Common Molecular Pathogenesis Of Pulmonary Fibrosis and Lung Cancer Metastasis

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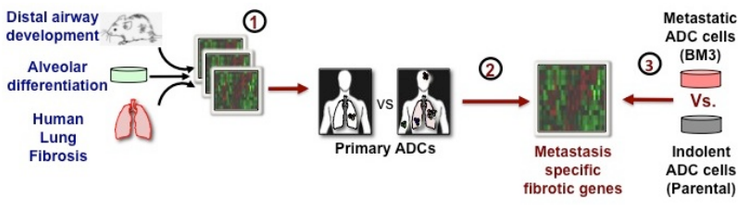
ABSTRACT

Lung adenocarcinoma (ADC) is the most common subtype of lung cancer, and can metastasize rapidly to distant organs even when diagnosed at early stages. Similar to ADC, idiopathic pulmonary fibrosis (IPF) also affects the distal airways. Striking clinical parallels have been documented between IPF and ADC independent of smoking history. Through bioinformatic analysis, we have identified a molecular class of ADCs that are similar to distal alveolar stem cells (DASCs) and which correlates with poor prognosis. Interestingly, this molecular subset of tumors is enriched for genes commonly deregulated in lung tissue from patients with exacerbated IPF. Among these genes are regulators of hyaluronic acid (HA), an extracellular glycosaminoglycan that is deposited in the lungs during fibrosis. Our integrated approach further reveals that expression of the HA receptor RHAMM (receptor for hyaluronan-mediated motility) is up-regulated in highly metastatic cells. Therefore, HA signaling may promote ADC progression in an injured tissue microenvironment. Accordingly, by using an experimental mouse model of lung fibrosis and ADC, we demonstrate that airway injury can enhance the transplantation and outgrowth of ADC cells. We are currently using this model to study how RHAMM contributes to ADC metastasis in a fibrotic microenvironment. Collectively, our results suggest that aberrant airway regeneration may provide a selective advantage to specific subpopulations of invasive lung cancer cells. Elucidating the link between fibrosis and ADC metastasis will provide insight into more effective strategies in the treatment of recurrent lung ADC.

BACKGROUND

- ADC arises in the peripheral airways and is composed of cells that resemble distal airway epithelial progenitors.
- IPF is a non-malignant chronic disease that also affects the distal airways and has a median survival of 2-5 years.
- IPF is the most common fibrotic disorder and is characterized by the formation of scar tissue in the lung, which may be attributed to impaired alveolar epithelium repair.
- Independent of smoking history, chronic IPF patients are up to 3 times more likely to develop lung cancer.
- Early stage non-small cell lung cancer patients with concurrent IPF have a poor prognosis.
- Peripheral lung tumors that contain a fibrotic stroma are frequently of the ADC subtype and progress more rapidly.
- Molecular mechanism behind the association between IPF and ADC is unknown.
- IPF microenvironment may allow a subpopulation of cells to gain a selective advantage.
- Independent of IPF, a metastatic cell may use similar molecular mechanisms or mimic an IPF-like microenvironment to gain a selective advantage.

COMPUTATIONAL PIPELINE



Step 1: A bioinformatic pipeline is used to stratify a cohort of primary lung ADCs into distinct classes based on gene expression profiles from lung IPF patients and normal lung development.

Step 2: A gene module that classifies poor prognosis ADC patients is identified. From this subset, a list of differentially regulated genes is generated for genes that are co-enriched in IPF patient samples.

Step 3: To enrich for genes expressed in metastatic tumor cells, candidates are filtered based on their expression patterns in highly metastatic human lung ADC cells (BM3) compared to indolent parental cells isolated from animal transplant models. This results in a list of metastasis specific fibrotic genes.

RESULTS

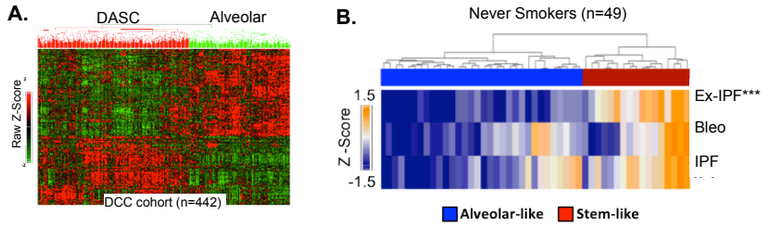
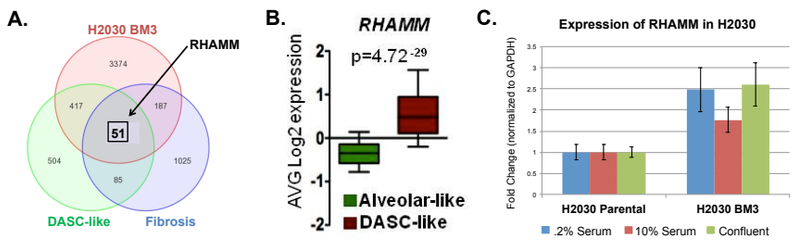


FIGURE 1: CORRELATION OF LUNG ADC AND FIBROSIS. (A) Heatmap of primary ADCs from the Director's Challenge Cohort (DCC) stratified into "DASC-like" or "alveolar-like" classes based on distal airway developmental genes. **(B)** Relative enrichment of IPF gene signature in DASC-like ADCs. For each never smoker patient tumor sample, a correlation coefficient score was calculated using the gene signature of lung tissue from patients with exacerbated IPF (Ex-IPF), stable IPF (IPF), or mice treated with bleomycin (Bleo). Heatmap represents the hierarchical clustering of Z transformed correlation scores. ***:p<0.0001 for enrichment in DASC-like tumors.



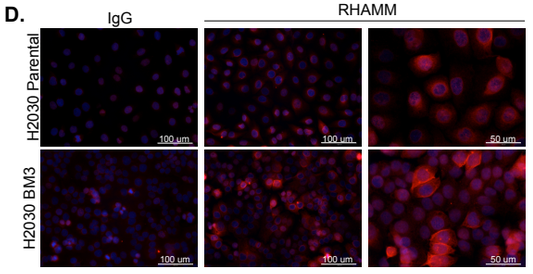


FIGURE 2: RHAMM EXPRESSION IN METASTATIC ADC CELLS. (A) 51 genes were commonly up-regulated in the DASC-like subset from multiple patient cohorts, metastatic (BM3) cells compared to indolent parental cells, and lung tissue from human with stable and exacerbated IPF. Among these genes RHAMM (aka CD168, HMMR) was identified for further investigation. **(B)** RHAMM is highly expressed in the poorly-differentiated DASC-like tumors. p-value by t-test. **(C)** RHAMM is overexpressed in a highly metastatic lung ADC cell line (H2030 BM3) and **(D)** is expressed at the cell surface of tumor cells.

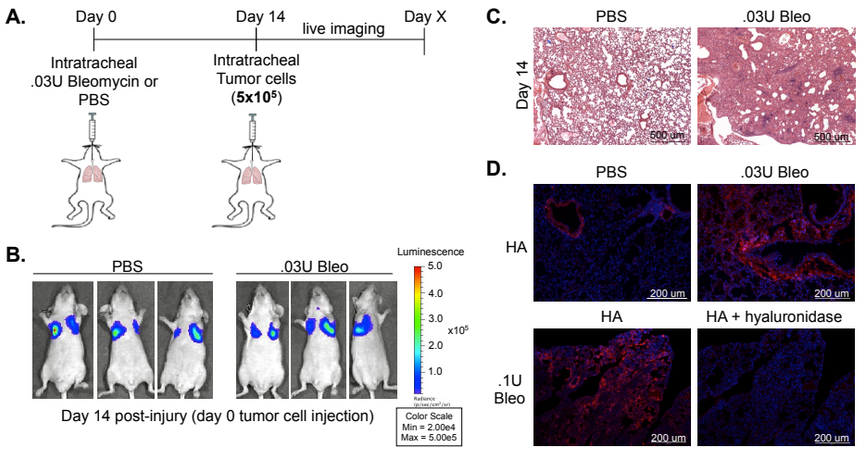


FIGURE 3: FIBROTIC ADC ORTHOTOPIC MOUSE MODEL. (A) Experimental design. To induce fibrosis, .03U of Bleomycin is intratracheally (IT) injected into athymic mice. At day 14, $5x10^5$ tumor cells that express luciferase are IT injected and imaged using IVIS Spectrum. **(B)** Mice are imaged immediately following tumor cell injection ensuring relatively similar delivery of tumor cells. **(C)** Lung H&E from mice IT injected with Bleo or PBS show fibrosis 14 days after injection. **(D)** Hyaluronic acid (HA) is increased in fibrotic tissues harvested from mice 14 days post–injury. Mice were injected with .03 or .1U of Bleomycin and stained with streptavidin conjugated HA binding protein.

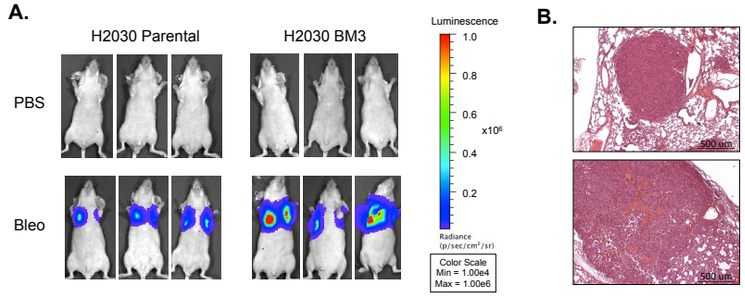
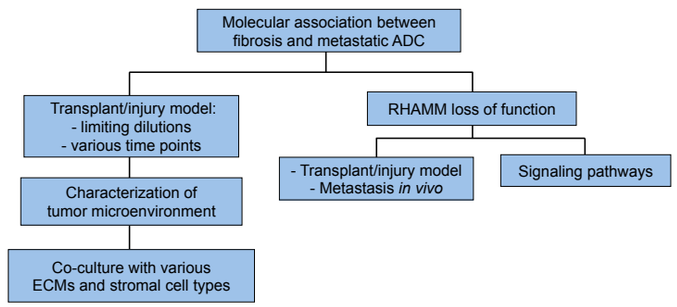


FIGURE 4: INJURY PROMOTES ADC TRANSPLANTATION. (A) Tumor cell transplantation is significantly enhanced in bleomycin treated animals. Representative images of mice imaged 14 days post tumor cell injection. **(B)** H&E sections of lungs harvested from mice injected with .03U Bleomycin and H2030 BM3 cells. **(C)** Incidence of transplantation with 5^{e5} tumor cells is indicated in parenthesis. The subsequent growth rate of of metastatic BM3s compared to their indolent parental cells is plotted based on bioluminescent activity. p-value determined by student's t-test. **(D)** DCC patient survival based on primary tumor median expression of RHAMM and the HA producing enzyme hyaluronan synthase 2 (*HAS2*).

CONCLUSIONS

- By using an integrated approach metastasis specific fibrotic genes were identified.
- RHAMM (aka HMMR, CD168) is up-regulated in fibrotic tissue, recurrent patient tumors and on the surface of a highly metastatic ADC cell population.
- RHAMM is a receptor for HA, a glycosaminoglycan that is produced by HA synthase 2 (HAS2) and deposited in the lung extracellular matrix during injury.
- Using a fibrotic mouse model we found that fibrosis increases ADC transplantation, and metastatic cells have more rapid growth rate compared to their indolent parental cells.
- These results suggest that metastatic cells are better suited to take advantage of the fibrotic microenvironment.
- High expression of HAS2 and RHAMM correlates with poor ADC patient prognosis indicating that this pathway may link the pathogenesis of ADC metastasis to fibrosis.

FUTURE DIRECTIONS



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