

Perspective

See related article by Zackular et al., p. 1112

Human Fecal Microbiome–Based Biomarkers for Colorectal Cancer

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Abstract

Colorectal cancer may develop slowly over years from precursor lesions, and thus screening combined with early diagnosis is the key to disease prevention. Recent studies have elucidated specific traits in the gut microbiome associated with colorectal cancer and suggested that the microbiome may be useful in screening for colorectal cancer purposes but failed to provide protocols that can be applied in a practical situation. A recent study by Zackular and colleagues, presented on page 1112, provides an important way forward here in showing that specific analysis of multiple aspects of the microbiome composition *in toto* provides reliable detection of both precancerous and cancerous lesions. This important achievement when combined with other noninvasive techniques promises to provide highly effective tools for early colorectal cancer diagnosis and its prevention. *Cancer Prev Res*; 7(11); 1108–11. ©2014 AACR.

Introduction: Problems with Current Screening Methodologies for Colorectal Cancer

Colorectal cancer is the third most common cause of cancer in the aging population of the world, and screening for colorectal cancer screening is set to become an integral part of the patient care. The latter goal is hampered by a paucity for reliable, noninvasive, cost-effective, and temporally expedient procedures with respect to colorectal cancer screening methods. The established screening modalities include the Guaiac fecal occult blood test (gFOBT), fecal immunochemical test (FIT), flexible sigmoidoscopy (FS), colonoscopy, CT-colonography, colon-capsule endoscopy, and different molecular markers and metabolites. The molecular markers are measured from the blood, urine, or fecal samples (1, 2). Of these, gFOBT and FIT are used for initial screening either in individual patients or larger population screening programs, followed by the colonoscopy, CT-colonography, or FS in the symptomatic patients. gFOBT is a commonly used screening modality for colorectal cancer that is based on the heme content of fecal matter and like many screening protocols, performed either annually or biannually. Although it has been shown that gFOBT can significantly reduce the colorectal cancer–related mortality in a screening setting (3), a major disadvantage

of the method is the false-positive results yielded by stool blood derived from diverticula, vascular lesions, or hemorrhoids. Furthermore, this method has poor sensitivity in diagnosing minor- or no-bleeding colorectal cancer lesions. Consequently, following population-based screening in the United States, other imaging screening modalities have to be applied before a suspicion of colorectal cancer is warranted (1). Better results are obtained by using the FIT method, another noninvasive colorectal cancer screening technology, which detects globin rather than heme. The FIT test provides a qualitative as well as quantitative measurement. The test is considered highly specific as it is targeted toward the human globin marker but does not detect food-derived globin proteins. A major shortcoming of this technology is that it is deficient in detecting proximal colorectal cancer because of prolonged luminal globin degradation before excretion in the feces, which hampers detection of this biomarker for such cancers. Nevertheless, among the many screening methodologies, gFOBT and FITs are the cheapest and most noninvasive options as compared with other time-consuming image technologies, especially considering large-scale population surveillance. Other molecular markers used, include the measurement of methylated DNA, for example, septin-9, NDRG4, BMP3, VIM, and TFP12. Gene-expression signatures (i.e., RNA) for colorectal cancer screening, such as matrix metalloproteinase-7 (MMP7) and prostaglandin 2 (PTGS2) genes, have also been used (1, 4). Importantly, a recent clinical trial has compared a noninvasive, multitarget stool DNA test with FIT in persons at average risk for colorectal cancer (5). The multitarget stool DNA test used in this study has consisted of molecular assays for aberrantly methylated *BMP3* and *NDRG4* promoter regions, mutant *KRAS*, and β -actin (a reference gene for human DNA quantity), as well as an

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immunochemical assay for human hemoglobin. This clinical trial has demonstrated that multitarget stool DNA testing detected significantly more cancers than did FIT, but had more false-positive results (5). Nonetheless, the recently (August 11, 2014) FDA-approved Cologuard (DNA-based colorectal cancer test) that identifies altered DNA and/or blood in stool is currently available in the United States and is expected to improve the early colorectal cancer detection.

Together with the human DNA-based colorectal cancer screening methods, several other proof-of-concept studies have been performed showing that the detection of specific human miRNAs isolated from feces or the quantification of the metabolites produced by the colorectal tumors and secreted into the blood stream (serum metabolites) could be useful to differentiate adenomatous to carcinoma stages in colorectal cancer (6). These methods, however, are still very much experimental and rely in part on both human studies and murine models. The serum metabolites have been able to differentiate the healthy to colorectal tumors (but with opposing results), whereas the experimentally induced colorectal cancers in murine models have been identified by the variation of metabolites secreted in the feces (7, 8). Though these approaches have been experimentally developed, as stand-alone technology they remain unproven and require gFOBT/FITs codetermination when implemented in regular screening. Overall, there is an increased demand of new screening methods that can be applied alone or together with gFOBT/FIT to increase the specificity and sensitivity to detect different colorectal cancer stages.

The Microbiome Reveals Early Cancer in the Colon

Within this context, new data published in this issue of the journal by Zackular and colleagues (9) are of great importance and provide valuable insight into this status of the colorectal cancer screening field. The authors have demonstrated that advanced analyses of the human fecal microbiome, which arises in the advent of adenoma toward carcinoma of the colon, can improve these colorectal cancer screening strategies. Three important aspects of the study need to be highlighted. First, the article represents a collaborative effort involving collection of samples from subjects in 4 locations: one in Canada and three in cancer research centers across the United States. Second, the research team has clearly distinguished the fecal microbiome composition of healthy ($n = 30$), adenoma ($n = 30$), and carcinoma ($n = 30$) groups. The analyses included Illumina MiSeq sequencing platform of the bacterial 16S rRNA gene, an approach allowing phylogenetic comparison and quantification of the bacterial diversity. Importantly, the groups have been compared individually resulting in the identification of bacterial operating taxonomic units specifically enriched or decreased in healthy versus adenoma clinical samples, healthy versus carcinoma, and adenoma versus carcinoma

samples. This demonstrated that the microbiome-based analyses can detect the presence of precarcinogenic and carcinogenic lesions. Third, the study demonstrates that the combination of data on the body mass index, a known clinical risk factor of colorectal cancer, gFOBT, and the microbiome data can provide an excellent discriminatory ability between healthy individuals to those with colonic lesions.

The Colon Cancer Microbiome in the Context of Human Intraluminal Ecology

It is estimated that at least 15%, but possibly more, of the cancer burden worldwide is attributable to known intestinal infectious agents (10) that are often resident in the human gut together with other members of the human intestinal microbiota. Such a vast bacterial community, comprising more than 1,000 bacterial phylotypes, affects various host functions. This includes that nutritional absorption, host metabolism, interactions with immune system, carcinogenesis, and tissue development are involved in many disease processes, including colorectal cancer (11). The first bacterium associated with colorectal cancer was *Streptococcus bovis*, in which patients with an infection due to this bacterium were found to have a very high prevalence of colorectal cancer (12). Furthermore, apart from its clearly established role in gastric carcinogenesis (13), *Helicobacter pylori* have since been associated with colorectal cancer. As well, intraepithelial *Escherichia coli* was found to be present in the colonic mucosa of tumor and normal tissue from patients with colorectal cancer (14). In recent years, several groups have appreciably expanded the evidence linking infectious agents to colonic disease development (reviewed in ref. 10). These microorganisms are thought to create a microenvironment more favorable to colorectal cancer development. Microbiome sequencing studies have proven very successful in uncovering novel candidate bacterial species in tumor and stool samples from patients with colorectal cancer. In particular, two North American studies in 2012 showed overrepresentation of *Fusobacterium nucleatum* in colorectal cancer tumors versus surrounding normal tissue (10, 15). *F. nucleatum* is a highly invasive, gram-negative anaerobic bacterium and part of the oral and gut commensal microbiota that has been linked to several diseases, such as appendicitis (16) and inflammatory bowel disease (17). This bacterium may contribute to colorectal cancer development by invading colonic mucosa and inducing local inflammation and increased expression of cytokines, leading to colorectal disease (18). More convincing evidence that *F. nucleatum* infection directly contributes to colorectal carcinogenesis rather than being a consequence of disease progression derives from two recent reports showing that *F. nucleatum* invasion, through its unique FadA adhesion, recruits tumor-infiltrating immune cells and generates an oncogenic/proinflammatory microenvironment conducive for colorectal neoplasia (18, 19). The importance of this bacterium to colorectal

cancer has also been demonstrated in a recent European human cohort study in which *F. nucleatum* has been identified as a novel risk factor for disease progression from adenoma to cancer (20). This finding seems to be supported in the study of Zackular and colleagues (9), in which *F. nucleatum* was enriched in the samples from the carcinoma compared with adenoma clinical groups. Furthermore, *F. nucleatum* and other bacteria relevant for intestinal inflammation, for example, *Porphyromanas*, *Lachnospiraceae*, and *Enterobacteriaceae*, were enriched in the fecal samples of patients with carcinoma as compared with healthy subjects. Importantly, an earlier study has also found that genotoxic member of *Enterobacteriaceae* (*E. coli*) has also been associated in human sporadic colon cancer (14). Moreover, potential commensal and health promoting bacteria affiliated with *Bacteroides* and *Clostridiales* were depleted in the colorectal cancer samples, indicating the importance of the intestinal dysbiosis in the colorectal cancer development. Furthermore, *Porphyromonas* and the other pathobionts associated with colorectal cancer are predominantly asaccharolytic, whereas some *Bacteroides* spp. and *Clostridiales* spp. can degrade complex plant polysaccharides such as starch, cellulose, xylans, and pectins providing succinic acid, acetic, butyric acid, and in some cases, propionic acid as the major end products that are important for colonic health. As most colorectal cancers may develop slowly over years from precursor lesions, screening and early colorectal cancer-associated intestinal dysbiosis-based diagnosis after the detection of specific intestinal pathogens might hold the key to disease prevention. Furthermore, efforts to detect and restore the reduced levels of beneficial bacteria depleted in the colorectal cancer, for example, *Bacteroides* and *Clostridiales*, need to be considered in future tests and prophylactic and dietary regimes targeting colorectal cancer.

Mechanisms Linking the Colon Cancer Microbiome to Tumor Progression

Typically, bacteria involved in cancer initiation and progression are able to induce a chronic inflammation in the colonic epithelium as a result of direct infiltration of the bacteria or by the production of metabolic by-products. The classical pathways activated by the dysbiosis and bacterial infiltration are via the Toll-like receptors (TLR) and NOD-like receptors (NLRP-3 and 6). The activation leading to plethora of signaling in the NLR pathway especially set up by the bacteria-like *Prevotellaceae* and the unculturable TM7 families, which activate the inflammasome via caspase-1/11, IL1 β , IL18, and IL22BP (21). The inflammasome activated in NOD2- and NLRP6-deficient mice promotes colorectal cancer with bacterial dysbiosis (22, 23), paracrine secretion by the tumor-associated fibroblasts, and activation of the IL6 pathway leading to tumor angiogenesis via expression and release of VEGF-A by the tumor cells (24). Microbial metabolites such as ssDNA, dsRNA, and lipopolysaccharide, activate the TLR

family of receptors and enhance the immunogenic influence on the colon leading to polyp formation. Further activation of Th17 cells secreting large amounts of IL17A has been shown to have protumorigenic effects by inducing chronic inflammation. The bacteria involved in these specific types of cell activation are the toxigenic *Bacteroides fragilis*, segmented filamentous bacteria, and flagellin-positive bacteria. These interactions lead to activation and secretion of IL17A, IL22, IL6, and TGF β by the Th17 and lamina propria dendritic cells in the intestine (21). Commensal bacterial like lactobacilli, bifidobacteria, and those belonging to the *Clostridium* genera are able to counteract the intestinal inflammation via the activation of T-regulatory cells causing an immunosuppressive niche in the intestine that can further affect tumor development. Apart from inflammasome activation leading to colorectal cancer, certain bacteria (specifically *Citrobacter rodentium*) are also able to induce epithelial-mesenchymal transition in the dedifferentiation of intestinal cells forming colonosphere in the *in vitro* culture models with murine colon cell lines (25). This group has established the tumor-mediated colonic hyperplasia murine models and studied the PI3K/AKT signaling leading to colorectal cancer via WNT- β -catenin and TLR-4-based NF- κ B activation pathways stimulated by *C. rodentium* (26). Most fundamental studies on the role of bacteria in colorectal cancer have been published using different animal models, and yet, data in patients with colorectal cancer have long been lacking on the causal relationships of the microbiome and intestinal carcinogenesis. Hence, the study by Zackular and colleagues (9), has added an important aspect about the *in vivo* relevance of different bacterial species associated with the colorectal cancer stages. Nevertheless, the newly highlighted pathobionts, like the members of *Porphyromanas* and *Lachnospiraceae*, still need to be examined in functional studies and validated in different models of colorectal cancer.

Future Studies

Ongoing efforts for large-scale colorectal cancer population-based screening studies require a standard, state-of-the-art, technologic platform for a cost-effective and reliable generation and data analyses. Furthermore, some limitations of the fecal microbiome retrospective analyses performed after colonoscopy preparations (Zackular and colleagues in ref. 9) need to be addressed in future microbiota-based prospective colorectal cancer studies before colonoscopy. Studying the human microbiome in relation with age, race/ethnicity, and different stages of tumor can help us create a fast and reliable colorectal cancer tests for use in nationwide colorectal cancer screening programs based on the human fecal microbiome. Apart from the potential to translate the knowledge to novel colorectal cancer screening tests, it is also becoming clear that the intestinal dysbiosis in general and specific commensal bacteria in particular play a key role in the carcinogenesis, tissue repair, cellular

dedifferentiation/redifferentiation, and other systemic diseases or protection against colorectal cancer. Future studies in these directions will pave the way in decoding the colorectal cancer—and other intestinal diseases—associated microbiota as a target to improve human health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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