WNT5a/5b Signaling Represses Functional Responses in Lung Epithelial Progenitors

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Background: Chronic obstructive pulmonary disease (COPD) represents a worldwide concern with high morbidity and mortality, and is believed to be driven in part by accelerated ageing of the lung. Although several studies indicate a role for non-canonical WNT signaling, in particular of WNT-5A and WNT-5B, in ageing and COPD, the precise impact of non-canonical WNT signaling on lung repair remains poorly understood. We hypothesized that WNT-5A/5B might impact on alveolar epithelial repair. Methods: Lungs were harvested for precision-cut lung slices (PCLS) or the generation of lung organoids. PCLS were treated with recombinant WNT-5A or WNT-5B (500 ng/mL) or vehicle control. Lung organoids recapitulate various features of the lung providing an in vitro model system for studying regenerative mechanisms of adult lung epithelium. Lung organoids were established by co-culturing epithelial cells ($EpCAM^+/CD45^-/CD31^-$) with CCL206 fibroblasts in Matrigel. Results: The gene expression levels of the non-canonical ligands WNT-5A and WNT-5B were increased in whole lung tissue from aged WT mice and their expression correlated with the senescence marker p16. In PCLS derived from young WT mice, treatment with WNT-5A had no effect on gene expression of the type I and type II epithelial cell markers Aqp5 and Sftpc; interestingly, WNT-5B treatment significantly decreased gene expression of Aqp5 (mean difference = 36.35% ± 10.93%, compared to vehicle control, $p < 0.05$,) and Sftpc (mean difference = 46.25% ± 5.76%, compared to vehicle control, $p < 0.01$). In addition, WNT-5B significantly decreased expression of the canonical WNT target gene, Axin2 (mean difference = 83.13% ± 9.65%, compared to vehicle control, $p < 0.05$). The number of organoids visible 7 days after culture was significantly decreased by WNT-5A and WNT-5B ($p < 0.05$). WNT-5A significantly decreased the number of airway type organoids ($p < 0.01$), whereas WNT-5B selectively repressed alveolar type organoid formation ($p < 0.05$). Immunofluorescence studies confirmed that numbers of acetylated-α $tubulin^+$ organoids were significantly decreased by both WNT-5A and WNT-5B, however, numbers of SPC^+ organoids were significantly decreased by WNT-5B ($p < 0.05$). Neither WNT-5A nor WNT-5B stimulation affected the size of lung organoids measured on day -14. Conclusions: Non-canonical WNT signaling is correlated with ageing. The non-canonical WNT ligands WNT-5A and WNT-5B repress gene expression of alveolar epithelial cell markers in lung slices, and functionally inhibit lung organoid formation. We speculate that such a mechanism may contribute to defective alveolar repair in ageing and COPD.

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