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Matrix Metalloproteinase MMP-12 Increases Intestinal Epithelial Tight Junction Permeability and Severity of Experimental Colitis

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Background: Matrix Metalloproteinase (MMP)-induced extracellular matrix remodeling modulates intestinal inflammation. MMP-12 is a human macrophage elastase capable of degrading basement membrane (BM). Defective intestinal barrier leading to increased intestinal permeability is an important pathogenic factor for intestinal inflammation. The role of MMP-12 in intestinal barrier function and intestinal inflammation remains unclear. We hypothesize that MMP-12 induces degradation of BM and helps macrophage transmigration thereby compromising intestinal barrier and augmenting intestinal inflammation. **Aim:** The aim of this study was to investigate the role of MMP-12 in intestinal epithelial tight junction (TJ) permeability and macrophage transmigration in experimental dextran sodium sulfate (DSS) colitis and in vitro Caco-2 cell model. Methods: Nine weeks-old wild type (WT) and MMP-12$^{-/-}$ mice were administered 3% DSS in drinking water for 7 days. An in vivo colonic recycling perfusion and in vitro epithelial cell model was used to study epithelial permeability. **Results:** DSS treatment resulted in a marked increase in MMP-12 protein expression in WT colon. DSS administration significantly increased the colonic permeability in WT but not MMP-12$^{-/-}$ mice ($p<0.01$). The loss of body weight, disease activity index, and histological lesion score of colitis was significantly attenuated in MMP-12$^{-/-}$ DSS group compared to WT DSS group ($p<0.01$). In immunohistochemical study, the BM laminin was significantly lost in WT DSS colon but not in MMP-12$^{-/-}$ DSS colon. The epithelial infiltration of macrophages in DSS colitis, as assessed by macrophage marker CD68 staining, was found to be significantly lower in MMP-12$^{-/-}$ mice than WT mice. To further investigate the role of MMP-12 in intestinal TJ permeability, human intestinal epithelial Caco-2 cells were co-cultured with phorbol myristate acetate activated, MMP-12 secreting human macrophage U937 cells. The co-culture resulted into progressive and significant decrease in Caco-2 transepithelial resistance (TER) and concomitant increase in paracellular inulin flux, indicating loss of Caco-2 TJ barrier. Furthermore, siRNA-induced knock down of MMP-12 expression in U937 macrophage cells attenuated loss of Caco-2 TER and increase in inulin flux after U937 co-culture. Also, siRNA-induced knock down of MMP-12 significantly prevented U937 macrophage transmigration across the Caco-2 cells. **Conclusion:** The clinical severity, colonic permeability, and macrophage infiltration in colitis was attenuated in MMP-12$^{-/-}$ mice. Macrophage derived MMP-12 increased intestinal epithelial permeability and enables macrophage transmigration in a cell culture model. These data suggest that MMP-12-induced macrophage transmigration and loss of intestinal epithelial TJ barrier contributes to the development of colitis.

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Long Noncoding RNA (LncRNA) H19 Regulates Gut Barrier Function by Altering microRNA-675 (MiR-675) Processing

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The effectiveness and stability of the gut epithelial barrier depend on specialized structures composing different intercellular junctions (IJs) including tight junctions (TJs) and adherens junctions (AJs). These IJ complexes are highly dynamic and their constituent proteins undergo continuously remodeling and turnover, but the exact mechanisms underlying the control of TJ/AJ expression remain unclear. Our previous studies demonstrate that the RNA-binding protein HuR associates with the TJ mRNAs and promotes TJ translation, thereby enhancing the epithelial barrier function. LncRNAs are defined as transcribed RNAs that are >200 nt in length but lack any extensive "Open Reading Frame". LncRNAs play important roles in a wide range of biological processes and are involved in many human pathological conditions. The lncRNA H19 is one of the first identified lncRNAs and recently shown to act as a precursor of miR-675 embedded in H19's first exon. This study tested the hypothesis that lncRNA H19 modulates TJ/AJ expression by altering miR-675 processing and this process is regulated by HuR. METHODS: Studies were conducted in Caco-2 cells, and the levels of lncRNA H19, miR-675-3p and miR-675-5p were measured by quantitative real-time PCR analysis. Functions of H19 were investigated by transfection with recombinant pCMV-SPORT6 vector containing wild-type H19 cDNA. Association of H19 with HuR was determined by mRNP/immunoprecipitation (IP) assays. Epithelial barrier function was assayed by transepithelial electrical resistance (TEER) and membrane-impermeable trace molecule FITC-dextran. RESULTS: H19 overexpression by transfection with the H19 expression vector decreased cellular abundances of ZO-1 and E-cadherin proteins (by >80%), although it failed to alter expression levels of claudin-1, claudin-3, claudin-5, JAM-1, ZO-2, and β-catenin. Ectopically expressed H19 disrupted the epithelial barrier function as indicated by a decrease in TEER values and an increase in the levels of paracellular flux of FITC-dextran. Increased H19 also increased the levels of miR-675-3p and miR-675-5p that repressed expression of ZO-1 and E-cadherin. HuR was found to directly bind the H19, whereas co-transfection with the H19 expression vector and the recombinant adenoviral plasmids containing HuR (AdHuR) not only prevented H19-induced stimulation of miR-675-3p and miR-675-5p processing but also abolished repression of ZO-1 and E-cadherin expression by H19. HuR overexpression by AdHuR also promoted the epithelial barrier function as shown by an increase in TEER and decrease in paracellular flux in cells overexpressing H19. CONCLUSIONS: These results indicate that 1) H19-induced IJ repression and subsequent barrier dysfunction are mediated via miR-675 and 2) HuR interacts with H19 and inhibits miR-675 processing from H19, thus protecting the barrier function.

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microRNA-375-KLF5 Regulation of Intestinal Epithelial CFTR Function

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We have recently demonstrated *in vitro* and *in vivo* a role for the IL-13/Type II IL-4 receptor (IL4Ra and IL13Ra1) and STAT-6 in the up regulation of intestinal epithelial Cystic Fibrosis Transmembrane Conductance Regulator (ATP-Binding Cassette Sub-Family C, Member 7) (CFTR)-dependent Cl^- conductance. In an attempt to identify the molecular pathways involved in this process, we performed miRNA microarray and qRT-PCR analyses of IL-13-stimulated intestinal epithelial cells and revealed downregulation of four miRNA's, miR-375, miR-212, miR-181a-2 and miR-145. To determine the involvement of miRNA-375 to IL-13-induced CFTR-mediated Cl^- conductance, we examined forskolin-induced Isc and Cl^- secretion in the human intestinal epithelial cell line (Caco2bbe) following overexpression of miR-375 (PreMIR-375) and antagonism of miRNA-375 (antagomir-375). Overexpression of miRNA-375 (40-fold induction) significantly abrogated IL-13-induced CFTR mRNA and protein expression and Cl^- secretion (CFTR mRNA Fold-induction: 50.2 ± 28.2 vs 6.6 ± 2.9 Forskolin response; 85.5 ± 17.1 vs 53.1 ± 9.6 (ΔIsc($\mu A/cm^2$)); mean ± SD; n = 6; PreMIR-control vs PreMIR375 respectively; $p < 0.01$). Conversely, antagonism of miR-375 amplified IL-13-induced CFTR mRNA and protein expression and CFTR-dependent Cl^- conductance (CFTR mRNA Fold-induction: 3.3 ± 0.4 vs 8.1 ± 1.4; Forskolin response; 96.4 ± 6.6 vs 114.7 ± 5.8; (ΔIsc($\mu A/cm^2$)); mean ± SD; n = 6; antagomir-control vs antagomir-375 respectively; $p < 0.01$). Analyses of the 3' untranslated region of the CFTR mRNA using TargetScan® (version 5.0) revealed that miR-375 was not predicted to interact with CFTR . Performing MiR-375 pulldown studies we revealed that miR-375 interacts with the transcription factor Kruppel like factor-5 (KLF5). Functional *in vitro* and *ex vivo* studies employing shRNA-KLF-5 technology revealed that miR-375 negative regulation of CFTR mRNA and protein expression and Cl^- secretion was dependent on KLF5. Moreover, intestinal epithelial specific-overexpression of KLF5 (Villin^{cre} KLF5Tg) enhanced CFTR-dependent Cl^- secretion in the small intestine of mice. Collectively, these findings indicate that the intestinal epithelial miR-375 is a key regulator of CFTR-dependent Cl^- secretion and gut homeostasis and mucosal immunity. These observations demonstrate a central role for the IL-13/ IL-13Ra1 pathway via miR-375/KLF-5 axis in the regulation of intestinal epithelial cell CFTR Cl^- secretion.

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Intracellular Calcium-Mediated Oxidative Stress Is a Common Denominator in the Mechanism of Tight Junction Disruption by Different Types of Stress in CaCo2 Cell Monolayers

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Epithelial tight junctions (TJs) form a major component of the intestinal mucosal barrier function, the disruption of which is involved in many gastrointestinal (GI) diseases. Various types of stress are known to disrupt the tight junctions and induce barrier dysfunction in the GI mucosa. Our recent studies indicated that the mechanism of TJ disruption by osmotic, chemical and stretch-induced stress involves a common signaling pathway involving rise in intracellular calcium $[Ca^{2+}]_i$ and activation of c-jun N-terminal kinase (JNK2) and Src kinase (c-Src). To further delineate the signaling mechanism we investigated the role of oxidative stress in this signaling cascade leading to TJ disruption. **Methods:** Caco-2 cell monolayers on transwell inserts were exposed to osmotic stress (0.6 M) or dextran sulfate sodium (DSS) (3% w/v) on the apical surface. Cell monolayers on Flexcell wells were subjected to cyclic stretch (12% stretch, 6 cycles/min). The barrier function was evaluated by measuring transepithelial electrical resistance (TER) and unidirectional flux of inulin. Integrity of TJs and adherens junctions (AJs) was analyzed by confocal immunofluorescence microscopy. Reactive oxygen species (ROS) production was measured by live cell imaging using MitoSOX, CM-H_2DCFDA, and Mitotracker as probes. Role of oxidative stress in TJ disruption was determined by evaluating the effect of antioxidants such as *N*-acetyl cysteine (NAC), l-nitroarginine methylester (l-NAME; NOS inhibitor), 1400W (iNOS inhibitor) or apocynin (NOX inhibitor), and the role of $[Ca^{2+}]_i$ was evaluated using BAPTA, a Ca^{2+} chelator. Activation of JNK2 and c-Src was evaluated by immunoblot analysis of JNK2^{pT183pY185} and c-Src^{pY418}. **Results:** Osmotic stress, DSS and cyclic stretch induced ROS production in a time dependent manner with a maximum level achieved within 30 min. Both MitoSox and H_2DCFDA showed localization of ROS in mitochondria as well as in non-mitochondrial regions, including nucleus. ROS production by all 3 types of stress was abrogated by pretreatment of cell monolayers with BAPTA, indicating the role of $[Ca^{2+}]_i$ in inducing oxidative stress. NAC and L-NAME, but not by 1400W or apocynin, blocked osmotic stress, DSS and cyclic stretch-induced barrier dysfunction and disruption of TJs and AJs. NAC and L-NAME also reduced stress induced activation of JNK2 and c-Src. **Conclusion:** These results indicate that oxidative stress mediates $[Ca^{2+}]_i$-induced activation of JNK2 and c-Src leading to disruption of TJs and AJs by different types of stress. Identifying common signaling mechanism in barrier dysfunction by multiple types of stress is essential to develop effective therapeutics as mutiple stresses are involved in the pathogenesis of GI diseases.

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Calcium-Sensing Receptor Stimulates Cl^-- and SCFA-Dependent but Inhibits Cyclic Nucleotide-Dependent HCO_3^- Secretion in Colon

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Bicarbonate (HCO_3^-) secretion is a well-established physiologic process that is closely linked to overall fluid and electrolyte movement in the mammalian colon. These present studies show that extracellular calcium-sensing receptor (CaSR), a fundamental mechanism for sensing and regulating ionic and nutrient compositions of extracellular milieu in the small and large intestine, regulates HCO_3^- secretion. Basal and induced HCO_3^- secretory responses to CaSR agonists were determined by pH stat techniques used in conjunction with short-circuit current measurements in mucosa from rat distal colon mounted in Ussing chambers.