Mesothelial Cells Exposed to Autoantibodies Display Upregulated Transcription Factors Involved in Collagen Pathways

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Abstract

Amphibole asbestos exposure has been linked to autoantibody production. Specifically, anti-mesothelial cell autoantibodies (MCAA) have been linked with fibrotic pleural disease in the asbestos contaminated vermiculite mining community of Libby, Montana. However, the exact intracellular mechanism of how these autoantibodies cause an increase in collagen deposition remains unknown. This study sought to gain insight into the signal transduction linking MCAA binding with collagen production by human mesothelial cells. In this study, transcription factor activation profiles were generated from human mesothelial cells treated with sera from the patients of Libby. Analysis of these profiles indicated significant differential expression in 15 of the 48 transcription factors analyzed compared to the sera cleared of all IgG. 11 of these transcription factors are associated with type 1 collagen deposition. These data suggest autoantibodies are directly involved in type 1 collagen deposition and may elucidate potential therapeutic targets for auto antibody mediated fibrosis.

Introduction

Vermiculite is a phyllosilicate that is widely used in insulation due to its ability to resist high temperatures. Libby, Montana was home to the largest vermiculite mine in the world prior to its closure. The vermiculite ore bed was contaminated with amphibole asbestos such that the community had years of exposure to the asbestos. Now, a high frequency of pleural disease has been observed by clinicians in Libby. High resolution CT scans show increased collagen deposition in the pleural cavity, which causes pain and dyspnea in the patients. Serum samples were taken from patients in the community and have been shown to have a high frequency of anti-mesothelial cell autoantibodies (MCAA). MCAA have been linked with collagen deposition associated specifically with pleural disease (Marchand 2012). In addition, MCAA induce collagen deposition by mesothelial cells in vitro (Serve 2013). The intracellular mechanism behind this collagen deposition has remained a mystery.

Human mesothelial cells were treated with sera provided by the patients in Libby. The sera had been shown to have high binding of mesothelial cell autoantibodies. Transcription factor activation profiles were generated to determine the pathway involved in collagen deposition. To ensure the autoantibodies were responsible for collagen deposition and no other factors in the sera were responsible, sera were cleared of the autoantibody and another profile was made and compared.

In order to develop a therapeutic treatment for this disease, understanding of the pathway causing the collagen deposition is vital. Knowing what transcription factors are involved in this mechanism is the first step towards this necessary understanding.

Materials and Methods

<u>Human Mesothelial Cell Culture:</u> Met5A cells (ATCC) were cultured in RPMI media with 5%FBS and antibiotics in a humidified 37ºC incubator, in 6-well plates. Cells were treated with 10µLs of MCAA+ serum for two hours. Control group was treated with 10µLs of 1X PBS.

<u>Serum Samples</u>- Obtained by the Libby Epidemology Research Program under an approved IRB protocol. All samples are stored at -80ºC until use. Serum was cleared of IgG using Pierce Protein G Agarose Beads, and then brought back to normal dilution and screened for IgG using SDS-PAGE.

<u>Generating Transcription Factor Profile:</u> Signosis TF Activation Profile Plate Array I, Catalog Number FA-1001. Elution of probe was performed in the Molecular Research Core Facility at ISU. Bio-Tek Synergy HT Microplate Reader was used to read the plates.

Results

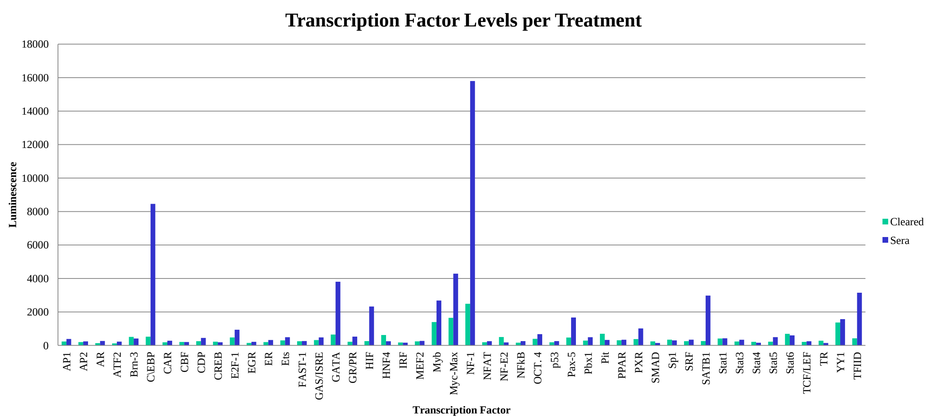

Figure 1. Graph summarizing the quantity of corresponding transcription factor made after treatment with autoantibody containing MCAA+ serum (dark blue) versus serum cleared of IgG (light blue). Nuclear extract was harvested from treated cells and applied to the appropriate oligonucleotide probes which had the appropriate promoter sequence. This ensured only active transcription factors were detected.

TF Symbol1	TF Title	Fold Change
AR	Androgen Receptor	1.933824
ATF2	Activating Transcription Factor 2	1.883333
C\EBP	Ccaat-Enhancer-Binding Proteins	16.16635
E2F-1	E2F Transcription Factor One	1.981013
GATA	GATA Transcription Factor	5.865948
GR/PR	Gastrin-releasing Peptide Receptor	2.423963
HIF	Hypoxia-inducible Factors	9.015504
Myb	Transcriptional Activator Myb	1.922636
Myc-Max	Myc-associated factor X	2.602307
NF-1	Nuclear Factor I	6.346324
Pax-5	Paired box protein	3.544681
PXR	Pregnane X Receptor	2.69496
SATB1	Special AT-rich Sequence-binding protein-1	11.45
Stat5	Signal Transducer and Actibator of Transcription 5	2.230769
TFIID	Transcription Factor II D	7.439716

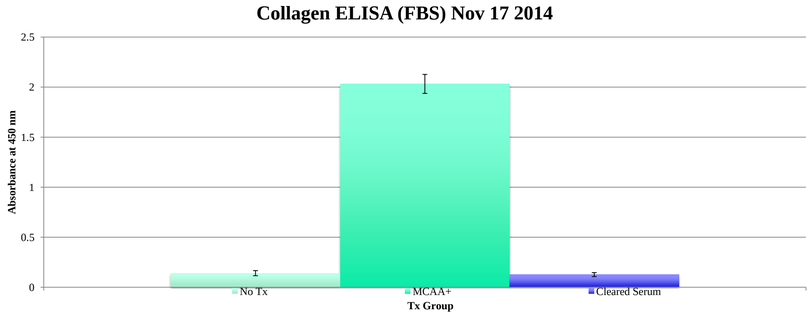

Figure 2. Graph displaying collagen deposition levels of human mesothelial cells treated with mesothelial cell autoantibody positive serum, serum cleared of all IgG, and untreated cells. This in vitro assay detected collagen deposited in the extracellular matrix.

<u>Results Summary:</u> Figure 1 clearly indicates multiple differentially expressed transcription factors between the whole serum and serum cleared of all IgG. Figure 2 shows that the serum containing the mesothelial cell autoantibody results in increased collagen deposition in vitro compared to the same serum cleared of all IgG. This information correlates with low transcription factor levels seen in the cleared serum in figure 1. The information in Table 1 highlights the most upregulated transcription factors and shows that 11 of them are directly involved in the binding of the COL1A1 promoter. This data suggests that the autoantibody is activating transcription factors associated with type 1 collagen deposition.

Table 1. A list of 15 transcription factors differentially expressed as indicated by the signifcant increase in fold change. 11 Transcription factors in blue have known binding sites to the COL1A1 promoter. To determine which transcription factors were involved in binding of the COL1A1 promoter the Champion ChiP Transcription Factor Search Portal was used.

Summary and Conclusions

- **Treatment of human mesothelial cells with serum containing anti-mesothelial cell autoantibodies resulted in differential expression of 15 of the 48 examined transcription factors, compared to cells treated with serum lacking MCAA.**
- **Of the 15 differentially expressed transcription factors 11 of them are known to be involved in collagen deposition.**
- **By analyzing TF activation for cells treated with complete MCAA+ serum versus serum with no IgG, we demonstrate which TF's are specifically activated by autoantibodies, rather than any other component in the serum.**
- **These results allow us to focus on pathways that involve the differentially upregulated TF's in order to determine the mechanism leading to collagen deposition by MCAA.**

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