Dysbiosis of the gut microbiota and colorectal cancer: the key target of molecular pathological epidemiology

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Microbiome is a dramatically advancing research field in the study of human cancers (1). Microbial distribution varies spatially and temporally from the mouth to the rectum during the individual's lifetime. These gut bacteria maintain intestinal homeostasis by regulating various biological functions such as mucosal barrier, immunity, and metabolic processes. Accumulating evidence indicates that the gut bacteria play a crucial role in human health (1), and the dysbiosis of the gut microbiota is associated with various disorders, including obesity, diabetes, inflammatory bowel disease, and many types of cancer especially colorectal cancer. According to emerging studies, several bacterial species [*Fusobacterium nucleatum* (*F. nucleatum*), *Bacteroides fragilis*, and *Escherichia coli*] appeared to have a critical role in the colorectal carcinogenesis (2,3). In the tumor microenvironment of colorectal carcinoma, there should be a complicated community of cancer cells, immune cells, and a great number of microorganisms. Experimental studies have shown that gut bacteria activate anti-tumor immunity and boost the efficacy of immunotherapy (4,5). Since the gut microbiota is likely influenced by food, lifestyle, antibiotics, and probiotics, a better understanding of the relationship between human cancer, immune system, and gut microbiome may promote clinical application including immunotherapy.

F. nucleatum is a common gram-negative anaerobe, which is one of the most prevalent bacteria in the oral cavity. *F. nucleatum* has been associated with inflammatory diseases including periodontitis, gingivitis, appendicitis, and liver abscess. Accumulating evidence suggests that the amount of *F. nucleatum* is remarkably higher in colorectal cancer tissue than matched normal colorectal mucosa (6-8). The fraction of colorectal cancer with *F. nucleatum* increases linearly along the large intestinal subsites from the rectum to the cecum, based on a continuum model of pathogenic influences of gut bacteria on colorectal carcinogenesis (9). An experimental study suggests that metastasis of *F. nucleatum*-present colorectal cancer might be prohibited by antimicrobial interventions (3). Human population-based studies indicate that an enrichment of *F. nucleatum* is associated with high-level microsatellite instability (MSI), advanced disease stage, low-level T-cell infiltration in colorectal carcinoma tissue (7), and worse clinical outcome (8). Collectively, *F. nucleatum* DNA in colorectal carcinoma tissue may serve as a prognostic biomarker, and provide insights for the establishment of new prevention and treatment strategies targeting bacteria in colorectal cancer. On the other hand, *Faecalibacterium prausnitzii* (*Fp*)

is an anaerobic bacterium of the most important butyrate-producing bacteria in the gut. *Fp* is inversely associated with the activity of inflammatory bowel disease, and the amount of *Fp* is reduced in colorectal cancer patients (10). The presence of members of *Bifidobacterium* genus (*Bb*) in the gut lumen may suppress colorectal carcinogenesis through prevention of enteropathogenic infection and inhibition of secondary bile acid production (11-13). *Fp* and *Bb* have gained a lot of attention in the field of colorectal cancer prevention.

In a recent article published in *Clinical Chemistry*, Guo and colleagues reported a simple fecal biomarker for detecting colorectal cancer utilizing two Chinese cohorts of 903 individuals (cohort I and cohort II) (14). To evaluate fecal dysbiosis, they first analyzed the fecal microbiota of patients with colorectal cancer (n=20) and healthy controls (n=20) by Illumina MiSeq sequencing. As a result, they found the increase of *F. nucleatum*, as well as the decreases of *Fp* and *Bb*. For further analyses, they measured the relative abundance of *F. nucleatum*, *Fp*, and *Bb* by quantitative polymerase chain reaction (PCR) in the stools of cohort I. Cohort I included 215 patients with colorectal cancer, 178 patients with benign colon disease, 100 patients with non-gastrointestinal cancer, and 156 healthy controls. The relative abundance of *F. nucleatum* was higher in colorectal cancer patients than in benign colon disease patients (P=0.043), non-gastrointestinal cancer patients (P<0.0001), and healthy controls (P<0.0001). Regarding fecal *Fp* and *Bb*, both relative abundances were lower in colorectal cancer patients than in non-gastrointestinal cancer patients (P=0.0001; P=0.05) and healthy controls (both P<0.0001). To investigate the association of dysbiosis with colorectal cancer, they focused on the ratio of *F. nucleatum* to *Fp* (*Fn/Fp*) and *F. nucleatum* to *Bb* (*Fn/Bb*). *Fn/Fp* was significantly higher in colorectal cancer patients than in non-gastrointestinal cancer patients and healthy controls (both P<0.0001). *Fn/Bb* was significantly higher in colorectal cancer patients than in the other three groups (all P<0.0001). As the next step, they evaluated the diagnostic performance of the microbial ratios, and investigated the antagonistic effect of *Fn* against two different indicator bacteria (*Fp* and *Bb*). *Fn/Bb* had a superior sensitivity of 85% and specificity of 92% in detecting colorectal cancer patients from healthy controls [area under the curve (AUC) =0.91]. The other AUCs were 0.88 for *F. nucleatum*, 0.74 for *Fp*, 0.87 for *Bb*, and 0.91 for *Fn/Fp*. The combination of *Fn/Bb* and *Fn/Fp* improved the diagnostic value (AUC =0.94). The AUCs to distinguish colorectal cancer patients from benign colon disease were 0.84 for *F. nucleatum*, 0.55 for *Fp*, 0.75 for *Bb*, 0.53 for *Fn/Fp*, 0.92 for *Fn/Bb*, and 0.92 for the combination of *Fn/Bb* and *Fn/Fp*. Moreover, they investigated these five AUCs to distinguish early stage colorectal cancer patients (stage I + II) from healthy controls. Each AUC was 0.64 for *F. nucleatum*, 0.75 for *Fp*, 0.69 for *Bb*, 0.82 for *Fn/Fp*, 0.88 for *Fn/Bb*, and 0.89 for the combination of *Fn/Bb* and *Fn/Fp*. The combination of *Fn/Bb* and *Fn/Fp* offered 60% specificity and 90% sensitivity in detecting only stage I of colorectal cancer (AUC =0.80). As a validation set, the cohort II including 152 patients with colorectal cancer and 102 healthy controls was utilized. The performance for identifying colorectal cancer was also confirmed in the validation cohort II. Finally, Guo and colleagues concluded that the ratio of *Fn* to the important bacterium, such as *Fp* or *Bb*, was identified as a useful biomarker for screening early colorectal cancer.

There have been great interests in developing non-invasive methods, such as a stool screening with safety, efficiency, convenience, and cost-effective. The effectiveness of guaiac fecal occult blood test and fecal immunochemical testing have been established to reduce both colorectal cancer incidence and mortality, thus allowing their use for many colorectal cancer screening programs. However, the issue is that the specificity of these techniques has been relatively low. Colon and rectal cancers represent heterogeneous sets of neoplasms with differing combinations of genetic and epigenetic alterations, the accrual of which is influenced by complicated interplay between tumor cells, host cells, and microorganisms. The analysis of mucosal tissues may be more appropriate to evaluate the mechanisms of microbiota which are physiopathologically involved in colorectal cancer development. However, considering that the invasiveness of classic endoscopic approaches or endoscopic biopsy, collection of mucosal samples is more challenging to conduct than that of fecal samples, particularly in healthy individuals. Consequently, new molecular colorectal cancer screening methods targeting microbiome in stools are required. A vast majority of studies on the diversity of the microbiota in human gut have been conducted on fecal samples to identify more competent diagnostic markers for colorectal cancer.

Shah and colleagues recently conducted the first microbiome-based meta-analysis for colorectal cancer to identify a common microbial marker in fecal samples, utilizing the results from nine studies including 79 colorectal adenomas patients, 195 colorectal cancer

patients and 235 controls (15). They emphasized a marked increase in *Parvimonas micra* ATCC 33270, *Streptococcus anginosus*, *Parabacteroides distasonis*, and other members of *Proteobacteria* in addition to the previously reported taxa such as *Fusobacterium* species. In their study, the AUC to distinguish colorectal cancer from controls was approximate 0.80. According to another recent study by Liang and colleagues, the quantification of *F. nucleatum* in stools by quantitative PCR could discriminate colorectal cancer from healthy controls with a high sensitivity of 78% and specificity of 80% (AUC =0.87) (16). Compare to fecal samples of healthy individuals, those of colorectal cancer patients have modifications in the microbiota composition, namely dysbiosis. Although *F. nucleatum* has continuously gained much attention for a possible role in initiating and progressing colorectal cancer, comprehensive analyses of the human microbiome ecosystem utilizing 16S ribosomal RNA (rRNA) next-generation sequencing (NGS) are strongly required. However, at this point, there is no consensus in terms of the dysbiosis of fecal samples for screening and diagnosis. Analyses utilizing quantitative PCR and 16S rRNA NGS are the most used approaches for investigating the composition of the gut bacterial community. However, it is challenging to define the gut dysbiosis because of some reasons. First, the gut microbial ecosystem is likely affected by a number of factors, such as race, age, sex, diet, lifestyle, medication, and supplemental use. Second, there is the diversity of methods including the regions of the 16S rRNA targeted (V1, V2, V3, V4, V5, and V6 regions) for the NGS approach. Due to the complexity of gut microbial ecosystem and methods, there is no consensus in terms of the dysbiosis observed in colorectal cancer compared to healthy individuals using quantitative PCR or 16S rRNA NGS in fecal samples. It is reasonable to assume that there is heterogeneity of microbiome in colorectal tumor microenvironment or stools of colorectal cancer patients. The microbial heterogeneity in colorectal cancer may make it challenging to define the microbial dysbiosis for screening and diagnosis of colorectal cancer. Further investigations are warranted to consider the microbial heterogeneity in colorectal cancer, not only for screening but also classification of colorectal cancer.

We argue that molecular pathological epidemiology (MPE) can establish a basis for individualized cancer prevention and treatments (17-20). MPE is a transdisciplinary integrative research field which has been derived from efforts to integrate the methodology of molecular pathology into population-based epidemiologic research. Conventional epidemiology typically investigates the relationships between epidemiologic exposures and a certain single disease entity based on the assumption that the single disease entity should represent a homogeneous process including disease course, etiologies, and pathogenesis. Beyond this conventional approach, MPE research attempts to decrypt differential associations of an exposure with several distinct subtypes classified by molecular or pathological features of the disease. Microbial MPE addresses etiologic heterogeneity according to subgroups of colorectal cancer classified by tumor tissue microbial profiling (21). Given the evidence that *F. nucleatum*-positive colorectal cancers may have some distinct features (22), it is of great interest to investigate the association of environmental factors with colorectal cancer in strata of tumor *F. nucleatum* status. Utilizing two large prospective cohorts, we reported that prudent diets (rich in whole grains and dietary fiber) are correlated with a lower risk for colorectal cancers with *F. nucleatum* but not ones without *F. nucleatum* (23). Specific nutritional components may induce intestinal inflammation, and the dietary inflammatory effects can be estimated based on an empiric dietary inflammatory pattern (EDIP) score. We found that higher EDIP scores were associated with increased risk of colorectal cancers with *F. nucleatum*, especially proximal colon cancers (24). These findings suggest that diet changes the gut microbiome to promote or inhibit colorectal carcinogenesis, and dietary interventions could be useful for cancer prevention and precision medicine. Emerging evidence also suggests potential roles of several bacteria such as *Bacteroides fragilis*, *Escherichia coli*, *Parvimonas micra*, *Fp*, and *Bb* on colorectal carcinogenesis. There has been an increasing understanding that colorectal cancer represents a heterogeneous group of tumor cells which develop through the multilevel accumulation of various combinations of molecular alterations in the colorectal epithelium. In fact, a huge number of microorganisms configure the bacterial ecosystem in gut lumen as well as tumor microenvironment, and those form a complicated network system through interactions (25). Emerging evidence indicates the heterogeneity of the microbial community in stool samples (15). Therefore, we seek the comprehensive analyses of the human microbiome ecosystem in stools utilizing 16S rRNA NGS to classify colorectal cancers into subtypes based on the heterogeneity of bacteria. If such a test is established, the fusion of MPE approach and screening test targeting bacteria can make it possible to advance our understanding of disease pathogenesis and

provide appropriate precision medicine to individuals.

In summary, the population-based study by Guo *et al.* supports the existing evidence on utility of fecal analyses targeting bacteria. Considering the heterogeneity of genetic and epigenetic changes in colorectal cancer, colorectal cancer may have the heterogeneity of microbiome in stools as well as in tumor microenvironment. Comprehensive analyses of the human microbiome ecosystem utilizing 16S rRNA NGS are strongly required, and the analyses must help us to understand colorectal carcinogenesis and subtypes of colorectal cancers more accurately. MPE research is a transdisciplinary integrative research field, which intrinsically pursue the addresses the disease heterogeneity in human population. We propose that MPE research combined with fecal bacterial analyses can play critical roles in providing rationales and discovering insights into precision medicine for colorectal cancer.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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