THE UNIVERSITY OF NEWCASTLE AUSTRALIA

School of Biomedical Sciences and Pharmacy

Examples of current projects being undertaken by UON Biomedical and Pharmacy researchers and HDR candidates.

Characterizing the impact miR-17-92, 150 or 155 in myofibroblast function and phenotype.

When fibrosis occurs in the major organs such as the lung, for example in idiopathic pulmonary fibrosis (IPF), it inevitably leads to organ failure and premature death of the afflicted individual. The development of fibrosis follows a similar pathway to normal wound healing, although there is chronic progression of the disease without resolution, suggesting the fine control of cellular functions that occur during wound healing is disturbed. Determining where this control is lost is paramount to preventing and treating this condition. To this end, we have shown that dysregulated activation the transcription factor STAT3 and aberrant expression of specific integrins characterize functionally different subsets of fibroblasts, the presence of which correlates with IPF progression. The question of what drives these phenotypically divergent cells and prolongs their lifespan remains unanswered. Our overarching hypothesis is that region-specific cues from the local microenvironment promote the development and survival of a specific population of lung fibroblasts that are critical for IPF pathology.

miRNAs are emerging as master regulators of differentiation and play a prominent role in a variety of diseases. Target prediction and validation for miRNA's is often problematic due to the number of potential mRNA targets that single prediction algorithms identify. To circumvent this, we firstly evaluated the literature and then compared results from three algorithms in the public domain miR-base v15 (http://www.mirbase.org/), Targetscan 4.2 (http://www.targetscan.org) and Pictar (http://pictar.mdc-berlin.de/).

In the initial studies in this aim, we are choosing to focus on 3 miRNA's that are involved in key facets of fibroblast biology and likely also prominently involved in the IPF. These miRNAs are miR-17-92, 150 and 155.

To understand the biology of these miRNA's in fibroblast differentiation and senescence, we will take a 2-pronged approach. Firstly, we will perform a time-course analysis of miRNA expression following exposure to activators of STAT3; IL-6, OSM and PDGF. To identify the temporal expression and role of these candidate miRNA's during fibroblast differentiation in vitro, we will analyse miRNA levels at strategic points ranging from 1-14 days in culture. Responses will be compared to hydrogen peroxide. Total RNA including miRNA will be isolated and changes in miRNA expression will be quantified by qRT-PCR using TaqMan Gene Expression Assays for the respective miRNAs. To investigate the effects of miR-17-92, 150 or 155 on fibroblasts, we will transfect primary cultures with either mature miRNA-specific LNA- modified antagomirs or their scrambled controls, at day 1. Initial studies will use Cy-3 or cy-5 labelled antagomirs to confirm uptake by cells and outcome measures be validated by qPCR analysis and immunohisto chemistry.

OVER 90%

OF OUR RESEARCH IS 'AT OR ABOVE WORLD STANDARD'

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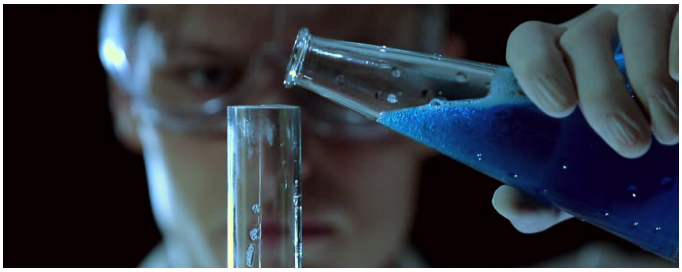

Indices of proliferation and differentiation will be assessed. To investigate whether gain-of-function of miR-17-92, 150 or 155 is sufficient to modulate the myofibroblast phenotype, we will overexpress these miRNAs using specific miRNA mimics. These are chemically synthesized, double-stranded RNAs which mimic mature endogenous miRNAs after transfection. For initial experiments we will introduce the mimics transiently into fibroblasts on day 1, 3 or day 7 and evaluate indices of proliferation or differentiation respectively.

Background:

Respiratory viruses are now emerging as important initiators of asthma, with up to 50% of cases of recurrent virus-induced wheezing in infancy developing asthma. In addition, repeated rhinovirus (HRV) infections during the first 2-3 yrs of life increases the risk of developing asthma between 4- and 26-fold, compared to 3-fold for allergen sensitization. Recent evidence shows that children admitted to hospital with pandemic H1N1 influenza were significantly more likely to have asthma, suggesting they also exacerbate existing disease. Collectively, these data indicate that children with asthma are at increased risk of infection and adverse outcomes from viral infections. Importantly, the primary target for respiratory viruses is the airway epithelium. We and others have shown that the asthmatic epithelium is abnormal and responds inappropriately to viral infections. It is more easily infected and has blunted production of anti-viral Interferons (β, λ). Understanding why the airway epithelium of asthmatics is so susceptible to virus infection is fundamental to developing therapeutic strategies against a myriad of respiratory diseases. We believe that proper epithelial wound healing is key.

We have shown that the epithelium of asthmatic subjects contains a greater proportion of resident stem cells, basal cells and less ciliated cells; i.e. it appears phenotypically immature. Importantly, this phenotype is maintained in vitro and correlates with aberrant responses to inflammatory stimuli as well as dysregulated differentiation, proliferation and wound healing.

Recent data suggests that endogenous small RNA's called microRNAs (miRNA) may play key roles in cellular differentiation and innate immunity. In endothelial cells, Kaposi's sarcoma-associated herpesvirus increases expression of miR-132 which then inhibits the expression of Interferon-inducible (anti-viral) proteins. Respiratory viruses such as rhinovirus and influenza are likely to do the same.

We hypothesize that respiratory viruses (HRV/Influenza in particular) interfere with specific miRNAs involved in epithelial differentiation and immunity. This denies proper regeneration and formation of an effective immune barrier but provides an environment favoring further viral replication. We further suggest that by restoring effective epithelial regeneration, efficient anti-viral defenses will develop

The student/s will work with faculty member and other research staff on this project which is part of a larger program investigating the mechanisms and consequences of aberrant repair in the lung. For this project, the student will be involved with establishing and maintaining primary cultures of human airway epithelial cells obtained from healthy donors as well as patients with asthma. He/she will also participate in designing experiments to investigate a number of cell functions. In this regard, he/she will be involved in fixing and staining of cells for microscopic analysis. The student will also learn more complex cell biology techniques such as transfection with reporter constructs and quantitative real time polymerase chain reaction. The student will be actively involved in tabulating, analyzing and interpreting the data generated. He/she will prepare the data for presentation at regular research in progress meetings and will have the opportunity to present significant research findings at appropriate national/international forums.

Title: Mechanisms controlling mucin production in chronic respiratory disease

Overview: Although a number of key proteins responsible for the regulation of mucin production are known, the extent of crosstalk between these proteins to control 'healthy' mucin production is not currently understood. Furthermore, how these pathways become dysregulated in diseases such as asthma and COPD remains a mystery. We have recently begun investigating two main regulatory pathways involved in mucus production. The first is the Notch signalling pathway which is well documented as a key regulator of epithelial architecture. The second is the Wnt/β-Catenin pathway which plays a role in regulating mucus production at the epithelium of mouse airways. The Jagged ligand (JAG1), responsible for Notch activation, has recently been shown to regulate epithelial cell architecture in the mouse airway, perturbation of which results in limiting goblet cell metaplasia. Interestingly, the Wnt/β-Catenin pathway has been shown to control expression of JAG1 in a range of tissues. Notch/JAG1 and β-catenin have independently been shown to control mucin production within the mouse airway, however equivalent studies are yet to be performed in relevant human tissues/cells. Furthermore, no studies currently link mucin production to Notch and Wnt/β-catenin crosstalk. The logical next step is to investigate key molecules within the Notch and Wnt/β-catenin signalling pathways of human AECs in order to elucidate the mechanisms behind mucin production, and finally how these differ in patients with chronic respiratory disease.

Hypothesis and Aims:

The Notch signalling pathway regulates mucin production within human AECs and inhibition of this pathway will result in a downregulation of mucin production. Furthermore, JAG1 production is dependent on activity of Wnt/β-Catenin signalling and as such, abrogating β-Catenin will limit downstream expression ofmucins via a Notch-mediated signalling pathway. In order to determine the underlying mechanisms of Notch signalling on mucin production within AECs we aim to firstly characterise the downstream effector molecules that influence expression of mucin genes in 'healthy' AECs. Secondly, this characterisation will be extended to include AECs from patients suffering from chronic asthma as well as COPD. Additionally, via pharmacological intervention as well as siRNA experiments exploiting the underlying mechanisms of mucin production within these patient populations we aim to identify novel therapeutic targets and as such modulate mucin overproduction.