The Effect of Galactosialidosis on Collagen Remodeling

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How you want to be treated.

Abstract

Background: Chronic obstructive pulmonary disease (COPD) is characterized by emphysematous destruction of lung parenchyma and small airway obstruction/obliteration. The most common causes for COPD are smoke exposure or Alpha-1 Antitrypsin deficiency, which are both thought to result in a protease and oxidant imbalance within the lung, leading to tissue destruction. We have also shown in our laboratory that parenchymal derived fibroblasts from COPD patients are defective in their ability to repair and remodeling collagen fibers, which are essential for normal tensile strength of tissues. An extremely rare genetic mutation that also causes emphysematous destruction within the lung is Galactosialidosis, a lysosome storage disease caused by loss of function of protective protein Cathepsin A (CTSA), which leads to a deficiency in Beta-galactosidase (GLB1) and Neuraminidase (Neu 1). Lehman et al., have previously shown the skin derived fibroblasts of Galactosialidosis patients demonstrate deficiencies in elastin and fibrilin-1 remodeling, but the effect on collagen fiber formation is unknown.

Hypothesis: The fibroblasts from our Galactosialidosis patient will have defective collagen I production and contraction compared to those of normal males.

Methods: Lungs were obtained from 37 year old male Galactosialidosis patient undergoing lung transplant surgery (St Paul's hospital), and three male donor lungs from the International Institute for the Advancement of Medicine. Lung fibroblasts were isolated from the airways, parenchymal and bullae of the diseased lung. Fibroblast from all donors were maintained in 10% FBS in DMEM media at 37ºC, and seeded on 4ug/ml collagen gels. The collagen gel contraction was visualized over 72 hours and the contraction calculated as a percent change in diameter. To determine, the fibroblasts were also imaged under the Multi-photon microscope to see their orientation.

Results: The collagen gels were analyzed and they contracted less than the average of the normal males' gels. In the Galactosialidosis patient ,the airway contracted 80%, the lower lobe contracted 89% and the bullae contracted 80% of the original well. The Small Airway surprisingly contracted the collagen by 92% of the starting area. From observing under It is also noted that the collagen observed from the Galactosialidosis donor had a lower peak intensity that those of the normal males.

Conclusion: The Galactosialidosis patient's cells acquired from the bullae and airway contracted collagen about 10% less than those of the normal males. However, the small airway and lower left lobe of the lung contracted collagen about the same as the normal donors. This result needs to be further studied to find reasons as to why.

Introduction

Chronic obstructive pulmonary disease (COPD) encompasses emphysematous destruction of lung parenchyma and small airway obstruction/obliteration. The most common causes for COPD are smoke exposure or Alpha-1 Antitrypsin deficiency (A1AT). A1AT deficiency is a genetic mutation that results in decreased A1AT that normally inhibits neutrophil elastase resulting in excessive proteolytic destruction of the alveolar lung tissue. In comparison to smoking associated emphysematous destruction of the lung which causes centrilobular emphysema, A1AT deficiency results in panlobular emphysematous break down of the Alveoli. It was recently observed in a double lung transplant patient from St Paul's Hospital with another genetic mutation, Galactosialidosis, that's his lungs appeared like that of a person with A1AT deficiency.

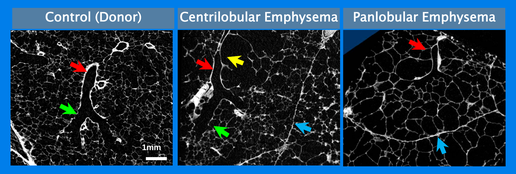


Figure 1. Representative micro computed tomography (microCT) images of a healthy terminal bronchiole (red arrow) and alveoli in comparison to Centrilobular and Panlobular emphysema. Centrilobular emphysema is the progression of emphysema starting from the terminal bronchiole downwards, while Panlobular emphysema is the nonspecific progression of emphysema in the alveoli. Images courtesy Dragos M. Vasilescu, Ph.D.

Galactosialidosis is a very rare disease that only about 100 people have been diagnosed with world wide. It is characterized by a mutation in the CTSA gene, which codes for the production of the protein Cathepsin A. Cathepsin A is a protein involved in storage and compartmentalization of lysosomes. The mutation in the CTSA gene causes a malformation of the protein. This incorrect form of Cathepsin A is unable to properly bind with Neuraminidase 1 and elastin binding Protein. The inability for Cathepsin A to bind with Neuraminidase 1 and elastin binding Protein leads to the production of a weaker form of elastin. The formation of weaker elastin might be a cause for deterioration of elastin in the alveolar wall, possibly leading to emphysema.

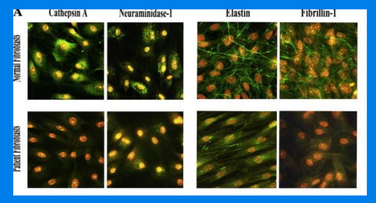


Figure 2. (*Lehman et al.*) This depiction shows results of a previous study done on skin cells of a Galactosialidosis donor's skin cells REF1. Here it was shown that elastin was deficient and there were less active fibers than in a normal person's fibroblasts. It also showed that Cathepsin A and Neuraminidase of a person with Galactosialidosis are less active than a normal person's.

Hypothesis & Aims

Fibroblasts from our Galactosialidosis patient will have defective collagen I production and contraction compared to those of normal males.

Aim1: Compare collagen I gel contraction abilities between Galactosialidosis affected fibroblasts in comparison to control donors.

Aim 2: Observe the differences in orientation of collagen fibers remodeled from our donor and control males

Table 1: Donor Cohort

Donor	Age	Sex
Normal	25	M
Normal	23	M
Normal	20	M
Galactosialidosis	37	M

Table 1. Donors are identified by gender and age.

Methods

Cell culture: Fibroblast cells derived from the regions lower left lobe, bullae, airway and small airway were awoken into a separate T-75 flask containing 12 mLs of 10% Fetal Bovine Serum (FBS) in DMEM or media, and included a 1% concoction of Penicillin, Streptomycin, and Fungizone (PSF). The cells were then grown to be 85-95% confluent. 3mLs of trypsin were added to each flask to detach the cells. The trypsin was diluted in 6mls of media and centrifuged at 1000 RPMs for 5 mins. The supernatant was removed from the cells and they were re-suspended in more media. Using the re-suspended cells, a count by placing 10uL of sample into a hemocytometer was performed to ensure 37,500 cells would be aliquoted.

Collagen gels: For testing collagen remodeling, rat tail collagen gels were made. A solution of 10% BSA and serum free DMEM (media) and were added to 6 wells of a sterile 12 well plate. The wells were incubated at 37ºC for a minimum of 30 minutes. The solution was then aspirated from the wells and a new solution of 10% rat tail collagen in serum free media was added to each of the 6 wells. This was incubated at 37ºC over night. Just before seeding, a pipette was traced around the edge of each well to dislodge the gels.

Seeding gels: Using the hemocytometer to provide an estimate of how many cells are in each sample, a calculation was performed to seed 500uL of 37,500 cells into each of the 6 rat tail collagen gels. The cells were undisturbed and allowed to Contract collagen for 72 hours at 37ºC.

Collagen Fixing: After the 72 hours period, the gels were washed and fixed for multiphoton microscopy and electron microscopy, and lysed for Western blotting.

Multiphoton Electron Microscopy: The Fibroblasts where imaged using a multiphoton machine. Their intensities were recorded and analyzed.

Results

Average contraction of fibrillar collagen from Galactosialidosis donor in comparison to normal parenchyma fibroblasts

Figure 3.

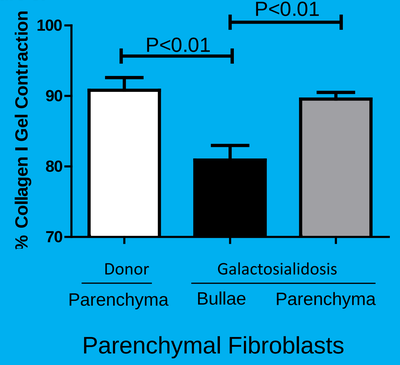


Figure 3. Galactosailidosis fibroblasts isolated from bullae are less efficient at collagen I gel contraction than donor fibroblats. Fibroblast cells from the bullae regions (black bar) contracted collagen significantly less than the donor (open) and non-diseased lung parenchyma (grey bar) from the Galactosialidosis patient. The data presented are the mean ± SEM, data were analyzed using a 1 way ANOVA with Turkey post test.

Average contraction of fibrillar collagen from Galactosialidosis donor in comparison to normal airway fibroblasts

Figure 4.

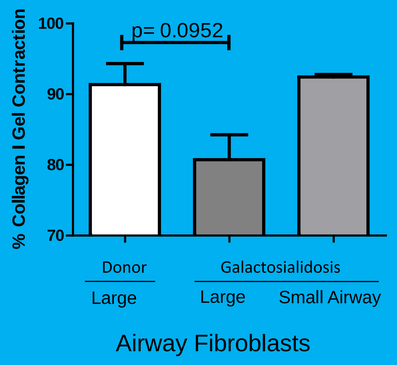


Figure 4. Galactosailidosis fibroblasts isolated from the airway are less efficient at collagen I gel contraction than donor fibroblast. Fibroblast cells from the diseased airway regions (grey) contracted collagen similarly to the donor (open) and non-diseased small airway (light grey bar) from the Galactosialidosis patient. The data presented are the mean ± SEM, data were analyzed using a 1 way ANOVA with Turkey post test.

Comparison of fibrillar collagen between normal and Galactosialidosis derived Parenchyma fibroblasts

Figure 5.

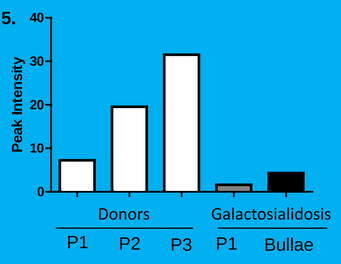
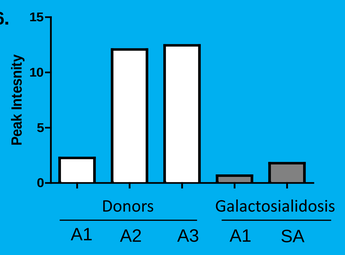


Figure 6.



Figures 5 & 6. Fibrillar collagen from all the diseased regions of the Galactosialidosis patient, had lower peak intensities than donor fibrillar collagen. The peak intensities of the donor (open) airway fibroblasts (A1, A2, A3) for Collagen I compare to the Galactosialidosis patient (grey).

Summary

1. Fibroblasts isolated from bullae of the Galactosialidosis diseased lung showed significantly less contraction of collagen I gels in comparison to fibroblasts derived from non-diseased areas of parenchyma, and normal donor derived fibroblasts.
2. Fibroblasts isolated from airways of Galactosialidosis patient were not different in their ability to contract collagen I.
3. The peak intensity of fibrillar collagen which relates to collagen remodeling in both the parenchyma and airway of the Galactosialidosis donor were significantly lower than that of normal males .

Future Aims

The lab aims to perform western blots on the gels to determine if the contractile machinery of fibroblasts are defective in our Galactosialidosis patient and to see if elastin production is also defective in lung derived fibroblasts.

Conclusion

The significance of defective collagen contraction and remodeling by fibroblasts obtained from bullae may indicate that these regions are more susceptible to defective repair following injury. The mechanisms involved in this process require further investigation to understand how the CTSA mutation in Galactosialidosis patients leads to defective collagen homeostatsis.

Acknowledgements

Dr. Tillie Hackett

>Jari Ullah
>Soheil Hajimohammadi
>Furquan Shaheen
>David Ahumada
>Soheil Hajimohammadi
>Dr. Stephanie Warner
>Dr. Dorota Stefanowicz
>Dr. Hyun-Kyoung Koo

Dr. Dragos M. Vasilescu

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None of the authors of this abstract presentation have any financial relationships to disclose

Funding