Development of *in vitro* systems based on Human iPSC-derived sympathetic neurons for the understanding of obesity-induced sympathetic neuropathy, and the leptin setpoint

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Project outline

Background: Sympathetic neurons in white adipose tissue constitute the efferent effector arm in the neuroendocrine loop of leptin action (1, 2). Obesity induces sympathetic axon remodelling and, ultimately, neuropathy that, in turn, worsens lipid mobilization and the metabolic adaptation that is conceptualized to rely on an upward shift in the equilibrium setpoint of leptin (reviewed in 2). This project builds on results obtained in my laboratory, some of which were generated by Novo Nordisk fellow Gitalee Sarker and which have been publicly announced at a Keystone conference (3) and in preparation for peer review. We discovered that endothelial-perineurial barrier cells that envelop sympathetic ganglia and peripheral axon bundles (sympathetic endothelial-perineurial cells – SEPCs) express the Leptin receptor (LepR) and the beta-2 adrenergic receptor (adr2b) to sense the balance between the afferent and the efferent arms in the neuroendocrine loop of leptin action (3). The discovery of SEPCs introduces a new element in the leptin setpoint theory that regulates the strength of the efferent sympathetic response in the periphery. Whether SEPCs secrete paracrine survival signals that affect sympathetic neurons is an open question and the primary focus of this project. To this end, *in vitro* co-culture systems that recapitulate in vitro neuro-perineurial interactions ought to be developed as primary cells (sympathetic ganglia) do not have the enabling scalability for a systematic approach to this problem. Recent advances in protocols for iPSC-derived sympathetic neurons established that co-culture with target cells promotes neuronal maturation (4,5) and holds promise for modelling *in vitro* the SPECs/sympathetic neuron cross-talk and the leptin setpoint.

Hypothesis: SEPCs secrete survival signals that, in response to the ratio of leptin and norepinephrine, maintain sympathetic neuron health and have a role in obesity-induced sympathetic neuropathy in white adipose tissues.

Aim 1: Optimization of Human iPSC-derived sympathetic neuronal cultures and their transformation for expression of fluorescent reporters and Designer receptors exclusively activated by designer drugs (DREADD) or channelrhodopsin.

Aim 2: Co-culture human iPSC-derived sympathetic neurons with human SPECs (Human Endothelial Cell lines recombinantly expressing $LepR^{+}ADRb2^{+}$) to ascertain if SEPCs modulate sympathetic neuron morphology for varying ratios of leptin and neural activation (via activation of DREADD by CNO).

Neuronal morphology will be monitored by the HTP Opera Phenix high-content imaging platform in the Wade-Martins lab.

Aim 3: Single-cell sequencing for the identification of genes differentially expressed by SEPCs in co-culture with iPSC-derived sympathetic neurons and stimulated with key doses of leptin/CNO (determined in Aim 2).

References:

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2. Martinez-Sanchez N, et al , Domingos AI. The sympathetic nervous system in the 21st century: Neuroimmune interactions in metabolic homeostasis and obesity. *Neuron*. 2022 Nov 2;110(21):3597-3626.
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4. Kirino, K., Nakahata, T., Taguchi, T. *et al.* Efficient derivation of sympathetic neurons from human pluripotent stem cells with a defined condition. *Scientific Rep* 8, 12865 (2018)
5. Oh Y et al., Lee G. Functional Coupling with Cardiac Muscle Promotes Maturation of hPSC-Derived Sympathetic Neurons. *Cell Stem Cell.* 2016 Jul 7;19(1):95-106

Supervisor's recent relevant publications:

Ana Domingos

1. The sympathetic nervous system in the 21st century: Neuroimmune interactions in metabolic homeostasis and obesity Martinez-Sanchez N, et al., **Domingos AI**. *Neuron*. 2022
2. Neuro-mesenchymal units control ILC2 and Obesity via a brain–adipose circuit. Cardoso, F.,. et al. **Domingos AI**, Veiga-Fernandes H. *Nature* 2021
3. Brain-sparing *sympathofacilitators* mitigate obesity without adverse cardiovascular effects. Mahú I, et al, Bernardes GJL, **Domingos AI**, *Cell Metabolism* 2020
4. Sympathetic neuron-associated macrophages contribute to obesity by importing and metabolizing norepinephrine. Pirzgalska RM, et al, Iannacone M, Spann NJ, Glass CK, **Domingos AI**. *Nature Medicine*. 2017
5. Sympathetic neuro-adipose connections mediate leptin-driven lipolysis. Zeng W, Pirzgalska RM, et al., Friedman JM, **Domingos AI**. *Cell*. 2015