miR on the wall: Investigating a role for miR-155 in lung disease

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Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease with no known cause and poor prognosis. It causes scarring (fibrosis) and stiffening of the lungs, making it difficult to breathe and can lead to respiratory failure, heart failure and other complications. Very little is known about the mechanisms of disease, but extensive research has identified a central role for rogue immune responses which promote the production and laying down of proteins which promote scarring.

The immune system, which acts to protect us from infectious agents and aids tissue repair, is made up of a complex network of cells, cell products and body tissues. One type of immune system cell is the macrophage, which can adopt a variety of roles in response to different stimuli in the body. Macrophages mediate healthy inflammation in response to bacterial infection, but also prevent excessive immune responses, by promoting wound healing and the remodelling (reconstruction) of damaged lung tissue. In IPF, macrophages have a pronounced remodelling ability and contribute to the excessive wound healing and scarring observed in this disease.

The DNA in our cells provides the instructions to make proteins and which genes (particular sequences of DNA coding for a particular protein) are actively expressed in which cells, to make which proteins, and in what amounts is tightly regulated. Some of our DNA encodes for short sections of RNA called microRNA (miR) which play a role in regulating the expression of other genes, and hence proteins, rather than coding for actual proteins.

A particular miR, called miR-155, is abundant in macrophages and is a key promoter of inflammatory responses by these cells. Recently, a new role for miR-155 in the prevention of tissue remodelling was identified. In an animal model, which induces fibrosis of the lung, mice lacking miR-155 developed more severe fibrosis than those with miR-155, indicating a protective role for miR-155 in fibrosis. Interestingly, macrophages from IPF patients have been shown to express decreased miR-155 and increased expression of genes characteristic of macrophages in a fibrotic state compared to healthy controls (see Figure 1). Better understanding of the role of miR-155 in macrophage-mediated fibrosis is of great importance.

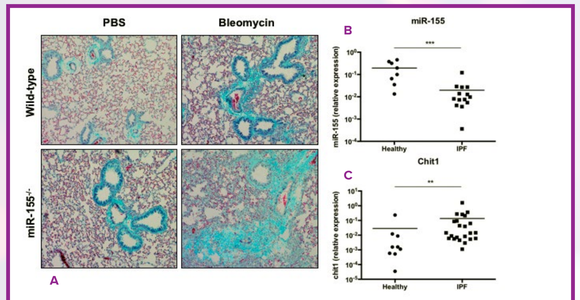

Figure 1 (A) Mice deficient in miR-155 (miR-155 -/-) have exacerbated disease (blue staining) in a model of lung fibrosis compared to control mice (WT). Bleomycin indicates mice that had received treatment to induce lung fibrosis, PBS was the control treatment. Macrophages from IPF patients have (B) lower miR-155 expression and (C) increased Chit1, a marker of fibrosis, than healthy controls.

What am I interested in?

While miR-155 has been well-characterised in the promotion of inflammation, its potential role in the regulation of lung fibrosis is less well-defined. I wanted to investigate the molecular pathways through which miR-155 acts in the regulation of remodelling in the lungs and identify whether dysregulated miR-155 might be used as a biomarker of IPF and potentially as a therapeutic target. In particular, I wanted:

1. To determine whether modulating miR-155 expression has an effect on the ability of macrophages to respond to pro-fibrotic stimuli;
2. To determine the characteristics of macrophages treated with miR-155 mimic;
3. To investigate whether molecular pathways associated with remodelling are affected by overexpression of miR-155 in macrophages.

What did I do?

I set up a model in the lab that represented the lung microenvironment. I isolated macrophages from human blood samples and grew them in the presence of particular chemicals which are found at high levels in the lungs. miR-155 was artificially overexpressed in these macrophages via delivery of a miR-155 mimic into the cells. After incubating these miR-155-treated macrophages for 72 hours in the presence of pro-fibrotic stimuli, I used a number of techniques to analyse any changes in pro-fibrotic mediators and gene expression in these cells. I looked for the presence and quantity of particular proteins and I looked at the level of expression of particular genes.

What did I find?

Pre-treating macrophages with miR-155 mimic profoundly altered the ability of these cells to respond to pro-fibrotic stimuli. Macrophages in which miR-155 was overexpressed produced significantly lower levels of two pro-fibrotic proteins which are highly associated with disease progression in IPF, namely TGF-β1 and CCL18 (see Figure 2). Analysis of expression of proteins on the surface of these cells showed that treatment with miR-155 mimic significantly reduced expression of two markers commonly used to characterise a fibrotic macrophage, namely CD206 and CD163 (see Figure 3). Interestingly, in this cell culture system, overloading of macrophages with miR-155 did not elicit an unwanted, spontaneous pro-inflammatory response. However, when these cells were further activated with an inflammatory stimulus, miR-155-treated macrophages produced increased levels of a pro-inflammatory marker and decreased levels of an anti-inflammatory marker, further indicating that these cells had shifted away from a fibrotic response (data not shown).

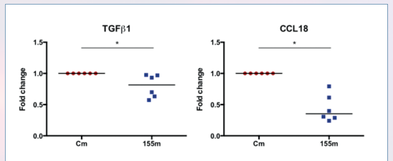

Figure 2 Treatment with miR-155 mimic significantly reduces gene expression of pro-fibrotic proteins, TGF-β1 and CCL18, compared to a control group (Cm).

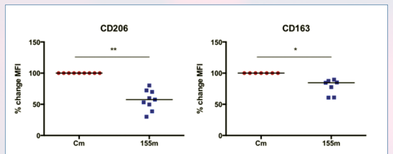

Figure 3 Macrophages treated with miR-155 mimic have reduced expression of remodelling-associated proteins, CD206 and CD163. % change MFI is a measure of the amount of protein expressed on the cell surface, as determined by a technique called flow cytometry.

What does it mean?

My findings may shed much needed light on the contribution of macrophages to the excessive tissue remodelling seen in IPF. The data suggests that miR-155 plays a crucial role in regulating how macrophages respond to the pro-fibrotic microenvironment of the lungs. Delivery of miR-155 mimic prevents macrophages from producing fibrotic mediators which are known to promote production of scar tissue in the lungs, hence, miR-155 may be a candidate therapeutic avenue for IPF. miRNAs reduce expression of their targets by preventing the translation of the target mRNA (the intermediary coding molecule, between a gene's DNA sequence and its protein product) into protein. Therefore, treating macrophages with miR-155 mimic may reduce the expression of the pro-fibrotic proteins in the lungs. Further elucidation of the proteins and pathways which miR-155 regulates may open up alternative therapeutic strategies.

Who am I?

I am in the final year of my PhD at the University of Glasgow, which I began in 2013 after completing my Honours degree in Biochemistry and Immunology at Trinity College Dublin. I have always had a strong interest in inflammation and how the immune system responds to stress and infection. In the future, I hope to continue my research career in the field of autoimmunity (i.e. when rather than the immune system protecting us from infectious agents, the body's immune system attacks itself).

I am grateful to the University of Glasgow and to Medical Research Scotland for their ongoing support. I would also like to thank Dr Mariola Kurowska-Stolarska and Professor Iain McInnes for their guidance and supervision during the course of this project.

Brian started his PhD project entitled *"miR-155 is a master switch controlling the balance between inflammation and fibrosis in chronic disorders"* in 2013 and, while principally, based at the University of Glasgow, supervised by Professor Iain McInnes, he is also working closely with an industrial collaborator.