Fibroblasts Affect the Phenotype of Normal Human Bronchial Epithelial Cells when Co-cultured in Three-Dimensional (3D) Organotypic Cultures

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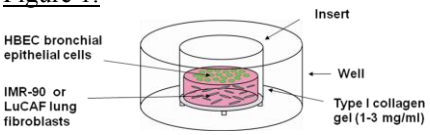
Statement of Purpose: The stromal microenvironment plays an important role in the development and progression of adult respiratory diseases. Pulmonary diseases such as asthma, fibrosis and cancer are thought to be the result of altered communications between the epithelial and stromal tissue compartments.[1-4] In order to study these epithelial and mesenchymal interactions, we have developed a three dimensional (3D) *in vitro* model of the human airway that mimics bronchial morphology and function. This model consists of a type I collagen matrix, normal human fetal lung fibroblasts (IMR-90) or primary human adult lung cancer-associated fibroblasts (LuCAFs), derived from lung cancer resective surgery, and a surface epithelium of normal human bronchial epithelial cells (HBECs).

Methods: HBECs were provided by Dr. Scott Randell (University of North Carolina, Chapel Hill, NC) and were cultured in bronchial epithelial growth medium (BEGM). IMR-90 (CCL-186) were obtained from American Type Culture Collection (ATCC; Manassas, VA). LuCAFs were derived from explanted lung tissues obtained from lung cancer resective surgeries. Fibroblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5.0% (v/v) fetal bovine serum (FBS). For 3D cultures (see Figure 1.), fibroblasts (IMR-90 or LuCAFs) were embedded in rat tail type I collagen (BD Biosciences; San Jose, CA) [5.0 x 10^5 cells/ml] and cast into 12-mm diameter Millicell PICM01250 cell culture inserts (Millipore; Billerica, MA). The fibroblasts were cultured for 7 days submerged in air liquid interface (ALI) medium. On day 7, 1.5 x 10^5 second passage (P2) HBECs were seeded onto each construct surface. The cell-seeded constructs were cultured at an air-liquid interface for a period of 4 – 7 days, or until complete (100%) confluence was reached, at which point the tissues were cultured for an additional 2 and 4 weeks in order to promote functional differentiation of the epithelium. Harvested tissues were fixed and embedded in paraffin, and tissue sections were analyzed by histology, immunohistochemistry and scanning electron microscopy (SEM). To determine whether fibroblasts secreted extracellular matrices (ECMs) of differing thickness or biomechanical compliance, IMR-90 and LuCAFs were seeded onto gelatin-coated glass coverslips, cultured for 8 days in DMEM supplemented with 5% (v/v) FBS and ascorbic acid [50 mg/ml], and then analyzed with confocal microscopy and atomic force microscopy (AFM). To determine whether fibroblasts secreted soluble factors that altered the phenotype of HBECs, conditioned medium from either IMR-90 or LuCAFs was applied to HBECs for a period of 48 hours, and the HBECs were analyzed for differentially expressed genes using qPCR focused arrays (SABiosciences; Frederick, MD).

Figure 1.



Results: When cultured at an air-liquid interface, the epithelial component of our model generated a well-differentiated pseudo-stratified bronchial epithelium that contained basal (P63-positive), ciliated (blue arrows), and non-ciliated, secretory (red arrows) epithelial cells (Figure 2.). Our research demonstrated that IMR-90 and LuCAFs differentially altered the phenotype of HBECs in distinct ways. IMR-90 allowed HBECs to form a surface epithelium, while LuCAFs promoted HBECs to invade the collagen gel forming both epithelial nodules (green asterisks) and cysts (Figure 3.). AFM data indicated that IMR-90-secreted ECMs were two-fold stiffer than ECMs secreted by LuCAFs. Furthermore, LuCAFs secreted soluble factors that induced HBECs to express genes associated with immune responses, apoptosis, mitosis, cell survival, differentiation and cancer.

Figure 2.

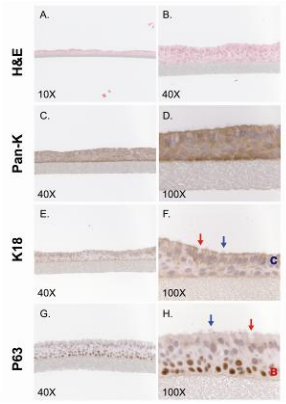
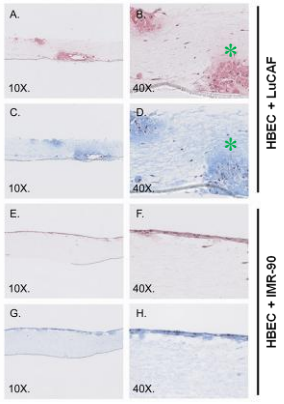


Figure 3.



Conclusions: In conclusion, our data suggest that LuCAFs may alter the HBEC phenotype by modifying biomechanical signals conveyed through the extracellular matrix (ECM). This supports the premise that a disruption in tissue homeostasis occurs in part due to changes within the stroma. We anticipate that our model will be of benefit to researchers and public health regulatory agencies by providing a novel tool with which to explore the origins of pulmonary diseases, test inhaled toxicants, and for the development and testing of potential diagnostic and therapeutic products.

References:

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