SRP Diabetes Postdoctoral Fellowship Program 2023

Project title: Investigating DNA damage as a driver of adipocyte senescence and inflammation in obese, hyperinsulinemic individuals

Background

Many metabolic diseases, including type 2 diabetes (T2D), strongly associate with obesity, making obesity one of the major health challenges facing the world today. Understanding how fat cells respond to obesity and contribute to pathological processes is more relevant than ever. A pro-inflammatory adipocyte secretory pattern is augmented by obesity and significantly pronounced in large adipocytes, however the underlying mechanisms connecting adipocyte hypertrophy and proinflammatory secretion are less understood[1]. One cellular program that can induce a pro-inflammatory secretory pattern is premature cellular senescence. Senescent cells alter their phenotype and are highly metabolically active, releasing increased levels of pro-inflammatory cytokines and chemokines[2,3]. This secretory profile, termed the senescence-associated secretory phenotype (SASP), is becoming well established as a negative regulator of tissue and whole-body health[4-7]. Recently we identified an unexpected, active cell cycle program in fully differentiated mature human adipocytes, which upon continued mitogenic stimulation (prolonged hyperinsulinemia) results in a pro-inflammatory, senescent phenotype[8].

Senescence has traditionally been considered an irreversible block of cell cycle progression, commonly induced by stress, oncogenic stimulation or DNA damage[9,10]. One source of cellular stress is reactive oxygen species (ROS), with increased ROS resulting in genomic and mitochondrial DNA damage[11]. Obesity in humans associates with increased oxidative stress, with plasma levels of lipid peroxidation markers significantly correlated to BMI and waist circumference[11]. Diabetes is associated with an inability to detoxify ROS[12]. In our recent study in *Nature Medicine* we show an increase in the adipocyte DNA damage response (γH2AX) as a function of a patient's BMI[8], in line with previous data suggesting an active DNA damage response in obesity[13-16]. Oxidative-induced DNA damage is repaired via the base-excision repair pathway, which is initiated by DNA glycosylases. The most commonly formed oxidative lesion, 8-oxoguanine (8-oxoG), is recognized and cleaved by the enzyme, 8-oxoG DNA glycosylase (OGG1). Deletion of OGG1 renders mice and humans susceptible to metabolic disease. Conversely, enhanced expression of the human OGG1 gene protects against diet-induced obesity, insulin resistance, and adipose tissue inflammation in mice[15,17]. Recent evidence that adipocyte senescence can be induced by modulating adipocyte DNA-damage responses[18], supports the call for experiments exploring the potential of using small molecules to repair DNA damage.

Objectives

Senescent cells, which can release factors that cause inflammation and dysfunction, increase in adipose tissue and associate with obesity and hyperinsulinemia. The proposed project will determine whether oxidative stress and DNA damage in human adipocytes drives adipocyte senescence, and the associated pro-inflammatory secretory profile. A small molecule approach to attenuate adipocyte DNA damage and reverse adipocyte senescence will be investigated.

Methodology

Senescence assays: Adipocyte senescence will be measured as detailed recently for adipocytes[8]. Senescence associated beta galactosidase quantification as well as the protein expression of cyclin D1 and CDK-4/6 inhibitors (p21 and p16) will be determined using quantitative confocal microscopy[8]. The SASP will be investigated at a transcript (single cell and bulk mRNA sequencing) and protein (targeted- WB, Elisa, O-Link and untargeted-mass spec) level.

Single cell RNA sequencing (scRNAseq): Due to the highly buoyant and fragile properties of mature human adipocytes, adipocyte scRNAseq has been difficult to achieve. We have developed a method to perform

scRNAseq on both freshly isolated mature human adipocytes as well as adipocyte nuclei. The postdoctoral fellow will have access to state-of-the-art methodology and datasets for probing adipocyte SASP and DNA damage at a single cell level.

DNA damage/oxidative stress measurement and inhibition: A small molecule activator of oxidative DNA repair proteins, OGG1a, will be administered to mature human adipocytes *in vitro* (in collaboration with Professor Thomas Helleday, KI). OGG1a produces a novel biochemical function in the OGG1 protein so that the repair of oxidative damage is increased 10-fold. We have established a unique culture system which recapitulates chronic hyperinsulinemia to generate an *in vitro* model where adipocyte senescence can be induced[8]. Using this model system, the postdoctoral candidate will measure the effectiveness of using an OGG1 activator to (i) reduce adipocyte DNA damage, (ii) reduce insulin induced adipocyte senescence and (iii) reduce the pro-inflammatory secretory profile of senescent fat cells.

Work plan

The work program performed by the postdoctoral fellow consists of 2 two experimental paradigms:

1. Isolate mature adipocytes from patient biopsies, quantitate adipocyte senescence and measure the DNA damage response and oxidative DNA damage. Tissue from lean, obese normoinsulinemic, obese hyperinsulinemic and diabetic individuals (type 1 and 2) will be investigated.
2. The ability of a small molecule activator to reduce adipocyte DNA oxidative damage *in vitro* will be investigated (preliminary data supports a reduction in adipocyte DNA damage). The effect of reducing DNA damage on baseline and hyperinsulinemia-induced adipocyte senescence, and associated SASP, will be determined.

Relevance of project for diabetes

With T2D increasing globally at an epidemic rate we are in dire need of a better understanding of disease pathogenesis and the development of novel treatment strategies. Adipose tissue senescence is fast emerging as a significant contributor to adipose tissue inflammation and metabolic disease. Clearance of senescent cells using senolytics has been shown to improve metabolic health[19-22]. Senolytics are small molecule compounds which selectively induce apoptosis in senescent cells. Whilst preliminary data from animal studies using senolytics is encouraging, the safe translation into human studies has been slower to demonstrate. A recent study demonstrates that continuous or acute removal of senescent cells in mice, results in a disruption of blood-tissue barriers, with subsequent liver and perivascular tissue fibrosis. Senescent cells were found to have important structural and functional roles in the organism and it was instead proposed that strategies aimed at delaying or preventing senescence could be powerful therapeutic tools[23]. *Given the strong association between adipose tissue senescence, T2D and metabolic disease, novel therapies aimed at reducing senescent cell buildup are of significant interest. This postdoctoral project will elucidate the role of obesity-associated DNA damage as a driving factor in promoting adipocyte senescence and adipose tissue inflammation. Important proof of concept validation, elucidating whether a small molecule approach can attenuate adipocyte senescence and inflammation, will also be conducted.*

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