PROJECT GRANT RECIPIENT AND FINAL REPORT

Funded by:	ARA Research Fund ($60,000)
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Intended Department:	Walter and Eliza Hall Institute of Medical Research (WEHI), Inflammation Division
Project:	Stopping blood vessel remodelling in vasculitis

We are very grateful for the grant funding received from Arthritis Australia in support of our project "Stopping blood vessel remodelling in vasculitis".

Study Overview

Vasculitis refers to a group of diseases characterized by their ability to cause inflammation to develop in and around blood vessels. While there are many forms of vasculitis (such as Kawasaki Disease, Takayasu's arteritis or Giant Cell arteritis), all share the potential to cause adverse remodelling of the inflamed blood vessels. In severe cases, this remodelling can lead to narrowing of the lumen, restricting blood flow to the downstream tissue. This process can lead to tissue-ischemia, which is the leading cause of morbidity and mortality for vasculitis patients. The aim of this study was to investigate the pathophysiology of adverse blood vessel remodelling and identify new treatment strategies to prevent this life-threatening pathology developing in vasculitis patients.

Study Methodology

Adverse remodelling is caused by the excessive accumulation of fibroblasts within the intimal layer of inflamed vessels. In this study, we investigated how such intimal fibroblasts develop during Kawasaki Disease (KD), a paediatric vasculitis typically involving the coronary arteries. To follow the origin of intimal fibroblasts, we performed lineage tracing studies in a murine model of KD, induced by the injection of *Candida albicans* water soluble complex (CAWS). We also used conditional genetic deletion strategies to identify intrinsic regulators of intimal fibroblast formation and analysed patient samples to study this population in human disease.

Major Project Findings

A summary of the major findings is given below. For a full description of these results, refer to the attached manuscript or https://www.biorxiv.org/content/10.1101/2023.12.15.571811v1 where the paper has been uploaded onto Biorxiv.

1. By using an array of lineage tracing systems in the CAWS mouse model of Kawasaki Disease, we have shown that the intimal thickening that emerges during vasculitis is caused by the migration and proliferation of smooth muscle cells (SMCs) into and within the intimal of inflamed vessels.

2. By using immunofluorescent microscopy, we have shown that migration of SMCs into the inflamed intima coincides with their activation of the mechanistic target of rapamycin (mTOR) signalling pathway.

3. By use Cre-LoxP genetic systems to selectively delete the mTORC1 subunit RAPTOR in SMCs, we have shown that genetically deleting mTOR signalling in SMCs completely abrogated their ability to migrate into the intima during vasculitis.

4. By using both genetic and pharmacological (i.e. rapamycin) strategies to inhibit mTOR, we have shown that stopping mTOR signalling reduces SMC proliferation and the severity of adverse blood vessel remodelling during vasculitis.

5. By analysing arterial sections (obtained during autopsy or biopsy) from patients with active Kawasaki Disease, Takayasu's arteritis or Giant Cell arteritis, we have shown that the mTOR signalling pathway is activated by the intimal fibroblasts that drive intimal hyperplasia in human disease.

6. Pending - We are in the midst of completing RNA-sequencing of SMCs to describe the molecular basis for SMCs activation and how mTOR signalling controls this event (this analysis is underway).

Summary of the study findings and their clinical significance

In total, our findings from this study reveal that the mTOR signalling pathway is an intrinsic, essential and druggable pathway which is activated in the intimal vascular fibroblasts that drive adverse blood vessel remodelling in vasculitis. We believe that these findings provide compelling rationale for using mTOR inhibitors as a novel therapeutic strategy in systemic vasculitis. Indeed, this study has prompted at Clinicians at the Royal Melbourne Hospital (RMH) to treat one Takayasu's arteritis patient with mTOR inhibitors to control their progressive stenotic disease (Ian Wicks, Personal Communication). This illustrates the clinical potential for this approach. We hope that these results will lead to the further clinical application and/or clinical trials of mTOR inhibitors to treat vasculitis patients in the near future.

Project Publications

The major findings from this study have formed the basis for the below manuscript, which is currently under External Peer Review at *EMBO Reports*. This manuscript is available on Bioxriv (see below link) and has been provided as an attachment with this report.

- ***mTOR signaling controls the formation of smooth muscle cell-derived intimal fibroblasts during vasculitis.*** Angus. T. Stock*, Sarah Parsons, Jacinta. A. Hansen, Damian. B. D'Silva, Graham Starkey, Aly Fayed, Xin Yi Lim, Rohit D'Costa, Claire. L. Gordon & Ian. P. Wicks*. Under Peer Review at *EMBO Reports.*
- The above manuscript is available online at: https://www.biorxiv.org/content/10.1101/2023.12.15.571811v1.

Presentations

- I have given an Oral presentation of this study at the **2023 ARA Victorian/Tasmanian ASM**. *NB - I was awarded the best Basic Science Presentation.*

- I have been invited to give an Oral Presentation of this study at the **2024 International Kawasaki Disease Symposium (iKDS)** in Montreal Canada.