(using pHrodo method) and ROS content (using H2DCFDA assays) ex vivo were also examined.

Results and discussion Circulating monocyte levels were significantly higher in IPF compared to healthy controls ($p < 0.001$) and correlated negatively with lung function (FVC $r = -0.6$, $p = 0.003$) and CT fibrosis score ($r = 0.45$, $p = 0.007$). IPF monocytes displayed higher M2:M1 ratio profile compared to healthy controls – with higher *IL10, CD163, IL1R2, FGL2* and lower *TNFa and CXCL10* gene expression. When these monocytes were differentiated to macrophages (MDMs) ex vivo, IPF macrophages showed a significantly higher level of M2 markers and CD14 expression, reduced phagocytosis and produce lower levels of ROS –supporting M2 and pro-repair phenotype.

Conclusions Our data show that circulating monocytes in IPF are elevated compared to age-matched controls, correlate positively with disease severity, and are different from those found in age-matched healthy controls. They have pro-repair, M2-like features and differentiate to pro-repair monocyte-derived macrophages and may contribute to the aberrant repair process in IPF.

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S51 MTOR REGULATES TGF-β INDUCED PRO-FIBROTIC GENE EXPRESSION IN PRIMARY HUMAN LUNG FIBROBLASTS

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Introduction TGF-β is a major pro-fibrotic cytokine with a critical role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). TGF-β drives fibroblast to myofibroblast differentiation and extracellular matrix synthesis. mTOR plays a critical role in regulating protein translation and is the catalytic subunit of two functionally distinct complexes, mTORC1 and mTORC2, which have differential sensitivities to rapamycin. The aim of this study was to delineate mTOR signalling in response to TGF-β in human lung fibroblasts and investigate the role of the mTOR pathway in TGF-β mediated myofibroblast differentiation and collagen synthesis.

Methods All human samples were obtained with informed, signed consent and with research ethics committee approval. Primary human lung fibroblasts (pHLFs) were grown from explant cultures. Cells were pre-incubated with varying concentrations of inhibitor before stimulation with TGF-β 1ng/ml. Collagen biosynthesis and αSMA expression were measured by a high-content imaging based molecular crowding assay. Gene expression was measured by qPCR. Western blots were performed to assess mTOR substrate phosphorylation.

Results TGF-β was found to stimulate the delayed and sustained induction of mTOR signalling in pHLFs and this signalling pathway was critical for mediating the late peak in TGF-β induced pro-fibrotic gene expression. Accordingly, active-site mTOR inhibition exerted pronounced inhibitory effects on pHLF collagen biosynthesis and myofibroblast differentiation. The induction of mTOR signalling in response to TGF-β was dependent on the canonical Smad pathway. In addition, potent and selective pharmacological agents demonstrated that TGF-β induced mTOR signalling was independent of PI3K/Akt activity, suggesting that mTOR is not activated through the prototypical linear PI3K/Akt axis downstream of TGF-β. Moreover, rapamycin-resistant mTOR signalling was found to be critical for TGF-β induced pro-fibrotic gene expression in pHLFs.

Conclusion mTOR is a an important pro-fibrotic signalling node downstream of TGF-β and a potential target for therapeutic intervention in IPF.

S52 SUBERANILOHYDROXAMIC ACID (SAHA) INHIBITS COLLAGEN DEPOSITION IN A TRANSFORMING GROWTH FACTOR β1-DRIVEN PRECISION CUT LUNG SLICE (PCLS) MODEL OF PULMONARY FIBROSIS

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Introduction and objectives Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive interstitial lung disease that is refractory to current treatment options. Transforming growth factor (TGF)-β1 is a key pro-fibrotic cytokine that plays a crucial role in IPF pathogenesis. Our group previously demonstrated distinct epigenetic modifications involved in repression of the antifibrotic gene cyclooxygenase-2 (COX-2) in fibroblasts from IPF (F-IPF) lungs compared with fibroblasts from non-fibrotic lungs (F-NL). Epigenetic drugs capable of inhibiting DNA and histone modifications may, therefore, represent a putative novel therapy. The aim of this study was to investigate the ability of 4 epigenetic inhibitors to regulate TGF-β-driven fibrosis in *ex vivo* mouse lung.

Methods A precision-cut lung slice (PCLS) model of fibrosis was established using the previously described[1] CC10-tTS-rtTA-TGFβ$_1$ transgenic (tgTGF-β$_1$) mouse. The model was first assessed by investigating PCLS overexpression of TGF-β$_1$ in response to stimulation of the transgene by doxycycline treatment. Gene expression of COX-2 and fibrotic markers including collagen were assessed after 4 days of treatment. The anti-fibrotic potential of 4 epigenetic inhibitors; BIX01294 (BIX, inhibitor of G9a histone methyltransferase), 3-deazaneplanocin A (DZNep, inhibitor of EZH2 histone methyltransferase), SAHA (inhibitor of histone deacetylases, HDACs) and Decitabine (DAC, DNA demethylating agent) was investigated. Viability of PCLS was assessed by MTT and Prestoblue® assay.

Results Treatment of PCLS from tgTGF-β$_1$ mice with doxycycline induced a concentration-dependent increase in global TGF-β$_1$, pro-fibrotic markers including collagen and pro-inflammatory COX-2, which was comparable to recombinant TGF-β$_1$ treatment. Treatment with three of the epigenetic inhibitors BIX01294, DZNep and DAC did not reduce the pro-fibrotic response following doxycycline treatment. However SAHA demonstrated a significant suppressive effect on COX-2 and collagen expression, while not directly affecting TGF-β$_1$ transgene expression.

Conclusions The data suggests that SAHA has the potential to reduce fibrosis in a TGF-β$_1$ driven model of pulmonary fibrosis. Further work is currently underway to assess the anti-fibrotic potential of this drug in tgTGF-β$_1$ animals.

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