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Epigenetic silencing of SOCS3 expression contributes to fibrosis in Crohn's disease

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Abstract/Background

Identified risk polymorphisms affecting the Jak-STAT3 pathway in patients with Crohn's disease could affect TGF-β1 and collagen I expression and in the pathway's negative regulator, SOCS3. Genetic factors, however, account for only ~25% of disease. Epigenetic events also shape gene expression. Recent experiments showed that autocrine IL-6 production in mesenchymal cells, subepithelial myofibroblasts (SEMF) and muscle cells, of patients with fibrostenotic Crohn's disease causes sustained Jak-STAT3 activity, excess TGF-β1 and Collagen I production and fibrosis. SOCS3 paradoxically decreased in these cells. We now identify epigenetic mechanisms that silence SOCS3 expression in SEMF of patients with fibrostenotic Crohn's disease. In a previous experiment, using isolated SEMF of normal ileum and affected ileum from patients with each Crohn's phenotype, inflammatory (Montreal B1), fibrostenotic (B2) and penetrating (B3), we confirmed decreased SOCS3 protein levels were unique to B2 patients. Expression of miR-19b increased in SEMF of affected ileum. SOCS3 transcriptional activity decreased after transfection of miR-19b mimic and increased when antagomiR-19b was expressed. Epigenetic silencing of SOCS3 in ileal SEMF of patients with fibrostenotic Crohn's disease occurs by increased miR-19b mediated inhibition of SOCS3.

Introduction

- Cytokines, including IL-6, are pivotal modulators of responses in inflammatory diseases and are secreted by numerous cells including activated intestinal subepithelial myofibroblasts.
- Binding of IL-6 activates the constitutively associated JAK-1 and JAK-2 resulting in cross-phosphorylation and docking sites for STAT3. Specific STAT3 residues are acetylated and phosphorylated and STAT3 translocates to the nucleus where it regulates transcriptional activity, including of TGF-β1 and COL1a1.
- A consensus STAT3 binding element is present in the 5'-UTR promoter region of the Suppressor of Cytokine Signaling-3 (SOCS3) gene (1).
- Normally activation by IL-6, via Jak-STAT3, induces expression of SOCS3, a crucial negative regulator of cytokine-induced Jak-STAT3 signaling.
- We recently demonstrated that in patients with Crohn's disease with a Montreal B2 fibrostenotic phenotype, that despite increased autocrine production of numerous pro-inflammatory cytokines, particularly IL-6, in mesenchymal cells of affected ileum, there is loss of the expected upregulation of SOCS3 protein.

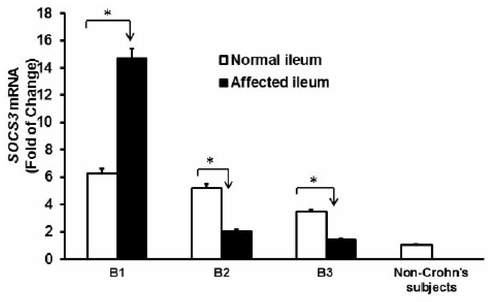


Figure 1. SOCS3 expression decreased in affected ileum of patients with stricturing (Montreal B2) and penetrating (B3) Crohn's disease compared to normal ileum in the same patient as well as non-Crohn's subjects. The opposite occurred in patient with inflammatory (B1) Crohn's disease. Values represent the mean ± SEM of 5-6 separate experiments. *denotes p<0.05 vs normal ileum in the same patient.

- In mesenchymal cells, subepithelial myofibroblast (SEMF) and smooth muscle cells, we observed sustained JAK-STAT3 signaling that accounted for the excess TGF-β1 and collagen I production from these cells that is a characteristic pathogenetic mechanism in fibrostenotic Crohn's disease (2).
- Epigenetic mechanisms are increasingly recognized as regulators of gene expression and disease. Genetic factors account for at most 25% of disease in Crohn's patients. One important epigenetic process regulating expression is small non-coding microRNAs (miRs) that target mRNA for degradation.
- A highly conserved STAT3 binding element is present in the promoter region of the miR17~92 cluster gene, C13orf25 (3). Position 1561-1568 of the SOCS3 3' UTR is an 8-mer sequence for miR-19a-5p and miR-19b-5p binding derived from this cluster (1).
- This work identifies one of three epigenetic mechanisms leading to the unexpected low levels of SOCS3 expression in patients with fibrostenotic Crohn's disease compared to other Crohn's disease phenotypes.
- SOCS3 expression is silenced in SEMF of patients with fibrostenosis via increased expression of miR-19a-5p and miR19b-5p that targets Socs3 mRNA for degradation.

Methods

Methods

- Mesenchymal cells were isolated from Crohn's disease patients
- Human studies were approved by the VCU Institutional Review Board
- Quantitative real-time PCR used to measure RNA transcripts with miR-19a-5p and miR-19b-5p primers
- Results for qRT-PCR were calculated using the $2^{-\Delta\Delta Ct}$ method based on U6 for miR amplification (remains stable across Crohn's phenotypes examined)
- Luciferase Reporter Assay was used to measure gene transcription.
- Cells were transfected with anti-miR-19b or pre-miR-19b.
- Statistic Analysis: values represent means ± SE of n experiments, where *n* represents the number of experiments on cells derived from separate subjects
- Statistical significance was determined by Student's t-test for either paired or unpaired data and was assumed for p<0.05
- Comparison between multiple groups was made using ANOVA with a Turkey test for post hoc comparisons

Participants

Demographics	Patient No. (% of total)
Age (years)	
under 20	2 (8)
20-29	9 (38)
30-39	7 (29)
40-49	4 (17)
50-59	1 (4)
over 60	1 (4)
Sex	
Male	9 (37)
Female	15 (63)
Race	
White	14 (58)
Black or African	9 (38)
Other/unknown	1 (4)
Non-Crohn's Subjects	6 (100)
CD Subjects Montreal Phenotype	
B1-non-stricturing, non penetrating	6 (33)
B2-stricturing	6 (33)
L1-ileal 4 (67%)	4 (67)
L2-ileo-colic 2 (33%)	2 (33)
B3-Penetrating	6 (33)

Results: miR-19b regulates SOCS3

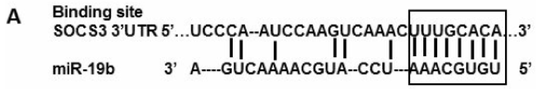


Figure A. Hybridization analysis of SOCS3 and miR-19b identified a conserved 8-mer seed sequences complementary for both has-miR-19a and hsa-miR-19b at position 1561-1568 of SOCS3 3' UTR.

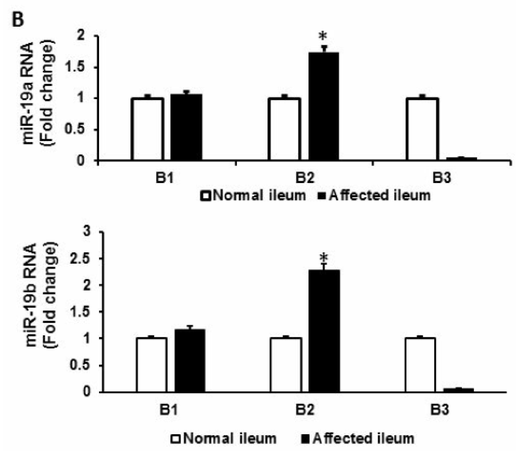


Figure B. miR-19a and miR-19b expression was unchanged or decreased in SEMF of affected ileum in patients with Montreal B1 and B3 phenotype Crohn's disease, respectively, compared to non Crohn's subjects. In contrast, miR-19b levels were significantly elevated in SEMF of affected ileum compared to normal ileum in patients with B2 fibrostenotic disease. * denotes p<0.05 vs normal ileum.

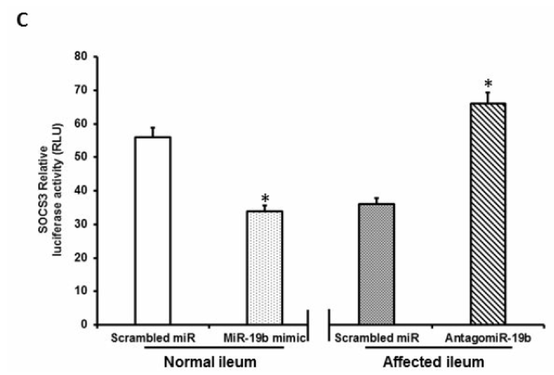
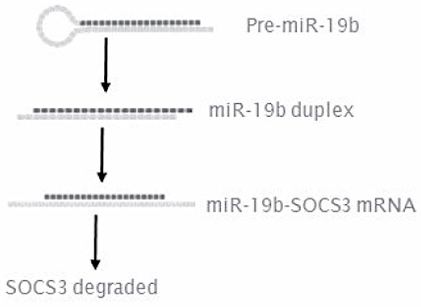


Figure C. Transcriptional activity of socs3 was decreased in SEMF of normal ileum of patients with fibrostenosis by transfection of miR-19b-5p mimic. Transcriptional activity of SOCS3 in SEMF of affected ileum was increased by transfection of antagomiR-19b-5p. Transcriptional activity was measured by dual luciferase-SEAP reporter assay. Values represent the mean ± SEM of 3-6 separate experiments. * denotes p<0.05 vs normal ileum (B), or control scrambled miR (C).

Conclusion

- Transcription of miR-19b-5p from the miR-17~92 targets SOCS3 mRNA for degradation
- The epigenetic events explored in this experiment, miRs, are linked not only to fibrostenotic Crohn's disease but also to development of fibrosis in other organs including the skin, heart, liver, lung, and kidney (4, 5, 6)
- Expression of miRs are altered in the fibroblasts of fibrotic organs including several members of the paralogous miR17~92 and miR106a~363 clusters, miR-21 and miR-29 (7-11). These resulting epigenomic alterations impact expression of pro-fibrotic genes such as collagen, TGF-β1, matrix metalloproteinases and α-smooth muscle actin resulting in their increased expression and development of fibrosis.
- In summary, identified genetic factors and susceptibility loci account for only 13.6% of disease variability and no more than 25% of the genetic risk in patients with Crohn's disease (12,13). Epigenomic changes can further contribute to the 'heritable' risk of developing fibrostenotic Crohn's disease. This data demonstrates one of the mechanisms of epigenetic silencing of SOCS3 that allow the sustained Jak-STAT3 activity that drives excess TGF-β1 and collagen expression and characterizes fibrostenotic Montreal B2 Crohn's disease.



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