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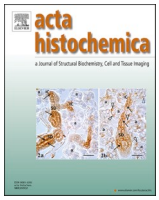

# Pulmonary 5-HT$_{2B}$ receptor expression in fibrotic interstitial lung diseases

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ABSTRACT

Pulmonary fibrosis is a severe condition in interstitial lung diseases (ILD) such as idiopathic pulmonary fibrosis (IPF) and systemic sclerosis-ILD, where the underlying mechanism is not well defined and with no curative treatments available. Serotonin (5-HT) signaling via the 5-HT$_{2B}$ receptor has been recognized as a promising preclinical target for fibrosis. Despite this, the involvement of the 5-HT$_{2B}$ receptor in fibrotic ILD is widely unexplored. This work highlights the spatial pulmonary distribution of the 5-HT$_{2B}$ receptor in patients with IPF and systemic sclerosis-ILD. We show that the 5-HT$_{2B}$ receptor is located in typical pathological structures e.g. honeycomb cysts and weakly in fibroblast foci. Together with immunohistochemistry and immunofluorescence stainings of patient derived distal lung tissues, we identified cell targets for 5-HT$_{2B}$ receptor interference in type II alveolar epithelial cells, endothelial cells and M2 macrophages. Our results emphasize the role of 5-HT$_{2B}$ receptor as a target in lung fibrosis, warranting further consideration in targeting fibrotic ILDs.

## 1. Introduction

Progressive fibrosing interstitial lung disease (PF-ILD) describes a phenotypic subset of interstitial lung diseases characterized by progressive and complex lung fibrosis. PF-ILDs represent a major health problem worldwide with a high number of affected individuals, owing to the incomplete knowledge of fibrotic pathogenesis, absence of appropriate and validated biomarkers, and current lack of effective disease-modifying therapeutic agents. Despite large gaps in knowledge, PF-ILDs have in common self-sustaining fibrosis, suggesting shared pathogenetic pathways (Lofdahl et al., 2020).

Idiopathic pulmonary fibrosis (IPF) is one of the most common types of ILD (Hutchinson et al., 2015; Wollin et al., 2019). IPF patients demonstrate large heterogeneity in their pulmonary manifestation of fibrosis and is generally not regarded as an inflammatory disease. Nonetheless, patients with a rapid progress or experiencing acute exacerbation have reported severe innate and adaptive inflammatory infiltrates where the extent of inflammation was correlated with yearly forced vital capacity (FVC) decline (Balestro et al., 2016). Another chronic disease with PF-ILD is systemic sclerosis (SSc). Most of these patients who develop severe restrictive lung disease do so in the first five years following symptom onset (Denton and Khanna, 2017; Khanna et al., 2020). SSc is an autoimmune disease characterized by microvasculopathy, immune dysregulation, chronic inflammation, and subsequent fibrosis of the skin and internal organs (Denton and Khanna, 2017). Nintedanib, a tyrosine kinase inhibitor with anti-fibrotic properties, has recently been approved for use in both IPF and SSc-ILD, reducing the annual rate of decline in forced vital capacity (FVC), which is consistent with overlapping disease mechanisms (Wollin et al., 2019; Distler et al., 2019).

Lung fibrosis is believed to be initiated by lung microdamage involving both endothelial and epithelial cells. One consequence of tissue injury is that circulating platelets become activated and release serotonin (5-hydroxytryptamine, 5-HT), activating different types of 5-HT receptors (G-protein coupled receptors) locally expressed on cells at the damaged site. When the regulating mechanisms fail in PF-ILD, an excessive production of extracellular matrix (ECM) takes place, which overtime develops into lung fibrosis and ultimately distortion of normal lung architecture with loss of respiratory function. Importantly, platelets are not the only source of 5-HT in the respiratory system. Mast cells

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transport and release 5-HT within injured tissue and resident pulmonary neuroendocrine cells are also known to synthesize and secrete 5-HT (Mann and Oakley, 2013). Increased systemic levels of 5-HT have shown to be increased during fibrosis in vivo (Fabre et al., 2008) and the finding is probably linked to an enhanced platelet degranulation at injured sites. Already in 1983, platelets were shown to be depleted in 5-HT in patients with inflammatory arthritic diseases such as SSc, systemic lupus erythematosus and rheumatoid arthritis (RA) (Zeller et al., 1983). More recently a paper by Hirigoyen et al. (2015) reported significantly reduced intraplatelet 5-HT content in patients with diffuse SSc compared with limited SSc and healthy control subjects, indicating increased platelet activation and 5-HT release in these patients (Hirigoyen et al., 2015). A positive correlation of serum 5-HT level with the modified Rodnan Skin Score (mRSS) was recently reported in a small SSc patient study (Petric et al., 2022).

A significant role of peripheral 5-$HT_{2B}$ receptors in fibrosis has been suggested, with the 5-$HT_{2B}$ receptor being upregulated in fibrotic tissues. Activation of the receptor leads to increased production of profibrotic mediators and modifies cell differentiation leading to excessive extracellular matrix synthesis (Mann and Oakley, 2013; Dees et al., 2011; Konigshoff et al., 2010).

In this study, we investigated the expression of the 5-$HT_{2B}$ receptor in different structures of distal lung as well as key target cells activated during tissue remodeling to understand the expression profile in PF-ILD's. We therefore investigated the expression of 5-$HT_{2B}$ receptor in endothelial, and epithelial cells and M2 macrophages and found that all cells express the receptor in remodeled lung. Therefore, this study confirms other studies of the involvement of 5-$HT_{2B}$ receptor in lung fibrosis and pinpoints that its expression is not confined to one cell type but is ubiquitously expressed by endothelial, epithelial and M2 macrophages in both IPF and SSc-ILD. This study further emphasizes the 5-$HT_{2B}$ receptor as a potential target to inhibit fibrosis in PF-ILDs.

## 2. Material and methods

### 2.1. Human material

Lung tissue explants or resected material from patients with IPF or SSc-ILD or from healthy donors were used to excise blocks of distal lung tissue for histology. All material used in the study was approved by the local Ethical Committee in Lund (Dnr 2015–891, 2018/12) and Gothenburg (Dnr 1026–15, 2008–413, 2022–01221–02). Individual patient and healthy donor characteristics are described in Table 1, showing a mean age ranging from 51.5 to 57.6 years between the groups with prior smoking history in some IPF patients and healthy individuals.

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board in Lund (Dnr 2015–891, 2018/12) and Gothenburg (Dnr 1026–15, 2008–413, 2022–01221–02). Informed consent was obtained from all subjects involved in the study or closest relative.

### 2.2. Immunohistochemistry

#### 2.2.1. DAB HRP substrate staining

Formalin-fixed paraffin embedded (FFPE) distal lung tissues were stained for the 5-$HT_{2B}$ receptor with adjacent tissue section stained with hematoxylin and eosin (HE), according to standardized procedures. For each group, three individuals were examined with two tissue blocks per individual. In brief, deparaffinized tissue sections were treated with low pH antigen retrieval solution (Agilent Dako, Santa Clara, CA, USA, cat no. K8005) at 97 $^0$C for 30 min. With Dako EnVision Dual Link System-HRP (Dako, cat no. K4065), sections were blocked with endogenous peroxidase block for 10 min and stained for 1 h with rabbit polyclonal anti-5-$HT_{2B}$ receptor antibody (Aviva system biology, San Diego, CA, USA, cat no. OAAF04758, 1:500). Bound antibodies were identified with labelled polymer-HRP and substrate chromogen according to manufacturer's instructions. Slides were counterstained with hematoxylin and dehydrated with standard procedures and mounted with Pertex.

**Table 1**
Patient characteristics.

| | Healthy | IPF | SSc-ILD |
|---|---|---|---|
| **Sex (male/female)** | 5/0 | 3/2 | 1/4 |
| **Age (mean ± SD)** | 52.2 ± 17.4 | 57.6 ± 5.0 | 51.5 ± 8.7 |
| **Smoking history** (never/former) | 2/3 | 2/3 | 4/0 |

#### 2.2.2. Dual HRP and ALP substrate staining

After antigen retrieval, as previously described, slides were blocked with BLOXALL blocking solution (Vector Laboratories, Burlingame, CA, U.S., cat no. SP-6000) for 10 min. After washing in TBS, slides were blocked with 2.5% normal serum (Vector Laboratories, cat no. MP-7401–15) for 20 min. After washing in TBS, primary antibody anti-5-$HT_{2B}$ receptor antibody (dilution 1:500, Aviva system biology) was added and incubated for 1 h. After wash steps in TBS, ImmPRESS HRP anti-rabbit polymer reagent (Vector Laboratories, MP-7401–15) was added and incubated for 30 min. Slides were then incubated with ImmPACT DAB EqV HRP substrate (Vector Laboratories, cat no. SK-4103) according to manufacturer's instructions. Unbound DAB substrate was washed away and second primary antibody rabbit polyclonal anti-CD206 (dilution 1:3000, abcam, Cambridge, U.K., cat no. ab64693) was added and followed the same protocol as described above, with the usage of ImmPRESS AP Horse anti-Rabbit IgG Alkaline Phosphatase (Vector Laboratories, cat no. MP-5401) and ImmPACT Vector Red Substrate (Vector Laboratories, cat no. SK5100). Slides were counterstained with Mayer's hematoxylin solution for 40 s, washed, dehydrated and mounted with Pertex according to standard procedure.

### 2.3. Immunofluorescence

Deparaffinized FFPE lung tissue sections were dual-stained for 5-$HT_{2B}$ receptor (Aviva system biology, dilution 1:100) in combination with mouse monoclonal anti-human CD31 (Agilent, cat no. M082301–2, dilution 1:100) or HT2–280 (Terrace Biotech, cat no. TB-27AHT2–280, dilution 1:200). Two individuals per group were examined including two tissue blocks per individual. In brief, tissue sections were treated with heat-induced antigen retrieval with low pH retrieval solution (Agilent Dako) as mentioned in previous section. Slides were treated for 1 h at room temperature with primary antibodies, diluted in PBS with 2% BSA. After washing steps in PBS, slides were incubated with secondary antibodies, diluted 1:200, with DAPI (Sigma-Aldrich, Saint Louis, Missouri, U.S., cat no. MBD0015), diluted to 1–2 μg/ml for 45 min at room temperature. Secondary antibodies: anti-mouse antibody for targeting HT2–280 (Thermo Fischer Scientific, Waltham, MA, U.S., cat no. A-21042), anti-mouse antibody for targeting CD31 (Thermo Fisher Scientific, cat no. A-31570) and anti-rabbit antibody for targeting 5-$HT_{2B}$ receptor (Thermo Fisher Scientific, cat no. A21246). Secondary antibody control was included in all runs with omitted primary antibody. After washing steps, slides were mounted with fluorescent mounting medium (Dako, cat no. S3023). All slides in this study were imaged with a semi-automated slide scanner Olympus VS120-L100-FL080 (Olympus, Tokyo, Japan) either in brightfield mode for immunohistochemistry (IHC) treated slides or in fluorescent mode for immunofluorescent antibody labeled slides. Representative images were acquired using QuPath (version 0.3.0(Bankhead et al., 2017) and ImageJ (1.53j, Wayne Rasband and contributors National institute of health, U.S.).

## 3. Results

### 3.1. 5-HT$_{2B}$ receptor distribution in distal lung tissue

Distal lung tissue derived from explanted lungs from healthy controls, and patients with IPF and SSc-ILD were examined for the localization of the 5-HT$_{2B}$ receptor using IHC with HRP-DAB detection accompanied with corresponding tissue sections stained with HE.

In healthy donors, the expression of the receptor was mainly distributed to bronchiolar epithelium, with positive expression in ciliated cells, as well as in vascular endothelial cells, smooth muscle cells and in alveolar septum (Fig. 1 A-D). In addition, alveolar macrophages demonstrated a granular receptor expression (Fig. 1 D, D1). The pulmonary distribution pattern found in healthy donors was similarly identified in patients with IPF (Fig. 1E-H1) and SSc-ILD (Fig. 1I-L1). In patients with IPF, the 5-HT$_{2B}$ receptor was distinctly found in suspected areas of alveolar type II (ATII) hyperplasia (Fig. 2A-B1) and in common pathological features such as honeycomb cysts (with bronchiolar and ATII epithelial cells) and weakly in fibroblast foci (Fig. 2 E-F2). Interestingly, a converging expression pattern of the same pathological structures was also seen in patients with SSc-ILD (Fig. 2 G-H1, K-N). Both IPF and SSc-ILD patients showed no clear expression of the 5-HT$_{2B}$ receptor in inflammatory infiltrates (Fig. 2 C-D, I-J).

### 3.2. Cell typing of 5-HT$_{2B}$ receptor expression

Cellular localization of the 5-HT$_{2B}$ receptor expression in lung tissue of healthy donors, IPF and SSc-ILD patients were made based on the morphological evaluation of IHC and HE stainings. To validate IHC evaluations in the vasculature and in further detail evaluate 5-HT$_{2B}$ receptor expression in specific cell types in the alveolar space, dual antibody labeling was performed combining the 5-HT$_{2B}$ receptor with CD31 (endothelial marker), HT2–280 (ATII cells) or M2 macrophages (CD206). With immunofluorescence, CD31 was co-localized with 5-HT$_{2B}$ receptor in vasculature structures in both healthy and diseased conditions (Fig. 3A), however, some vessels lacked the co-staining of CD31 and 5HT$_{2B}$ receptor on endothelial cells. The 5-HT$_{2B}$ receptor was in addition localized to ATII cells with co-localization with HT2–280 marker (Fig. 3B). Interestingly, the expression of 5-HT$_{2B}$ receptor was found in the bronchiolar epithelial cells and ATII cells lining honeycomb cysts in IPF and SSc-ILD.

With dual HRP and ALP detection systems, CD206 expression converged with the expression of the 5-HT$_{2B}$ receptor, confirming the receptor expression in macrophages with the specific identification in the M2 phenotype (Fig. 4). The M2 phenotype was primarily localized to alveolar macrophages with increased macrophage infiltration in SSc-ILD and IPF, in comparison to lung tissue from healthy donors.

## 4. Discussion

Shared biological pathways have been described in the development and progression of lung fibrosis in IPF and SSc-ILD (Lofdahl et al., 2020), justifying a closer examination of promising converging disease targets for therapeutic interventions. In this study, we have characterized the expression of 5-HT$_{2B}$ receptor in SSc-ILD and IPF, a receptor recognized for its pro-fibrotic effects (Lofdahl et al., 2016; Lofdahl et al., 2018; Lofdahl et al., 2018) and its ability as a potential therapeutic target in disease. In distal lung tissue, the 5-HT$_{2B}$ receptor was expressed on several cell types and structures, of which typical fibrotic features found in SSc-ILD and IPF patients, such as honeycomb cysts and fibroblastic foci, showed positive receptor expression.

The myofibroblast is considered the primary ECM-secreting cell during wound healing and fibrosis, releasing excessive amounts of collagens and other ECM proteins as well as TGF-β, resulting in an autocrine stimulation of fibroblasts. Myofibroblasts are generated from a variety of sources including fibroblasts, epithelial and endothelial cells as well as bone-marrow derived hematopoietic progenitor cells (Ortiz-Zapater et al., 2022; Zhao et al., 2018). Several mediators, including TGF-β and 5-HT can induce differentiation into myofibroblasts (Dees et al., 2011; Lofdahl et al., 2016). Activated fibroblasts and myofibroblasts situated in the fibroblastic foci mark an area of extensive and active remodeling. A potential source of fibroblasts has been suggested to originate from epithelial cells through EMT, where TGF-β1 is a major driver in the differentiation process, possibly acting as a second messenger to 5-HT (Dees et al., 2011; Chaturvedi et al., 2018; Willis et al., 2007). Studies have shown that 5-HT$_{2B}$ receptor antagonism inhibits 5-HT and TGF-β1 driven myofibroblast differentiation and lung fibrosis in vivo (Lofdahl et al., 2016).

Within the distal lung, ATII cells have been described to undergo significant phenotypic and functional changes upon fibrotic lung injury. ATII cells play a crucial role in lung repair/regeneration after injury and has lately been considered a therapeutic target. The suggested plasticity of ATII cells with the ability to undergo epithelial-mesenchymal transition (EMT) and their active role in repair response highlight these cells as possible sources for myofibroblast in lung fibrosis (Willis et al., 2007). Loss of normal function of ATII cells and development of a pro-fibrotic phenotype have been demonstrated to contribute to the development of idiopathic pulmonary fibrosis (IPF) where ATII cells were shown to secrete profibrotic mediators including TGF-β, IL-1β and WNT ligands (Ruaro et al., 2021; Lehmann et al., 2018). Bronchiolar epithelial cells and ATII cells were shown to express the 5-HT$_{2B}$ receptor, which was also detected in honeycomb cysts in IPF and SSc-ILD. EMT has also been demonstrated to be activated by M2 macrophages, a subtype involved in tissue repair,(Zhu et al., 2017) and also a cell type strongly implicated in lung fibrosis. The polarization of macrophages to a type 2 phenotype can be triggered by 5-HT$_{2B}$ receptor activation (de las Casas-Engel et al., 2013).

In efforts to understand the mechanisms resulting in lung fibrosis, pulmonary macrophages have been implicated with a key role in the fibrotic process. Pulmonary fibrosis has been proposed to be regulated by macrophage plasticity i.e. M1/M2 polarization (Kishore and Petrek, 2021) and M2 macrophages have been shown to be a rich source of profibrotic mediators such as TGF-β and chemokine (C-C motif) ligand 18 (CCL18) that promote the trans-differentiation of resident fibroblasts into myofibroblasts (Roofeh et al., 2020). Changes in the expression of M2 genes have recently been observed in tocilizumab-treated SSc patients (Khanna et al., 2016). Furthermore, serum concentrations of CCL18 were reduced suggesting that effects on M2 macrophages could be related to the observed effects on lung function (de las Casas-Engel et al., 2013; Khanna et al., 2016). A potential role for M2 macrophages in fibrosis has also been suggested as nintedanib, in clinical use for IPF and SSc-ILD, inhibits M2 differentiation of human monocytes in vitro and reduces M2 macrophage counts in vivo (Huang et al., 2017).

Moreover, activated endothelial cells may further amplify the fibrotic response by secreting platelet activating factors, fibrinogen and von Willebrand factor, which further activates platelets resulting in release of mediators such as 5-HT (Ruaro et al., 2021; Lehmann et al., 2018). Endothelial dysfunction and vasculopathy develop early in SSc, with Raynaud's phenomenon as a typical vascular manifestation (Varga and Marangoni, 2017). A common comorbidity in several ILDs is pulmonary arterial hypertension, where hypoxic responses have been linked to 5-HT receptors. The histological expression of 5-HT$_{2B}$ receptor in both vascular endothelial cells and smooth muscle cells creates a responsive serotonergic signaling platform that during microvascular injury contributes to fibrotic involvement and vascular dysfunction in SSc-ILD and IPF (Gagermeier et al., 2005).

In conclusion, although the small samples size, with difficulties in adjusting for risk factors e.g. smoking history and gender differences, this study provides further detailed knowledge of the involvement of serotonergic signaling in lung fibrosis. Promising targets for future intervention may center on the alveolar interstitium with ATII cells and endothelial cells, together with macrophage and fibroblast activation. A

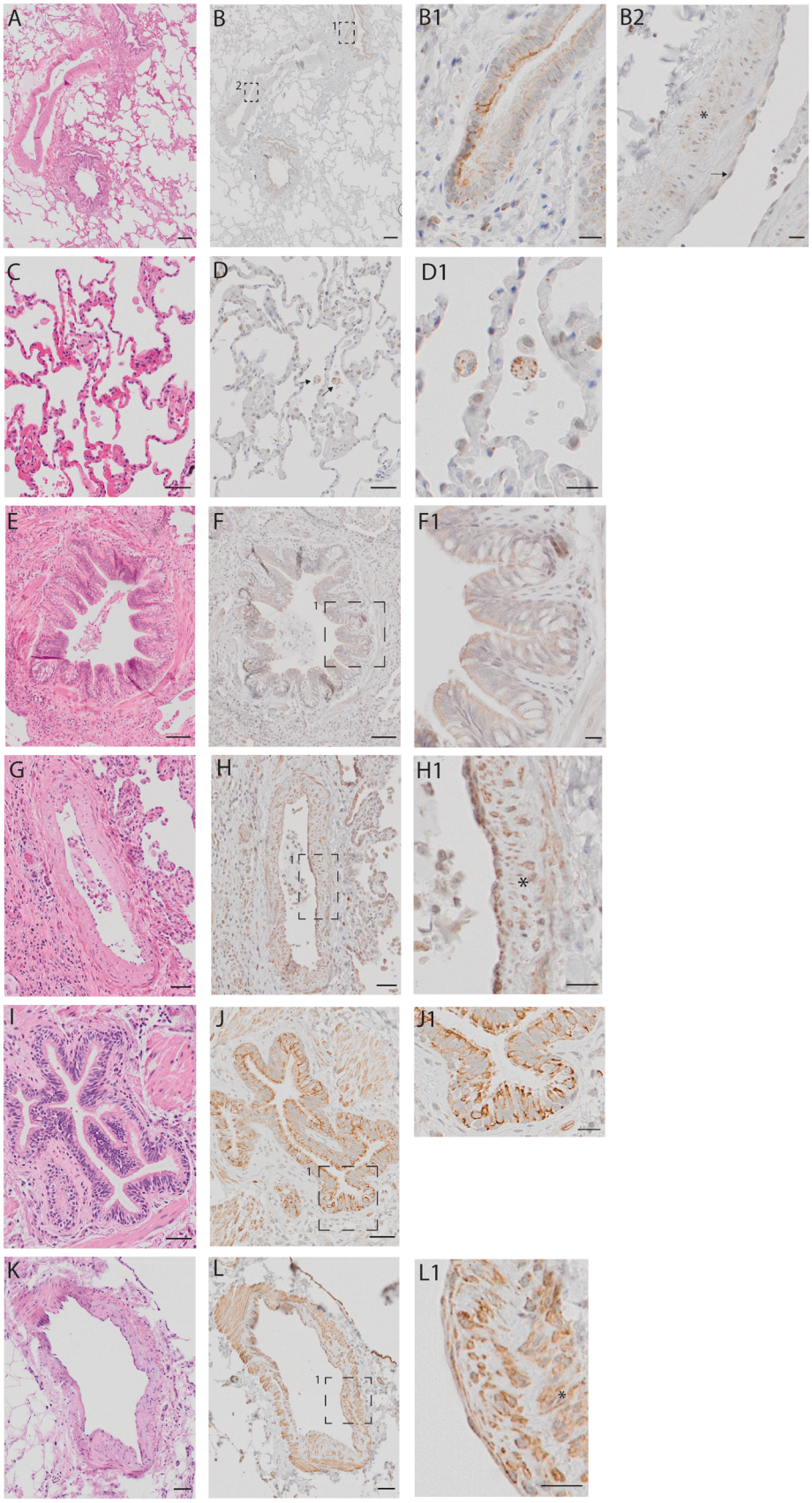


**Fig. 1. 5-HT$_{2B}$ receptor expression in distal lung tissue.** Lung expression of the 5-HT$_{2B}$ receptor, visualized with IHC-DAB staining (brown color), was in healthy donors detected in airway epithelial cells (A- B1), smooth muscle cells (B2, *) and endothelial cells (B2, arrow), in alveolar septum (C) and alveolar macrophages (D-D1 (arrows)). All images are shown together with HE stainings of corresponding tissue sections. Similar expression patterns were detected in IPF (airways (E-F1), vessels (G-H1) and smooth muscle cells (H1, *) and in SSc-ILD (airways (I-J1, vessels (K-L1) and smooth muscle cells (L1, *). Scale bar: 500 μm (A-B), 50 μm (C-L) with enlarged images 20 μm (B1–2, D1, F1, H1, J1, L1).

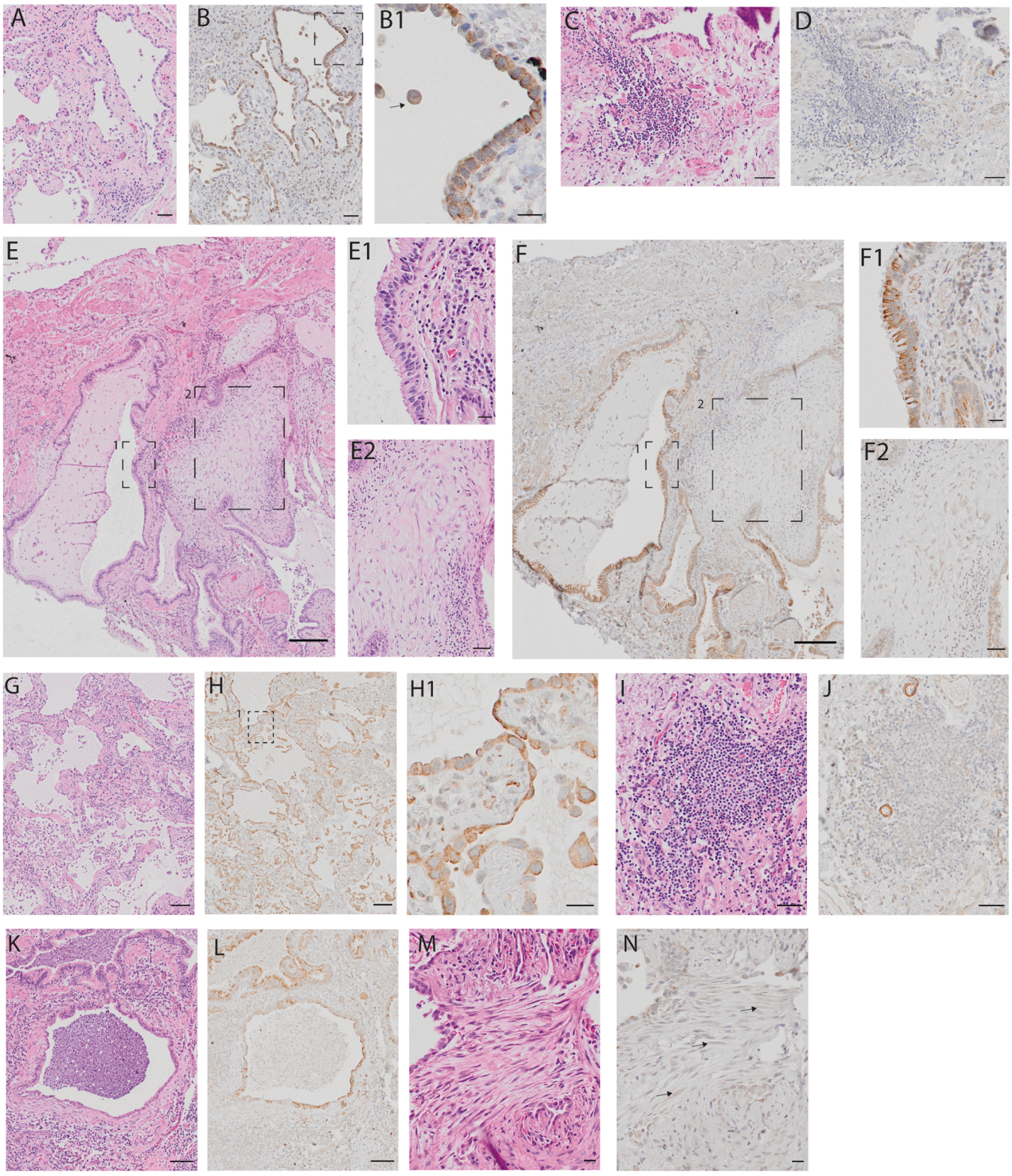


**Fig. 2. 5-HT$_{2B}$ receptor expression in remodeled distal lung tissue.** In pathological structures, 5-HT$_{2B}$ receptor was detected in restructured alveolar compartments marked with suspected ATII hyperplasia in IPF (A-B1) and SSc-ILD (G-H1), along with positive receptor expression in alveolar macrophages in IPF (B1, arrow). Low to devoid receptor expression was observed in inflammatory infiltrates in both IPF (C-D and SSc-ILD (I-J). Honeycomb cysts in IPF expressed the 5-HT$_{2B}$ receptor in ciliated epithelial cell lining the lumen of the cysts (E-E1, F-F1) as well as a weak expression in fibroblasts in fibroblast foci (E-E2, F-F2), which was also seen in SSc-ILD (honeycomb cyst K-L; fibroblast foci M-N, arrow = suspected fibroblasts). Images are representative based on the examination of three individuals per group with two separate tissue blocks per individual. Scale bar: 200 μm (E, F), 100 μm (G-H), 50 μm (A-D, I-N,), 20 μm (M-N, B1, E1–2, F1–2, H1).

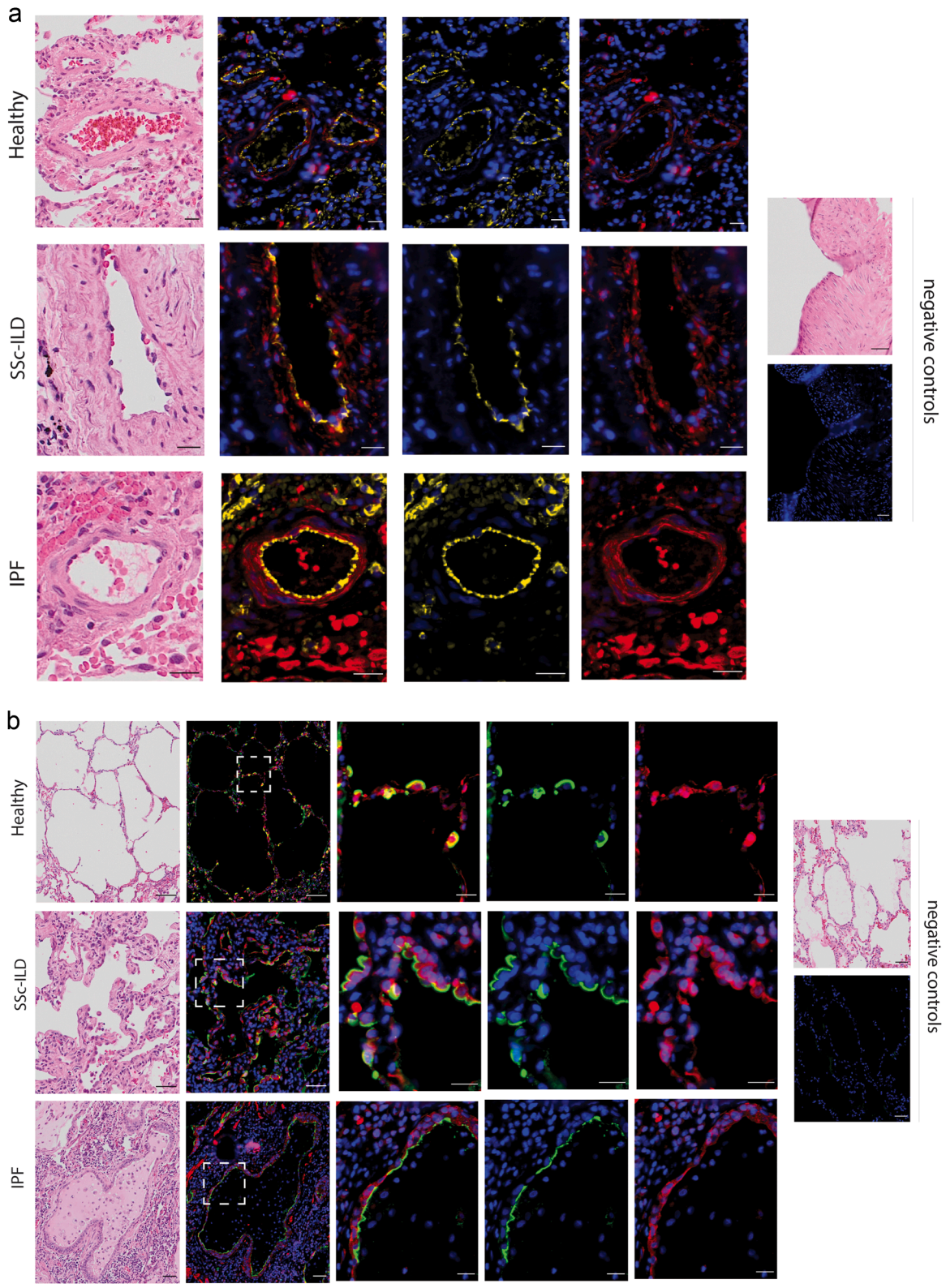


**Fig. 3. Expression of the 5-HT$_{2B}$ receptor on ATII cells and endothelial cells.** In lung tissue derived from heathy donors, SSc-ILD and IPF patients, endothelial cells (CD31$^{+}$ cells, yellow) were shown to express the 5-HT$_{2B}$ receptor in all groups (A). HT2–280$^{+}$ cells (green), identifying ATII cells, were shown to express the 5-HT$_{2B}$ receptor (red) (B). In IPF and SSc-ILD, the merged expression was seen in cells lining honeycomb cysts. Negative controls, with omitted primary antibodies, showed no clear antibody signal in examined structures. All images are shown together with HE stainings of corresponding tissue sections. Images are representative based on the examination of 2–3 individuals per group with either 1 or 2 separate tissue blocks per individual. Scale bars: images with HT2–280; healthy= 100 μm, SSc-ILD and IPF = 50 μm, with enlarged images = 20 μm. Images with CD31, scale bar = 20 μm and images with negative controls, scale bar = 50 μm.

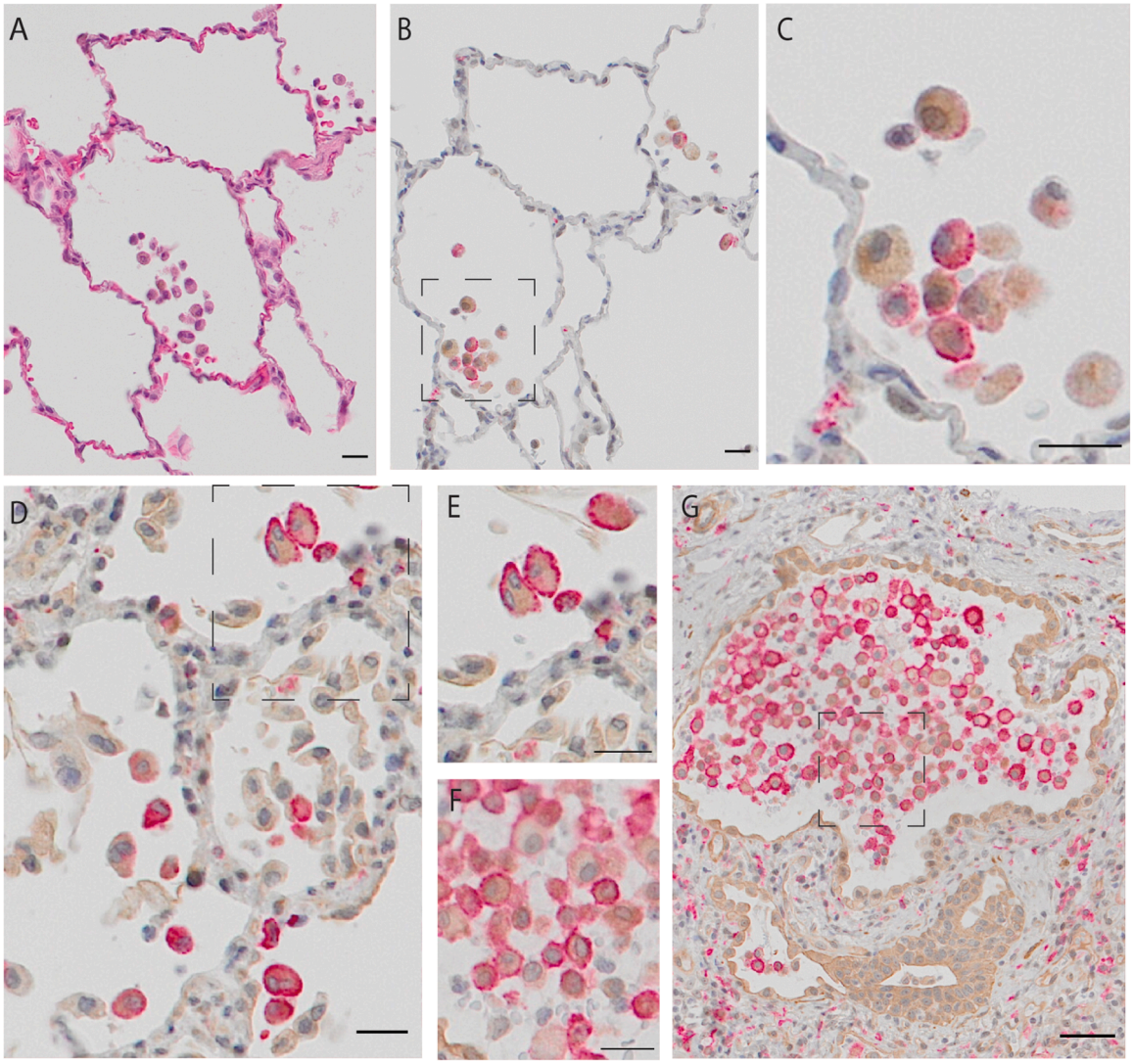


**Fig. 4. M2 macrophages express the 5-HT$_{2B}$ receptor in healthy and diseased lung tissue.** M2 macrophages (CD206$^{+}$, red) were visualized in distal lung tissue from healthy donor (A-C), IPF (D-E) and SSc-ILD (F-G) patients. M2 macrophages co-expressed the 5-HT$_{2B}$ receptor (brown). Scale bars. 20 μm (A-F), 50 μm (G). n = 2 individuals per group with either 1 or 2 separate tissue blocks.

distinct expression of the 5-HT$_{2B}$ receptor in these cells in both IPF and SSc-ILD patients together with a suggested 5-HT$_{2B}$-mediated fibrotic mechanism support further evaluation of this promising target in PF-ILDs.

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## CRediT authorship contribution statement

**Anna Lofdahl:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Annika Nybom:** Methodology, Formal analysis. **Jenny Wigen:** Writing – original draft, Writing – review & editing. **Göran Dellgren:** Resources, Funding acquisition. **Hans Brunnstrom:** Formal analysis. **Christina Wenglen:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Funding acquisition. **Gunilla Westergren-Thorsson:** Conceptualization, Investigation, Resources, Writing – review & editing, Funding acquisition.

## Conflict of interest

The authors declare no conflict of interest, except C.W. that declare employment by AnaMar AB, a company developing 5-HT$_{2B}$ receptor antagonists for therapeutic purposes.

## Data Availability

Data will be made available on request.

## References

Balestro, E., Calabrese, F., Turato, G., Lunardi, F., Bazzan, E., Marulli, G., et al., 2016. Immune inflammation and disease progression in idiopathic pulmonary fibrosis. PLoS One 11 (5), e0154516.

Bankhead, P., Loughrey, M.B., Fernandez, J.A., Dombrowski, Y., McArt, D.G., Dunne, P. D., et al., 2017. QuPath: open source software for digital pathology image analysis. Sci. Rep. 7 (1), 16878.

Chaturvedi, S., Misra, D.P., Prasad, N., Rastogi, K., Singh, H., Rai, M.K., et al., 2018. 5-HT2 and 5-HT2B antagonists attenuate pro-fibrotic phenotype in human adult dermal fibroblasts by blocking TGF-beta1 induced non-canonical signaling pathways including STAT3: implications for fibrotic diseases like scleroderma. Int. J. Rheum. Dis. 21 (12), 2128–2138.

Dees, C., Akhmetshina, A., Zerr, P., Reich, N., Palumbo, K., Horn, A., et al., 2011. Platelet-derived serotonin links vascular disease and tissue fibrosis. J. Exp. Med 208 (5), 961–972.
Denton, C.P., Khanna, D., 2017. Systemic sclerosis. Lancet 390 (10103), 1685–1699.
Distler, O., Highland, K.B., Gahlemann, M., Azuma, A., Fischer, A., Mayes, M.D., et al., 2019. Nintedanib for systemic sclerosis-associated interstitial lung disease. New Engl. J. Med. 380 (26), 2518–2528.
Fabre, A., Marchal-Somme, J., Marchand-Adam, S., Quesnel, C., Borie, R., Dehoux, M., et al., 2008. Modulation of bleomycin-induced lung fibrosis by serotonin receptor antagonists in mice. Eur. Respir. J. 32 (2), 426–436.
Gagermeier, J., Dauber, J., Yousem, S., Gibson, K., Kaminski, N., 2005. Abnormal vascular phenotypes in patients with idiopathic pulmonary fibrosis and secondary pulmonary hypertension. Chest 128 (6 Suppl), 601S.
Hirigoyen, D., Burgos, P.I., Mezzano, V., Duran, J., Barrientos, M., Saez, C.G., et al., 2015. Inhibition of angiogenesis by platelets in systemic sclerosis patients. Arthritis Res Ther. 17, 332.
Huang, J., Maier, C., Zhang, Y., Soare, A., Dees, C., Beyer, C., et al., 2017. Nintedanib inhibits macrophage activation and ameliorates vascular and fibrotic manifestations in the Fra2 mouse model of systemic sclerosis. Ann. Rheum. Dis. 76 (11), 1941–1948.
Hutchinson, J., Fogarty, A., Hubbard, R., McKeever, T., 2015. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. Eur. Respir. J. 46 (3), 795–806.
Khanna, D., Denton, C.P., Jahreis, A., van Laar, J.M., Frech, T.M., Anderson, M.E., et al., 2016. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. Lancet 387 (10038), 2630–2640.
Khanna, D., Tashkin, D.P., Denton, C.P., Renzoni, E.A., Desai, S.R., Varga, J., 2020. Etiology, risk factors, and biomarkers in systemic sclerosis with interstitial lung disease. Am. J. Respir. Crit. Care Med. 201 (6), 650–660.
Kishore, A., Petrek, M., 2021. Roles of macrophage polarization and macrophage-derived miRNAs in pulmonary fibrosis. Front Immunol. 12, 678457.
Konigshoff, M., Dumitrascu, R., Udalov, S., Amarie, O.V., Reiter, R., Grimminger, F., et al., 2010. Increased expression of 5-hydroxytryptamine2A/B receptors in idiopathic pulmonary fibrosis: a rationale for therapeutic intervention. Thorax 65 (11), 949–955.
de las Casas-Engel, M., Dominguez-Soto, A., Sierra-Filardi, E., Bragado, R., Nieto, C., Puig-Kroger, A., et al., 2013. Serotonin skews human macrophage polarization through HTR2B and HTR7. J. Immunol. 190 (5), 2301–2310.
Lehmann, M., Buhl, L., Alsafadi, H.N., Klee, S., Hermann, S., Mutze, K., et al., 2018. Differential effects of Nintedanib and Pirfenidone on lung alveolar epithelial cell function in ex vivo murine and human lung tissue cultures of pulmonary fibrosis. Respir. Res 19 (1), 175.
Lofdahl, A., Rydell-Tormanen, K., Muller, C., Martina Holst, C., Thiman, L., Ekstrom, G., et al., 2016. 5-HT2B receptor antagonists attenuate myofibroblast differentiation and subsequent fibrotic responses in vitro and in vivo. Physiol. Rep. 4, 15.
Lofdahl, A., Wenglen, C., Rydell-Tormanen, K., Westergren-Thorsson, G., Larsson-Callerfelt, A.K., 2018. Effects of 5-Hydroxytryptamine Class 2 Receptor Antagonists on Bronchoconstriction and Pulmonary Remodeling Processes. Am. J. Pathol. 188 (5), 1113–1119.
Lofdahl, A., Rydell-Tormanen, K., Larsson-Callerfelt, A.K., Wenglen, C., Westergren-Thorsson, G., 2018. Pulmonary fibrosis in vivo displays increased p21 expression reduced by 5-HT2B receptor antagonists in vitro - a potential pathway affecting proliferation. Sci. Rep. 8 (1), 1927.
Lofdahl, A., Tornling, G., Wigen, J., Larsson-Callerfelt, A.K., Wenglen, C., Westergren-Thorsson, G., 2020. Pathological insight into 5-HT2B receptor activation in fibrosing interstitial lung diseases. Int. J. Mol. Sci. 22, 1.
Mann, D.A., Oakley, F., 2013. Serotonin paracrine signaling in tissue fibrosis. Biochim. Biophys. Acta 1832 (7), 905–910.
Ortiz-Zapater, E., Signes-Costa, J., Montero, P., Roger, I., 2022. Lung fibrosis and fibrosis in the lungs: is it all about myofibroblasts? Biomedicines 10, 6.
Petric, M., Perkovic, D., Bozic, I., Marasovic Krstulovic, D., Martinovic Kaliterna, D., 2022. The levels of serum serotonin can be related to skin and pulmonary manifestations of systemic sclerosis. Medicina (2), 58.
Roofeh, D., Lescoat, A., Khanna, D., 2020. Emerging drugs for the treatment of scleroderma: a review of recent phase 2 and 3 trials. Expert Opin. Emerg. Drugs 25 (4), 455–466.
Ruaro, B., Salton, F., Braga, L., Wade, B., Confalonieri, P., Volpe, M.C., et al., 2021. The history and mystery of alveolar epithelial type II cells: focus on their physiologic and pathologic role in lung. Int. J. Mol. Sci. 22, 5.
Varga, J., Marangoni, R.G., 2017. Systemic sclerosis in 2016: dermal white adipose tissue implicated in SSc pathogenesis. Nat. Rev. Rheuma 13 (2), 71–72.
Willis, B.C., Borok, Z., TGF-beta-induced, E.M.T., 2007. mechanisms and implications for fibrotic lung disease. Am. J. Physiol. Lung Cell Mol. Physiol. 293 (3), L525–L534.
Wollin, L., Distler, J.H.W., Redente, E.F., Riches, D.W.H., Stowasser, S., Schlenker-Herceg, R., et al., 2019. Potential of nintedanib in treatment of progressive fibrosing interstitial lung diseases. Eur. Respir. J. 54, 3.
Zeller, J., Weissbarth, E., Baruth, B., Mielke, H., Deicher, H., 1983. Serotonin content of platelets in inflammatory rheumatic diseases. Correl. Clin. Act. Arthritis Rheum. 26 (4), 532–540.
Zhao, Y., De Los Santos, F.G., Wu, Z., Liu, T., Phan, S.H., 2018. An ST2-dependent role of bone marrow-derived group 2 innate lymphoid cells in pulmonary fibrosis. J. Pathol. 245 (4), 399–409.
Zhu, L., Fu, X., Chen, X., Han, X., Dong, P., 2017. M2 macrophages induce EMT through the TGF-beta/Smad2 signaling pathway. Cell Biol. Int. 41 (9), 960–968.