Longitudinal MRI of Progressive Pulmonary Fibrosis in a Transgenic, TGF-Alpha-Induced Mouse Model

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Target Audience: Lung Imaging, Preclinical Imaging

Purpose: Idiopathic pulmonary fibrosis (IPF) is a spatially heterogeneous, progressive, and inevitably fatal disorder, for which there are no approved medical therapies that can reverse disease progression. Because IPF etiology is poorly understood, mouse models play a vital role in studying the biological mechanisms underlying disease progression and in assessing potential therapies. Unfortunately, the histological and biochemical assays used to study murine pulmonary fibrosis require mice to be sacrificed and lungs to be excised and sectioned or homogenized. Thus, the spatial and temporal information commonly available from these models is limited. To provide regional information over time, MRI has shown promise for non-invasively examining lung fibrosis in small animals. However, all previous murine MRI studies have focused on fibrosis models that involve acute insults such as bleomycin[1,2], which do not exhibit the pathological progression seen in IPF. Moreover, acute insults generate intense inflammation, which does not typically occur in human disease[3]. Thus, while imaging studies of these models show methodological promise, they are of limited use for elucidating IPF biology.

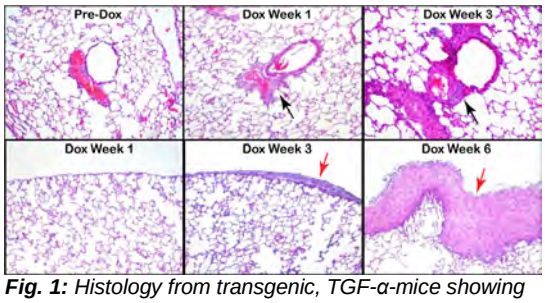

Fig. 1: *Histology from transgenic, TGF-α-mice showing progressive, vascular-adventitial fibrosis (top, black arrows) and pleural fibrosis (bottom, red arrows).*

Here we report the first longitudinal imaging study of a non-inflammatory and progressive model of IPF. Specifically, we examined a doxycycline (Dox) regulatable, transgenic mouse model[4] that overexpresses transforming growth factor alpha (TGF-α) under control of a lung-epithelial-specific promoter gene. When treated with Dox, mice display progressive pathology that mimics key aspects of non-inflammatory human pulmonary fibrotic disease, including extracellular matrix deposition and fibrotic lesion migration from the vascular adventitia and subpleural region into the interstitium (Fig. 1). When Dox administration ceases, pathological changes begin to resolve, thus testing MRI's ability to assess biologically relevant fibrosis progression and resolution in a single animal model.

Methods: Fibrosis was generated in human-TGF-α expressing mice (FVB/NJ background) over 7 weeks by providing Dox-treated food (62.5 mg/kg, Sigma Aldrich). Multi-slice, GRE MRI (BW=150kHz, TE/TR=0.85/104ms, α=45°, NEX=8, slice thickness= 0.75mm, resolution=160μm×160μm) was performed at 11 time points (pre-Dox treatment, once/week on Dox, once/week for 3 weeks post-Dox) in free-breathing, isoflurane-anesthetized mice (n=4). Images were acquired with respiratory and cardiac gating at end expiration (acquisition time ~15 min), using a 7-T Bruker Biospec system. After the final MRI session, animals were euthanized, and lungs were prepared for histology.

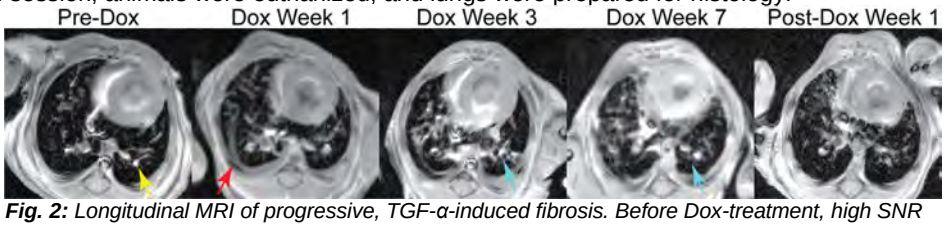

Fig. 2: *Longitudinal MRI of progressive, TGF-α-induced fibrosis. Before Dox-treatment, high SNR was observed from only larger vasculature (yellow arrow). After 1 week on Dox, high-SNR structures were observed extending from the pleura into the interstitium (red arrow). Over time, most of these regions thickened and, high-SNR was also observed surrounding the pulmonary vasculature (Weeks 3 and 7, blue arrows). By Post-Dox Week 1, high-SNR structures began to resolve.*

Results and Discussion: Prior to treatment, images from all animals displayed low signal-to-noise ratio (SNR) from the lung parenchyma (SNR~6) and high SNR only from larger pulmonary vasculature (Fig. 2, Pre-Dox). After 1 week on Dox, high SNR was observed extending from the pleura into the interstitium. These regions typically thickened in Weeks 2–7, and high SNR was also observed extending from the pulmonary vasculature. Consistent with the physiology of restrictive lung disease, lung volume decreased during treatment in all animals. After Dox-treatment (e.g., Post-Dox Week 1), the total volume of high-SNR regions decreased and lung volume increased in all animals, consistent with published functional, biochemical, and histological studies[5,6]. Additionally, longitudinal MRI of fibrosis progression allowed previously unrecognized dynamic processes to be observed. That is, transient structures were observed forming and resolving during Dox-treatment in all animals (e.g., Fig. 3). These results suggest that fibrosis progression in this model involves a spatially heterogeneous, dynamic interplay between the lung's healing processes and pathological remodeling that can only be detected and studied through longitudinal imaging.

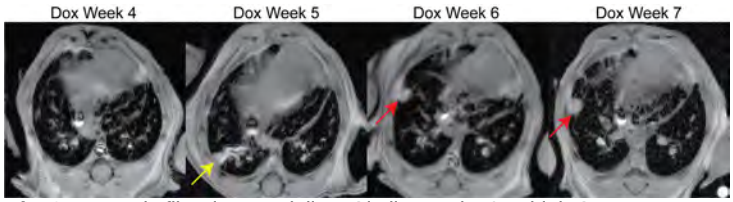

Fig. 3: *Dynamic fibrotic remodeling. Similar to Fig. 2, a high-SNR structure protruding from the sub-pleural region in Week 6 persisted into Week 7 (red arrows). In contrast, a second structure (yellow arrow) formed by Week 5 and resolved by Week 6, indicating that fibrosis progression in this model involves a previously unknown, dynamic component.*

Conclusions: The formation and disappearance of high-SNR regions in lung MRI is consistent with the patterns of pleural and adventitial fibrosis proliferation and resolution known to occur in this model. However, MRI also revealed dynamic remodeling that was previously unknown. Together, these results argue that MRI can non-invasively assess pulmonary fibrosis progression and resolution in mouse models and provide new insights into the biology of pulmonary fibrosis.

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References: [1] C Egger, et al., *Am J Physiol-Lung, C* 306 (12), L1064-L1077 (2014). **[2]** RE Jacob, et al., *J MRI*, 31 (5) 1091-1099 (2010) **[3]** A Moeller, et al., *Int J Biochem Cell B*, 40 (3), 362-382 (2008) **[4]** WD Hardie, et al., *Am J Physiol-Lung C,* 286 (4) L741-L749 (2004) **[5]** WD Hardie, et al., *Am J Resp Cell Mol,* 37 (3), 309-321 (2007) **[6]** TD Le Cras, et al., *Am J Pathol,* 176 (2), 679-686 (2010)