Title: Epidermal growth factor receptor (EGFR) activation is required for TGFβ1-induced epithelial-mesenchymal transition (EMT) in idiopathic pulmonary fibrosis (IPF)

Dr. Hoda 33477 Tawel mhxst@nottingham.ac.uk MD , Dr. William 33495 Coward mszwxrc@exmail.nottingham.ac.uk, Dr. Karl 33496 Deacon Mszkd2@exmail.nottingham.ac.uk and Prof. Linhua 33497 Pang mszlp@exmail.nottingham.ac.uk. 1 Respiratory Biomedical Research Unit / Clinical Sciences Building, University of Nottingham / Nottingham City Hospital, Nottingham, United Kingdom, NG5 1PB.

Body: Introduction: One of the possible sources of myofibroblasts accumulation in IPF is the trans-differentiation of the injured alveolar epithelial cells to myofibroblasts through EMT process. Studies so far suggest that TGFβ1 and EGFR signalling cascades are activated during lung fibrosis. We hypothesized that TGFb1 signalling requires EGFR activation to induce EMT during lung fibrosis. Material and methods: To address the role of EGFR cascades in TGFβ1-induced EMT, immortalized human bronchial epithelial cells (iHBECs) were treated with or without TGFβ1 (2ng/ml), different concentrations of EGF and EGFR tyrosine kinase inhibitor (AG1478) for 3 days. Expression of the epithelial marker E-cadherin and the mesenchymal marker α-smooth muscle actin (αSMA) was examined by flow cytometry, Western blot and, immunocytochemistry staining. EGFR and Smad2 phosphorylation was measured by Western blot. Results: Stimulation of iHBECs with TGFβ1 down-regulated E-cadherin expression and increased αSMA expression. Treatment of the iHBECs with EGF also induced EMT in a concentration-dependent manner and potentiated TGFβ1-induced EMT. Inhibition of EGFR with 2µM AG1478 reduced TGFβ1-induced Smad2 phosphorylation and EMT. TGFβ1 stimulation also induced EGFR expression and phosphorylation which was prevented by AG1478. The data suggests an important role of EGFR activation in TGFβ1-induced EMT.Conclusions: The study has demonstrated that iHBECs undergo EMT in response to TGFβ1 stimulation signified by E-cadherin down-regulation and expression of αSMA and that EGFR signalling is required in TGFβ1-induced EMT.