Introduction

Asthma is the most common chronic condition in children worldwide. This chronic inflammatory disorder appears as airway hyperresponsiveness and inflammation and is often characterized by pathological modification of the airway structure. The structural changes are often called airway remodeling and include: epithelial changes, sub-epithelial fibrosis and deposition of abnormal extracellular matrix components in the basement membrane and in particular collagen. Airway remodeling is a fundamental feature of asthma, linking inflammation and structural changes.

Methods and Procedures

Quantification of Airway Remodeling in Normal and Asthmatic patients

Astable laser beam emitted from an Argon-iodine laser was used for the experiments. The laser beam was driven through a three stage spectrometer. The spectrometer separated the incident beam into its wavelength components using a grating. The spectrometer was optimized for the laser wavelength and each image detected at this specific emission wavelength band was a data point in the spectral graph. The laser output was attenuated using neutral density filters and the average power transferred to the sample was below the damage threshold of the samples. The laser beam was focused on the tissue sample using a microscope objective. The forward direction was captured using a non-de-scanned detector. For thickness measurements, an image analysis algorithm based on structure tensor analysis was used to calculate the degree of variation in thickness in normal and asthmatic airways.

Hypothesis

We hypothesize that the structural orientation of fragmented collagen and the structural orientation of basement membrane thickness variation will be different in normal and asthmatic airways.

Specific Aim

To evaluate whether the structural orientation of fragmented collagen and the structural orientation of basement membrane thickness variation will be different in normal and asthmatic airways.

Results

Figure 1: Determining the correct counter stain for collagen analysis with SHG imaging

Collagen SHG

Method 1:

Method 2:

Method 3:

Figure 2: A) Forward scatter image of an airway where Collagen fibers are visualized using SHG microscopy. B) Comparison of sub-epithelial collagen fibers orientation coherency in normal and asthmatic airways. By performing an unpaired t-test on collagen fiber orientation coherency, we demonstrate that asthmatic airways have significantly less organized collagen fibers compared to normal airways.

Figure 3: Basement membrane thickness variation is different in normal and asthmatic airways.

A) Aperio image snapshot of an airway and the local thickness output of a traced basement membrane B) Colour histogram showing the frequency of different colours observed C) Numerical conversion of the thickness D) Normal distribution of the results E) Averages and variations of basement membrane thickness in normal and asthmatic airways. By using this algorithm, basement membrane average thickness and its variation was compared and it was seen that these terms are bigger in asthmatic airways.

Analysis

Collagen orientation, thickness of subepithelium and thickness of basement membrane were determined using the spectral capacity of the system and each image analyzed at the specific emission wavelength band provided a data point in the spectral graph. This collagen parameter was measured on 3 different regions of interest on 6 different sample in each group.

Summary

In comparison, asthmatic airways have...

- Less structured orientation coherency of sub-epithelial collagen compared to non-asthmatic airways.
- Trend towards greater and more variable basement membrane thickness than non-asthmatic airways.

Future Studies:

- Determine the ability of airway fibroblasts from asthmatic and non-asthmatic donors to remodel collagen 1 gels by developing a collagen 1 gel contraction bio-assay.

References


Abstract

Asthma is a chronic inflammatory disease of the airways associated with hyperresponsiveness and airflow obstruction. This characteristic pathologic feature of asthma is due to remodeling of the airway epithelium, submucosa, and smooth muscle in the airway wall. Structural changes are evident as thickening of the basement membrane, remodelling of extracellular matrix components, and increased fibrosis.

Hypothesis

It is hypothesized that structural changes in asthmatic baseline membrane thickness will be different from normal. This will be demonstrated through structural modification of asthmatic airways compared to normal airways.

Specific Aim

The specific aim of this study is to compare fibrillar collagen arrangement and thickness variation in asthmatic and normal baseline membrane to the structural modifications and thickness variation in normal and asthmatic baseline membrane.

Methods and Procedures

High resolution Aperio images of airways stained with Massons Trichrome and an antibody labeled with DAB. These sections were then analyzed using a wavelength scan for the second harmonic generation (SHG). A color analysis algorithm was designed to examine the structural orientation of basement membrane structures by light microscopy with Hematoxolin, Massons Trichrome and an anitbody labeled with DAB. These sections stained with hemotaxolyn enable a strong SHG signal in forward direction was captured using a non-de-scanned detector.

Figure 1: Immunohistochemistry studies require removal of paraffin by autodigestion and counter staining of biological tissues to identify different structures within patient samples. Human lung airway sections were stained to visualize tissue structures by light microscopy with Hematoxolin, Massons Trichrome and an antibody labeled with DAB. These sections were then analyzed using a wavelength scan for the second Harmonic signal of Collagen 1 structures compared to autofluorescence. Our data demonstrate that only tissue sections stained with hematoxolyn enable a strong SHG signal compared to autofluorescence.

Methods and Procedures

Methods: Immunohistochemistry studies require removal of paraffin by autodigestion and counter staining of biological tissues to identify different structures within patient samples. Human lung airway sections were stained to visualize tissue structures by light microscopy with Hematoxolin, Massons Trichrome and an antibody labeled with DAB. These sections were then analyzed using a wavelength scan for the second Harmonic signal of Collagen 1 structures compared to autofluorescence. Our data demonstrate that only tissue sections stained with hematoxolyn enable a strong SHG signal compared to autofluorescence.

Results: Using our image analysis algorithm, we found that there was a trend towards smaller basement membranes in asthmatic airways. We found that the variations in basement membrane thickness was similar between asthmatic and non-asthmatic airways.

Conclusion

Future studies will determine the effect of Collagen remodeling on the airway structure. We hypothesize that structural modifications of basement membrane thickness in asthmatic airways will be different from normal airways.

Figure 2: Sub-epithelial collagen is more disorganized in asthmatic airways based on the structure tensor analysis

A. Normal

B. Asthma

Table 1: information on the donors used (n=10 samples).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Average Age</th>
</tr>
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<tbody>
<tr>
<td>Asthma</td>
<td>34.37</td>
</tr>
<tr>
<td>Normal</td>
<td>37.17</td>
</tr>
<tr>
<td>Total</td>
<td>19.12</td>
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</tbody>
</table>

Figure 3: Basement membrane thickness variation is different in normal and asthmatic airways.

A. Normal

B. Asthma

Methods and Procedures

High resolution Aperio image scan of an airway section

A. Forward scatter image of an airway where Collagen fibers are visualized using SHG microscopy. B) Comparison of sub-epithelial collagen fibers orientation coherency in normal and asthmatic airways. By performing an unpaired t-test on collagen fiber orientation coherency, we demonstrate that asthmatic airways have significantly less organized collagen fibers compared to normal airways.

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