The WNT receptor Frizzled-8 in pulmonary remodelling and inflammation
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Chapter 3

Functional interactions of WNT-5B with decorin in chronic obstructive pulmonary disease

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive and complex inflammatory lung disease, resulting in airflow obstruction that is not fully reversible. COPD is mainly caused by small irritating particles, for instance from cigarette smoke, combined with a genetic susceptibility [1-3]. The major pathological processes in COPD are chronic bronchitis, remodelling of the small airways and parenchymal destruction [1, 4].

An important player in tissue remodelling is transforming growth factor (TGF)-β. TGF-β levels are increased both in bronchial and alveolar epithelial cells of COPD patients [5, 6]. TGF-β is mainly produced in an inactive form and is activated by inflammation [7]. Active TGF-β is pro-fibrotic and induces myofibroblast differentiation as well as aberrant production of extracellular matrix (ECM) proteins [8]. The ECM comprises a tight network of many collagens, fibronectin, elastic fibers and proteoglycans that function as structural backbone for cells, but can also influence cell growth, proliferation and signalling [9-11]. Fibroblasts are the main producers of ECM and TGF-β is the main regulator of ECM composition. For instance, stimulation with TGF-β increases fibronectin and collagen deposition by fibroblasts [12, 13], whereas it decreases decorin production [14].

Decorin is a small, leucin-rich proteoglycan. It can be excreted in soluble form, but can also be a part of the ECM and therefore stay associated to the cell. Decorin is not only a structural part of the ECM, playing an important role in collagen cross-linking, but is also an important regulator, because of its ability to bind cell surface receptors, cytokines and growth factors [15]. Furthermore, decorin can bind the active form of TGF-β via its protein core [16]. In this way, it can attenuate TGF-β activity, thereby diminishing its effects on remodelling [16-18]. Van Straaten et al. found that decorin expression was reduced in the peribronchiolar area of patients with severe COPD compared with the control group and with patients with pulmonary fibrosis [19]. Noordhoek et al. showed that basal decorin production in cultured fibroblasts from patients with severe emphysema is higher compared to mild emphysema. Nevertheless, TGF-β and basic fibroblast growth factor (bFGF) reduce decorin production to a larger extent in cultured fibroblasts from patients with severe emphysema than in those with mild emphysema [14]. Similarly, Hallgren et al. found that basal decorin production did not differ between fibroblasts of COPD grade 4 patients and non-COPD controls, however TGF-β induced a decrease of decorin in fibroblasts of COPD patients only and not in fibroblasts of non-COPD controls [20]. In addition, this decrease was found in fibroblasts of the central, but not the distal airway [20]. Interestingly, it has been suggested that decorin may contribute to the prevention of tissue remodelling, because overexpression of decorin inhibits the biological availability of TGF-β and decorin in turn can inhibit TGF-β function [16-18]. In this way, TGF-β and decorin are part of a negative feedback loop, where they inhibit each other’s function. Accordingly, TGF-β levels are increased in the lungs of COPD patients, while decorin expression is reduced [10], which may thus amplify TGF-β signalling and airway remodelling.

The wingless/integrase 1 (WNT) signalling pathway also is known to interact with TGF-β [21-23, chapter 2] and has recently been shown to play a role in inflammation and remodelling in the lung [23-27, chapter 4]. WNT ligands are secreted glycoproteins that function as growth factors and can interact with Frizzled receptors (FZD) to activate various pathways. The canonical signalling pathway is dependent on β-catenin and the noncanonical pathways signal mainly via calcium (WNT/Ca²⁺ pathway) or to the
RhoA/c-Jun N-terminal kinase (JNK; WNT/planar cell polarity (PCP) pathway) [28-30].

We previously showed that the noncanonical ligands WNT-5A [31] and WNT-5B [24] are upregulated in COPD. Importantly, WNT-5B can mimic TGF-β-induced fibroblast activation [chapter 2], implying a role for WNT-5B in airway remodelling. It was shown that decorin can directly bind WNT-1-induced secreted protein (WISP)-1 [32], thereby inhibiting its function. Whether decorin is able to bind WNT growth factors and thus can inhibit their function has not been described. In this study, we investigated functional interactions of WNT-5A and WNT-5B with decorin.

Methods

Cell culture

MRC-5 human lung fibroblasts [33] were cultured at 37°C in a humidified atmosphere with 5% CO₂ in Ham's F12 medium supplemented with 10% (v/v) foetal bovine serum (FBS), 2 mM L-glutamine, 50 μg/ml streptomycin, 50 U/ml penicillin and 1.5 μg/ml amphotericin B. Prior to stimulation, cells were grown to confluence in 6-well cluster plates and placed in supplemented medium with 0.5% (v/v) FBS for 24 hours.

Cell stimulation

Subsequently, cells were stimulated for 24 hours (mRNA expression studies) or 48 hours (protein expression studies) with recombinant human TGF-β1, recombinant human/mouse WNT-5A, recombinant human WNT-5B, decorin from bovine articular cartilage or recombinant human hepatocyte growth factor (HGF). In case of combined stimulation with decorin and WNT-5B, decorin was added 30 min prior to WNT-5B stimulation. After stimulation, medium was collected and stored at -20°C until further analysis. Cells were lysed for mRNA isolation or washed once with ice cold Hank's balanced salt solution (HBSS; composition [mg/l]: KCl 400, KH₂PO₄ 60, NaCl 8000, NaHCO₃ 350, Na₂HPO₄·1H₂O 50, glucose 1000; pH 7.4) and lysed for protein analysis.

mRNA isolation

The mRNA of the stimulated fibroblasts was isolated with TRI Reagent® Solution (Ambion) according to the manufacturer's instructions. The amount of mRNA in all samples was determined using spectrophotometry (ND-1000, NanoDrop, ThermoScientific, Wilmington, DE, USA). Equal amounts of mRNA (1μg) were then reverse transcribed according to the manufacturer’s instructions (Promega Benelux b.v., Leiden, the Netherlands) and the cDNA was stored at -20°C until further use. mRNA expression was determined using real-time PCR, which was performed with the Illumina Eco Personal QPCR System (Westburg, Leusden, the Netherlands). The sequences of the specific primers used for determining genes of interest are listed in table 1. Real-time PCR data were analyzed using the comparative cycle threshold (ΔCt) method. Ct is the amplification cycle number. The cycle parameters were denaturation at 95°C for 30 seconds, annealing at 59°C for 30 seconds, and extension at 72°C for 30 seconds for 40 cycles. The amount of target gene was normalized against the endogenous housekeeping gene 18S ribosomal RNA (designated as ΔCt). Experimental conditions were normalized against basal condition (designated as ΔΔCt). Relative differences were determined using the equation 2⁻¹(ΔΔCt).
### Table 1 - Human primers used for the determination of specific genes of interest.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>decorin</td>
<td>5‘-AAT TGA AAA TGG GGC TTT CC-3’</td>
<td>5‘-GCC ATT GTC AAC AGC AGA GA-3’</td>
</tr>
<tr>
<td>fibronectin</td>
<td>5‘-TCG AGG AGG AAA TTC CAA TG-3’</td>
<td>5‘-ACA CAC GTG CAC CTC ATC AT-3’</td>
</tr>
<tr>
<td>sm-α-actin</td>
<td>5‘-GAC CCT GAA GGA CCC GAT AGA AC-3’</td>
<td>5‘-GGG CAA CAC GAA GCT CAT TG-3’</td>
</tr>
<tr>
<td>WNT-5B</td>
<td>5‘-TGA AGC AGA TTG ACA GCT T-3’</td>
<td>5‘-AGT AGG GTT CCC TGA TTC CAG T-3’</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>5‘-CGC CGC TAG AGG TGA AAT TC-3’</td>
<td>5‘-TTG GCA AAT GCT TTC GCT C-3’</td>
</tr>
</tbody>
</table>

**Western Blot analysis**

Cells were lysed in sodium dodecyl sulfate (SDS)-lysis buffer (composition: 62.5 mM Tris, 2% (w/v) SDS, 1 mM NaF, 1 mM NaVO₃, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 7 mg/ml pepstatin A; pH 6.8). To homogenize the samples, the lysate was passed through a syringe (5 ml) with a needle (B-Braun, 0.6x25mm) five times. Protein concentration was determined using a Pierce® BCA protein determination assay according to the manufacturer’s instructions (Thermo Fisher Scientific Inc., Rockford, IL, USA). Equal quantities of protein were subjected to electrophoresis on polyacrylamide gels and transferred to nitrocellulose membranes and subsequently analyzed for proteins of interest using specific primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. By using enhanced chemiluminescence reagents, bands were recorded in the G:BOX iChemi gel documentation system equipped with GeneSnap image acquisition software (Syngene, Cambridge, UK). Band intensities were quantified by densitometry using analysis software Image Studio™ Lite (LI-COR Biosciences, Lincoln, NE, USA).

**Immunocytochemistry on smooth muscle α-actin**

MRC-5 human lung fibroblasts were grown to confluence on Lab-Tek™ borosilicate chamber slides and changed to supplemented medium with 0.5% (v/v) FBS for 24 hours prior to stimulation. Cells were stimulated for 48 hours with recombinant WNT-5B with or without prior addition of decorin, fixed for 15 minutes at room temperature (RT) in cytoskeletal buffer (CB; composition: 10 mM MES, 150 mM NaCl, 5 mM EGTA, 5 mM MgCl₂ and 5 mM glucose; pH 6.1) containing 3% paraformaldehyde (PFA). Cells were then permeabilized by incubation for 5 minutes at RT in CB containing 3% PFA and 0.3% Triton X-100. Fixed cells were blocked for 2 hours at RT in Cyto-TBS buffer (20 mM Tris base, 154 mM NaCl, 2.0 mM EGTA and 2.0 mM MgCl₂; pH 7.2) containing 1% bovine serum albumin (BSA) and 2% normal donkey serum and subsequently incubation overnight at 4°C with primary antibody (mouse anti-smooth muscle(sm)-α-actin, diluted 1:100) in Cyto-TBS containing 0.1% Tween 20 (Cyto-TBST). Incubation with Cy3-conjugated secondary antibody (donkey anti-mouse, diluted 1:100) was done for 2 hours at RT in Cyto-TBST. Nuclei were stained with Hoechst 33342. After staining, coverslips were mounted using ProLong Gold antifade reagent (Invitrogen, Paisley, UK) and analyzed using an Olympus AX70 microscope equipped with digital image capture system (ColorView Soft System with Olympus U CMAD2 lens).
Binding experiment
Protein A/G beads were coated overnight at 4°C with decorin from bovine articular cartilage (10 µg) or 1% BSA. Subsequently, beads were blocked with 1% BSA in plain Ham’s F12 at 4°C for one hour, after which increasing concentrations of recombinant WNT-5B was added to either decorin-coated beads or BSA-coated control beads. The beads were incubated at 4°C overnight. Next day, medium was collected and 20 µl loading buffer was added to the beads. The beads were heated to 95°C for 10 minutes to release bound protein and subsequently centrifuged. Fifteen µl of the supernatant was then subjected to electrophoresis on polyacrylamide gels and transferred to nitrocellulose membranes and subsequently analyzed for WNT-5B protein expression as described for Western Blot analysis.

Materials
Recombinant human TGF-β1, recombinant human/mouse WNT-5A and recombinant human WNT-5B were obtained from R&D Systems (Minneapolis, MN, USA). Human/mouse anti-WNT-5B (1:250; ab93134) was purchased from Abcam (Cambridge, UK). Anti-human α-sm-actin mouse monoclonal antibody (1:1000; A2547), horseradish-peroxidase (HRP)-conjugated secondary antibodies goat anti-rabbit (1:2000; A0545), rabbit anti-mouse (1:2000; A9044) and rabbit anti-goat (1:10000; A8919) were purchased from Sigma (St. Louis, MO, USA). Anti-human decorin (H-80) rabbit polyclonal IgG (1:200; sc-22753), anti-human fibronectin (C20) goat polyclonal IgG (1:500; sc-6952) and anti-human GAPDH mouse monoclonal IgG1 (1:1000; sc-47724) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals used were of analytical quality.

Statistical analysis
All Real-Time PCR data were log transformed before statistical analysis. For comparison between two conditions, a two-tailed Student’s t-test was used. For comparisons between multiple conditions, two-way ANOVA was used, followed by a post hoc analysis using the Student-Newman-Keuls multiple comparisons test. For the binding experiment, a Wilcoxon Signed Rank test was used. P < 0.05 was considered significant.

Results
Growth factors reduce decorin expression in human lung fibroblasts
We first investigated the effect of WNT-5A and WNT-5B on decorin expression in MRC-5 human lung fibroblasts, using TGF-β1 as a positive control. We observed that TGF-β1, WNT-5B and to a lesser extent WNT-5A were able to reduce decorin mRNA and protein expression (figures 1A-E). The reduction of decorin mRNA expression had an EC50 for TGF-β1 of 0.06 ng/ml and a maximal response at 2 ng/ml (0.48 ± 0.059 fold decrease; figure 1C), an EC50 for WNT-5A of 69.47 ng/ml and a maximal response at 300 ng/ml (0.60 ± 0.17 fold decrease; figure 1D) and an EC50 for WNT-5B of 68.70 ng/ml and a maximal response at 500 ng/ml (0.54 ± 0.08 fold decrease; figure 1E).
Figure 1: TGF-β1, WNT-5A and WNT-5B decrease decorin expression. MRC-5 human lung fibroblasts were treated for 24 hours (mRNA) or 48 hours (protein) with recombinant TGF-β1, WNT-5A or WNT-5B as described in the methods section. (A) Decorin mRNA expression in MRC-5 human lung fibroblasts after 24 hours of stimulation with TGF-β1, WNT-5A or WNT-5B. Data represent mean ± s.e.m. of 5-11 independent experiments. (B) Decorin protein expression in MRC-5 human lung fibroblasts after 48 hours of stimulation with TGF-β1, WNT-5A or WNT-5B. Data represent mean ± s.e.m. of 4 independent experiments. (C) Concentration dependent decrease of decorin mRNA expression in MRC-5 human lung fibroblasts after 24 hours of stimulation with increasing concentrations of TGF-β1 (EC50: 0.06 ng/ml). Data represent mean ± s.e.m. of 10 independent experiments. (D) Concentration dependent decrease of decorin mRNA expression with increasing concentrations of WNT-5A (EC50: 69.47 ng/ml). Data represent mean ± s.e.m. of 4-7 independent experiments. (E) Concentration dependent decrease of decorin mRNA expression with increasing concentrations of WNT-5B (EC50: 68.70 ng/ml). Data represent mean ± s.e.m. of 4-9 independent experiments. ** p < 0.01 * p < 0.05 compared to basal conditions using a two-tailed Student’s t-test.
Functional interactions of WNT-5B with decorin in COPD

WNT-5B-induced fibroblast activation is reduced in the presence of decorin
Decorin is able to bind TGF-β [16], thereby reducing its activity. We have previously shown that WNT-5B but not WNT-5A can mimic TGF-β-induced fibroblast activation [chapter 2]. We hypothesized that decorin could also bind WNT-5B and reduce its effect on fibroblast activation. We observed that after 48 hours of stimulation, WNT-5B-induced fibronectin and sm-α-actin protein expression was attenuated in the presence of decorin (figures 2A-C). After 4 hours of stimulation with WNT-5B, sm-α-actin mRNA did not yet show an induction. Sm-α-actin mRNA expression was induced after 8, 16 and 24 hours of WNT-5B stimulation and not attenuated in the presence of decorin (figure 2D). Fibronectin mRNA expression did not yet show a clear induction after 4 and 8 hours of stimulation with WNT-5B. Fibronectin mRNA expression was induced after 16 and 24 hours of stimulation with WNT-5B and not attenuated by addition of decorin (figure 2E). These data indicate that WNT-5B-induced fibroblast activation is reduced in the presence of decorin; however regulation of protein expression does not correlate with mRNA expression.

Decorin is able to bind recombinant WNT-5B
We next investigated via which mechanism decorin can inhibit effects of WNT-5B on fibronectin and sm-α-actin protein levels. Based on the structure of WNT-5B, we hypothesized that decorin is able to bind to WNT-5B. We therefore performed a pull-down with protein A/G beads and showed that beads coated with decorin captured more WNT-5B than control beads coated with BSA (figures 3A-B). This indicates that decorin can directly bind to WNT-5B.

The MET receptor is not involved in decorin-induced effects in human lung fibroblasts
Next, we investigated whether other mechanisms may contribute to the inhibitory role of decorin on WNT-5B-induced effects is inactivation via binding. In addition to its regulatory role in capturing cytokines and growth factors, decorin can also antagonize cell surface receptors. One of these is the receptor tyrosine kinase MET [34]. The only known ligand of this oncogenic receptor is HGF. We observed that the mRNA of the MET receptor is expressed in MRC-5 human lung fibroblasts with an average Ct-value of 26.8 ± 0.45 (n = 3). We stimulated MRC-5 human lung fibroblasts with HGF and the combination of HGF and WNT-5B to investigate whether this receptor plays a role in WNT-5B-induced effects on fibroblast activation. HGF alone did not have an effect on fibronectin protein expression and the combination of HGF and WNT-5B had the same effect as WNT-5B alone (figure 3C). These data show that the MET receptor is not involved in WNT-5B-induced effects on fibroblast activation.
Figure 2: Decorin inhibits WNT-5B function. MRC-5 human lung fibroblasts were stimulated with decorin, WNT-5B or their combination for different time points as described in the methods section. (A) The effect of decorin, WNT-5B or their combination after 48 hours of stimulation on fibronectin protein expression MRC-5 human lung fibroblasts. Data represent mean ± s.e.m. of 4 independent experiments. (B) The effect of decorin, WNT-5B or their combination after 48 hours of stimulation on sm-α-actin protein expression in MRC-5 human lung fibroblasts. Data represent mean ± s.e.m. of 4 independent experiments. (C) The effect of decorin, WNT-5B or their combination after 48 hours of stimulation on sm-α-actin stress fiber formation.
in MRC-5 human lung fibroblasts. Cells were stained for sm-α-actin (red) and DNA (Hoechst 33342; blue). Pictures were taken at 100× magnification. (D) The effect of decorin, WNT-5B or their combination after 4, 8, 16 or 24 hours of stimulation on fibronectin mRNA expression in MRC-5 human lung fibroblasts. Data represent mean ± s.e.m. of 4 independent experiments. (E) The effect of decorin, WNT-5B or their combination after 4, 8, 16 or 24 hours of stimulation on sm-α-actin mRNA expression in MRC-5 human lung fibroblasts. Data represent mean ± s.e.m. of 4 independent experiments. *** p < 0.001 ** p < 0.01 compared to basal conditions, #### p < 0.001 # p < 0.05 compared to the stimulated control, $$$ p < 0.001 $$ p < 0.01 compared to inhibited control (one-way repeated measures ANOVA with Student-Newman-Keuls multiple comparisons test; data is tested per time point; figures D-E). Abbreviations in figure: DCN, decorin.

**Figure 3: Decorin is able to bind WNT-5B.** Pull-down with protein A/G beads was performed as described in the methods section. (A) Representative blot of increasing concentrations of WNT-5B protein expression after pull-down with BSA-coated or decorin-coated beads. (B) Quantification of WNT-5B protein expression after pull-down of 500 ng/ml WNT-5B with BSA-coated or decorin-coated beads. Data represent ± s.e.m. of 4 independent experiments. * p < 0.05 compared to BSA-coated beads with WNT-5B (Wilcoxon Signed Rank test). MRC-5 human lung fibroblasts were stimulated with decorin, WNT-5B or HGF for 48 hours as described in the methods section. (C) Fibronectin protein expression after 48 hours of stimulation with decorin, WNT-5B, HGF or different combinations. *** p < 0.001 ** p < 0.01 compared to basal conditions, # p < 0.05 compared to the stimulated control, $$ p < 0.01 compared to inhibited control (one-way repeated measures ANOVA with Student-Newman-Keuls multiple comparisons test). Abbreviations in figure: DCN, decorin.
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Discussion
This study shows for the first time an intricate interaction between the proteoglycan decorin and the growth factor WNT-5B. WNT-5B has negative effects on remodelling by activating fibroblasts and decorin is able to reduce WNT-5B-induced fibroblast activation. We propose that this occurs via direct binding to WNT-5B. Furthermore, both TGF-β and WNT-5B reduced decorin mRNA and protein expression in human lung fibroblasts. Hence, a similar negative feedback loop occurs as we previously observed for decorin and TGF-β [14 and figure 4].

Remodelling of the small airways contributes importantly to COPD pathophysiology. Fibroblasts produce ECM proteins such as fibronectin, collagens, proteoglycans, laminins, and elastins, thereby contributing to the repair process in healthy tissue. Many studies have suggested that intrinsic dysfunctions/defects in fibroblasts from patients with COPD lead to abnormal remodelling [14, 20]. TGF-β and WNT-5B are key players in structural airway changes such as myofibroblast differentiation and aberrant production of ECM proteins [7, 12, 13, 35, chapter 2] and both are increased in COPD [19, 20, 24]. Whereas TGF-β increases the expression of the ECM protein fibronectin and myofibroblast differentiation marker sm-α-actin, TGF-β is known to reduce the expression of the ECM proteoglycan decorin [14, 35]. Decorin has a protective function in COPD, as it reduces remodelling by inhibiting growth factors such as TGF-β via direct binding [16, 18]. In this way, decorin in the ECM can function as a depot for growth factors. Thus, TGF-β and decorin also form a negative loop, inhibiting each other’s expression and function. We have previously shown that WNT-5B is able to mimic TGF-β-induced fibroblast activation [chapter 2]. Here we show that WNT-5B is also able to reduce decorin expression. In addition, decorin is able to inhibit WNT-5B-induced fibroblast activation, indicating a similar negative feedback loop as previously seen with TGF-β. Interestingly, WNT-5B is a TGF-β target gene in lung fibroblasts [24], further contributing to this interaction. This is an important finding, as it suggests that restoring decorin levels to normal or preventing its downregulation can be protective against remodelling processes in COPD.

Next to the structural role of decorin in the ECM and its regulatory role in capturing cytokines and growth factors, decorin is also able to antagonize cell surface receptors, notably the MET receptor [34]. We investigated whether this receptor contributes to fibroblast activation in our model. Our results show that activation of the MET receptor does not play a role in (WNT-5B-induced) fibroblast activation, indicating that the main mechanism contributing to the inhibitory role of decorin on WNT-5B-induced effects is inactivation via binding.

WNT-5A and WNT-5B are both known to regulate remodelling processes in the airways. We observed a more profound effect of WNT-5B compared to WNT-5A on decorin expression, which is in line with previous results, where we found that WNT-5B, but not WNT-5A is able to mimic TGF-β-induced fibroblast activation [chapter 2].

Whereas we clearly observed that decorin inhibits WNT-5B function in fibroblasts as demonstrated by a reduction in fibronectin and sm-α-actin protein expression, the results for mRNA expression of the fibronectin and sm-α-actin genes were less straightforward. A mechanism that could explain these observed differences between the regulation of WNT-5B function on mRNA and protein level is receptor internalization of the FZD8 receptor. It is known that decorin itself is able to inhibit signalling effects by
stimulating internalization of receptors as is shown by Zhu et al. for the epidermal growth factor receptor (EGFR) [37]. Upon internalization, the downstream effects of the activated EGFR are first amplified, followed by a reduction of these effects. We previously showed that WNT-5B has its effect on fibroblast activation via the FZD8 receptor [chapter 2]. One possible explanation for the difference between the effect of decorin on fibronectin and α-smooth muscle actin mRNA versus protein expression could therefore be that decorin bound to WNT-5B can bind to the FZD8 receptor, upon which the whole complex is internalized. If this mechanism functions in the same manner as Zhu et al. Show for the EGFR, this would explain the initial increase in mRNA expression, while the ultimate protein expression is decreased. The exact mechanism that underlies the discrepancy between mRNA and protein expression and whether receptor internalization plays a role herein needs to be further investigated.

Next to its role in remodelling, WNT-5B also has an important pro-inflammatory role, as this growth factor can induce interleukin (IL)-6 and chemokine ligand (CXCL)8 secretion by human lung fibroblasts [31]. It is not clear yet whether decorin is also able to inhibit these WNT-5B-induced inflammatory effects and this needs to be further investigated.

Summarizing, we show that decorin reduces WNT-5B-induced fibroblast activation, presumably via direct binding of WNT-5B to decorin although additional mechanisms may also play a role. Since decorin production is reduced in COPD, insufficient binding of WNT-5B to decorin might lead to hyperactivation of ECM production. In addition, WNT-5B expression is increased in COPD patients, further augmenting ECM production. This implies that restoration of decorin levels to normal in COPD patients will reduce growth factor signalling and thereby halt the ECM overproduction.

Figure 4: proposed interaction between the ECM proteoglycan decorin and WNT-5B-induced fibroblast activation. Decorin is able to reduce TGF-β-induced effects [16]. We show that decorin is able to reduce WNT-5B-induced effects on fibroblast activation. Illustrated are two possible mechanisms via which this reduction could be established.

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1 From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016. 2016; Available at: http://www.goldcopd.org/.


