PNAS LETTER

Reply to Chong et al.: Investigating TGFβ1 biological activities using genetically engineered mouse strains

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In their letter (1), Chong et al. informed us about their research (2), which thematically overlaps with figure 4 of our investigation on the role of extracellular histones in the TGFβ1/IL-27 balance in fibrotic lung remodeling (3). Both studies utilized Cre-lox mouse strains for a platelet-specific deletion of TGFβ1 in bleomycin-induced fibrosis. Chong et al. did not observe differences in lung fibrosis severity despite using an extensive panel of endpoints, contrasting with our findings highlighting the importance of platelet-derived TGFβ1 in solid organ fibrosis.

Chong et al. (1) speculated about possible explanations for these divergent findings and acknowledged using a different *Tgfb1* floxed mouse strain (4, 5). Specifically, they used *B9d2*^{exon4}*Tgfb1*^{exon1} floxed mice, which have both exon 1 of *Tgfb1* and exon 4 of the adjacent gene *B9d2* ("*stumpy*"; NM_172148) flanked with loxP sites (4, 6). At the time that these mice were generated, the proximity of the *B9d2* gene to the *Tgfb1* gene was not appreciated.

B9D2 is essential for mammalian ciliogenesis (6), highly abundant in the lungs, and expressed in hematopoietic cells and platelets. Loss-of-function mutations in *B9d2* are associated with Meckel Syndrome and Joubert Syndrome, which can include organ fibrosis, suggesting B9D2 may have properties that protect against fibrosis (7). Thus, dual deletion of profibrotic TGFβ1 and potentially antifibrotic B9D2 could rebalance the fibrotic response to resemble that of wild-type mice. Further studies are needed to explore the significance of B9D2 in lung fibrosis.

Moving forward, we propose refining gene targeting approaches. A new conditional TGFβ1 strain recently provided by Jackson Laboratories (C57BL/6J-*Tgfb1*^{em2Lutzy}/Mmjax; exon 3 floxed) could be crossed with the *Gp1bα*-Cre deleter for highly specific gene ablation in megakaryocytes and platelets (8).

In addition to sex differences, age may be a confounding factor in human idiopathic pulmonary fibrosis (IPF) and healthy control cohorts (2), as TGFβ1 concentrations inversely correlate with age (9). While we agree that micro-computed tomography (CT) has advantages over histology for quantifying interstitial lung disease, it also has limitations, such as difficulty in reliably distinguishing fibrosis from inflammation.

Platelets contain 40 to 100 times more TGFβ1 than other cell types (10), are locally activated during pulmonary fibrosis, and local TGFβ1 overexpression results in lung fibrosis (11). We find that the current evidence strongly supports a role of platelet-derived TGFβ1 in the development and progression of pulmonary fibrosis.

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