LA TROBE UNIVERSITY

Scientific laboratory projects

2019

HBS3IRP Independent Research in Human Physiology

CONTENTS

1. Impact of stroke on the vasculature in mice overexpressing NOX4-oxidase

Advisor: Dr Michael De Silva

Overview

Oxidative stress contributes to brain and vascular injury after stroke. NOX4-oxidase is an important source of reactive oxygen species in both health and disease. Preliminary data from our laboratory suggests that mice overexpressing NOX4-oxidase only in endothelial cells (inner lining of blood vessels)(NOX4-Tg mice) have poorer functional outcomes. This project aims to identify some of the changes that may contribute to the poorer outcome in NOX4-Tg mice.

References / readings

- Ritchie RH, Drummond GR, Sobey CG, De Silva TM, Kemp-Harper BK (2017). The opposing roles of NO and oxidative stress in cardiovascular disease. Pharmacological Research, 116: 57-69.

  **background and sections on ischemic stroke*

- Casas AI, Geuss E, Kleikers PWM, Mencl S, Herrmann AM, Buendia I, Egea J, Meuth SG, Lopez MG, Kleinschnitz C, Schmidt HHHW (2017). NOX4-dependent neuronal autotoxicity and BBB breakdown explain the superior sensitivity of the brain to ischemic damage. Proc Natl Acad Sci U S A. 114:12315-12320.

2. Is thymosin β4 (Tβ4) expression altered in skeletal muscle myoblasts when they are cultured in different developmental stages?

Advisor: Dr Caroline Taylor

Overview

Skeletal muscle myoblasts are able to be grown in different functional stages. They are normally grown as single cell myoblasts but can be stimulated to become multicellular myotubes. Myotubes are a differentiated form of myoblasts and more closely resemble mature muscle. Our lab also grows myoblasts as 3-dimensional spheroids for implantation studies.

Thymosin β4 is a small protein that is expressed by all cell types and it has been proposed to have a number of biological effects. One of its roles is in regulation of the cell cytoskeleton and expression levels may change when the shape of the cell is altered.

This project will examine the expression of Tβ4 in myoblasts, myotubes and 3D spheroids of different sizes using real-time PCR.

References / readings

- Kuzan A (2016) Thymosin β as an actin-binding protein with a variety of functions. Advances in Clinical and Experimental Medicine; 25(6): 1331-1336.

3. Do skeletal myoblasts produce interleukin-18 (IL-18)?

Advisor: Dr Caroline Taylor

Overview

In the last few years, a number of studies have detected the proinflammatory cytokine interleukin-18 in the blood of individuals suffering from muscle conditions such as sarcopenia. It is however, unclear whether this IL18 is associated with the skeletal muscle itself or some other cell type such as inflammatory cells or blood vessels.

This project will examine the expression of IL-18 in C2C12 mouse myoblast cells grown under to different conditions.

References / readings

- Li, C. W., Yu, K., Shyh-Chang, N., Li, G. X., Jiang, L. J., Yu, S. L., … Li, D. J. (2019). Circulating factors associated with sarcopenia during ageing and after intensive lifestyle intervention. Journal of cachexia, sarcopenia and muscle, 10(3), 586–600. doi:10.1002/jcsm.12417
- Helmers SB, Bruton M, Loell I, Ulfgren AK, Gracie AJ, McInnes IB, Lundberg IE (2018) Expression of interleukin-18 in muscle tissue of patients with polymyositis or dermatomyositis and effects of conventional immunosuppressive treatment. Rheumatology (Oxford). 57(12):2149-2157

4. The role of the IL-18/IL-18R signalling axis on vascular remodelling in a mouse model of abdominal aortic aneurysms

Advisor: Dr Antony (Bill) Vinh

Overview

Abdominal aortic aneurysms (AAA) affect approximately 13% of the adult population aged over 65, and ruptured aneurysms can cause massive internal bleeding, which is usually fatal. There are currently no pharmacological approaches to prevent or control AAA expansion, with surgical intervention as the only option, which always includes a degree of risk. While the pathogenesis of AAA formation remains unclear, inflammation has recently been implicated in the remodelling of blood vessels leading to expansion and eventual rupture. An important inflammatory pathway involved in innate immunity is the inflammasome, which functions to release pro-inflammatory cytokines in response to various pathogen and stimuli. Interleukin (IL)-18 is an inflammasome-derived cytokine that binds to its cognate IL-18R receptor to induce further inflammation in target cells such as T cells. Surprisingly, we have striking new data that IL-18R-knockout mice showed significantly greater incidence of AAA formation compared to wildtype (WT) mice using a mild model of AAA formation (KO: 87% vs WT: 9%). We now aim to further examine the mechanisms associated with the greater incidence in the IL-18R-KO mice. Using histolopathological and immunohistochemical staining we will examine the composition of vessels from WT, IL-18-KO and IL-18R-KO within healthy sections and sections with AAAs. Specifically, we aim we examine elastin degradation, extracelluar matrix deposition (collagen) and macrophage infiltrates. By understanding the role of IL-18R in the formation of AAAs, we may identify novel therapeutic targets to pharmacologically control AAA progression and/or rupture.

References / readings

- Golledge, J, Norman, PE. Current status of medical management for abdominal aortic aneurysm. *Atherosclerosis*. 2011;217:57–63
- Davis, FM, Daugherty, A, Liu HS. Updates of Recent Aortic Aneurysm Research. *Arteriosclerosis, Thrombosis and Vascular Biology*. 2019;39(3) e83–e90
- Latz, E. The inflammasomes: mechanisms of activation and function. *Current Opinion in Immunology*. 2010;22(1):28-33