and functionally characterize human white adipocyte subpopulations *ex vivo*

Methodology

The project involves a large set of techniques and approaches, which will be applied to both clinical samples and cell models. **In Aim 1**, we will perform spatial transcriptomics on WAT samples obtained from subjects before and two hours after a hyperinsulinemic euglycemic clamp. From these studies, the postdoc will determine single cell transcriptional responses in relationship to spatial/histological features using advanced bioinformatic and imaging pipelines that we have set up in collaboration with Dr. Ståhl. Subjects are recruited within a larger study of individuals with or without type 2 diabets followed before/after bariatric surgery (NCT01727245). In **Aim 2**, the postdoc will use plasma membrane markers of different adipocyte subtypes to isolate and characterize the fat cell subpopulations as described below.

Work plan (including description of the work the postdoc fellow will perform)

Aim 1 (months 1-6): To understand how adipocyte heterogeneity is linked to disease, we will apply spatial transcriptomics to a larger cohort of individuals with insulin resistance/type 2 diabetes and determine the effects of insulin stimulation *in vivo* by clamp. By comparing the results with published transcriptional profiles of the insulin response in WAT, we will first evaluate how well the spatial transcriptomics data captures the insulin-regulated transcriptome and second, determine how the insulin response differs between different adipocyte subtypes in relation to insulin sensitivity and/or type 2 diabetes. This will allow us to answer key questions, *e.g.* is the number of insulin-responding cells fewer in insulin resistant states or is the response attenuated in most cells? It will also allow us to determine the impact of cell size and spatial arrangements (*i.e.* neighborhood analyses) in relation to other cell types.

Aim 2 (months 6-24): Functional studies of individual fat cell subpopulations requires assays separating cells based on the expression of marker proteins. Standard FACS procedures commonly used for this are not suitable as isolated adipocytes are too fragile to survive the sorting procedure. To circumvent this hurdle, we have developed a completely novel approach where we complex antibodies against plasma membrane marker proteins to dense sepharose beads and then incubate these complexes with mature fat cells. Following incubations, the suspension is overlayered on a continuous OptiPrep gradient (0-25%) containing iodixanol, a non-ionic, non-toxic and metabolically inert density gradient agent. This separates adipocytes based on density (*e.g.* buoyant adipocytes not bound by beads float, while bead-bound cells sink) and different fractions are collected using a tube slicer. Functional assays already established in the laboratory, including adipokine secretion, glucose uptake/*de novo* lipogenesis and lipolysis, are thereafter performed. More advanced analytical approaches, *e.g.* metabolomics, proteomics and bioenergetic measures, will be done in collaboration with Drs. Natalie Krahmer (Helmholtz Zentrum München), and Myriam Aouadi (KI). Altogether, these techniques will for the first time allow us to map and functionally characterize human adipocyte subtypes *ex vivo* and establish their relationship to clinically relevant metabolic phenotypes.

Relevance of project for diabetes

Adipocyte heterogeneity and its link to insulin sensitivity is a novel concept in WAT biology. A better understanding of the mechanisms causing adipocyte heterogeneity is essential in order to link fat cell subpopulations to insulin resistance, type 2 diabetes and atherosclerotic cardiovascular disease. To the best of our knowledge, this is the first time that adipocyte heterogeneity will be studied in a completely unbiased and systematic approach. The combination of unique human cell models and beyond-state-of-the-art transcriptional studies of carefully phenotypes clinical samples from subjects with or without type 2 diabetes facilitates the translatability of the findings generated within the proposal. The results identified herein may lay the foundation for future development of risk markers and therapies targeting WAT.

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