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Metagenomic Analysis of the Dynamic Changes in the Gut Microbiome of African American With Colorectal Cancer

Hassan Brim, Edward L. Lee, Adeyinka O. Laiyemo, Scot E. Dowd, Hassan Ashktorab

Hassan Brim, Edward Lee, Scot Dowd, Adeyinka Laiyemo, 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 cultivatable 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 nextera library preparation kits. Sequencing was performed on each sample. For all samples an average of 20 million reads were obtained. Paired Sequencing reads were joined and entered into MG-RAST for metagenome analysis. The data was compared to M5NR using a maximum e-value of 1e-5, a minimum identity of 60 %, and a minimum alignment length of 15 measured in amino acids for protein databases. Results: Metagenomes were 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 Microviridae (bacteriophage) 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= 0.05) 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 primarily related to phage capsid 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

Lihong Wang, Hailong Cao, Sari Acra, D. Brent Polk, Fang Yan

Background & Aims. The mucus layer coating the gastrointestinal tract serves as the first line of intestinal innate defense. Intestinal commensal microbiota regulates mucin production by goblet cells and requires mucus for binding sites and energy source. Probiotics have been shown to promote production of mucins by goblet cells to enhance host innate defense against infection. p40, a Lactobacillus rhamnosus GG (LGG)-derived soluble protein, ameliorates intestinal injury and colitis and preserves barrier 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 *Egfr*^{wa2} (EGFR kinase defective) mice on C57BL/6 background were gavaged with pectin/zein beads containing p40 (10 µg/day) with or without co-treatment with 3% DSS in drinking water for 4 days. Colonic tissues were prepared for carnoy fixation and epithelial cell isolation. EGFR activation was detected by Western blot analysis, mucin production by MUC2 immunostaining and PAS staining, and *Muc2* and *Spdef* (a down-stream target of Notch signaling) gene expression by real-time PCR. **Results.** p40 activated EGFR in LS174T cells in a concentration dependent manner with the peak activation at 1 h-treatment. p40 (50 ng/ml) stimulated *Muc2* gene expression from 1h to 24 h treatment, with the peak at 6 h-treatment (4.12±1,01 fold), as compared to un-treated cells. Increased mucin production by p40 treatment was confirmed by immunostaining with anti-MUC2 antibody and PAS staining. No effect of p40 on *Spdef* gene expression was found in LS174T cells. Down-regulation of EGFR expression by EGFR siRNA transfection or inhibition of EGFR kinase activity by AG1478 abolished p40's effects on up-regulation of *Muc2* gene expression and MUC2 protein level in LS174T cells. Furthermore, p40 treatment increased goblet cell number (1.89±0.56 fold) and thickness of the mucus layer (2.01±0.49 fold) in the colon of wt, but not *Egfr*^{wa2} mice. In addition to reduction of colitis as reported before, p40 stimulated mucin production in the colon of DSS-treated wt, but not *Egfr*^{wa2} mice. **Conclusion.** These studies demonstrate that p40 promotes mucin production, via activation of EGFR, but not through the Notch pathway. Defective mucin production has been reported in the pathogenesis of IBD. Thus, p40 regulated mucus protection may contribute to innate defense for maintaining health and prevention of intestinal inflammatory diseases.

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Pharmacokinetics and Tolerability of iOWH032, an Inhibitor of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Chloride Channel, in Normal Volunteers and Cholera Patients

Ullrich Schwertschlag, Amresh Kumar, Sonali Kochhar, Robert Ings, Yuhua Ji, Eugenio L. de Hostos, Robert Choy, Mohammed Salam, Wasif A. Khan, Patricia Lin

Amresh Kumar*, Sonali Kochhar*, Robert Ings**, Yuhua Ji**, Eugenio de Hostos**, Robert Choy,** Mohammed Salam***, Wasif Khan***, Patricia Lin**, Ullrich Schwertschlag**. PATH, New Delhi (India)* and South San Francisco, CA (USA)** and icddr,b Dhaka (Bangladesh)*** iOWH032 is a synthetic low molecular weight inhibitor of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. This novel compound is being developed for the treatment of cholera toxin-induced secretory diarrhea; however, the effects of secretory diarrhea on the pharmacokinetic properties of small-molecule therapeutics have been poorly characterized. Thus, the present study was conducted to compare the pharmacokinetic profiles of a single dose of 300 mg iOWH032 in healthy American volunteers (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. Each of the plasma profiles 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 Bangladeshis. 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 Bangladeshi and cholera patient populations at 4.8 ± 2.6 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 22,668 ± 10,362 ng*h/mL for healthy Bangladeshis and 6,250 ± 4910 ng*h/mL for patients with cholera. For both the healthy Bangladeshi and cholera patient groups, less than 5% of the AUC∞ was in the extrapolated portion of plasma profile, consistent with the relatively short half-life (t½) which was similar 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 doubled in the cholera patients compared to the healthy Bangladeshis. The Tmax values would suggest that the rate of absorption was similar for the two Bangladeshi population groups and the similar t½ indicates 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, Fayez 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. Genomic-wide FXR binding assay showed that FXR binding site was detected in the promoter region of NHE8 gene in the mouse intestine, and this binding was dramatically increased after FXR ligand GW4064 treatment. However, the role of FXR on NHE8 gene expression has not been characterized. Methods: Methods: Human intestinal epithelial cells (Caco2 cells) were treated with 1 nM and 5 nM FXR agonist GW4064. 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 NHE-8 protein in Caco-2 cells, and Real-time PCR was used to quantitate NHE8 mRNA expression in control or GW4064 treated cells. Promoter reporter assays were used to analyze NHE8 gene promoter activity in Caco2 cells. Results: GW4064 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 GW4064 treatment. Conclusion: We observed that the intestinal NHE8 mRNA abundance and protein expression were indeed decreased by GW4064 in Caco2 cells. GW4064 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

Hua Xu, Jing Li, Anthony McCoy, Fayez K. Ghishan

Introduction: Butyrate is a major metabolite in colonic lumen arising from bacterial fermentation of dietary fiber. Butyrate has been shown to stimulate electroneutral Na absorption through its regulation on NHE3. Although NHE8, the newest addition of intestinal NHE family, is involved in sodium absorption in the intestinal tract, whether butyrate alters NHE8 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 before 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. Gel mobility shift assays (GMSAs) were used to identify the promoter sequences and the transcriptional factor(s) involving in butyrate-mediated regulation. Results: Western blots showed that NHE8 protein expression was increased about three folds after butyrate treatment. Real-time PCR data demonstrated that NHE8 mRNA abundance was also increased 200% by butyrate treatment. Transfection with the human NHE8 promoter reporter constructs showed that butyrate treatment increased reporter gene expression by 150%. GMSA results indicated that butyrate stimulates NHE8 expression by enhancing Sp3 transcriptional factor binding on NHE8 basal promoter region. Conclusion: Butyrate stimulates NHE8 expression in human intestinal epithelial cells, which further supports the role of butyrate in enhancing the process of sodium absorption in the intestine.

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Salivary Interleukin (IL) 5 and 4 Are Increased in Eosinophilic Esophagitis and Correlate With Esophageal Eosinophilia in Children

Girish Hiremath, Anthony Olive, Sridevi Devaraj, Robert J. Shulman, Carla M. Davis

Background Eosinophilic esophagitis (EoE) is a chronic, clinicopathological condition affecting about 42 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