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

Emily T. Marshall  
Virginia Commonwealth University

Chao LI  
Virginia Commonwealth University

John F. Kuemmerle  
Virginia Commonwealth University

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Abstract/Background

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 the production and phosphorylation of STAT3. Specific STAT3 residues are acetylated and phosphorylated and STAT3 translocates to the nucleus where it regulates transcriptional activity. In mesenchymal cells, subepithelial myofibroblast (SEMF) and smooth muscle cells of patients with Crohn's disease increases expression in SEMF of patients with fibrostenotic Crohn's disease. In a previous experiment, using miR-19b transfected normal ileal and affected ileal from patients with Crohn's phenotype, inflammatory (Montreal B1), fibrostenotic (B2) and penetrating (B3), we confirmed decreased SOCS3 expression 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 antagoniR-19b was expressed. Epigenetic silencing of SOCS3 in ileal SEMF of patients with fibrostenotic Crohn's disease occurs by increased miR-19b mediated degradation of SOCS3.

Methods

Inflammatory cells were isolated from Crohn's disease patients. Human specimens were approved by the VCU Institutional Review Board. Cells were transfected with anti-miR-19b or pre-miR-19b. Quantitative real-time PCR used to measure RNA transcripts with miR-19a-5p, miR-19b-5p, miR-9-3p, miR-125b-5p, miR-19a-3p, miR-19b-3p and miR-9-5p 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 phenotype Crohn's disease, respectively. Comparison between multiple groups was made using ANOVA with a Bonferroni correction. Statistical significance was determined by Student's t-test for either paired or unpaired data and was assumed for p<0.05.

Results: miR-19b regulates SOCS3 expression in SEMF

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

Because miR-19b regulates SOCS3 expression in SEMF of affected ileum in patients with Montreal B1 and B3 phenotype Crohn's disease, respectively, compared to non Crohn's subjects and in contrast, miR-19b levels were significantly elevated in SEMF of affected ileum in patients with B2 fibrotic disease. * denotes p<0.05 vs normal ileum.

Conclusion

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

Emily Marshall, Chao Li1, John F. Kuenmmerle1,2

Departments of Medicine1, Physiology and Biophysics2, and VCU Program in Enteric Neuromuscular Sciences

Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA

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