Functions of Lung Parenchymal Versus Airway Derived Fibroblasts in Collagen Matrix Remodeling and Contraction

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Abstract

Rationale: Asthma is characterized by inflammation and structural remodeling of the airways. This remodeling includes thickening of the basement membrane, an increase in smooth muscle mass, excess mucus production, and increased collagen deposition. The fibroblast is the main cell able to produce, remodel and contract collagen, which is important for providing the tensile strength of connective tissue. It is unknown whether fibroblasts from asthmatic airways differ in their ability to remodel and contract collagen compared to normal donors, and if this is a factor in the excessive deposition of collagen seen in asthmatic airways.

Method: Parenchymal and airway fibroblasts were obtained from donor lungs deemed not suitable for transplant from both asthmatic and non-asthmatic individuals. These cells were cultured in 10% FBS in DMEM with added antibiotic/anti-mycotic. Cells between passages 3 and 5 were seeded onto collagen-1 gels prepared in serum-free DMEM at approximately 37,500 cells per well to allow gel contraction at a measurable rate. Gel contraction over time was then quantified as a percentage of initial gel area at 72 hours using ImageJ software. ANOVA and Student's t-tests were performed with significance obtained when $p < 0.05$. Collagen remodeling was determined using multi-photon microscopy through second harmonic signal, with higher signal emitted from more densely remodeled collagen fibers. Gels were then analyzed for protein levels using western-blot analysis.

Results: It was determined that seeding 37,500 cells per well provided a measurable gel contraction rate which could be recorded between 0-72 hours. Upon completion of the 72 hours, analysis of the contraction abilities of different fibroblasts from 20 donors showed that parenchymal and airway fibroblasts, even within the same donor, had significantly different rates of gel contraction. At every time point parenchymal fibroblasts were significantly faster at contracting collagen-1 gels as compared to airway fibroblasts ($p=0.004$). Comparing airway fibroblasts obtained from asthmatics to those obtained from non asthmatics (n=10), non asthmatic airway fibroblasts significantly contract collagen more than asthmatic airway fibroblasts ($p=0.017$). Multi-photon analysis for remodeled collagen found that non asthmatic airway fibroblasts remodeled collagen to a greater extent than asthmatic airway fibroblasts, generating a significantly stronger second harmonic signal than gels remodeled by asthmatic airway fibroblasts ($p=0.033$).

Conclusion: Parenchymal fibroblasts are significantly faster at collagen-1 gel contraction than airway fibroblasts within asthmatic donors. Within non asthmatic donors, both cell types show similar rates of contraction. Furthermore, non asthmatic airway fibroblasts significantly contract collagen-1 gels more than fibroblasts obtained from asthmatic airways. However, no statistical difference was determined between asthmatic parenchymal fibroblasts and non asthmatic parenchymal fibroblasts. In order to determine why asthmatic airway fibroblasts could not contract collagen gels as efficiently as their non asthmatic counterparts, western blot analysis for the proteins involved in collagen remodeling and contraction will be conducted.

Introduction

Asthma is a complex pulmonary disease affecting roughly 300 million individuals globally. It is characterized by coughing, wheezing, and shortness of breath due to airflow obstruction and inflammation of airways. Asthmatic airways also display structural remodeling, including accumulation of sub-epithelial fibroblasts and deposition of excess collagen. Collagen is an extracellular matrix protein secreted by fibroblasts which contracts and relaxes during biological processes such as wound healing. It is unknown whether fibroblasts from various regions of the lung differ in their ability to contract collagen and whether fibroblasts from asthmatic individuals differ in contraction ability than those from non asthmatic individuals. It is also unknown whether asthmatic fibroblasts express higher or lower levels of the proteins involved in collagen remodeling and contraction as compared to non asthmatic derived fibroblasts. These proteins include integrins, integrin linked kinase, and alpha smooth muscle actin, all of which allow fibroblasts to remodel and contract collagen.

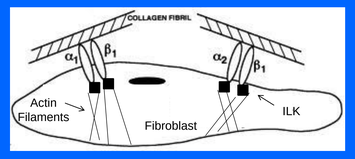

Figure 1. Depiction of the collagen fiber remodeling and contraction process performed by fibroblasts cells within connective tissue. Proteins significant to the process are highlighted including integrins, integrin linked kinase (ILK), and actin filaments.

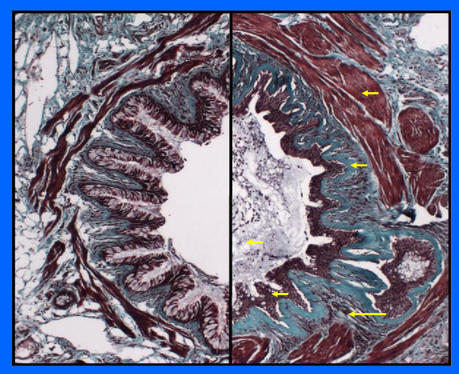

Figure 2. Representation airway sections from a 11 year old non-asthmatic male donor (left panel) and age matched male fatal asthmatic (right panel), stained with masons trichrome. Yellow arrows indicate the remodeling features associated with the disease which include increased smooth muscle mass, sub-epithelial fibrosis and thickening of the basement membrane, mucus plugging and infiltration of inflammatory cells and dysplasia of the airway epithelium.

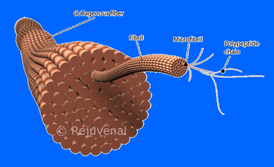

Figure 3. Collagen in its possible structural forms. Generally existing in loose fibrils in the extra cellular matrix, collagen can be remodeled into dense fibers to allow more contraction during wound repair. Collagen fibers of higher density emit a stronger Second Harmonic Generation signal observed with a multi-photon microscope than non-remodeled loose collagen fibrils, allowing a comparison of remodeling degree and density of collagen gels.

Hypothesis

Airway and parenchymal fibroblasts have different rates of collagen contraction and remodeling. These processes may be altered in asthma, contributing to airway remodeling.

Table 1: Donor Cohort

Subject	Number of Individuals	Average Age† (Range)	Sex (M/F)
Non Asthmatics	10	18.9 (5-43)	(7/3)
Asthmatics	10	21.2 (10-36)	(5/5)

Table 1. Donors are identified by disease, average age of group, age range, and sex. †Students t-test was performed between average age of donors and found no statistical difference between the two patient cohorts.

Methods

Both parenchymal and airway fibroblasts used in this study were obtained from donor lungs deemed not suitable for transplant from the International Institute for the Advancement of Medicine. Fibroblasts from both regions of the lung in asthmatic and non-asthmatic individuals were analyzed in order to determine differences in collagen gel remodeling and contraction.
After extraction tissue, the cells were then cultured in a media of 10% FBS in DMEM with added antibiotic/anti-mycotic. Cell media would be replaced every two days to allow optimal conditions for cell proliferation. Once the fibroblasts had reached a passage between passages 3 and 5 and adequate (>70%) confluency, they were then seeded onto collagen I gels.

For this study, collagen I gels prepared from rat-tail collagen I in serum-free DMEM were incubated overnight and then seeded with 37,500 fibroblasts per well in a 12 well plate. This quantity of cells was found to display gel contraction at the most measurable rate. Collagen gel contraction at the end point of 72 hours was then quantified as a percentage of initial gel area using ImageJ software. Through this software, the entire well area and collagen gel area could be determined and quantified. By then comparing the area of the gel to that of the well, a percentage of contraction could be obtained. ANOVA and Student's t-tests were then performed to compare the contractile abilities of the fibroblasts to one another, with significance obtained when $p<0.05$.

To further explain any differences in contractile abilities of the fibroblast, collagen gels were then analyzed for protein levels using western-blot analysis. The proteins being blotted for included integrins such as αvβ1 which fibroblasts use to attach to collagen, integrin linked kinase (ILK) which attaches integrins to actin filaments, and smooth muscle actin which provides the mechanical force required to contract the collagen fibers.

The remodeled and contracted gels were then used to analyze fibrillar collagen formation and remodeling. The non-centrosymmetric structural organization of collagen fibrils enabled them to be imaged with Second Harmonic Generation (SHG) microscopy. The orientation of the collagen fibers within the images was quantified to determine how well the structures were organized in a single direction. TEM images of the same tissues were also used to observe the differences in the collagen organization, to help explain why some cells remodeled and contracted collagen gels more than others.

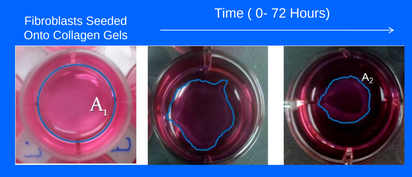

Figure 4. Fibroblast induced collagen I gel contraction. Collagen I gel area decreased over time due to remodeling and contraction as highlighted in blue.

Results

Collagen I Gel Contraction Abilities of Parenchymal Vs Airway Fibroblasts

Figure 5A

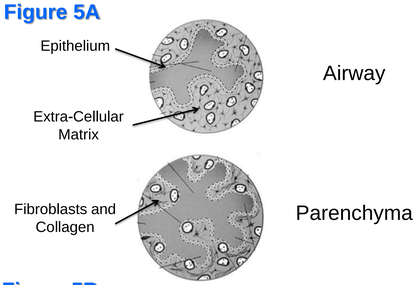

Figure 5B

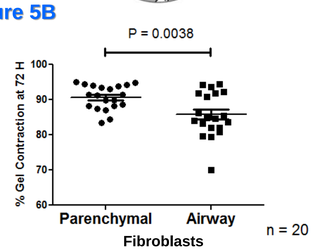

Figure 5A. Comparison between a cross section of an airway and of a parenchymal unit of the lung. Both structures differ greatly in role of the epithelium, extra cellular matrix, and number of fibroblasts and collagen fibers.

Figure 5B. Parenchymal fibroblasts significantly contract collagen 1 gels more than airway fibroblasts at 72 hours ($p = 0.0038$).

Collagen I Contraction Abilities of Non-Asthmatic Vs Asthmatic Fibroblasts

Figure 6A

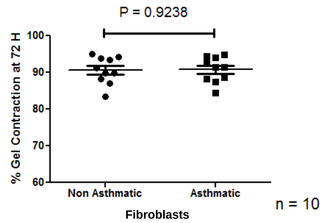

Figure 6B

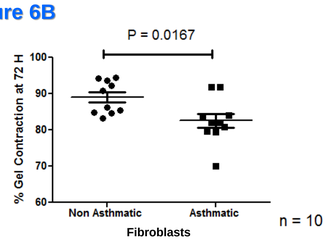

Figure 6A. Non-asthmatic parenchymal fibroblasts do not significantly contract collagen I gels more than asthmatic parenchymal fibroblasts ($p = 0.924$).

Figure 6B. Non asthmatic airway fibroblasts significantly contract collagen I gels more than asthmatic airway fibroblasts ($p = 0.0167$).

Fibrillar Collagen Remodeling Analysis by Second Harmonic Signal Generation

Figure 7A

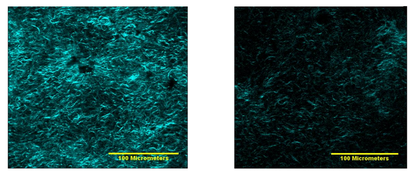

Figure 7B

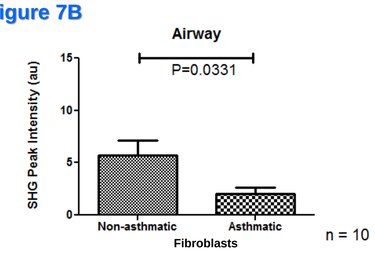

Figure 7A. Multi-photon images of second harmonic signal generated from collagen I gels remodeled by non-asthmatic airway fibroblasts (left) and asthmatic airway fibroblasts (right). Higher density of collagen I gel leads to a stronger emission of second harmonic signal.

Figure 7B. Non asthmatic airway fibroblasts significantly generate a stronger second harmonic signal intensity than asthmatic airway fibroblasts ($p=0.033$).

Summary

- Parenchymal fibroblasts significantly contract collagen I gels more than airway fibroblasts within asthmatic donors. Within non asthmatic donors, both cell types show similar rates of contraction.
- Non asthmatic airway fibroblasts significantly contract collagen I gels more than fibroblasts obtained from asthmatic airways. No difference was determined in contractile abilities of parenchymal fibroblasts regardless of disease.
- Non asthmatic airway fibroblasts significantly remodel collagen fibrils into more dense collagen fibers as compared to asthmatic derived fibroblasts.

Future Directions

- In order to determine why asthmatic airway fibroblasts could not contract collagen I gels as efficiently as their non asthmatic counterparts, western blot analysis for the proteins involved in collagen remodeling and contraction will be conducted.
- A difference in these protein levels may help to explain why asthmatic airway fibroblasts do not remodel the collagen fibrils as densely and then do not contract the gels as much as non asthmatic airway fibroblasts.

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