CIGARETTE SMOKE-INDUCED PULMONARY INFLAMMATION BECOMES SYSTEMIC BY CIRCULATING EXTRACELLULAR VESICLES CONTAINING WNT5A AND INFLAMMATORY CYTOKINES

PhD Thesis

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INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD)

COPD is an irreversible condition of airflow limitation characterized by inflammation and impaired respiratory gas exchange. The prevalence of COPD is approximately 10% among individuals older than 40 years of age. Furthermore, based on recent statistics, COPD is the seventh and fourth highest cause of disability and death worldwide, respectively. The main cause of COPD is tobacco smoking, but other factors, such as air pollution, occupational hazards, and infections can also initiate the disease. Despite all the therapeutic efforts, COPD is irreversible and progressive in nature. Although COPD is recognized primarily as a lung disease, it has systemic manifestations and the comorbidities affect the heart, pancreas, skeletal muscle. To suppress systemic inflammation, glucocorticoid therapy is used to treat acute exacerbations of the disease. Apart from the limited efficacy, side effects occur including histone deacetylase regulated resistance to corticosteroids. To find a permanent cure for COPD, more research on drug target identification and disease pathomechanism is required.

The role of Wnt5a and PPAR gamma in the inflammatory processes of the lung

The Wnt signaling pathway plays important role in embryonic development, tissue homeostasis and regulates cell fate specification, cell migration, cell adhesion and cell polarization. WNT ligand family is composed of 19 distinct Wnt secreted glycoproteins in humans. WNT signaling is separated into canonical and non-canonical signaling. Wnt5a is one of the most implicated non-canonical Wnt molecule in carcinogenic and inflammatory processes.

Peroxisome proliferator-activated receptor (PPAR) - gamma was identified as a central molecule in inflammation. PPAR gamma, a member of the ligand-activated nuclear hormone receptor super family, controls gene transcription associated with lipid metabolism, adipogenesis, inflammation, and metabolic homeostasis, making PPAR gamma an emerging anti-inflammatory and anti-oxidative target gene. Regulation of PPAR gamma, however, is essentially Wnt signaling dependent. Mostly, if the canonical Wnt pathway is up-regulated, PPAR gamma is down-regulated. PPAR gamma is also a non-canonical Wnt pathway target. Wnt5a is also a strong pro-inflammatory molecule that has been reported as a potent activator of cigarette smoke-induced inflammatory cytokine expression. Cigarette smoke (CS) is also an initial trigger of macrophage activation and macrophage polarization toward the classical M1 and the alternative M2 lineages. The anti-inflammatory activity of PPAR gamma is associated with inflammatory disorders of the lung leading to the hypothesis that treatment with PPAR gamma agonists can suppress inflammation and reverse CS-induced emphysema. Macrophage

polarization is a multifactorial process and is associated with PPAR gamma in COPD. We hypothesized that although both canonical and non-canonical Wnt signaling regulate PPAR gamma, the non-canonical Wnt signaling pathway turns into dominant upon CS exposure. We also theorized that CS exposure induced local lung inflammation which becomes systemic *via* delivery of inflammatory messages in extracellular vesicles.

The role of Wnt signaling in the lung regeneration

Mechanical injury of the alveoli induced by ventilation or loss of gas exchange surface due to cigarette smoke or aging could both be treated if the process is understood and the appropriate molecular targets are identified for drug development.

The alveolar surface of the lung has a significant physiological regeneration capacity. Type II alveolar epithelial cells (ATII) have been suspected to act as progenitor cells in the alveoli and function as stem cells and show clonal proliferation in response to injury. The alveolar surface area is mostly covered by flat and thin Type I alveolar epithelial cells (ATI) that die by apoptosis upon injury. The cuboid ATII cells are more resistant to injury, they proliferate, migrate and spread over the basement membrane then transdifferentiate into ATI cells. Recent organ regeneration studies suggest that reactivation of developmental mechanisms occur during injury repair involving BMP, FGF, Notch and Wnt signaling pathways.

The Wnt/beta-catenin signaling is necessary for alveolar morphogenesis. Wnt ligands, such as Wnt5a, Wnt7b and most Frizzled (Fzd) receptors are crucial in the regulation of lung development. During aging deregulated Wnt ligand composition can alter alveolar epithelial differentiation and can give rise to modified molecular microenvironments that promote emphysema and other diseases. Based on the above, we hypothesized that Wnt signaling is necessary for the induction and regulation of ATII-to-ATI transdifferentiation (TD). To investigate our hypothesis, cellular transformation of primary human ATII cells was studied *in vitro* and data was compared to gene expression of ATI and ATII cells freshly isolated from primary human lung tissues. The effects of the identified Wnt ligands were tested in three-dimensional (3D) human lung aggregate cultures to confirm their roles in the TD process and in the regulation of their downstream targets recognized by artificial neural network (ANN) analysis.

AIMS OF THE STUDY

1. Does the cigarette smoke induce increased level of the non-canonical Wnt5a molecule and trigger inflammatory environment specific for COPD?
2. Is the elevated level of Wnt5a detected in COPD patients' sera which might explain the development of systemic inflammation?
3. Does the Wnt signaling pathway regulate pulmonary regeneration and epithelial TD processes, and if so, how?

MATERIALS AND METHODS

Materials

Cigarette smoke extract (CSE) was diluted in cell culture media to a final concentration of 0.5%. Recombinant human Wnt5a (rhWnt5a) protein was used at a concentration of 1 μg/ml. PPAR gamma agonist, rosiglitazone (RSG), and antagonist, GW9662 were used at 10 μM concentrations each.

Collection of human serum samples

Human blood samples were collected from the Division of Pulmonology, 1st Department of Internal Medicine, Medical School, University of Pecs. A total of 5 COPD patients and 5 age-matched control samples were collected. Blood samples were clotted for 30 min at room temperature. Samples were then centrifuged at 1,500 rpm for 10 min. Serum samples were stored at -80°C until used for further analysis.

Animals

CS Exposure of Mouse Lungs

C57BL/6 male mice were housed in the animal facility of the Department of Pharmacology and Pharmacotherapy at the University of Pecs. Mice were exposed to CS using 3R4F research cigarettes (College of Agriculture, University of Kentucky; Lexington, KY, USA) in a manual smoking system. The exposures were performed 2 × 30 min/day with two cigarettes per occasion.

Microvesicle/Exosome Homing Study

BALB/C mice were housed in the animal facility of the Department of Immunology and Biotechnology at the University of Pecs. Microvesicles/exosomes isolated from supernatants of Wnt5a-A549 cell lines were fluorescently labeled with DiI. Native and fluorescently labeled microvesicles/exosomes were injected into the tail veins of mice. Control mice were injected with non-fluorescent exosomes.

Mouse lung cell isolation

3 mice/group were anesthetized. Abdominal aorta was intersected, and mice were perfused through right ventricle. To initiate the fine digestion trypsin was applied and PBS was used to wash out trypsin thereafter. Pulmonary lobes were dissected into smaller pieces and digested in collagenase-dispase for 50 min with continuous stirring. Digested lung cells were filtered with 70 μm cell-strainer.

Cell sorting

Single cell suspensions of mouse lungs were labeled with anti-mouse CD45-FITC produced at the University of Pecs, Department of Immunology and Biotechnology and anti-mouse EpCAM1 (G8.8 anti-rat-PE). Cell sorting was performed in a FACSAria III cell sorter. The following populations were collected: $EpCAM^{-}CD45^{-}$, $EpCAM^{+}CD45^{-}$, $EpCAM^{+}CD45^{+}$, and $EpCAM^{+}CD45^{-}$. The purity of sorting was above 99%.

Preparation of cigarette smoke extract (CSE)

CS of two 3R4F research grade cigarettes were bubbled into RPMI using a peristaltic pump. CSE solution was filtered and considered as 100% stock and diluted with culture media to 0.5% for further experiments. The pH of each CSE solution was measured as a mean pH 7.28. The OD of the inner particles of the CSE was measured spectrophotometrically at the wavelength of 360 nm and compared to the control media.

Cell lines and primary cells

Human adenocarcinoma A549 cell line and transgenic Wnt5a overexpressing-A549 (Wnt5a-A549) cell line were cultured in complete Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS. Primary human small airway epithelial cells (SAEC), normal human lung fibroblast cells (NHLF) and normal human lung microvascular endothelial cells (HMVEC-L) were cultured in small airway epithelial cell growth medium (SAGM), fibroblast

growth medium, and endothelial growth medium. All the above cell cultures were incubated at 37°C in humidified atmosphere containing 5% CO2.

Isolation of primary human monocytes

Macrophages were obtained from healthy blood donors. Peripheral blood mononuclear cells (PBMCs) were isolated by standard density Ficoll-Paque gradient centrifugation. Positive selection of monocytes was performed by $CD14^+$ MACS colloidal superparamagnetic microbeads conjugated with monoclonal anti-human CD14 antibodies using MACS MultiStand, OctoMACS. Monocyte purity was regularly>85%. Macrophage differentiation was induced by incubation with 10 nM PMA, for 5 h, at 37°C in humidified atmosphere containing 5% CO_2.

Three-dimensional (3D) human lung aggregates

Three-dimensional lung aggregate tissues were generated from NHLF (40%), SAEC (30%) and HMVEC-L (30%) primary cells in the absence or presence of peripheral monocyte-derived macrophages. Cell suspensions were dispensed onto a low attachment 96-well U-bottom plate and were centrifuged at 600 *g* for 10 min. Tissue aggregates were maintained in 2:2:1 ratio of endothelial cell growth medium, SAGM, and fibroblasts growth medium. In the various experiments, aggregates were treated with 0.5% CSE, 1 μg/ml and 0,1ug/ml rhWnt5a, rhWnt4 and rhWnt7a for 48 h.

RNA isolation, cDNA synthesis and Quantitative RT-PCR

Total RNA was prepared using NucleoSpin RNAII kit with on-column DNase digestion. Random hexamer primers of the high capacity RNA to cDNA kit was applied. For cDNA synthesis 1 μg of total RNA was used. For gene expression analysis, quantitative RT-PCR was performed using SensiFAST SYBR Green reagent on ABI StepOne and StepOnePlus instruments. Data were analyzed using StepOne software and expression changes were normalized to beta-actin housekeeping gene.

TaqMan array

Total RNA was isolated from human monocytes. TaqMan master mix combined with the cDNA samples was loaded onto the Human WNT Pathway, Fast 96-well TaqMan Array plate. Gene expression was analyzed using ABI StepOnePlus instrument.

Immunohistochemistry and fluorescent staining

Deparaffinization of formalin-fixed paraffin-embedded mouse lung tissues were performed and antigen retrieval was performed in citrate buffer. 3D human lung tissue aggregates were removed from the 96-well plate and embedded into Cryomount medium and frozen to −80ºC. Sections were made using Leica CM1950 cryostat. After a drying step, sections were fixed in cold acetone for 10 min. Fixed slides were blocked in PBS containing 5% BSA for 20 min. Primary antibodies were rat anti- human Wnt5a (1:100, Clone 442625, R&D Systems, Minneapolis, USA), anti-AQ5 rabbit polyclonal IgG (1:100, sc-28628, Santa Cruz Biotechnology, Dallas, USA) and anti-CD51 polyclonal goat antibody (1:100, PA5-47096, Thermo Fisher Scientific, Waltham, USA). Secondary antibodies were Alexa Fluor 488 donkey anti-rat IgG polyclonal antibody (1:200, Thermo Fisher Scientific, Waltham, MA, USA), goat anti-rabbit IgG antibody (1:2000, Alexa Fluor 568, ab175471, Abcam Plc, Cambridge, United Kingdom), anti-mouse IgG 1:200, Alexa Fluor 488, Thermo Fisher Scientific, Waltham, USA) Nuclei were counterstained with DAPI (1:1000, Serva, Heidelberg, Germany). Fluorescent images were captured using Olympus IX81 fluorescence microscope (Shinjuku, Tokyo, Japan) equipped with CCD camera and analysis software. Images were processed and analyzed with ImageJ.

Flow Cytometric Bead Array (CBA) cytokine assay

Production of pro-inflammatory cytokines IL-6 and IL-8 were measured in cell culture medium using the multiplexed flow CBA Human Inflammatory Cytokines Kit (BD Biosciences, San Jose, CA, USA). After 48h culture medium/each sample of 3D aggregate cell cultures were collected. The samples were diluted four times in the kit's assay buffer. Diluted samples were added to fluorescent cytokine capture bead suspension, and human inflammatory cytokine-phycoerythrin detection reagent. Samples were then incubated for 3 h at room temperature. Labeled beads were measured using FACS Canto II flow cytometer with BD FACS DIVA software V6 and data were analyzed by FCS Express V3 software.

EV isolation from human serum samples

Extracellular vesicles were isolated from human serum samples of 5 COPD patients and 5 healthy controls using the Total Exosome Isolation kit (from serum). Samples were then centrifuged at 300 × *g* for 30 min at 4ºC. The supernatant was centrifuged with 2,000 × *g* for 30 min at 4ºC to collect oncosomes in the pellet. The supernatant was then mixed with total exosome isolation reagent. Samples were incubated at 2–8°C for 30 min, then centrifuged at

14,000 × *g* for 10 min. The pellet was collected and resuspended in cold EV resuspension buffer and stored at −80ºC until further analysis.

EV isolation from A549 and Wnt5a-A549 cell lines

Extracellular vesicles were isolated from cell culture media using total exosome isolation kit (from cell culture media). After culturing the A549 and Wnt5a-A549 cell lines FBS-free medium was added to the cultures for 2 days. The supernatant was centrifuged at 300 × *g* for 10 min at 4ºC to remove the cells. Then the supernatant was centrifuged with 2,000 × *g* for 30 min at 4ºC to collect megasomes/oncosomes in the pellet. Finally, total exosome isolation reagent (for cell culture media) was added in 0.5 volumes. Samples were incubated at 4°C overnight and then centrifuged at 10,000 × *g* for 1 h at 4°C. The pellets containing EV-s were collected and stored at −80°C.

Staining and localization of EV-s (exosomes)

Exosomes were centrifuged for 1 h at 4°C. Pellets were incubated in DiI for 30 min at 37°C, then centrifuged with 10,000 × *g* for 1 h at 4°C. BALB/c mice were injected with equal amount of stained or unstained exosome suspension in 200 µl of PBS. After 20 h of exosome injection lung, liver, thymus, and the spleen were removed for fluorescence imaging of EV accumulations by the IVIS Lumina III *In Vivo* Imaging System using the Living Image Software (IVIS Imaging Systems). Fluorescence intensity was compared to fluorescence intensity of unstained control organs.

Electron microscopic detection of exosomes

Exosomes isolated from the cell culture supernatant were fixed in 2.5% glutaraldehyde aqueous solution. Exosomes were embedded in the EM grid. Samples were treated with 5% uranyl-acetate at pH 7 for 5 min. The grid was examined using Morgagni 268D transmission electron microscope at 80 kV. Images were acquired using an integrated MegaView III digital camera.

Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)

Oncosome and exosome samples were loaded to the Aviva Systems Biology human WNT5A ELISA Kit. The plate was pre-coated with Wnt5a-specific antibody. Samples were incubated for 2 h at 37ºC ont he plate, then the biotinylated Wnt5a-specific detector antibody was applied for 1 h at 37ºC. Samples were incubated with Avidin-HRP conjugate for 1 h at 37°C. The wells were washed and TMB substrate was added for 15 min at 37°C in the dark. The reaction was

then stopped and the color reaction was measured at 450 nm absorbance. Results were calculated against the standard curve.

Isolation/Sorting and in vitro TD of human pulmonary epithelial cells

Human primary lung epithelial cells were isolated from tissue samples from lobectomy patients with normal lung function. For lung tissue digestion trypsin, DNase and foetal calf serum (FCS) were used. Isolated cells were processed for fluorescent activated cell sorting (FACS). Freshly isolated lung cells were washed with PBS containing 0.1% BSA and 0.1% Na-Azide, then antibodies were added for 30 minutes. EpCAM-FITC, CD208-PE and Podoplanin-APC conjugated antibodies were used to differentiate between AT1-like ($EpCAM^+$ $Podoplanin^+$ $CD208^-$ population) and AT2-like ($EpCAM^+$ $Podoplanin^-$ $CD208^+$ population) epithelial cells. Cells were sorted with a Beckman-Coulter MoFlo XDP high-speed cell sorter. Pulmonary epithelial cells were cultured in DCCM-1 medium containing 10% FCS for 3-6 days. On day 3 and day 6, cells were processed for microarray analysis or real-time qPCR.

Microarray analysis

cDNA of cultured cells of patients (days 3 and 6 of culturing) was fragmented and fluorescently labeled using the GeneChip WT terminal Labeling Kit. cDNA was hybridized to Human Gene 1.0 ST arrays. Probe cell intensity data (CEL) from the microarrays were analysed using the Expression Console software with the default settings of the RMA-sketch workflow.

Protein ANalysis THrough Evolutionary Relationships (PANTHER)

The PANTHER Classification System was designed to classify proteins and their genes in order to facilitate high-throughput analysis.

Artificial neural network (ANN) analysis

Evaluation of Wnt signaling pathway on AT2-to-AT1 TD was carried out using ANN software. Gene expression data were obtained with Affymetrix array using pooled cDNA samples of AT2 cells as controls compared to AT1 cells. Mean sensitivity was determined and set as to 1.0.

Statistical analysis

Statistical analysis was performed with GraphPad version 6 software. Data are presented as mean ± SEM, and statistical analysis was performed using the independent samples *t*-test and one-way ANOVA with Bonferroni correction. $p < 0.05$ was considered as significant.

RESULTS

CS and CSE trigger Wnt5a up-regulation in both mouse and human lung tissues

After CS exposition, C57BL/6 mice lungs developed emphysema-like tissue damage that correlated with decreased number of $EpCAM^+$ epithelial cells and increased number of $CD45^+$ cells. Both the epithelial cell enriched and the CD45+ cell populations showed elevated Wnt5a mRNA levels. Wnt5a protein levels were also increased. To test the effects of CS on the human lung, *in vitro* human lung aggregate cultures were exposed to CSE for 48 h and up-regulated mRNA and protein levels of Wnt5a were detected only if monocytes were present.

Increased inflammatory cytokine production in the lung after CS exposure is associated with the presence of macrophages

Both in the presence or absence of macrophages, significant increase of IL6 peptide levels were detected compared to untreated controls. Both IL-6 and IL-8 peptide levels increased significantly in the monocyte containing tissue cultures after rhWnt5a (1 μg/ml, for 48 h) treatment indicating a sequential activation of pro-inflammatory cytokine and Wnt5a production. Wnt signaling TaqMan Array analysis of CSE exposed blood monocytes have shown an up-regulation of characteristic non-canonical Wnt signaling molecules including Wnt5a, Wnt5b, Wnt11 as ligands and an inhibitor of the canonical Wnt pathway, Dickkopf 2 (DKK-2). Reduced transcription of TLE2 and secreted frizzled-related protein 5 was also observed, demonstrating deregulation of Wnt signaling in macrophages as a direct effect of CS exposure. To study how PPAR gamma is involved in the CS induced Wnt5a up-regulation as well as consequent pro-inflammatory cytokine production, human lung aggregate cultures were exposed to Wnt5a and CSE in the presence or absence of PPAR gamma agonist, rosiglitazone (RSG), and inhibitor (GW9662). The M1 phenotype is mostly characterized by pro-inflammatory cytokines IL-6, IL-8, IL-23, and IL-1β, while M2 is specified by anti-inflammatory cytokines IL-10 and TGF-beta. Inhibition of the anti-inflammatory PPAR gamma by GW9662 reduced the gene expression of IL-10 - the characteristic cytokine marker of the anti-inflammatory M2 phenotype. Further analysis showed significant increase of PPAR gamma in CSE exposed human blood monocyte-derived macrophages after 3 h, however 48 h of CSE exposure reduced the transcript level of PPAR gamma to the control levels indicating that expression of the anti-inflammatory PPAR gamma would not keep up with the prolonged inflammatory stimuli.

Chronic CS exposure increases Wnt5a protein levels in EV-s of COPD patients' sera

While Wnt5a was not detectable in sera of neither COPD patients nor age-matched healthy volunteers, oncosomes isolated from sera of COPD patients contained similar levels of Wnt5a as measured in the Wnt5a overexpressing Wnt5a-A549 cell line. Similarly, exosomes contained high levels of pro-inflammatory cytokines with an extremely high level of IL-8. To investigate whether these concentrated "cellular messages" can reach other organs, fluorescently labeled EVs were followed and the experiment provided evidence that inflammatory cytokine containing exosomes can reach and become concentrated in the liver, lungs, spleen, and thymus of test animals.

Wnt signaling pathways are the most active during ATII-to-ATI TD *in vitro*

1527 genes were analyzed by an Affymetrix array with the help of the Protein ANalysis THrough Evolutionary Relationships program (PANTHER) where the studies revealed that 73 genes belonged to the Wnt pathway. mRNA levels of Wnt4, Wnt5a and Wnt7a ligands and Fzd1, Fzd2 and Fzd7 receptors showed significant changes using Affymetrix array and these results were confirmed by qRT-PCR analysis. The *in vitro* detected gene expression changes were compared to mRNA levels of freshly isolated primary human ATI and ATII cells. Alveolar type identity of the sorted cell populations was confirmed by qRT-PCR analysis using differentiation markers SFTPC for ATII cells and AQP5/RAGE for ATI cells. Although no significant differences were detected in SFTPC levels, AQP5 and RAGE were significantly higher in the sorted ATI than in the ATII population. While there was no remarkable difference in mRNA levels of Wnt receptors, all three Wnt ligands, Wnt4, Wnt5a and Wnt7a proved to be significantly higher in the freshly isolated primary ATI cells than in ATII cells.

Three dimensional (3D) aggregate cultures confirm a role of Wnt ligands in TD

Recombinant human Wnt4 or recombinant human Wnt5a treatment in *in vitro* 3D lung aggregate cultures downregulated SFTPC both at message and protein level. Treatment of rhWnt4, rhWnt5a and rhWnt7a triggered downregulation of SFTPC message levels, but only Wnt7a treatment increased the AQP5 both at mRNA and protein levels. To investigate Wnt7a induced intracellular signaling activity, lung aggregate tissue sections were stained for beta-catenin and phospho-beta-catenin. Although Wnt7a exposure increased beta-catenin levels, beta-catenin phosphorylation levels decreased significantly, indicating the complexity of intracellular signal regulation that is involved in the TD process leading to AQP5 expression.

ANN analysis of microarray data reveals Wnt pathway targets during AT2-to-AT1 TD

The ANN analysis highlighted different molecules such as thrombospondin1 (THBS1), transglutaminase 2 (TGM2), integrin alpha V (ITGAV), epithelial membrane protein 2 (EMP2), cytochrome P450 family 4, subfamily B, polypeptide 1 (CYP4B1) and ankyrin repeat domain 1 (ANKRD1) as targets of the altered Wnt signaling pathway activity during the ATII-to-ATI TD process. THBS1, EMP2, ITGAV, CYP4B1 and ANKRD1 mRNA levels were significantly increased and TGM2 significantly decreased during the TD process. To link genes identified by the ANN analysis to specific Wnt ligands, 3D aggregate co-cultures were treated with rhWnt4, rhWnt5a and rhWnt7a for 48 h. Only ITGAV mRNA was downregulated following rhWnt5a and not by rhWnt4 or rhWnt7a treatment. However, rhWnt5a resulted in increased ITGAV protein levels in the mesenchymal fibroblast core of the aggregate, while in the outer epithelial cell layer of the aggregate ITGAV protein levels significantly decreased.

SUMMARY

Does the cigarette smoke induce increased level of the non-canonical Wnt5a molecule and trigger inflammatory environment specific for COPD?

1. CS induced the expression of non-canonical Wnt5a molecule and simultaneously suppressed the canonical Wnt pathway. Significant decrease of the $EpCAM^+$ epithelial cells have been detected that correlated with emphysema and decreased gas exchange surface.
2. The non-canonical Wnt5a was associated with activation of pro-inflammatory processes during M1 phenotype macrophage differentiation and pro-inflammatory cytokine production was up-regulated upon CS exposure. In parallel, inflammatory environment was also marked by the increased number of the $CD45^+$ leukocyte population of immune cells and also the reduction of the anti-inflammatory PPAR-gamma in the lung.

Is the elevated level of Wnt5a detected in COPD patients' sera which might explain the development of systemic inflammation?

3. According to our results we concluded that during cigarette smoking both Wnt5a and pro-inflammatory cytokines can be carried to every organ in EVs in COPD patients and

thus not just the inflammation can become systemic but also the inhibition of the anti-inflammatory activity of PPAR gamma making COPD a complex disease.

Does the Wnt signaling pathway regulate pulmonary regeneration and epithelial transdifferentiation processes, and if so, how?

4. Our results show that Wnt4, Wnt5a and Wnt7a ligands are negative regulators of SFTPC in the process of ATII-ATI TD, besides Wnt7a acts as an inducer of AQP5. However, the only gene that was directly affected by an individual Wnt ligand, was ITGAV. Thus, we hypothesize that ITGAV and not directly Wnt ligands are responsible for regulation of SFTPC levels.

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Total impact factor: **33,825**

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