Quantification of Airway Collagen I Remodeling in Asthma

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Abstract

Introduction: In this research we studied asthmatic human airway remodelling in context of collagen morphology using optical imaging methods. Airway remodeling is a fundamental feature of asthma, linking inflammation with airway hyperresponsiveness (AHR). Remodeling involves all tissues of the airway wall including the epithelial, submucosal, and smooth muscle layers as well as vascular structures. The asthmatic airway also shows evidence of fibrosis, with deposition of abnormal extracellular matrix components in the basement membrane in particular collagen I.

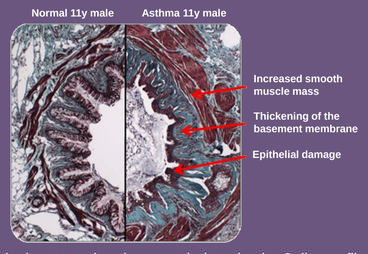
Methods: Asthmatic and non-asthmatic subjects were used to analyze airway basement membrane and fibrillar collagen formation and remodeling. The noncentrosymmetric structural organization of collagen fibrils enables it to be imaged with Second Harmonic Generation (SHG) microscopy. The orientation of 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. Airway and parenchymal fibroblasts from subjects were isolated and used in collagen I gel contraction assays to observe the function of the fibroblast cells in collagen formation and remodeling.

Results: In asthmatic patients, basement membrane is thicker compared to non-asthma and as the airway size gets bigger, the basement membrane gets thicker**.** In the large airways of asthmatic patients there is a higher level of disorganization in fibrillar collagen, whereas in small airways the fibrillar collagen organization is not different. Asthmatic airway fibroblasts do not contract collagen I gels as efficiently as non-asthmatic fibroblasts, potentially due to deficient remodeling of collagen I.

Conclusion: There are considerable differences in the morphology of collagen in asthmatic tissues compared to non-asthma due to airway remodelling and fibroblast cells affect fibrillar collagen formation and remodelling. Expanding our understanding of collagen deposition and organization in disease creates potential opportunities for therapeutic intervention.

Introduction

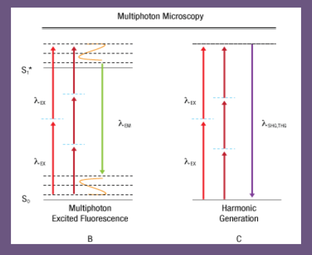
Asthma is a chronic inflammatory disease of the airways. The disease normally appears as airway hyperresponsiveness and inflammation and is often characterized by pathological modification of the airway structure. The structural changes are termed airway remodeling and include: epithelial damage, sub-epithelial fibrosis and deposition of abnormal extracellular matrix components in the basement membrane, in particular collagen.



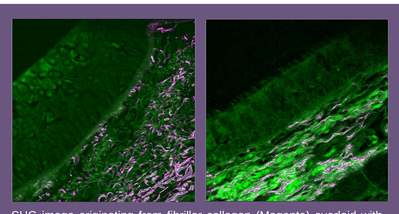
Collagen is the most abundant protein in animals. Collagen fibres are composed of a rod structure ~300 nm in length and 1.5 nm in diameter. The fibres are made up of three polypeptide strands of collagen, which are coiled together into a right-handed coil to form a triple helix .

MPE and SHG Microscopy

Multi-photon excitation (MPE) microscopy uses pulsed long-wavelength light to excite flurophores within the specimen being observed. The fluorophore absorbs the energy from two long-wavelength photons which must arrive simultaneously in order to excite an electron into a higher energy state, from which it can decay, emitting a fluorescence signal.



In addition to multiphoton fluorescence excitation, the ultrashort femtosecond laser is also efficient in producing the non-linear polarization effect of second harmonic generation (SHG) from its interaction with non-centrosymmetric biological structures, such as collagen and myosin.



SHG image originating from fibrillar collagen (Magenta) overlaid with the MPEF image (green) for the representative images of non-asthmatic(right image) and asthmatic(left image) airways.

Hypothesis

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

Specific Aim

To compare fibrillar collagen arrangement and basement membrane thickness variations in non-asthmatic and asthmatic airways and to determine if this alteration affects fibroblast function.

Analysis

To investigate the differences in the orientation, collagen fibrils organization was analyzed in the images by Structure Tensor analysis and using the local orientation and isotropic properties (coherency and energy) of every pixel of the image.). To measure corresponding orientation features (coherency and energy) within specified ROIs in the subepithelium, OrientationJ plugin was used which is based on structure tensors (Rezakhaniha, 2012). The coherency indicates if the local image features are oriented or not: C is 1 when the local structure has one dominant orientation and C is 0 if the image is essentially isotropic in the local neighbourhood. Pixels with higher energy values correspond to less isotropic and more clearly oriented structures. To infer the preferred orientation of structures present in the image and investigate changes in directional orientation of the fibers, fourier component analysis for directionality was performed using Directionality plug-in in ImageJ and based on the frequency distribution, circular histogram of local orientation distribution was generated.

Samples

	Gender (m/f)	Average Age (yrs)
Asthma	7 / 5	20.8
Non-asthma	7 / 3	17.4

(m=male, f=female, yrs=years)

Results

Basement membrane is thicker and more variable in asthmatic airways

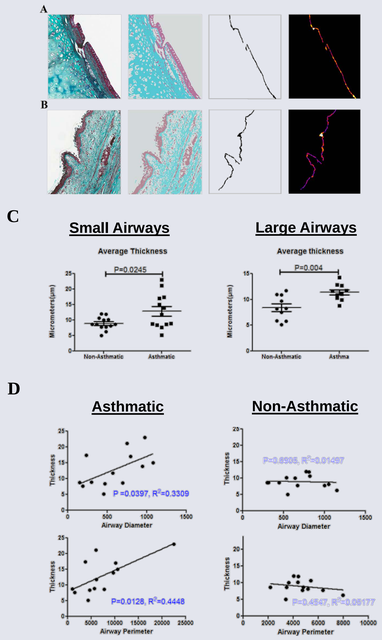


Figure 1: Basement membrane thickness analysis: Massons Trichrome stained images of (A) non-asthmatic and (B) asthmatic human airways were used for the basement membrane morphology assessment. Post-processed image makes it possible to select the basement membrane based on its color without the need to trace it. Local thickness plugin in Fiji was used to assess the average thickness and thickness variations throughout the layer. (C) Comparison of average basement membrane thickness in asthmatic and non-asthmatic patients in large and small airways. (D) Correlation of basement membrane thickness to the airway diameter and parameter in asthmatic and non-asthmatic patients

The organization level is different in sub-epithelial collagen of asthmatic airways

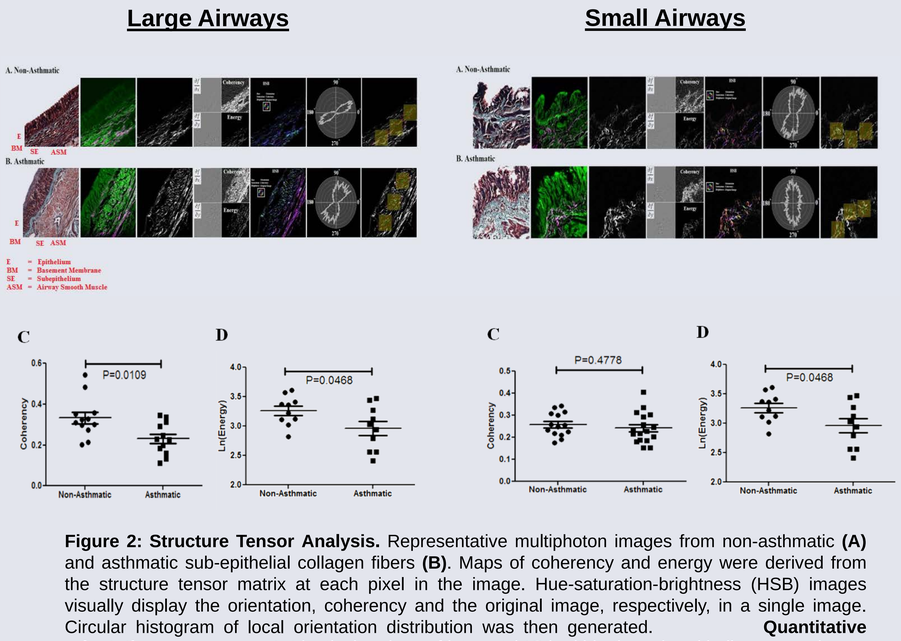


Figure 2: Structure Tensor Analysis. Representative multiphoton images from non-asthmatic **(A)** and asthmatic sub-epithelial collagen fibers **(B)**. Maps of coherency and energy were derived from the structure tensor matrix at each pixel in the image. Hue-saturation-brightness (HSB) images visually display the orientation, coherency and the original image, respectively, in a single image. Circular histogram of local orientation distribution was then generated. **Quantitative orientation measurement:** Identical regions of interest (ROI) within the sub-epithelium were chosen for this analysis. Corresponding orientation features from specified ROIs are defined. The weighting function in this mode is the ROI window with uniform weights. **(C)** Comparison of sub-epithelial collagen fibers orientation coherency and **(D)** energy in non-asthmatic compared to asthmatic airways. By performing an unpaired t-test on collagen fiber orientation coherency and energy we demonstrate that asthmatic airways have significantly less organized collagen fibers compared to non-asthmatic airways in large airways.

Differential collagen remodeling by airway and parenchymal fibroblasts

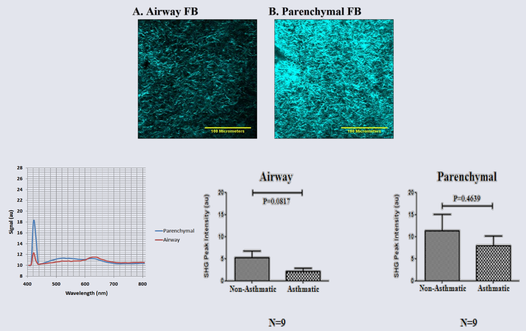


Figure 3: Representative two photon excitation microscopy images of Collagen gel seeded with asthmatic parenchymal and airway fibroblasts. Spectral graph shows SHG peak generated from the fibrillar collagen. SHG peak intensity was measured across the samples and was compared between the groups.

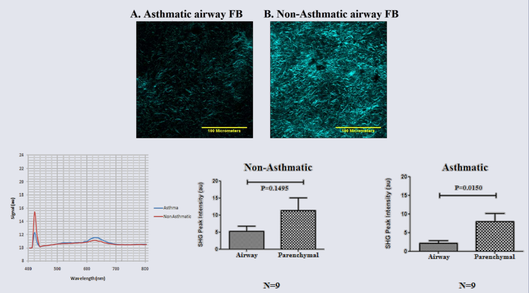


Figure 4: Representative two photon excitation microscopy images of Collagen gel seeded with non-asthmatic and asthmatic airway fibroblasts and SHG peak generated from the fibrillar collagen. SHG peak intensity was measured across the samples and was compared between the groups.

Summary

- In asthmatic patients, basement membrane is thicker compared to non-asthma and as the airway gets bigger, the basement membrane gets thicker**.**
- In the large airways of asthmatic patients there is a higher level of disorganization in fibrillar collagen, whereas in small airways the fibrillar collagen organization is not different.
- Asthmatic airway fibroblasts do not contract collagen I gels as efficiently as non-asthmatic fibroblasts, potentially due to deficient remodeling of collagen I.
- The remodeling of fibrillar collagen as well as basement membrane modification is important as collagen structure plays an essential role in the ability of fibroblasts to maintain airway structure during homeostasis and repair.

References

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