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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Microgeographic Proteomic Networks of the Human Colonic Mucosa and Their Association With Inflammatory Bowel Disease

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SUMMARY

By using metaproteomics of the human colonic mucosal surface, we found evidence for a proteomic ecology of millimeter-scale protein networks distinguished by functional specialization and cell source predominance, and their relative abundance across colonic regions and in health vs quiescent inflammatory bowel disease.

BACKGROUND & AIMS: Interactions between mucosal cell types, environmental stressors, and intestinal microbiota contribute to pathogenesis in inflammatory bowel disease (IBD). Here, we applied metaproteomics of the mucosal–luminal interface to study the disease-related biology of the human colonic mucosa.

METHODS: We recruited a discovery cohort of 51 IBD and non-IBD subjects endoscopically sampled by mucosal lavage at 6 colonic regions, and a validation cohort of 38 no-IBD subjects. Metaproteome data sets were produced for each sample and analyzed for association with colonic site and disease state using a suite of bioinformatic approaches. Localization of select proteins was determined by immunoblot analysis and immunohistochemistry of human endoscopic biopsy samples.

RESULTS: Co-occurrence analysis of the discovery cohort metaproteome showed that proteins at the mucosal surface clustered into modules with evidence of differential functional specialization (eg, iron regulation, microbial defense) and cellular origin (eg, epithelial or hemopoietic). These modules, validated in an independent cohort, were differentially associated spatially along the gastrointestinal tract, and 7 modules were associated selectively with non-IBD, ulcerative colitis, and/or Crohn's disease states. In addition, the detailed composition of certain modules was altered in disease vs healthy states. We confirmed the predicted spatial and disease-associated localization of 28 proteins representing 4 different disease-related modules by immunoblot and immunohistochemistry visualization, with evidence for their distribution as millimeter-scale microgeographic mosaic.

CONCLUSIONS: These findings suggest that the mucosal surface is a microgeographic mosaic of functional networks reflecting the local mucosal ecology, whose compositional differences in disease and healthy samples may provide a unique readout of physiologic and pathologic mucosal states. *(Cell Mol Gastroenterol Hepatol 2016;2:567–583; http://dx.doi.org/10.1016/j.jcmgh.2016.05.003)*

Keywords: Inflammatory Bowel Disease; Mucosal; Networks; Ecology; Metaproteomics.

The intestinal mucosa plays diverse and critical roles in nutrient uptake, host defense, and local and systemic endocrinology.[1–4] The functional state of the mucosa in health and disease can be affected profoundly by its interplay with environmental metabolic stressors and luminal intestinal microbiota.[4,5] Studying the mucosal–luminal interface (MLI) and how it is changed in disease is difficult because of the many dimensions of complexity of this ecosystem.[6,7] The analytic challenge is central to the pathogenesis of inflammatory bowel disease (IBD), which is a multifactorial process involving genetic susceptibility, environmental factors, and microbiota.[8,9]

Metaproteomics is an emerging technology to address this challenge. The metaproteome of the mucosal surface is a composite of human and microbial products, skewed for proteins devoted to translation, energy, carbohydrate metabolism, and antimicrobial defense.[10–16] Focusing on the metaproteome recovered by lavage from the MLI of healthy

[†]Deceased.

Abbreviations used in this paper: **ANOVA, analysis of variance; CD, Crohn's disease; HBD, human β-defensin; HD5, human alpha defensin 5; HNP, human neutrophil peptide; HPLC, high-performance liquid chromatography; IBD, inflammatory bowel disease; IHC, immunohistochemistry; MALDI, matrix-assisted laser desorption/ionization; MFN, mucosal functional network; MLI, mucosal–luminal interface; MS/MS, tandem mass spectrometry; NLME, nonlinear mixed-effect model; PVCA, principal variance component analysis; TOF, time of flight; UC, ulcerative colitis; WGCNA, weighted correlation network analysis.**

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human subjects, we previously observed that the predominant feature is intersubject variation, with an additional significant intestinal segmental pattern of the surface metaproteome distinguishing the distal and proximal colon.[10] A similar segmental difference was observed in foundational studies of the mucosal bacterial composition.[17,18] In view of emerging concepts of intestinal ecology, this suggests that the metaproteome reflects the human contribution to the mucosal habitat.[19] With this foundation, this study applies metaproteomic analysis to characterize the disease-related features of the MLI in ulcerative colitis (UC) and Crohn's disease (CD).

Materials and Methods

Mucosal Lavage Sample Collection and Analysis

The overall study design is shown in the flowchart in Figure 1. The demographics of the study population and the sample characteristics are summarized in Table 1. All enrolled subjects at both Cedars Sinai Medical Center and the University of California Los Angeles Ronald Reagan Medical Centers were consented to participate in research studies approved by the Institutional Review Board. CD and UC subjects were recruited from those undergoing surveillance colonoscopy; lavage samples were taken from mucosal sites that were endoscopically normal. Non-IBD control subjects were recruited from patients undergoing colonoscopy for colorectal screening. For each patient, 6 colonic regions were sampled and collected by endoscopic lavage.

All sample collections and processing followed the preanalytic proteomic pipeline previously detailed.[10] The demography of an independent control data set of 205 mucosal lavage samples from 38 non-IBD subjects, used as a validation cohort for this study, was reported previously.[10]

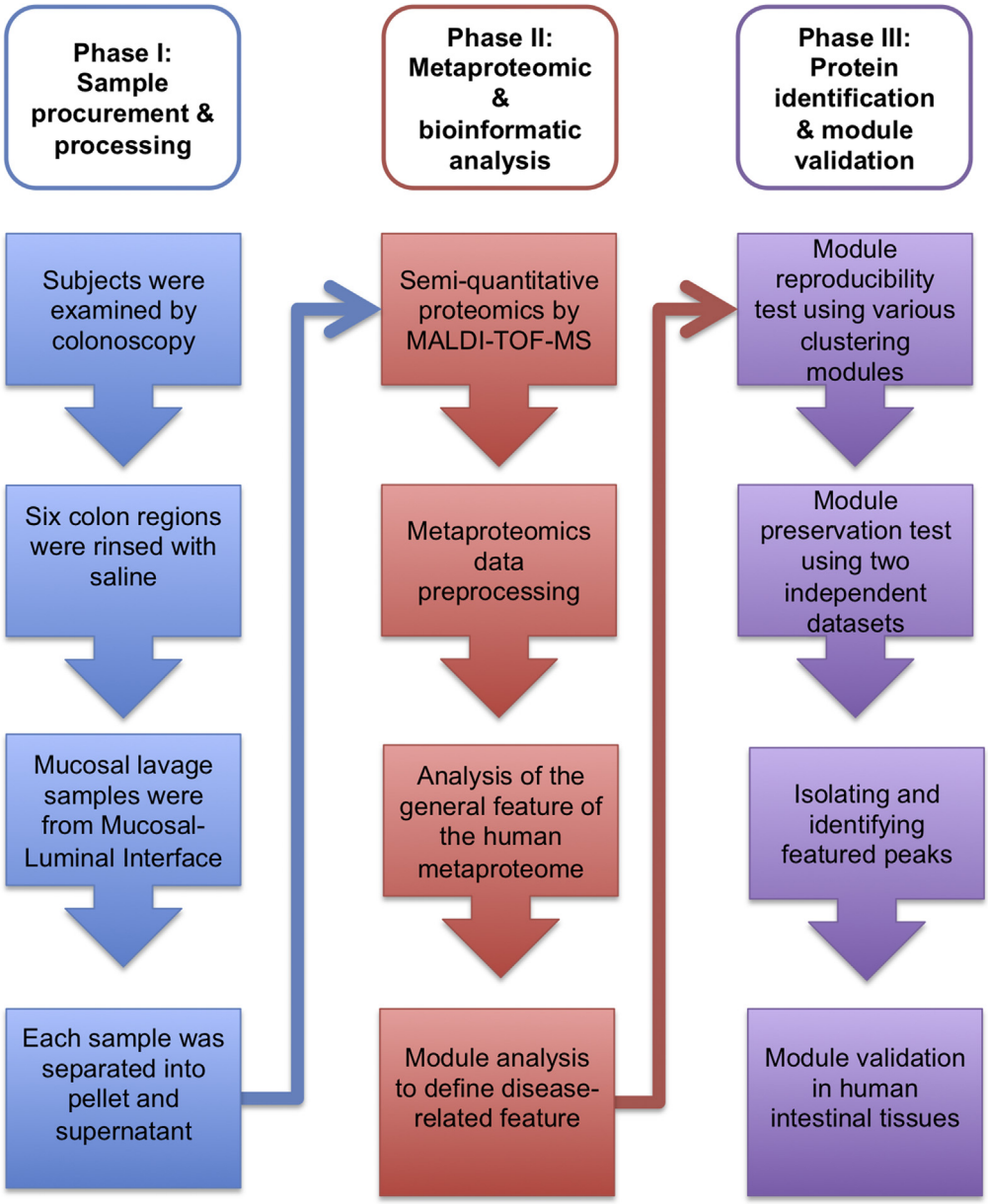

Figure 1. Flowchart of metaproteomic analytic pipeline.