WNT/β-catenin signalling is upregulated in the COPD bronchial epithelium

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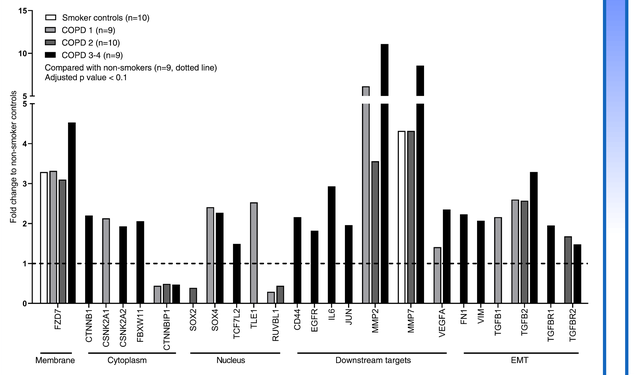
Introduction. Chronic obstructive pulmonary disease (COPD) is a devastating and irreversible disorderof the airways and alveoli of the lungs, representing the third cause of mortality worldwide. Although developmental pathways, particularly WNT, have been demonstrated to participate to the development of distal features of COPD (emphysema), mechanisms driving the aberrant phenotype of COPD at the airway epithelium (AE) level remain elusive.

Hypothesis. The canonical WNT pathway is dysregulated in the COPD AE and contributes to its phenotype.

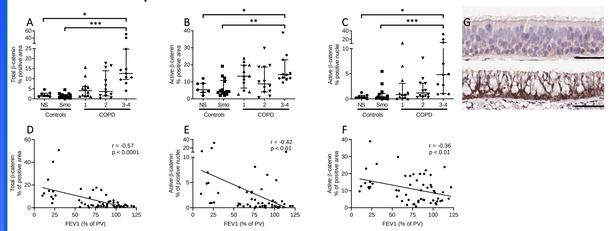
Methods. We performed a WNT-targeted RNA sequencing (RNA-seq) of 100 canonical WNT-related genes in air/liquid interface-reconstituted (ALI-)AE from 47 patients (9 non-smoker controls, 10 smoker controls, 9 mild COPD, 10 moderate COPD and 9 very severe COPD). Quantification of active and total β-catenin was performed in immunostained proximal AE (n=56). In addition, WNT pathway was *in vitro* activated or inhibited in ALI-AE from 6 COPD patients, respectively using CHIR99021 or XAV939 to assess its role in broncho-epithelial differentiation.

WNT targeted RNA-sequencing

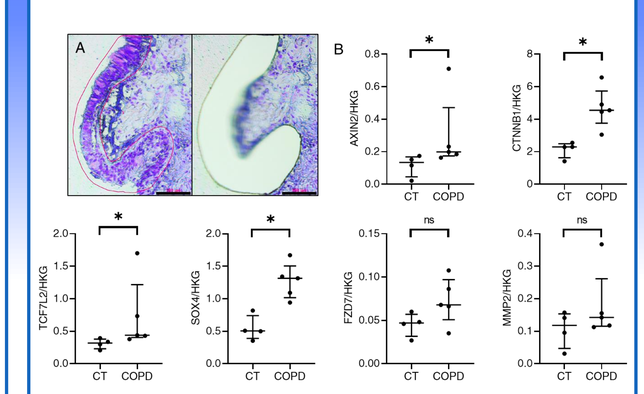


WNT/β-catenin pathway components are upregulated in COPD epithelium. This graph shows the significantly modified genes in targeted RNA-seq (adjusted p-value < 0.1), as compared with non-smoker controls (dotted line). Genes are shown according to their localisation in the WNT cascade and depict a global WNT activation in COPD that is more pronounced in very severe disease.

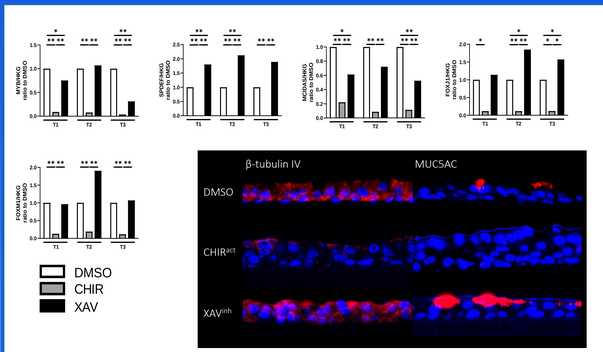
Active and total β-catenin



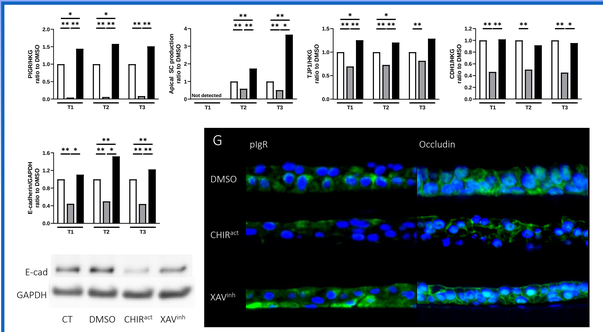
Total, active and intranuclear β-catenin staining is increased in the COPD bronchial epithelium. Total (A), active (B), and nuclear (C) β-catenin staining were increased in the airways from severe COPD patients compared with non-smokers and smokers controls. A significant correlation between total (D), active (E) and nuclear (F) β-catenin, and disease severity evaluated by the FEV1 (% of predicted values, PV) was also found (D). G, typical picture of control and very severe COPD epithelium (G, upper and lower panels, respectively).



Components of WNT pathway components are increased in the COPD epithelium. Using laser microdissection of severe COPD (n=5) and non smokers (n=4), as depicted in A, we show an increased gene expression of AXIN2, CTNNB1, TCF7L2 and SOX4 in the COPD airway epithelium, all being components of the canonical WNT pathway. No difference was found for FZD7 and MMP2.

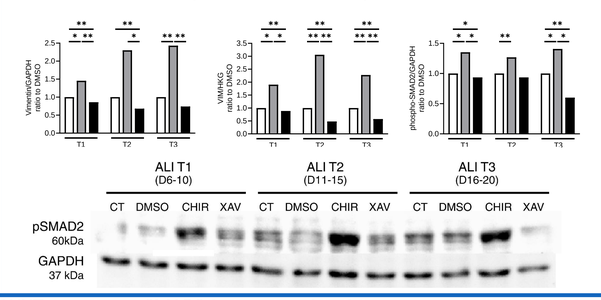


WNT activation alters epithelial differentiation. *In vitro* WNT activation and inhibition on COPD-deriver ALI-AE (n=6) was applied using CHIR99021 and XAV939, respectively. WNT activation disrupted the differentiation towards predifferentiated (MYB), goblet (SPDEF, MUC5AC), ciliated (MCIDAS, FOXJ1, β-tubulin) and club cells (FOXM1). WNT inhibition, conversely, induced commitment towards goblet and ciliated cells, partially reversing COPD epithelial phenotype.



WNT activation alters epithelial polarization. WNT activation (CHIR 99021) decreased the gene and protein expression of tight- and adherens junctions components TJP1, CDH1 (E-cadherin) and occludin. It also repressed the expression of PIGR and of its secretory component (SC) release, witnessing both impaired epithelial barrier function and polarization. WNT inhibition improved CDH1, TJP and PIGR expression.

WNT modulation and epithelial-to-mesenchymal transition



WNT regulates SMAD2-induced epithelial-to-mesenchymal transition (EMT). WNT activation and inhibition respectively increased and decreased vimentin and phospho-SMAD2 expression, suggesting that WNT plays a role in TGF-β driven EMT in COPD. No difference could be obsereved regarding TGF-β protein as it was not detectable in cultures (not shown).

Conclusions. We here demonstrate, both *in situ* in surgical and microdissected tissue, and *in vitro* in ALI-cultures (RNA-sequencing), that the WNT/β-catenin pathway is upregulated in the COPD proximal epithelium, therefore contributing to the development and/or persistence of cardinal COPD features at this level. Thus, epithelial differentiation, polarity and barrier function were impaired upon WNT activation, while EMT features were induced by WNT activation, possibly through TGF-β signalling. Conversely, WNT inhibition reversed these abnormalities. These results suggest that the WNT canonical pathway could represent a target for future therapies in COPD, along with other less explored developmental pathways such as Notch and Sonic Hedgehog, maintaining hopes of reversing the disease course.

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