Persistent ERBB-YAP Signaling Delays Jamming of IPF Airway

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ABSTRACT

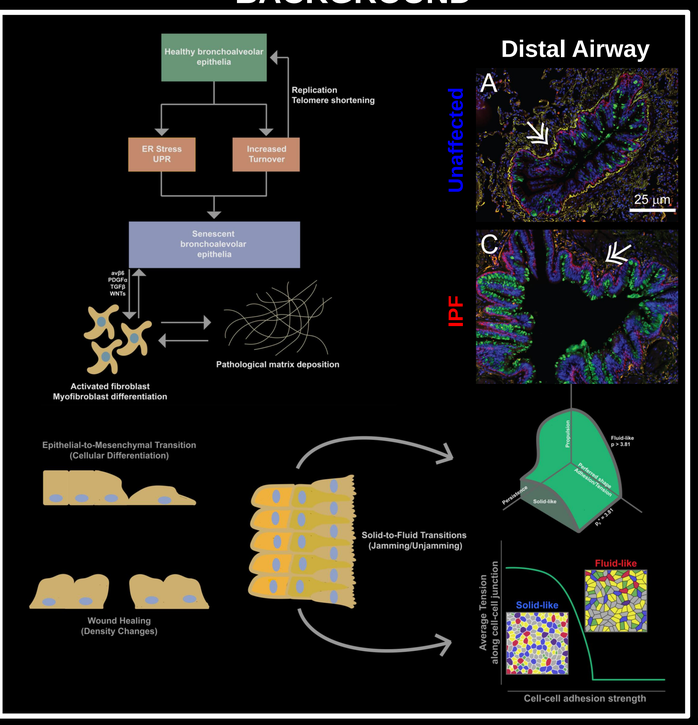
Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and ultimately fatal lung disease. IPF pathogenesis is thought to be driven by both airway and alveolar epithelial dysfunction. This epithelial dysfunction results in aberrant repair pathway activation, bronchiolization of the distal lung, and excessive mucus production. However, the origin of this dysfunction and the ability to propagate such dramatic structural changes is yet fully understood. A method by which large-scale structural reorganization occurs in epithelia is through the temporally-regulated jamming transition; a phase-change in which an epithelial monolayer transitions from an unjammed, fluid-like state to a jammed, solid-like state. This migratory program occurs without cellular differentiation into non-epithelial phenotypes and without density loss. However, the involvement of this physical transition in IPF pathogenesis has yet to be established.

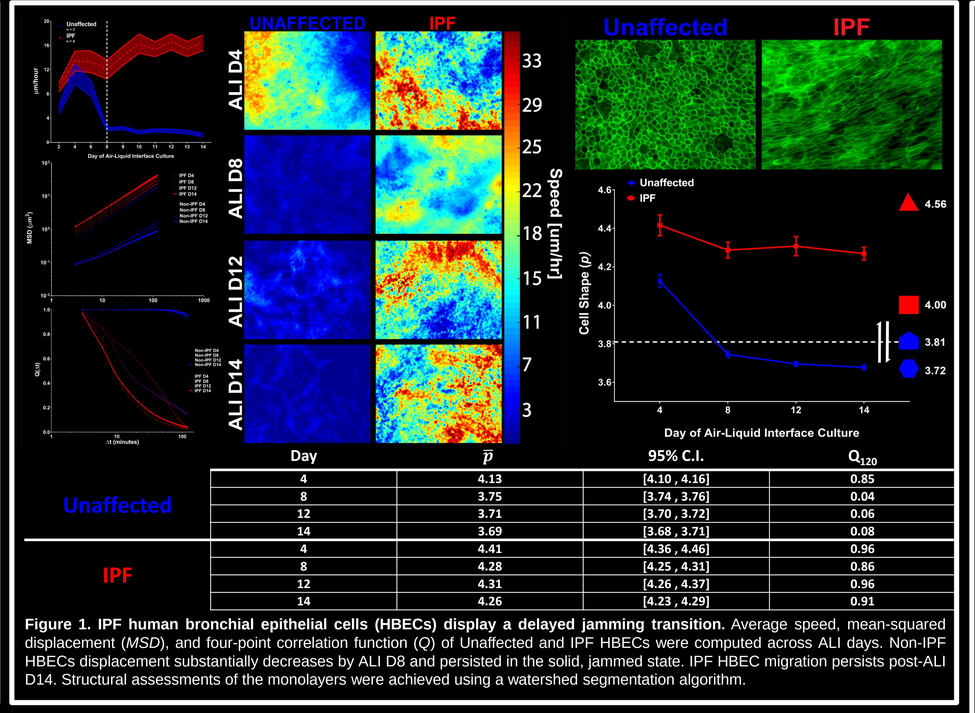
Methods: Primary human bronchial epithelial cells (HBECs) from patients with and without IPF were maintained in air-liquid-interface (ALI) throughout epithelial differentiation. To assess the jamming transition in IPF HBECs time-lapse imaging was utilized at varying time points. Time-lapse imaging was quantified via particle image velocimetry (PIV) to generate velocity fields. Computation of dynamic measurements were completed in MATLAB. Structural assessment of the monolayer was achieved by using a watershed algorithm in Python. Cell boundaries were stained with F-actin and perimeter-to-area and aspect ratios were computed for all cells within a given field of view.

Results: Jamming occurs by day 8 in unaffected patient HBECs, while IPF HBEC jamming occurs much later, around day 28 of culture. The gene signature of the unjammed state indicates an activation of the ERBB-YAP signaling axis. This signaling axis is temporally-correlated with the jamming transition at the protein level and is persistently activated in the IPF samples (throughout the unjammed state). Treating unaffected patient HBECs with either an EGFR ligand (amphiregulin) or YAP activator (XMU-MP-1) results in a spontaneous acquisition of an unjammed phenotype. Furthermore, chronic treatment results in cellular phenotypes similar to those in IPF cultures. Structural assessments of the jamming transition were concordant with dynamic measurements.

Conclusions: IPF HBECs display atypical dynamic measurements that are the result of chronic activation of the ERBB-YAP signaling axis. Activation of this axis in unaffected patient samples results in the acquisition of an 'IPF-like' phenotype. While, inhibition of this signaling axis results in IPF HBECs undergoing a jamming transition.

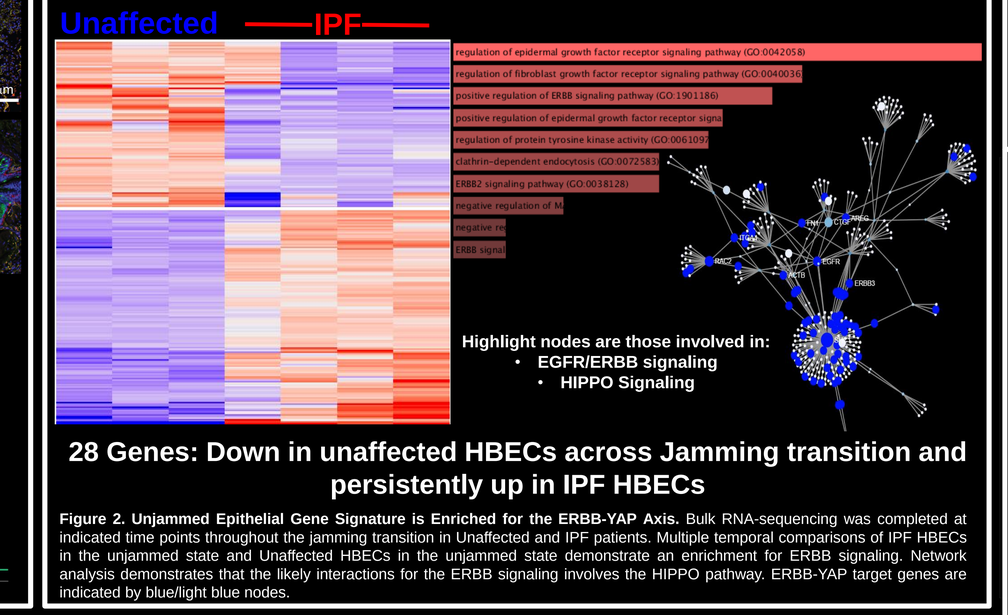
BACKGROUND





Day	$\bar{p}$	95% C.I.	Q_{120}
Unaffected 4	4.13	[4.10 , 4.16]	0.85
Unaffected 8	3.75	[3.74 , 3.76]	0.04
Unaffected 12	3.71	[3.70 , 3.72]	0.06
Unaffected 14	3.69	[3.68 , 3.71]	0.08
IPF 4	4.41	[4.36 , 4.46]	0.96
IPF 8	4.28	[4.25 , 4.31]	0.86
IPF 12	4.31	[4.26 , 4.37]	0.96
IPF 14	4.26	[4.23 , 4.29]	0.91

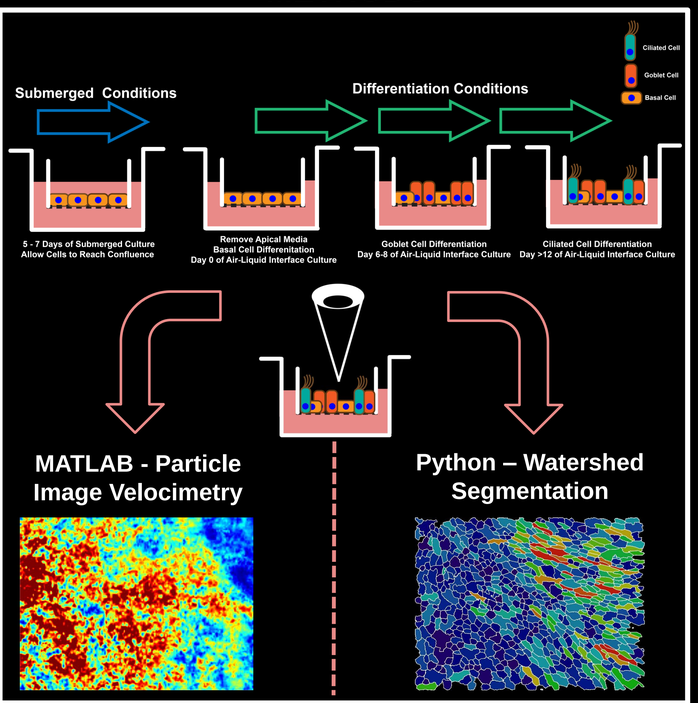
Figure 1. IPF human bronchial epithelial cells (HBECs) display a delayed jamming transition. Average speed, mean-squared displacement (*MSD*), and four-point correlation function (*Q*) of Unaffected and IPF HBECs were computed across ALI days. Non-IPF HBECs displacement substantially decreases by ALI D8 and persisted in the solid, jammed state. IPF HBEC migration persists post-ALI D14. Structural assessments of the monolayers were achieved using a watershed segmentation algorithm.



28 Genes: Down in unaffected HBECs across Jamming transition and persistently up in IPF HBECs

Figure 2. Unjammed Epithelial Gene Signature is Enriched for the ERBB-YAP Axis. Bulk RNA-sequencing was completed at indicated time points throughout the jamming transition in Unaffected and IPF patients. Multiple temporal comparisons of IPF HBECs in the unjammed state and Unaffected HBECs in the unjammed state demonstrate an enrichment for ERBB signaling. Network analysis demonstrates that the likely interactions for the ERBB signaling involves the HIPPO pathway. ERBB-YAP target genes are indicated by blue/light blue nodes.

METHODS



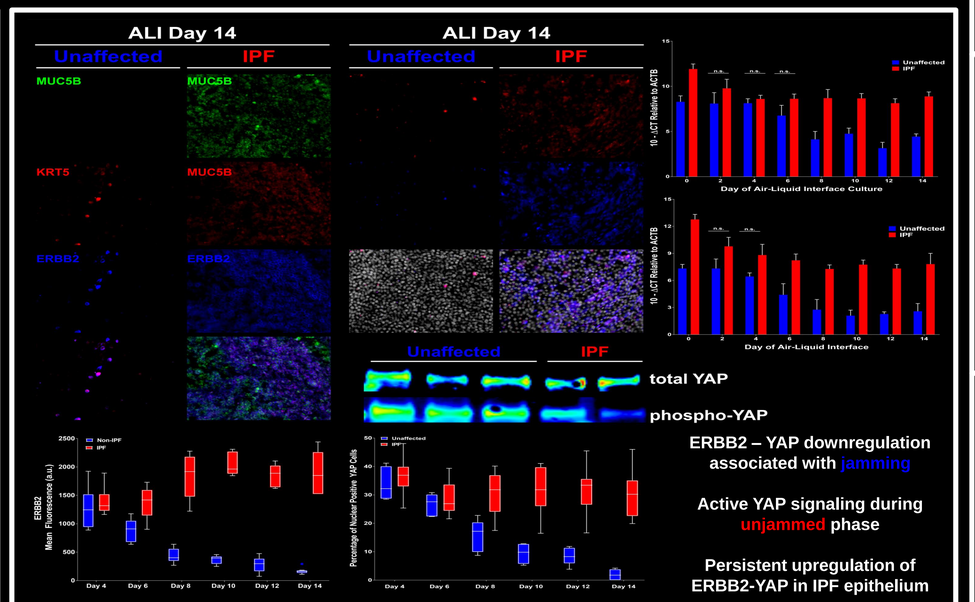


Figure 3. EGFR-YAP Correlate Temporally with the Jamming Transition. Immunofluorescence staining of the ERBB2 receptor, a member of the ERBB family, is demonstrated to be increased in the unjammed state of both unaffected and IPF patient samples. Accumulation of nuclear YAP (active YAP) in unaffected and IPF HBECs is associated with the unjammed state and result gene expression differences between unjammed and jammed states. Western blot demonstrates protein level differences between active (total YAP) and inactivate (phosphor-YAP) YAP between unaffected and IPF HBECs.

RESULTS

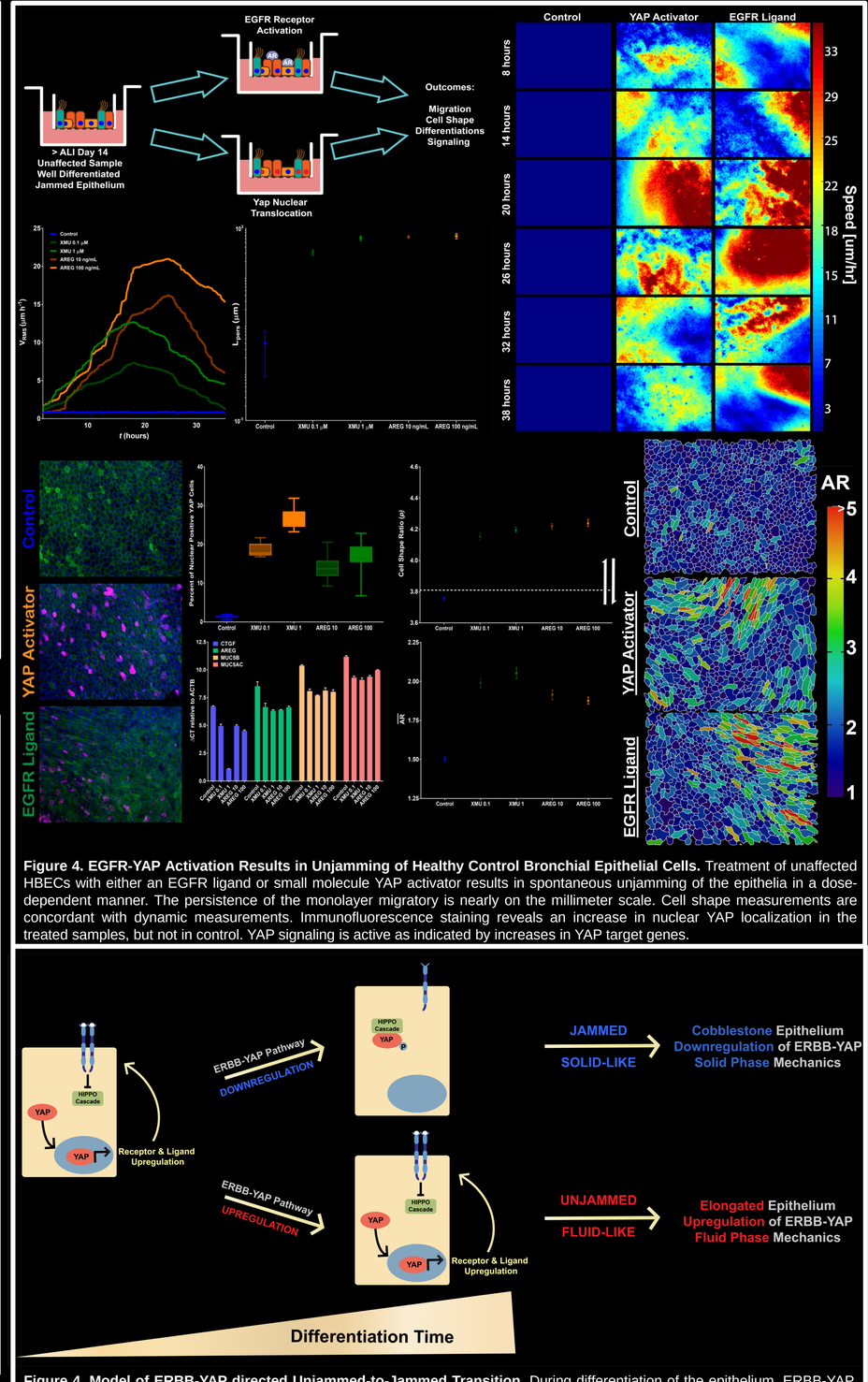


Figure 4. EGFR-YAP Activation Results in Unjamming of Healthy Control Bronchial Epithelial Cells. Treatment of unaffected HBECs with either an EGFR ligand or small molecule YAP activator results in spontaneous unjamming of the epithelia in a dose-dependent manner. The persistence of the monolayer migratory is nearly on the millimeter scale. Cell shape measurements are concordant with dynamic measurements. Immunofluorescence staining reveals an increase in nuclear YAP localization in the treated samples, but not in control. YAP signaling is active as indicated by increases in YAP target genes.

Figure 4. Model of ERBB-YAP directed Unjammed-to-Jammed Transition. During differentiation of the epithelium, ERBB-YAP signaling is active. This activation decreases as the monolayer matures and differentiates into more terminal cell-types. However, in IPF there is a retention of this ERBB-YAP signaling even when cells have fully differentiated into terminal phenotypes (goblet and ciliated cells). Activation of the ERBB-YAP axis in healthy, unaffected HBECs results in a phase change to an unjammed state as well as an acquisition of an 'IPF-like' cellular phenotype.

DISCUSSION

- IPF HBECs have a delayed jamming transition (dynamically and structurally)
- The unjammed state has a strong gene signature for the ERBB-YAP signaling axis that is persistently activated in IPF HBECs
- The jamming transition is temporally correlated with the dampening of ERBB-YAP signaling in unaffected HBECs, but is continuously active in IPF HBECs
- Activation of the ERBB-YAP axis results a phase change from a jammed-to-unjammed state in unaffected HBECs

FUTURE DIRECTIONS

- Inhibit ERBB-YAP signaling cascade to force a jamming transition in IPF HBECs
- Assess temporal dynamics of IPF HBECs through ERBB-YAP inhibitor
- Rescue experiments
- Epithelia-fibroblast cross talk during jamming and unjamming

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