The hexosamine biosynthetic pathway mediates extracellular matrix remodeling in idiopathic pulmonary fibrosis

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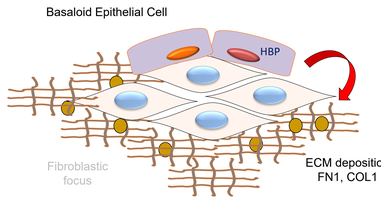
Abstract

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal interstitial lung disease that disproportionately affects veterans due to deployment-related exposures such as burn pit emissions.

IPF arises from chronic epithelial injury that activates stress pathways and drives formation of fibroblastic foci.

Fibroblastic foci disrupt alveolar architecture and promote excessive extracellular matrix (ECM) deposition.

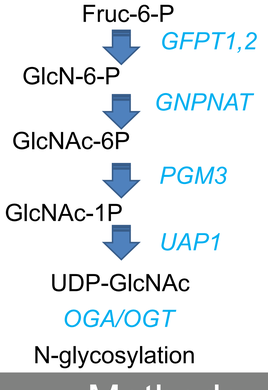
Of the ECM components associated with fibroblastic foci, fibronectin and hyaluronan (HA) accumulates in areas of active remodeling.



Fibronectin and HA constitute key scaffolds for ECM formation. How these initiators are produced are not fullyn understood. Here, we investigate the hexosamine biosynthetic pathway (HBP) as a key intracellular regulator of ECM deposition in IPF.

Background: The HBP

The HBP shunts intracellular fructose-6P into UDP-GlcNAc for protein N glycosylation.



Methods

RNA Seq of Human IPF/scRNA Seq of mouse bleomycin injury
GFPT2 knockout by shRNA/ GFPT2 genomic deletion by CRSPR

Results

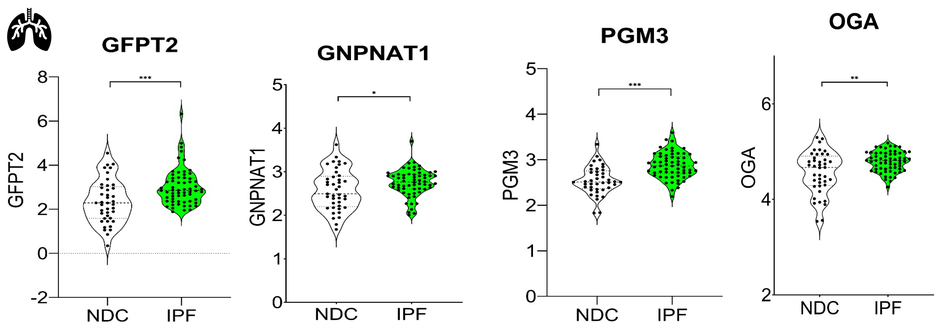


Fig. 2. Bulk RNA seq for HBP in Human IPF. NDC, nondiseased controls. **, P<0.01. Each symbol is an independent patient. The HBP core pathway is upregulated.

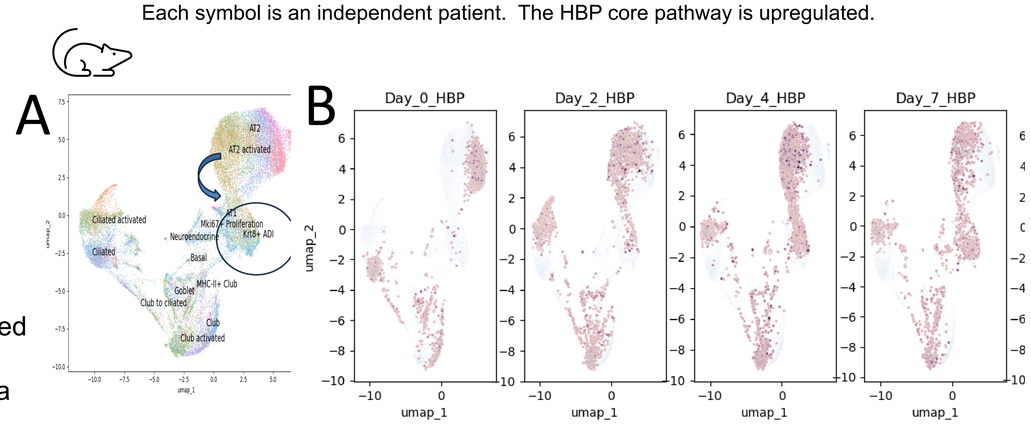


Fig. 3. scRNA seq of HBP activation in bleomycin mouse. A, Umap of epithelial types after bleomycin injury. AT2 cells transition into pathogenic ADI cells. B, Time course of HBP activation after bleomycin injury. Colors indicate HBP pathway expression. Note rapid activation of HBP in ADI cells 2-4 d after injury.

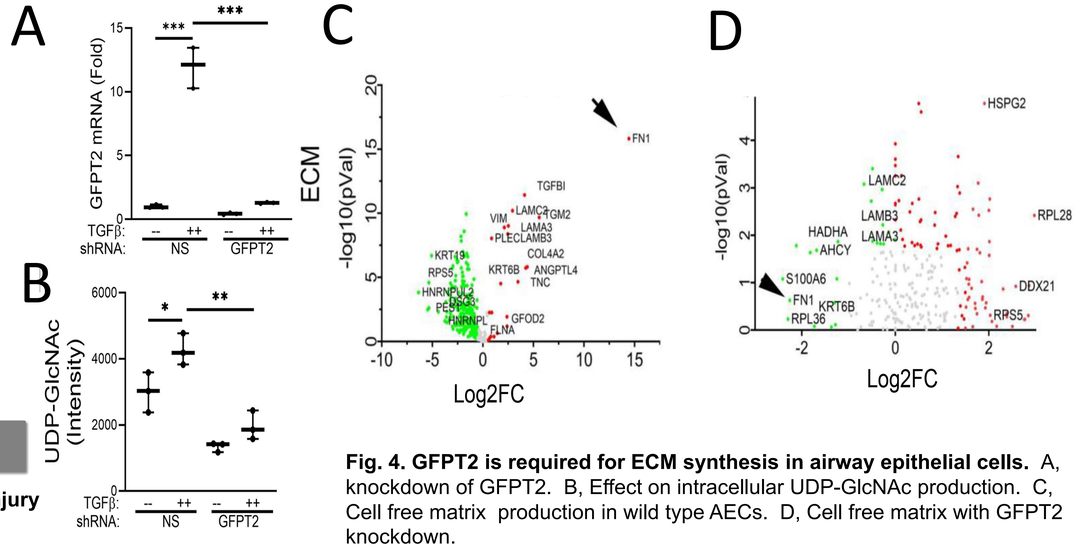


Fig. 4. GFPT2 is required for ECM synthesis in airway epithelial cells. A, knockdown of GFPT2. B, Effect on intracellular UDP-GlcNAc production. C, Cell free matrix production in wild type AECs. D, Cell free matrix with GFPT2 knockdown.

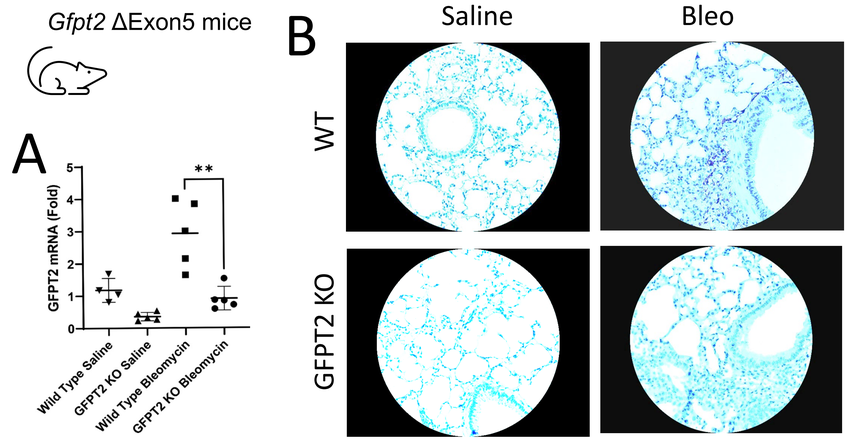


Fig. 5. GFPT2 knockout (KO) mice. Gfpt2 DExon5 mice were developed. A, Gfpt2 mRNA. B, Immunohistochemistry for GFPT2 expression in WT and KO mice. Note dramatic upregulation of GFPT2 in alveoli after bleomycin administration

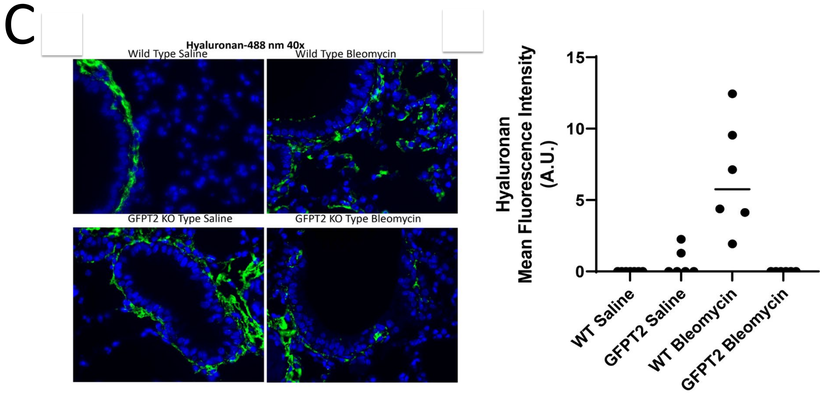


Fig. 6. Hyaluronan (HA) deposition is reduced in GFPT2 KO. A, HA staining. B, quantiation of HA fluorescence. Note dramatic reduction in HA in GFPT2 CKO.

Conclusions

- The HBP is activated in human IPF and in pathogenic epithelial cells where it is required for ECM secretion.
- GFPT2 is an inducible and rate-limiting enzyme for UDP-GlcNAc production
- GFPT2 KO reduces epithelial FN secretion and reduces HA production and accumulation in alveoli in vivo.
- These studies provide biological validation for targeting the HBP in fibrosing lung disease.

Future work will focus on GFPT2 in ECM structure and lung function.

Acknowledgements

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