UNDERSTANDING THE CELLULAR AND MOLECULAR BASIS OF MACROLIDE THERAPY FOR AIRWAY DISEASE
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Research Background

Investigator

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Co-Investigators

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Macrolide antibiotics, in particular azithromycin, have become widely used therapeutically for patients with airway diseases such as cystic fibrosis and post-transplant obliterative bronchiolitis (OB) accumulating evidence clearly demonstrates improvements in clinical relevant endpoints with their use. However, their mechanism of action is unknown.

Bronchiolitis obliterans syndrome (BOS), the clinical surrogate of obliterative bronchiolitis (OB), and the major cause of death after lung transplantation, is thought to be caused by a defective repair process that results in scarring of the small airways in the transplanted lung(s).

Although chronic injury and dysregulated repair of airway epithelium is thought to underlie OB pathogenesis, limited direct supporting evidence exists, particularly in the small airways. Successful airway epithelial repair requires carefully orchestrated but transient expression of multiple genes including matrix metalloproteinases (MMP) and integrins, with abnormal expression associated with defective repair, hyperplasticity and airway remodelling.

The researchers recently demonstrated up-regulation of MMP-2 and MMP-9 expression in OB affected airway epithelial cells, and thus are investigating whether other key MMPs, integrins and b-catenin would be abnormally up- or down regulated in OB epithelium.

Research Objectives

Any findings from this proposal in conjunction with our existing research platform will allow for preclinical assessment of other anti-rejection drugs as well as being able to segregate their effects on small and large airways. Similarly, and as equally important, we are ideally situated to investigate and discover at the cellular level just how these drugs work and thus be able to identify new therapeutic targets. With additional drug discoveries, and increasing existing drug effectiveness we aim that the current research will ultimately increase the long term survival of lung transplant recipients, which currently remains at a very low 20%.

Research Progress

The last quarter has been productive both in terms of sampling and data generating. Since commencing this project the research team has successfully obtained 25 small and large airway epithelial samples from 16 transplant patients (11 males).

The median age is currently 54 (range, 23-64 years). The team has been able to successfully grow these cells and subsequently wound them. The ability of each of these different types of cells to repair was then analysed (Fig. 1, below). Photo micrographs have subsequently been collated and the rate of repair quantified.

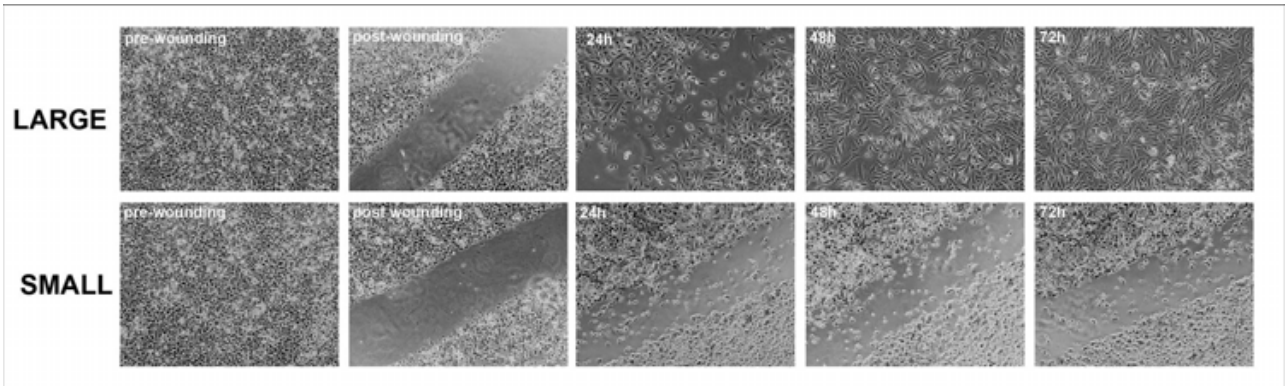


Figure 1

Results generated from this series of experiments have clearly shown a significant difference in the ability of the small airway epithelial cells to repair from large airway epithelial cells (Fig. 2).

The team has discovered that epithelial cells taken from the large airway completely repair when wounded and that the rate of repair is comparable to control large airway epithelial cells from non-transplanted lungs.

However, and most interestingly, the team has observed that epithelial cells derived from the small airways were unable to completely repair over the same period. In fact, it appeared they were unable to trigger the repair process at all (Fig.2, below).

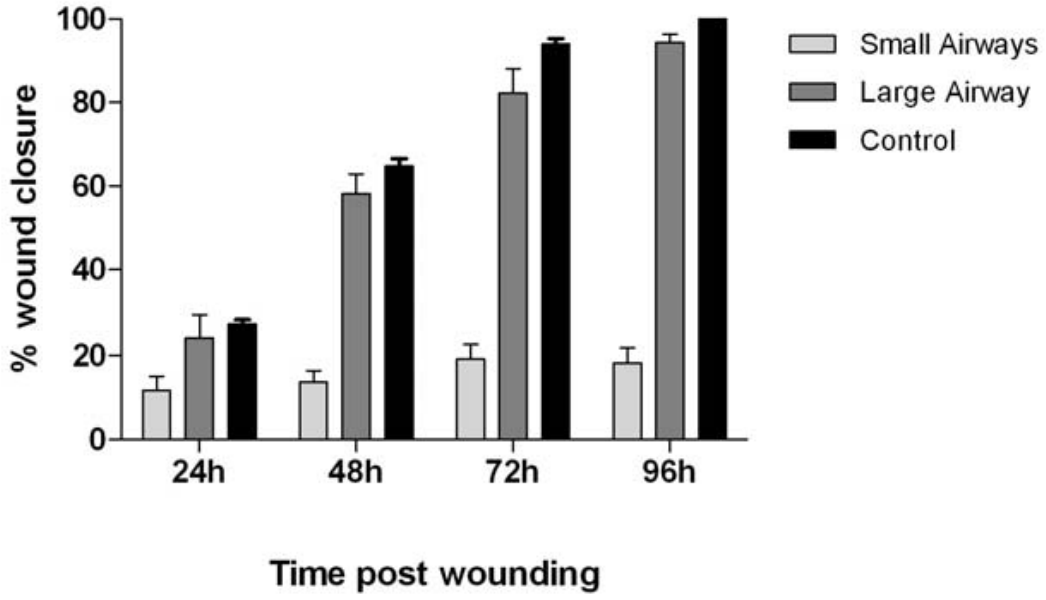


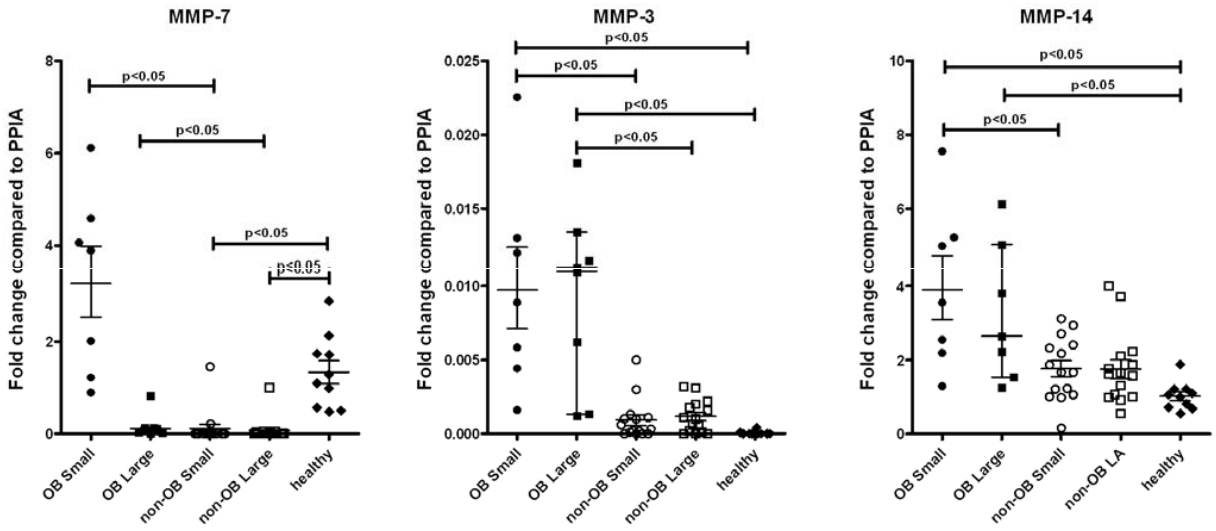
Figure 2

The researchers are currently extending these exciting observations and are aiming to identify why these cells are unable to repair.

However, these results remain the first to functionally show an abnormal repair capacity in transplant airway epithelial cells.

In addition, the team has also been able to look into the expression of a number of markers that corroborate these above findings (Fig. 3, overpage). The researchers have been able to separate those patients with OB to those without, and then separate out any difference observed between the small and large airway.

Preliminary results generated, have found that there is indeed abnormally low expression of the markers needed for normal repair (MMP-7, MMP-3, MMP-14, b-catenin) and high expression of particular markers are implicated with chronic injury and abnormal repair (integrin-b6, integrin-b8).



The team is currently validating all these initial observations. Once validated the next aim is to try and identify the factors regulating repair in these cells and how this translates to the functional observations the research team makes.

Ultimately, the team will endeavour to see if the effects can be reversed, namely through azithromycin addition.

Research Sharing

A number of public presentations on the research have been conducted acknowledging the McCusker Charitable Foundation's support including:

- Thoracic Society of Australia and New Zealand (TSANZ), 20012.

  Banerjee, B., Musk, M., Hopkins, P., Stick SM., Chambers DC., Kicic, A., (2012). Direct evidence of chronic epithelial injury and dysregulated repair in small and large airways of lung transplant patients. The Thoracic Society of Australia and New Zealand Annual Scientific Meeting. *Canberra, ACT. Australia.*

- International Society of Heart and Lung Transplantation Annual Meeting. (ISHLT), 20012.

  Banerjee, B., Musk, M., Hopkins, P., Stick SM., Chambers DC., Kicic, A., (2012). Direct evidence of chronic epithelial injury and dysregulated repair in lung allograft. International Society of Heart and Lung Transplantation Annual Scientific Meeting. *Prague, Czechoslovakia.*

The following manuscripts highlighting this research have been submitted for publication by the research team and acknowledge the McCusker Charitable Foundation for their financial assistance.

- Banerjee, B., Musk, M., Sutanto, E.N., Yerkovich, S.T., Hopkins, P.M.A., Knight, D.A., Lindsey-Temple, S., Stick, S.M., Chambers D.C., **Kicic A** (2012). Enhanced allograft small airway epithelial to mesenchymal transition is inhibited by azithromycin and mycophenolate. *American Journal of Transplantation.* (Revision submitted).

- Banerjee, B., Stick, S.M., Chambers D.C., **Kicic A** (2012). Models for studying the role of epithelial to mesenchymal transition (EMT) in bronchiolitis obliterans syndrome (BOS). *Journal of Transplantation.* (In preparation).