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**Paper:** Dysregulated Lung Commensal Bacteria Drive Interleukin-17B Production to Promote Pulmonary Fibrosis through Their Outer Membrane Vesicles. Yang et al. 2019. *Immunity* 50, 692-706.

**Running Title:** Don't Dys the B team: Dysregulated lung bacteria in fibrosis induce pro-inflammatory IL-17B.

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Idiopathic pulmonary fibrosis (IPF) is a severe disease with no cure, and a median survival rate of < 5 years (1). Other research indicates the cytokine IL-17 is part of the pro-fibrotic inflammatory response (2). The critical influence of the gut microbiome in IL-17 immune responses has been established (3), yet the role of the lung microbiota in lung inflammation, and specifically IL-17 responses that promote IPF, is overlooked. In their *Immunity* paper, Yang et al used the bleomycin model of fibrosis to determine if IL-17B, one of six IL-17 cytokines, caused lung fibrosis, and if the lung microbiome regulated pro-fibrotic IL-17 production.

They report that depletion of the microbiome with broad-spectrum antibiotics alleviated fibrosis and rescued mortality. The authors sequenced 16s DNA from lungs of control and bleomycin treated mice and found bleomycin caused dysbiosis. The researchers narrowed their results to three species, *B. ovatus* (B.o), *B. stercoris* (B.s), and *P. melaninogenica* (P.m), that were increased in the bleomycin group. Inoculation of antibiotics+bleomycin treated mice with any of these bacteria restored the severity of fibrosis. The researchers further determined that bacterial outer-membrane vesicles (OMV) isolated from B.s, P.m, or the lungs of bleomycin treated mice cause more severe fibrosis when transferred into antibiotics + bleomycin treated recipients, and LPS packaged in the OMV mediated the fibrotic pathogenesis. This is a novel finding, and it would be interesting to know if these bacteria or something equivalent is found in IPF, and what role bacterial OMV play in IPF as OMV have been shown to be potent inducers of pro-inflammatory responses and cell death elsewhere (4)

All of the microbiome-associated effects were found to be partially mediated by IL-17. Knocking out either IL-17A or B resulted in a 25% mortality rate in bleomycin, as opposed to 50% in WT, which suggests that both IL-17A and B are important drivers of fibrosis, but neither is solely responsible. The authors determined the signaling mechanism used by IL-17B and described downstream targets that are similar to IL-17A, which begs the question: why produce redundant IL-17 cytokines? One answer is that while IL-17 is strongly associated with T cells, the authors find, surprisingly, that IL-17B is produced by innate immune cells. This suggests complementary function in major the branches of the immune system.

Yang et al., produced wide scope of data that opens further avenues for investigation. More than one new finding was described in their work. However, there were some limitations. Microbiome depletion consisted of oral and intranasal antibiotics, and oral antibiotics alone had no effect on bleomycin pathogenesis. Yet it was a curious omission not to include a group that was only treated with intranasal antibiotics and leave the gut microbiome intact. The authors also claim the IL-17B producing innate immune cells are alveolar macrophages, but the markers used are not sufficient to confidently label that population (5). Further study of these IL-17B producing cells *in vitro* and better characterization *in vivo* is needed. Even with these limitations, their work shows a new role for lung bacteria that promote fibrosis, describes mechanistic detail of IL-17B signaling and OMV, and provides evidence of innate immune cells producing IL-17. Correlation exists between fibrosis in humans and dysbiosis in the lung, thus pre-clinical studies such as this provide insight into potential mechanisms of a poorly understood disease (6).

## References

1. Ley B, Collard HR, King TE, Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011;183(4):431-440.
2. Hams E, Armstrong ME, Barlow JL, Saunders SP, Schwartz C, Cooke G, Fahy RJ, Crotty TB, Hirani N, Flynn RJ, et al. IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis. *Proc Natl Acad Sci U S A* 2014;111(1):367-372.
3. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012;336(6086):1268-1273.
4. Vanaja SK, Russo AJ, Behl B, Banerjee I, Yankova M, Deshmukh SD, Rathinam VAK. Bacterial outer membrane vesicles mediate cytosolic localization of I $\psi$ s and caspase-11 activation. *Cell* 2016;165(5):1106-1119.
5. Misharin AV, Morales-Nebreda L, Mutlu GM, Budinger GR, Perlman H. Flow cytometric analysis of macrophages and dendritic cell subsets in the mouse lung. *Am J Respir Cell Mol Biol* 2013;49(4):503-510.
6. O'Dwyer DN, Ashley SL, Gurczynski SJ, Xia M, Wilke C, Falkowski NR, Norman KC, Arnold KB, Huffnagle GB, Salisbury ML, et al. Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *Am J Respir Crit Care Med* 2019;199(9):1127-1138.

**Paper:** PU.1 controls fibroblast polarization and tissue fibrosis. Wohlfahrt et al. 2019. *Nature* 566, 344-349

**Running Title:** PU.1, the missing switch behind fibrotic fibroblast polarization?

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Wohlfahrt et al., in their recent *Nature* publication, have identified PU.1, an ETS family transcription factor, as an essential regulator of the genetic switch between extracellular matrix producing profibrotic fibroblasts, as seen in idiopathic pulmonary fibrosis (IPF), and catabolic pro-inflammatory fibroblasts, as seen in rheumatoid arthritis. PU.1 has previously been implicated in diseases such as acute myeloid leukemia, Hodgkin lymphoma and Alzheimer's. However, a link between PU.1, fibroblasts and fibrosis has been inferred by a resistance to thioacetamide-induced fibrosis, and apparent scar-free wound healing, in PU.1 deficient mouse models (1, 2).

The authors report that PU.1, long known for its fundamental role in hematopoiesis and immune system development (3), is highly expressed in matrix producing fibrotic fibroblasts (isolated from pulmonary fibrosis patients), but is silenced by epigenetic mechanisms in resting (isolated from healthy controls) and inflammatory fibroblasts (isolated from patients with asthma) by repressive histone methylation and elevated expression of miR-155 respectively. This finding is consistent with the emerging role of miRNAs and/or epigenetic regulation in the pathogenesis of IPF. Manipulations of *SPI1* (gene encoding PU.1 in humans) in the phenotypically different fibroblasts examined polarized fibroblasts towards a pro-fibrotic gene signature by binding to the promoters of pro-fibrotic genes (e.g COL1A1 and ACTA2) and induced a pro-fibrotic phenotype supporting the suggested link between PU.1, fibroblasts and fibrosis suggested in previous publications.

Wohlfhart and colleagues report that PU.1 is associated with a network of pro-fibrotic factors including members of the TEAD-HIPPO, canonical TGF- $\beta$ -SMAD and AP1 signaling pathways. Other transcription factors linked to fibrosis such as SNAI2 were found to bind in close proximity to PU.1-binding sites within the genome and may contribute to the recruitment of transcription factors and the switch towards a fibrotic phenotype. Of note, many of the transcription factors identified differ from those previously reported to interact with PU.1 in shaping cell phenotype in macrophages and immune cells.

Interestingly, a recent report found that overexpression of *Spi1* in fibroblasts induced senescence (4). The question of what is driving abnormally high numbers of fibroblasts into senescence in IPF remains unanswered. Transcription factors, such as STAT3 and NF $\kappa$ B, known to cooperate and interact with PU.1 have been implicated in fibroblast senescence in IPF (5). Potentially PU.1, acting as a genetic switch, driving fibroblast polarization towards a fibrotic phenotype may also lead or contribute to the accumulation of senescent fibroblasts in the fibrotic lung.

The authors utilized the heterocyclic dication DB1976 to show that inhibition of PU.1 was efficacious in preventing uncontrolled fibrosis in murine models without perceptible inhibition of haematopoietic cell differentiation. Using DB1976, the authors report that it is possible to safely interfere with a transcription factor, such as PU.1, and enable the restoration of tissue homeostasis. This study supports the work of Antony-Debre et al. who also recently demonstrated that inhibition of PU.1, in experimental models of acute myeloid leukemia, was therapeutically beneficial in decreasing tumor burden and increasing survival (6). In combination these studies present proof of concept that PU.1 has potential as a therapeutic strategy in a number of conditions currently poorly served by available therapeutic modalities.

## References

1. Liu Q, Zhang Y, Yang S, Wu Y, Wang J, Yu W, Liu Y. Pu.1-deficient mice are resistant to thioacetamide-induced hepatic fibrosis: Pu.1 finely regulates sirt1 expression via transcriptional promotion of mir-34a and mir-29c in hepatic stellate cells. *Biosci Rep* 2017;37(6).
2. Martin P, D'Souza D, Martin J, Grose R, Cooper L, Maki R, McKercher SR. Wound healing in the pu.1 null mouse--tissue repair is not dependent on inflammatory cells. *Curr Biol* 2003;13(13):1122-1128.
3. Rothenberg EV, Hosokawa H, Ungerback J. Mechanisms of action of hematopoietic transcription factor pu.1 in initiation of t-cell development. *Front Immunol* 2019;10:228.
4. Delestre L, Cui H, Esposito M, Quiveron C, Mylonas E, Penard-Lacronique V, Bischof O, Guillouf C. Senescence is a spi1-induced anti-proliferative mechanism in primary hematopoietic cells. *Haematologica* 2017;102(11):1850-1860.
5. Waters DW, Blokland KEC, Pathinayake PS, Wei L, Schuliga M, Jaffar J, Westall GP, Hansbro PM, Prele CM, Mutsaers SE, et al. Stat3 regulates the onset of oxidant-induced senescence in lung fibroblasts. *Am J Respir Cell Mol Biol* 2019.
6. Antony-Debre I, Paul A, Leite J, Mitchell K, Kim HM, Carvajal LA, Todorova TI, Huang K, Kumar A, Farahat AA, et al. Pharmacological inhibition of the transcription factor pu.1 in leukemia. *J Clin Invest* 2017;127(12):4297-4313.

**Paper:** Yu G. *et al.* Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. *Nature Medicine*. 24, 39-49 (2018)

**Running Title:** An anti-fibrotic role for thyroid hormone in lung fibrosis

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While metabolomic studies have implicated dysregulated cellular metabolism in the pathogenesis of Idiopathic Pulmonary Fibrosis (IPF) (1, 2), the molecular mechanisms by which aberrant metabolic signaling pathways contribute to lung fibrosis are not well-understood. In their recent work published in *Nature Medicine*, Yu et al. found a marked increase in expression and activity of thyroid hormone (TH)-activating enzyme iodothyronine deiodinase 2 (DIO2) in lungs of IPF patients, potentially reflecting either TH deficiency or increased cellular metabolic requirements. *DIO2* gene expression was negatively correlated with disease severity, suggesting an adaptive role of DIO2 in IPF. Consistent with this observation in patients, in mouse studies, (a) genetic deletion of *DIO2* exacerbated bleomycin-induced lung fibrosis, (b) aerosolized delivery of TH led to resolution of fibrosis in the bleomycin and inducible transforming growth factor  $\beta$ 1 (TGF  $\beta$ 1) models, and (c) oral delivery of sobetirome, a thyromimetic drug, decreased established lung fibrosis in the bleomycin model. Mechanistic studies in peroxisome proliferator-activated receptor  $\gamma$  co-activator 1a (*Ppargc1a*)- or PTEN-induced putative kinase 1 (*Pink1*)- knockout mice and type II alveolar epithelial cells (AECs) revealed that TH acts through the PGC-1 $\alpha$  and PINK1 pathways to attenuate mitochondria-regulated cell death pathways in type II AECs. A limitation here is the lack of evaluation of the effect of TH on cell types other than type II AECs; experiments utilizing mice with knockdown of the thyroid hormone receptor (TR) in different fibrosis-relevant cell types would be beneficial in this regard. Nevertheless, taken together, their data make a strong case for an antifibrotic role of TH in type II AECs in IPF.

In 2015, Bueno et al. found that there is prominent accumulation of dysmorphic and dysfunctional mitochondria in type II AECs in lungs of IPF patients, which is associated with low

expression of PINK1(3). The current study by Yu et al. shows upstream regulation of PINK1 by TH-mediated signaling and renders support to the idea that dysfunctional mitochondria in type II AECs play an important role in pathogenesis of IPF. More recently, Bueno et al. reported that endoplasmic reticulum (ER) stress-effector activating transcription factor 3 (ATF3) can repress PINK1 expression in type II AECs and lead to dysregulation of mitochondria homeostasis (4). Given that ER stress is a critical pathogenic mechanism in lung fibrosis(5), further studies aimed at determining the relationship between TH signaling, ER stress, and mitochondrial function could be enlightening.

The only two FDA-approved drugs nintedanib and pirfenidone (and saracatinib, which was recently granted orphan drug designation for IPF by FDA) target fibroblasts that are the effector cells in IPF. Since Yu et al. provide evidence for a beneficial effect of the thyromimetic sobetirome on the function of epithelial cells, which are important in the initiation of fibrosis, it is tempting to speculate about a potential role of thyromimetics in early stages of the disease or in combination therapy. Also, given that mitochondrial dysfunction is a hallmark of cellular aging, an open question is whether thyromimetics could prove beneficial in other aging-related conditions. However, it is important to keep in mind that animal models often fail to predict human responses to drugs (6), and extensive studies including those on dosing, delivery, safety, and efficacy would be critical in fully elucidating the clinical potential of thyromimetics in IPF.

## References:

1. Kang YP, Lee SB, Lee JM, Kim HM, Hong JY, Lee WJ, Choi CW, Shin HK, Kim DJ, Koh ES, et al. Metabolic profiling regarding pathogenesis of idiopathic pulmonary fibrosis. *J Proteome Res* 2016;15(5):1717-1724.
2. Zhao YD, Yin L, Archer S, Lu C, Zhao G, Yao Y, Wu L, Hsin M, Waddell TK, Keshavjee S, et al. Metabolic heterogeneity of idiopathic pulmonary fibrosis: A metabolomic study. *BMJ Open Respir Res* 2017;4(1):e000183.
3. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, Kamga C, Corey C, Herazo-Maya JD, Sembrat J, Lee JS, et al. Pink1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *J Clin Invest* 2015;125(2):521-538.
4. Bueno M, Brands J, Voltz L, Fiedler K, Mays B, St Croix C, Sembrat J, Mallampalli RK, Rojas M, Mora AL. Atf3 represses pink1 gene transcription in lung epithelial cells to control mitochondrial homeostasis. *Aging Cell* 2018;17(2).
5. Burman A, Tanjore H, Blackwell TS. Endoplasmic reticulum stress in pulmonary fibrosis. *Matrix Biol* 2018;68-69:355-365.
6. Moore BB, Lawson WE, Oury TD, Sisson TH, Raghavendran K, Hogaboam CM. Animal models of fibrotic lung disease. *Am J Respir Cell Mol Biol* 2013;49(2):167-179.