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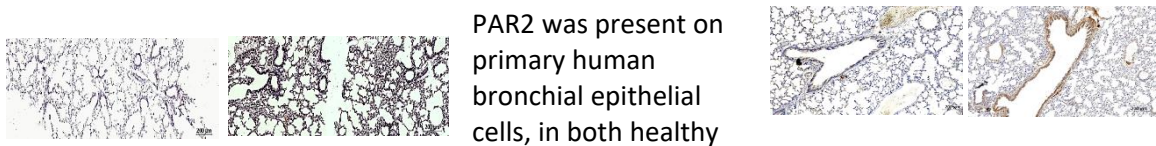
Proteinase activated receptor-2 induced autophagy dysregulation

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Lungs from patients with chronic obstructive pulmonary disease (COPD) display hallmarks of premature ageing including reduced autophagy, contributing to cellular senescence. The mechanisms underlying dysregulated lung autophagy remain unclear and under researched. While proteinase activated receptor-2 (PAR2) is a potential therapeutic target for inflammatory conditions, with documented roles in lung pathology, a role for this receptor in lung ageing is yet unexplored.

To investigate this, primary human bronchial epithelial cells from healthy (HBEC) and COPD patient donors (DHBE) were stimulated with PAR2 activators (SLIGKV, FLYGRL, trypsin) and inhibitors and autophagic flux quantified through fluorescent imaging and FACS analysis of an autophagosomal marker (CYTO-ID detection kit). Western blotting was used to analyse expression of autophagy-related genes to confirm findings. Parallel experimentation in human epithelial cell lines (A549 and BEAS-2B) provided supporting data, with immunohistochemistry (IHC) used to determine expression of autophagy markers, LC3 and ATG7, in PAR2 knock out murine tissue. PAR2 expression was assessed by immunofluorescence (IF).



PAR2 was present on primary human bronchial epithelial cells, in both healthy

and COPD patient donors and epithelial cell lines. Autophagic vesicles were successfully detected and modulated by appropriate autophagy control stimuli. PAR2 expression, assessed alone or in combination with activating synthetic peptide, resulted in reduction in autophagic flux within airway epithelial cultures. Further,

immunohistochemical analysis of ATG7 (n=3, P= ≤0.005) and LC3 (n=6, P=0.05) in PAR2 knock out murine lung indicated an involvement of PAR2 in regulating autophagy, as both markers were significantly upregulated (Figure 1).

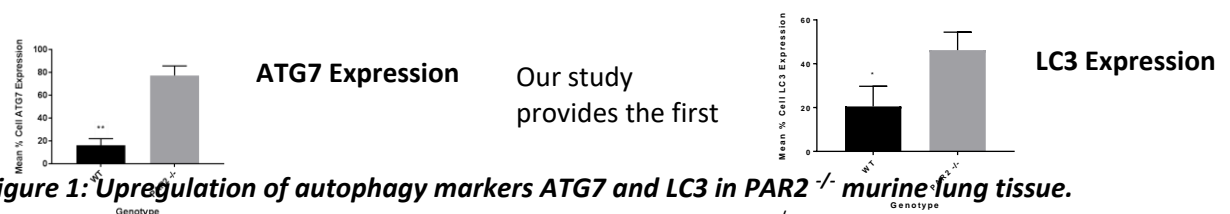


Figure 1: Upregulation of autophagy markers ATG7 and LC3 in PAR2^{-/-} murine lung tissue.

Autophagic marker expression was significantly increased in PAR2^{-/-} murine lung tissue. LC3 was significantly upregulated specifically in the airways, with ATG7 upregulated in the lung parenchyma.

data suggesting a role for PAR2 in the regulation of autophagy in lung airway epithelia, indicating a possible novel and targetable pathological mechanism underlying conditions such as COPD.