



## Estrogen Signaling and MicroRNAs in Lung Fibrosis Sex, Hormones, and Rock Scars

Substantial epidemiological evidence suggests that sex impacts the prevalence, susceptibility, and severity of chronic lung diseases. Whether this is directly related to sex hormones is currently unknown. On the other hand, it is well known that sex hormones regulate lung development, physiology, and pathology (1). In particular, idiopathic pulmonary fibrosis (IPF), a progressive fibrotic lung disease of unknown etiology, is more prevalent and has a worse prognosis in males than in females (2). However, differences in survival in both humans (3) and experimental models (4) are still controversial. Sex differences exist in other chronic lung diseases, including idiopathic pulmonary arterial hypertension (5), lymphangiomyomatosis, and collagen vascular disease-associated interstitial lung diseases (6) that primarily affect young to middle-aged women, supporting a role for estrogen signaling in both disease development and progression (7). These sex differences underline the importance of considering sex hormones in the prevention and treatment of chronic lung diseases.

In this issue of the *Journal*, Elliot and colleagues (pp. 1246–1257) study the role of estrogen signaling in the pathogenesis of lung fibrosis and attempt to explain sex differences in disease development and prognosis (8). They describe the results of an elegant series of experiments and report the following major findings:

1. ER $\alpha$  (estrogen receptor  $\alpha$ ) expression was upregulated in IPF lung tissue and fibroblasts at both the mRNA and protein levels.
2. IPF lung fibroblasts exhibited increased responsiveness to estrogen compared with controls, and blockage of ER diminished this effect. *In vivo* pharmacologic inhibition and mimicry of ER $\alpha$  and ER $\beta$ , respectively, attenuated bleomycin-induced lung fibrosis, as assessed by biochemical and histological analyses of fibrotic lungs.
3. Mechanistic data demonstrated that ER $\alpha$  antagonism exerted its antifibrotic properties through negative regulation of profibrotic kinase-controlled signal transduction pathways, including Smad2 and AKT (protein kinase B). ER $\beta$  agonism appeared to exert minimal effects on the regulation of the Smad-mediated TGF $\beta$  (transforming growth factor  $\beta$ ) canonical pathway.
4. The antifibrotic microRNAs let-7a (lethal-7a) and let-7d, which are known to be mediators of the ER-signaling pathway and to be reduced in IPF lungs (9), downregulated ER $\alpha$  and TGF- $\beta$  and increased Smad7 mRNA expression, respectively, in IPF lung myofibroblasts.
5. Mice with genetic ablation of the AF2 (activation function 2) functional domain of the estrogen ligand-binding domain developed lung fibrosis in response to bleomycin, which also occurred in

wild-type littermates, suggesting that ligands other than estrogens may be responsible for bleomycin-induced lung fibrosis.

6. IGF-1 (insulin-like growth factor 1), a known stimulator of the ER-signal transduction pathway, was upregulated in both IPF lungs and myofibroblasts and mediated ER transcriptional activity only in the IPF lung fibroblasts.

The idea of treating lung fibrosis with hormone analogs is both challenging and attractive. Our study group recently showed that IPF lungs are locally hypothyroid, and aerosolized administration of active thyroid hormone (T3) as well as systemic delivery of sobetirome, a thyroid-hormone receptor agonist, alleviated experimental lung fibrosis through restoration of alveolar epithelial cell mitochondrial homeostasis (10). In a similar way, a first-line antidiabetic drug called metformin exerts antifibrotic properties through positive regulation of the AMPK (adenosine monophosphate-activated protein kinase)-controlled metabolic pathway, rendering lung myofibroblasts more prone to apoptosis (11). Interestingly, and relevant to the concept of the current study, administration of danazol, a weak androgen, was shown to reconstitute telomere length in patients with manifestations of short-telomere syndrome, including bone marrow failure and pulmonary fibrosis (12). Dehydroepiandrosterone, a steroid prohormone that decreases with age, was shown to be significantly decreased in patients with IPF (an aging-related disorder) and exhibited strong antifibrotic properties through pleiotropic mechanisms such as apoptosis, proliferation, and differentiation of lung myofibroblasts (13). Finally, vitamin D, another steroid prohormone with a vital role in bone and muscle health, was found to be deficient in patients with IPF, was associated with worse prognosis, and exerted antifibrotic properties both *in vitro* and *in vivo* (14). The story of drug repurposing is rapidly expanding in chronic lung diseases, including IPF. Unfortunately, retrospective analyses of pirfenidone trials showed no effect of metformin use in patients with IPF, indicating that personalized-medicine approaches need to be applied to increase therapeutic likelihood (15). Whether hormone supplementation is effective for lung fibrosis *per se* or its associated comorbidities remains to be determined. Large prospective, randomized clinical trials in biologically enriched and well-defined cohorts of patients with IPF are required to address this issue. Local delivery of hormone analogs may provide clinical benefit with minimal adverse events and may lead to novel therapeutic strategies.

Although the results are cause for enthusiasm, this study has some limitations that should be adequately discussed:

1. The exact mechanism that regulates ER activation is currently unknown. Although the authors performed a thorough investigation of the ER-signal transduction pathway, genomic and nongenomic regulators of the ER pathway require further validation to identify potential therapeutic candidates. The suggested linkage of the ER pathway with microRNA mimicry appears vague and complicated given that microRNAs are undruggable targets due to their pleiotropism and complexity.
2. The phenomenon of ER-mediated attenuation of bleomycin-induced lung fibrosis does not seem to be cell specific. The authors focused on fibroblast biology; however, the effects of ER-signaling

©This article is open access and distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives License 4.0 (<http://creativecommons.org/licenses/by-nc-nd/4.0/>). For commercial usage and reprints, please contact Diane Gern (dgern@thoracic.org).

Originally Published in Press as DOI: 10.1164/rccm.201908-1535ED on August 22, 2019

modulation in other cell types that are known to be involved in lung fibrogenesis, including structural (epithelial) and immune (macrophages and T cells) cells, need to be better clarified.

3. ERs are ubiquitously expressed receptors that are involved in physiological and pathological processes, including carcinogenesis, and therefore the side effects of modulating their activity require further consideration. In addition, pulmonary hypertension and embolism have been associated with oral contraceptives through estrogen-induced obliterative and thrombotic vascular lesions, and estrogens exert proinflammatory and oxidative stress effects (1).

Despite the above concerns, this study represents the first attempt to provide a pathogenic linkage between sex hormones and lung collagen deposition, and to explain the female survival bias in lung fibrosis. Linking ER and other hormone analogs with accelerated aging, senescence, and metabolic disorders, which are often seen in patients with fibrotic lung disease, would be an interesting future approach—the best is yet to come. ■

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

Argyris Tzouveleakis, M.D., M.Sc., Ph.D.  
Demosthenes Bouros, M.D., Ph.D.  
Medical School  
National and Kapodistrian University of Athens  
Athens, Greece

## References

- Carey MA, Card JW, Voltz JW, Germolec DR, Korach KS, Zeldin DC. The impact of sex and sex hormones on lung physiology and disease: lessons from animal studies. *Am J Physiol Lung Cell Mol Physiol* 2007; 293:L272–L278.
- Olson AL, Swigris JJ, Lezotte DC, Norris JM, Wilson CG, Brown KK. Mortality from pulmonary fibrosis increased in the United States from 1992 to 2003. *Am J Respir Crit Care Med* 2007;176:277–284.
- Han MK, Murray S, Fell CD, Flaherty KR, Toews GB, Myers J, et al. Sex differences in physiological progression of idiopathic pulmonary fibrosis. *Eur Respir J* 2008;31:1183–1188.
- Gharaee-Kermani M, Hatano K, Nozaki Y, Phan SH. Gender-based differences in bleomycin-induced pulmonary fibrosis. *Am J Pathol* 2005;166:1593–1606.
- Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Survival in patients with idiopathic, familial, and anorexigen-associated pulmonary arterial hypertension in the modern management era. *Circulation* 2010;122:156–163.
- Gleicher N, Barad DH. Gender as risk factor for autoimmune diseases. *J Autoimmun* 2007;28:1–6.
- Gupta N, Finlay GA, Kotloff RM, Strange C, Wilson KC, Young LR, et al.; ATS Assembly on Clinical Problems. Lymphangioloeyomatosis diagnosis and management: high-resolution chest computed tomography, transbronchial lung biopsy, and pleural disease management. An official American Thoracic Society/Japanese Respiratory Society clinical practice guideline. *Am J Respir Crit Care Med* 2017;196:1337–1348.
- Elliot S, Periera-Simon S, Xia X, Catanuto P, Rubio G, Shahzeidi S, et al. MicroRNA let-7 downregulates ligand-independent estrogen receptor-mediated male-predominant pulmonary fibrosis. *Am J Respir Crit Care Med* 2019;200:1246–1257.
- Pandit KV, Corcoran D, Yousef H, Yarlagadda M, Tzouveleakis A, Gibson KF, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2010;182:220–229.
- Yu G, Tzouveleakis A, Wang R, Herazo-Maya JD, Ibarra GH, Srivastava A, et al. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. *Nat Med* 2018;24:39–49.
- Rangarajan S, Bone NB, Zmijewska AA, Jiang S, Park DW, Bernard K, et al. Metformin reverses established lung fibrosis in a bleomycin model. *Nat Med* 2018;24:1121–1127.
- Townsley DM, Dumitriu B, Liu D, Biancotto A, Weinstein B, Chen C, et al. Danazol treatment for telomere diseases. *N Engl J Med* 2016; 374:1922–1931.
- Mendoza-Milla C, Valero Jiménez A, Rangel C, Lozano A, Morales V, Becerril C, et al. Dehydroepiandrosterone has strong antifibrotic effects and is decreased in idiopathic pulmonary fibrosis. *Eur Respir J* 2013;42:1309–1321.
- Tzilas V, Bouros E, Barbayianni I, Karampitsakos T, Kourtidou S, Ntassiou M, et al. Vitamin D prevents experimental lung fibrosis and predicts survival in patients with idiopathic pulmonary fibrosis. *Pulm Pharmacol Ther* 2019;55:17–24.
- Spagnolo P, Kreuter M, Maher TM, Wuyts W, Bonella F, Corte TJ, et al. Metformin does not affect clinically relevant outcomes in patients with idiopathic pulmonary fibrosis. *Respiration* 2018;96:314–322.

Copyright © 2019 by the American Thoracic Society



## ⊗ The Fibrosis Burden of Systemic Sclerosis

Interstitial lung disease (ILD) tends to occur early in systemic sclerosis (SSc), places a significant burden on the patient, and is a leading cause of death (1–4). Although there is a well-recognized association between SSc and ILD, it is still not clear who will develop ILD, and if ILD does indeed develop, which patients will experience significant progression and over what period that progression might occur. Our current understanding of the

incidence and progression of this disease comes from several well-designed clinical trials, including SLS I (Scleroderma Lung Study I) and SLS II (3–5).

SLS I included 158 patients from 13 centers with symptomatic lung disease and either BAL evidence of alveolitis or any ground-glass opacities on computed tomography (CT), and with FVC between 45% and 85% predicted (3). Approximately 90% of the patients had some ground-glass opacity and 89% had evidence of fibrosis on high-resolution CT of the chest; 73% of the placebo group had worsening of FVC over a 12-month period. In that study, treatment with cyclophosphamide was found to have a modest effect on lung function decline. In the follow-on SLS II study, there was an 11% mortality at 1 year, which was due to progressive ILD, even in patients receiving cyclophosphamide (4).

⊗This article is open access and distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives License 4.0 (<http://creativecommons.org/licenses/by-nc-nd/4.0/>). For commercial usage and reprints, please contact Diane Gern ([dgern@thoracic.org](mailto:dgern@thoracic.org)).

Originally Published in Press as DOI: 10.1164/rccm.201907-1438ED on August 7, 2019