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Metagenomic Analysis of the Dynamic Changes in the Gut Microbiome of African American With Colorectal Cancer
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Hassan Brim, Edward Lee, Scott Dowd, Adeyinka Layemjo, Hassan Ashktorab Background: Linkage of specific bacterial markers to colorectal pathogenesis has been hampered by limited knowledge of the colonic microbiota and changing bacterial classification schemes. More than 80% of the colonic microbiota is not cultivable and can only be assessed through metagenomic analysis. Aim: To perform a metagenomic analysis of 10 colon cancer tumors and their matched normal tissues. Methods: Metagenome sequencing was conducted on the illumina 2x150bp sequencing platform. Briefly DNA was prepared and libraries created using standard library preparation kits. Sequencing was performed in each sample. For all samples an average of 20 million reads were obtained. Paired Sequencing reads were joined and entered into MG-RAST for metagenomic analysis. The data was compared to M5NR using an average e-value of 4e-3, a minimum identity of 60%, and a minimum alignment length of 15 measured in amino acids for protein databases. Results: Metagenome was compared using best hit classification in two groups (normal and tumor). Based upon this best hit classification at the kingdom level in MG-RAST it was found, using one-way ANOVA, that tumor samples had a significantly higher classification related to Microbiota (Bacteroidetes) specifically related to Enterobacteria phage with a P = 0.0028. The other notable significant difference was the archaea. It was found at the class level that methanobacteria had higher classification hits in the normal samples (p = 0.03). Based upon functional gene abundance classification it was found that phage (P=0.007) as well as iron acquisition and metabolism genes (P=0.035) were significantly different between the groups. Further analysis showed that as expected from the taxonomic classification data that tumor samples had higher levels of phage related functional classifications particularly related to phage capsule proteins. Other notable findings consisted of bacterial protein acetylation and deacetylation genes that were found to be significantly higher in normal tissue compared to tumor.

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Upregulation of Mucin Production by a Probiotic-Derived Protein Through Activation of EGF Receptor
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Background & Aims. The mucus layer coating the gastrointestinal tract serves as the first line of defense against luminal antigens. Intestinal commensal bacteria regulate mucin production by goblet cells and requires mucus for binding sites and energy source. Probiotics have been shown to promote production of mucus by goblet cells to enhance host innate defense against infection. p40, a Lactobacillus rhamnosus GG (LGG)-derived soluble protein, an obligate commensal intestinal operon, is known to function through activation of EGF receptor (EGFR) in intestinal epithelial cells. Since EGFR signaling has been shown to regulate mucin production in goblet cells, the purpose of this study was to investigate the effects and mechanisms of p40 regulation of mucin production. Methods. LS174T cells, a human colon cancer cell line with secretory cell characteristic, were treated with p40 (25 to 200 ng/ml) for 30 min to 24 h in the presence or absence of EGFR kinase inhibitor, AG1478 (300 nM) or knock out of EGFR using siRNA method. Wt and Egfrwa2 (EGFR kinase defective) mice on C57BL/6 background were gavaged with pectin/zein beads containing p40 (N=56), healthy Bangladeshi volunteers (N=8), and Bangladeshi cholera patients (N=12) with severe diarrhea. In addition, the safety of iOWH032 was assessed in these different populations. Plasma samples were collected pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, 24, 36, and 48 hours after dosing for analysis using a validated LC-MS/MS analytical method. All of the plasma samples of intact iOWH032 was evaluated by a non-compartmental pharmacokinetic analysis using WinNonlin 5.2 (Pharsight, Sunnyvale, CA). There were no pronounced differences in pharmacokinetic parameters between healthy Americans and healthy Bangladeshi. However, the maximum plasma concentration of iOWH032 (Cmax) was lower for Bangladeshi cholera patients (482 ± 388 ng/mL) than for the healthy Bangladeshis (1275 ± 491 ng/mL). The time to maximum plasma levels (Tmax) was similar for both the healthy Bangladeshis and cholera patient populations at 4 h ± 2 h and 3.8 ± 1.6 h after dosing, respectively. The mean AUC measured to infinite time (AUC∞) reflected the mean Cmax values and was 22680 ± 10362 ng/mL for healthy Bangladeshis and 6250 ± 4910 ng/mL for patients with cholera. For both the healthy Bangladeshis and cholera patient groups, less than 5% of the AUC∞ was in the extrapolated portion of plasma profile, confirming the linearity of the data for both the healthy Bangladeshis (8.5 ± 1.5 h) and cholera patients (8.2 ± 1.4 h). It was also noteworthy that the variability on these parameters was approximately double in the cholera patients compared to the healthy Bangladeshis. The Tmax values would suggest that the rate of absorption was similar for the two Bangladeshis population groups and the data may indicate that the disease state has no effect on the clearance of iOWH032. The most likely explanation for the lower exposure and increased variability (Cmax and AUC) of iOWH032 in the cholera patients is the increased and variable gastrointestinal transit time.

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The Role of Bile Acid Receptor FXR Activation on NHE8 Expression Regulation
Hua Xu, Jing Li, Meena Ananthanarayanan, Fayek K. Ghishan
Introduction. Farnesoid X receptor (FXR) is a highly expressed nuclear receptor in the liver and the intestine, and it plays critical roles in regulating bile acid and lipid homeostasis. Genetic-wide FXR binding assay showed that FXR binding site was detected in the promoter region of NHE8 gene in the murine intestine, and this binding was dramatically induced after FXR ligand GW40046 treatment. However, the role of FXR on NHE8 gene expression has not been characterized. Methods: Human intestinal epithelial cells (Caco2 cells) were treated with 1 nM and 5 nM FXR agonist GW40046. Total RNA was isolated using Trizol reagent, and total cell lysate was prepared in RIPA buffer. Western blotting was used to analysis the expression of NHE8 protein in Caco-2 cells, and Real-time PCR was used to quantify NHE8 mRNA expression in control or GW40046 treated cells. Promoter reporter assays were used to analyze NHE8 gene promoter activity in Caco2 cells. Results: GW40046 treatment decreased NHE8 protein expression by 60% and NHE8 mRNA expression by 43% in Caco2 cells. Transfection with the promoter construct containing NHE8 FXR response region showed that promoter reporter gene expression was repressed 33% in Caco2 cells after GW40046 treatment. Conclusion: We observed that the intestinal NHE8 mRNA expression and protein expression were indeed downregulated by GW40046 in Caco2 cells. GW40046 inhibited NHE8 gene expression by reducing promoter activation through FXR binding on NHE8's promoter region. These results suggest that NHE8 is subject to the regulation of bile acids in the intestine, which may shed new light on a role of NHE8 in bile acid and lipid homeostatic regulation.

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Modulation of NHE8 by Butyrate in Human Intestinal Epithelial Cells
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Introduction. Butyrate is a major metabolite in colonic lumen arising from bacterial fermentation of dietary fiber. Butyrate has been shown to stimulate electrogenic Na absorption through its regulation on NHE3. Although NHE8, the newest addition of intestinal NHE family, is involved in sodium ion transport, the intestinal NHE8 Na transport activity and expression in intestinal epithelial cells is unknown. This study investigated the role of butyrate on NHE8 expression in human intestinal epithelial cells. Methods: Human intestinal epithelial cells (Caco2 cells) were treated with 10 nM butyrate for 16 hours isolating protein and RNA. Total RNA was isolated using Trizol reagent. Total cell lysate was prepared in RIPA buffer. Western blotting was then used to analysis the expression of NHE8 protein in Caco-2 cells, and Real-time PCR was used to measure NHE8 mRNA expression level. Promoter reporter assays were used to analyze NHE8 gene promoter activity in Caco2 cells. Results: GW40046 treatment decreased NHE8 protein expression by 60% and NHE8 mRNA expression by 43% in Caco2 cells. Transfection with the promoter construct containing NHE8 FXR response region showed that promoter reporter gene expression was reduced to 33% in Caco2 cells after GW40046 treatment. Conclusion: We observed that the intestinal NHE8 mRNA expression and protein expression were indeed downregulated by GW40046 in Caco2 cells. GW40046 inhibited NHE8 gene expression by reducing promoter activation through FXR binding on NHE8's promoter region. These results suggest that NHE8 is subject to the regulation of bile acids in the intestine, which may shed new light on a role of NHE8 in bile acid and lipid homeostatic regulation.

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Salivary Interleukin (IL) 5 and 4 Are Increased in Eosinophilic Esophagitis and Correlate With Exophagel Eosinophilia in Children
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Background: Eosinophilic esophagitis (EoE) is a chronic, clinicopathological condition affecting about 4 per 100,000 children in United States with a 56% increase in yearly prevalence rate. Current invasive methods used to diagnose and monitor EoE are associated with significant potential risks and costs. Saliva testing to diagnose EoE and monitor its course offers the advantages of being noninvasive and less expensive than conventional tests. We