EDITORIAL

Human Intestinal Microbiota and Colorectal Cancer: Moving Beyond Associative Studies

See "BMI1 and MEL18 promote colitis-associated cancer in mice via REG3B and STAT3," by Liu X, Wei W, Li X, et al on page 000; and "Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice," by Wong SH, Zhao L, Zhang X, et al, on page 000.

Human surfaces and cavities are populated by numerous microbial communities, including bacteria, viruses, archaea, and fungi, which form a complex interactive network between themselves and the host. These inter-kingdom interactions are the result of millions of years of co-evolution, and are an intrinsic part of host health and disease balance. For example, intestinal bacterial communities have been associated with pathologic conditions such as inflammatory bowel diseases, colorectal cancer, obesity, and liver cirrhosis by 16S rRNA gene or whole genome metagenomic sequencing analysis.[1] An important concept emerging from these correlative studies is that a homeostatic equilibrium must exist among the bacterial community and the host to maintain health. According to this hypothesis, disruption of intestinal microbial equilibrium has the capacity to alter the homeostatic network, thereby eliciting deleterious host responses as observed in inflammatory bowel disease and CRC.

In support of this hypothesis, the microbial community varies between tumor and normal flanked tissue in CRC patients,[2,3] distal versus proximal tumors, and adenoma to adenocarcinoma progression.[4,5] Interestingly, differences in luminal intestinal biota may potentially serve as noninvasive CRC biomarkers when paired with either whole genome metagenomic,[6,7] or 16S rRNA sequencing analysis.[8] Thus, a core microbial component likely drives homeostatic signaling, and the identification of these microorganisms could prove invaluable for both prevention and therapeutic intervention. A number of studies using preclinical models colonized with selected bacterial candidates (eg, *Fusobacterium nucleatum*, *Bacteroides fragilis,* adherent invasive *Escherichia coli*) identified from microbial genomic work, have shed light on mechanisms (eg, inflammation, genotoxicity) by which bacteria could promote intestinal carcinogenesis[9–11] or even modulate therapeutic response in preclinical models.[12] Despite this, however, the evidence for the microbial community as a whole playing a functional role in intestinal carcinogenesis is unclear. In this issue of *Gastroenterology*, Wong et al[13] demonstrated the carcinogenic properties of microbial communities obtained from CRC patients, using fecal microbiota transfers into preclinical models (Figure 1).

In this study, the authors used 2 different approaches to dissect the impact of biota on intestinal carcinogenesis: wild-type mice treated with wide spectrum antibiotics and then exposed to the procarcinogenic compound axozymethane (AOM) and germ-free mice; both models colonized with stools from a pool of either 5 CRC patients or 5 healthy controls. In the antibiotic experiment, both the prevalence and number of colonic polyps were significantly higher in mice associated with CRC biota compared with healthy biota or control (no human biota) after 9 weeks. Interestingly, germ-free mice associated with CRC or healthy biota for up to 32 weeks failed to develop polyps, although increased intestinal proliferation was observed in the CRC biota condition when compared with healthy controls as measured by PNCA staining. Taxonomic analysis using 16S rRNA gene sequencing, performed in colonized AOM or germ-free mice, showed decreased microbial diversity and increased relative abundance of *F nucleatum*, *Peptostreptococcus anaerobius*, *Peptostreptococcus stomatis*, *Parvimonas micra*, *Solobacterium moorei*, and *Gemella morbillorum* in CRC biota compared with healthy biota.

On the host side, CRC-biota increased interleukin (IL)-17a, IL-22, and IL-23a messenger RNA accumulation, suggesting a Th17 profile, and Cxcr1 and Cxcr2, which are indicative of immune cell recruitment. Accordingly, abundance of $CD4^+$ interferon-γ^+ (Th1), and $CD4^+$ IL-17^+ (Th17) immune cells in intestinal tissues was significantly increased in mice colonized with CRC biota compared with healthy biota control mice. Finally, a number of oncogenic factors such as aurora kinase A, cell division cycle 20, and B lymphoma Mo-MLV insertion region 1 homolog (BMI1) were induced in colonic tissues of mice associated with CRC biota compared with healthy biota.

These findings have established the functional impact of the microbial community on the development of intestinal carcinogenesis, and have identified a potential microbial carcinogenic core. This important contribution reveals a series of new questions. For example, because luminal CRC biota promotes a low level of polyps in AOM-treated mice, and only proliferative signals in GF mice, it may be important to investigate host response to carcinogenic microorganisms obtained from mucosal tissues. The compositional profile of the mucosal community varies along the carcinogenic progression,[4] and is different than that in the luminal compartment.[14] Moreover, microbial organization, such as biofilm forming communities, may also be an important component of carcinogenesis, because 90% of right-sided tumors contain a biofilm positive community compared with <15% of left-sided tumors.[15]

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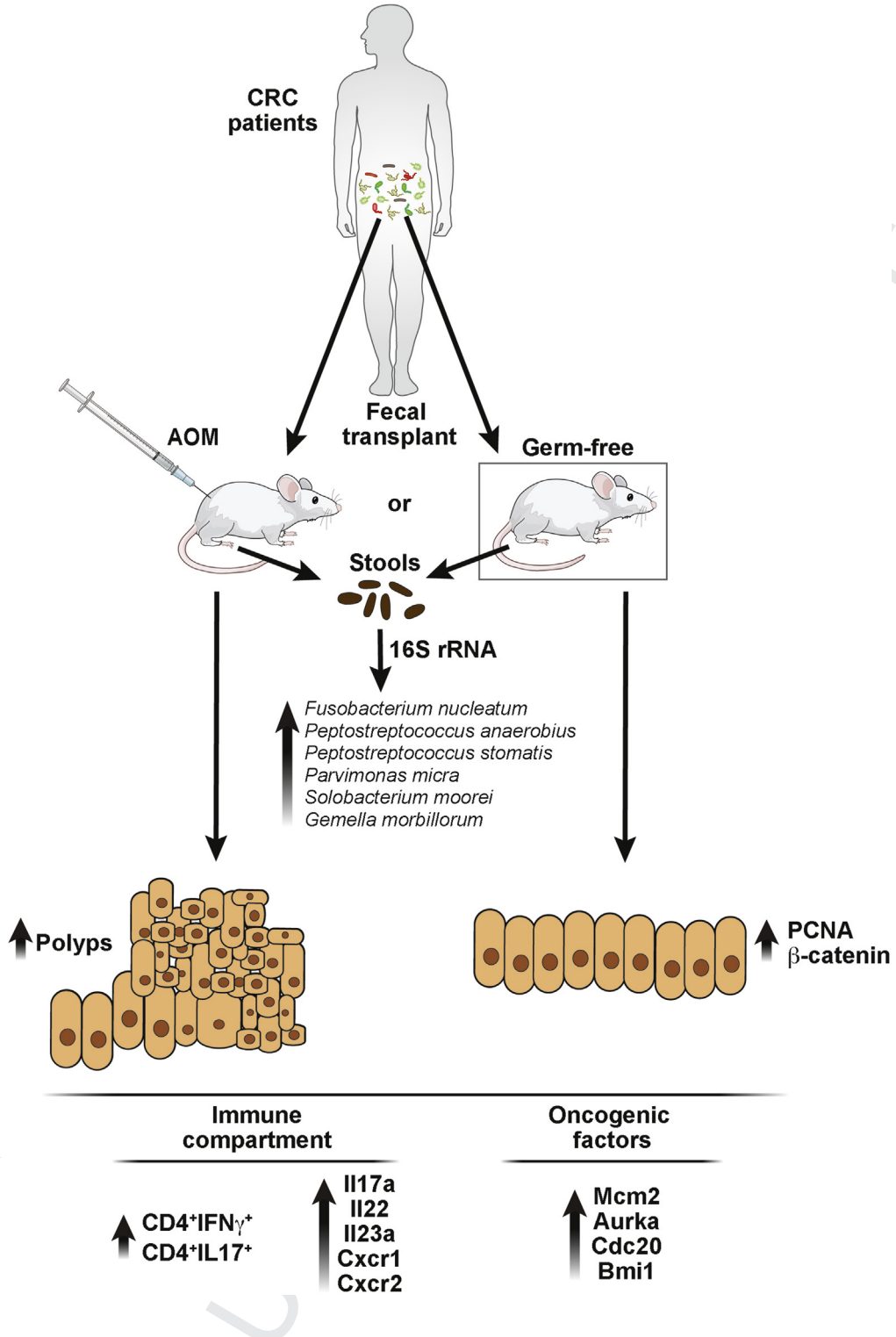

Figure 1. Colorectal cancer (CRC) biota promotes neoplastic lesions after oral transfer into mice. Introduction of stools from CRC patients into either axozymethane (AOM)-exposed or germ-free mice promotes the development of intestinal polyps or proliferative response respectively. Microbial composition present in the stools of mice reveals increased relative abundance of bacterial species forming networks in CRC patients. Colonic tissues from CRC biota associated mice showed increased immune cell recruitment, as well as immune and oncogenic gene expression.

Therefore, understanding the functional impact of these microorganisms in carcinogenesis would be important. Animal model selection is another important element to consider when studying host responses to mucosal or luminal bacterial communities. The authors used antibiotic-pretreated mice exposed to AOM to study host response to human biota. A confounding element in this approach is the presence of the murine biota in the housing facility environment, which likely resulted in the generation of a hybrid human–mouse biota ecosystem that may have influenced outcomes. Because a longitudinal microbial composition study was not performed in this cohort, the stability of the transplanted microbial community is unknown. Although the germ-free approach avoided this cross-contamination

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