Molecular hallmarks of lung cellular senescence in the bleomycin-induced and spirometry-confirmed mouse model of IPF

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Background & Aim

There is increasing evidence for senescent cells to play a contributory role in the pathogenesis of idiopathic pulmonary fibrosis (IPF).

This study aimed to characterize molecular markers of pulmonary senescence across the disease spectrum in the bleomycin-induced (BLEO) and spirometry-confirmed mouse model of IPF.

Methods

10-12 weeks old C57BL/6JRj male mice received either a single intratracheal instillation of bleomycin (1.5 mg/kg, 50 µL) or saline (CTRL) at study day 1. BLEO-IPF animals were randomized into study groups based on body weight loss at day 6 post-BLEO and terminated at specified time points (Fig. 1).

Terminal pulmonary endpoints included *(1)* spirometry (flexiVent) for expiratory/inspiratory capacity; *(2)* biochemical analysis for hydroxyproline (HP) content; *(3)* quantitative histomorphometry for markers of cellular senescence (p21) and fibrosis (PSR, Col1a1); *(4)* AI-based Ashcroft fibrosis scoring; *(5)* transcriptome signatures of cellular senescence.

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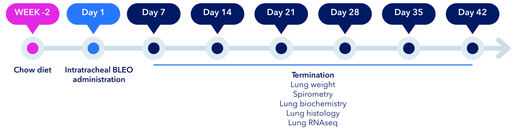
1 Study outline



Group	Animal	Name	Number of animals
1	CTRL	CTRL	10
2	BLEO-IPF	D7	10
3	BLEO-IPF	D14	11
4	BLEO-IPF	D21	12
5	BLEO-IPF	D28	12
6	BLEO-IPF	D35	12
7	BLEO-IPF	D42	9

Figure 1. Study outline and group overview

2 Lung weight and HP

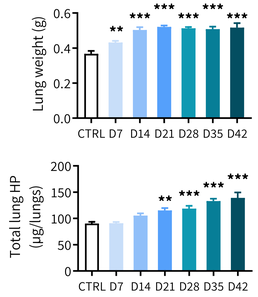


Figure 2. Metabolic and biochemical parameters in BLEO-IPF mice. **(A)** Terminal lung weight (g). **(B)** Terminal lung total HP. Dunnett's test one-factor linear model. **p<0.01, and ***p<0.001 vs. CTRL group.

3 Pulmonary function

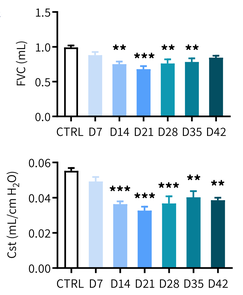


Figure 3. Pulmonary function testing in BLEO-IPF mice. **(A)** Forced vital capacity (FVC). **(B)** Static compliance (Cst). Dunnett's test one-factor linear model. **p<0.01, and ***p<0.001 vs. CTRL group.

4 Histological markers of fibrosis

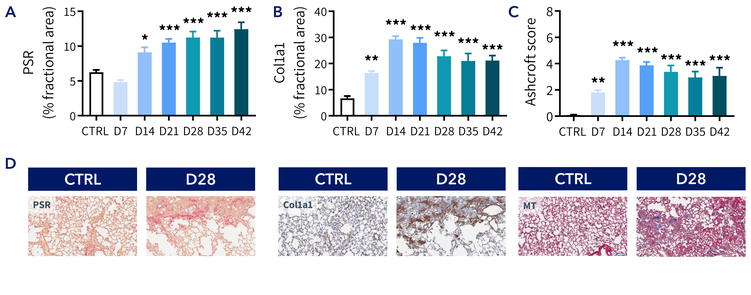


Figure 4. Lung quantitative histological markers of fibrosis. Histomorphometric assessments of PSR and Col1a1 were performed by conventional image analysis. Data were calculated as proportionate (%) area of histological staining (mean ± SEM). **(A)** % fractional area of PSR-stained fibers; **(B)** % fractional area of Collagen-1α1. Histopathological Ashcroft score were determined by GHOST deep learning-based image analysis. **(C)** Ashcroft score by Gubra Histopathological Objective Scoring Technique (GHOST). Dunnett's test one-factor linear model. *p<0.05, **p<0.01 and ***p<0.001 vs. CTRL group. **(D)** Representative photomicrographs of lung histological stainings.

5 Histological marker of lung senescence

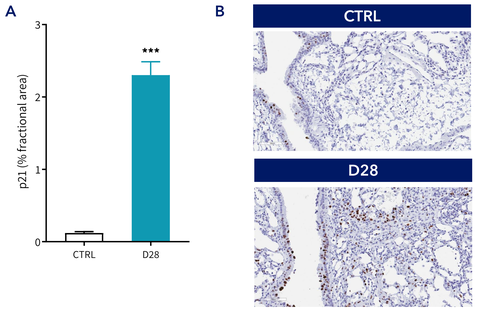


Figure 5. Lung quantitative histological marker of cellular senescence Histomorphometric assessment of p21 expression was performed by conventional image analysis. Data were calculated as proportionate (%) area of histological staining (mean ± SEM). **(A)** % fractional area of p21. **(B)** Representative photomicrographs of lung histological stainings. Dunnett's test one-factor linear model. ***p<0.001 vs. CTRL group.

6 Transcriptomic profile for lung senescence

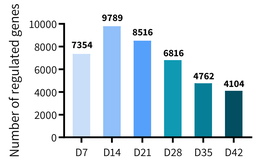
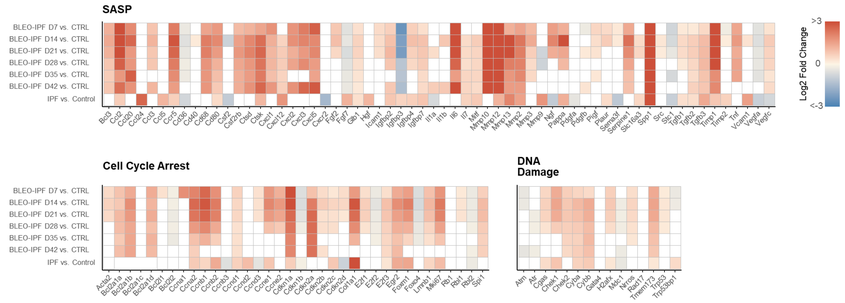


Figure 6. BLEO-IPF mice show marked upregulation of a wide array of lung senescence-associated genes. **(A)** Total number of differentially expressed genes (DEGs) during disease progression. **(B)** Candidate genes associated with the senescence-associated secretory phenotype (SASP), cell cycle arrest and DNA damage (log2-fold change compared to CTRL mice). Blue color gradients indicate significantly (p<0.05) down-regulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to CTRL group.



Conclusion

- BLEO-IPF mice demonstrate progressive increase in lung weight and total hydroxyproline content
- BLEO-IPF mice demonstrate reduced pulmonary expiratory and inspiratory function.
- BLEO-IPF mice demonstrate increased lung levels of quantitative histological markers of fibrosis
- BLEO-IPF mice demonstrate increased lung level of p21, a well-established histological marker of cellular senescence
- BLEO-IPF mice demonstrate marked lung transcriptome perturbations, including upregulation of a wide array of cellular senescence-associated genes

The single-installation BLEO-IPF mouse model represents a translational preclinical model for profiling potential senotherapeutics for IPF