ImmunoTools *special* Award 2015

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Investigating the Effect of Pro-fibrotic Mediators on the Expression Phosphorylation and SUMOylation Status of the Transcription Factor Elk1

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrotic lung disease. The survival rates are poor, with five-year survival rates worse than the majority of cancers, and currently there are limited treatments options available. IPF is characterised by excessive extracellular matrix (ECM) production and deposition within the airspaces (fibrosis), leading to rapidly deteriorating lung function and ultimately death. The pathogenesis of IPF is poorly understood and increased understanding of the mechanisms driving fibrosis is critical if new and effective treatments are to be developed.

Elk1 is a member of the ternary complex factor (TCF) subfamily of transcription factors. It is commonly thought of as a transcriptional activator but recent evidence suggests that it also has dynamic repressor functions, and is capable of limiting the transcription of target genes. The C-terminal domain of Elk1 contains several phosphorylation sites, which are phosphorylated by the MAP kinases ERK1/2. Phosphorylation of these sites enables Elk1 to activate transcription. Conversely, the R-motif of Elk1 contains two SUMOylation sites, which when SUMOylated enables Elk1 to repress transcription. Phosphorylation of Elk1 by ERK1/2 causes the SUMO groups to dissociate from the R domain, effectively “switching off” the repressor functions while activating the C-terminal domain.

αvβ6 integrins are fundamental to the development and progression of IPF, making them an attractive therapeutic target in the treatment of IPF. Recently I have obtained preliminary data suggesting a novel and fundamental role for Elk1 in repressing the β6 subunit of the αvβ6 integrin (ITGB6) in the lung, leading to repression of αvβ6 expression. Using both *in vitro* and *in vivo* methods my preliminary data suggests that loss of Elk1 leads to enhanced expression of αvβ6 integrins and exaggerated pulmonary fibrosis in response to lung injury. Furthermore, using human lung tissue

from IPF patients and non-fibrotic controls, I have shown for the very first time that expression of Elk1 is reduced in IPF, which may help explain the dramatic increase in expression of αvβ6 integrins frequently observed in IPF.

The mechanisms responsible for decreased Elk1 expression in Elk1 are currently unclear but an area of active research for me. It is possible that the ongoing fibrotic environment within the lungs of IPF patients drives the reduction in Elk1, leading to enhanced αvβ6 expression and potentiate the fibrotic response. To investigate this hypothesis I am investigating the effect of a variety of pro-fibrotic mediators thought to be involved in the pathogenesis of IPF on both the expression of, and the phosphorylation of, Elk1, in both lung epithelial cells and fibroblasts. Cells isolated from human IPF and non-fibrotic lung, and from murine lung tissue obtained following an in vivo model of lung fibrosis, are utilised. I am using a variety of recombinant proteins from ImmunoTools (including Fibroblast growth factor, Interleukin 1β, VEGF, among others) to stimulate the cells. I am then investigating the effects on Elk1 expression using a combination of western blotting to assess Elk1 protein expression, and quantitative Polymerase chain reaction to assess Elk1 mRNA expression, over various time points. Additionally, I am assessing the effect of the panel of pro-fibrotic mediated on the phosphorylation of Elk1 by western blotting using a phospho-specific Elk1 antibody.

The effect of pro-fibrotic mediators on Elk1 expression and phosphorylation has not previously been investigated. These studies will therefore shed light on the potential mechanisms driving decreased expression of Elk1 in IPF, and potentially highlight new targets for the development of novel, efficacious therapies for this devastating disease.