What can gut bacteria tell us about colorectal cancer?

Harold Tjalsma*

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One of the salient features of colonic malignancies is their continuous physical interaction with a dense bacterial population that consists of more than 10^3 different species [1,2]. In this respect, it may not sound so surprising that both clinical studies and experimental models have directly or indirectly linked the intestinal microbiota, or specific members thereof, to the development of colorectal cancer (CRC) [3]. Several types of evidence provide important information on the impact of host–microbiota interactions during disease development. First, the high bacterial density in the large intestine ($\sim 10^{12}$ cells/ml) compared with the small intestine ($\sim 10^2$ cells/ml), is paralleled by an approximate 12-fold increase in cancer incidence in the human colon [4]. Second, inflammatory bowel disease (IBD) patients with a reduced intestinal barrier function, who are thus associated with increased intestinal exposure to microbes, have an approximate five-fold increased risk for CRC [5]. Third,

clinical studies have shown that the use of NSAIDs can reduce human CRC by as much as 75% [6]. Fourth, mice that are genetically susceptible to CRC develop significantly less tumors under germ-free conditions [7]. Fifth, it has been shown that toxins from specific gut bacteria can induce DNA damage and increase inflammation, cell proliferation and cancer progression [8,9]. Sixth, bacterial metabolism can generate oxygen radicals that induce DNA damage and chromosomal instability [10]. Seventh, bacterial metabolism of dietary factors was shown to result in the formation of genotoxins and carcinogens [3,11]. Finally, certain infections with opportunistic gut pathogens are specifically associated with human CRC [12].

CRC-associated microbiota

Despite this compelling body of evidence, our understanding on the interactions between gut bacteria and developing tumors is still in its infancy. Until

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*Department of Laboratory Medicine (830), Nijmegen Institute for Infection, Inflammation & Immunity (N4i) and Radboud University Centre for Oncology (RUCO), Radboud University Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands; Tel.: +31 24 3618947; Fax: +31 24 3541743; h.tjalsma@labgk.umcn.nl

“...it was demonstrated that enterotoxigenic *Bacteroides fragilis* increased tumor development through the increased expression of IL-17 by T lymphocytes...”

a short while ago, the complex nature of the gut microbiota has hampered its detailed analysis. However, upon the recent emergence of next-generation sequencing technologies, it became possible to map the human microbiota in a culture-independent and high-throughput manner [1,2,13]. Combined efforts revealed that although there is large variation from person to person, the composition of gut bacteria is relatively stable throughout adult life [14,15]. Very recently, the microbial composition of both CRC tissue and adjacent nonmalignant biopsy samples from different patient populations was explored by such new sequencing approaches [16–18]. The most striking similarities that were observed concerned the enrichment of *Fusobacterium* spp. in CRC tissue samples [16–18], while *Streptococcus* [17] and *Coriobacteria* were also reported to be enriched in cancerous tissues [16]. Surprisingly, the nonmalignant biopsy samples were shown to be enriched by potentially pathogenic *Enterobacteria*, such as *Salmonella*, *Citrobacter*, *Cronobacter* and *Shigella* species [16,17], and *Bacteroides* species [17,18]. These findings make it tempting to speculate that the indigenous microbial composition, as revealed by the nonmalignant biopsy samples, may be a more relevant reflection of a microbiota population that confers a high risk to CRC. Consequently, the species found to be overrepresented in cancerous tissues may instead have had a competitive advantage in this newly developed microenvironment and thereby outcompeted the bacteria that were initially present at these sites. Although these deep-sequencing studies revealed an intriguing first glimpse of the bacterial species that are physically associated with CRC, it has to be realized that these approaches based on the simple presence or absence of bacteria clearly have their limitations as discussed elsewhere [19].

Bacterial drivers of CRC

The question now is, can we already use these microbiota profiles to gain insight in host–microbe interactions during CRC? To arrive at new testable hypotheses, it was recently proposed that a bacterial counterpart of the genetic driver–passenger model for CRC may exist [19,20]. In this scenario, a subset of gut bacteria, such as *Enterobacteria* or enterotoxigenic *Bacteroides fragilis* (ETBF) species (known as ‘drivers’ or ‘alpha-bugs’) drive persistent

inflammation, increase cell proliferation and/or induce DNA damage through the production of genotoxic substances (e.g., colibactin or fragylisin). Eventually, such bacterial drivers may contribute to the initiation of premalignant lesions and subsequent accumulation of mutations during the adenoma–carcinoma sequence. Importantly, both the enterobacterium *Citrobacter rodentium* and ETBF have already been implicated in CRC initiation in a mouse model for CRC [8,21]. More specifically, ETBF was shown to be directly genotoxic to colonic epithelial cells, and to cause cell proliferation and permeabilization of the intestinal barrier. Furthermore, it was demonstrated that ETBF increased tumor development through the increased expression of IL-17 by T lymphocytes (Th17) in the lamina propria [21]. These findings suggest that ETBF induces a persistent Th17 inflammatory response, which may push the colonic epithelium from a state of inflammation towards one of carcinogenesis.

Bacterial passengers of CRC

However, microbiota-associated initiation of CRC seems to tell only half of the story. Once tumorigenesis has been initiated, intestinal changes that favor the proliferation of opportunistic bacteria (‘passengers’) will be induced. Such bacterial passengers are gut bacteria that are relatively poor colonizers of a healthy intestinal mucosa, but gain a competitive advantage in the tumor microenvironment, which provides them with the possibility of outcompeting initial bacterial drivers of CRC. The physiological alterations during colon carcinogenesis that contribute to the formation of this selective colonic niche, include local shifts in metabolite profiles, colonic barrier function and rupture and bleeding of the colonic epithelium [22]. Based on recent microbiota sequencing studies, *Fusobacterium* has emerged as the most common passenger bacterium that benefits from this altered CRC microenvironment [16–18]. Nevertheless, despite the fact that this bacterium seems to proliferate in tumor tissue and even in CRC metastases, *Fusobacterial* infections have never been systematically linked to intestinal malignancies. Much more literature on host–pathogen interactions is available for another gut bacterium that was assigned as passenger. This concerns *Streptococcus gallolyticus*, which was previously known as *Streptococcus bovis* biotype I [23]. A recent meta-analysis

revealed that approximately 43% of patients infected with *S. gallolyticus* (mostly endocarditis) had colonic adenomas and 18% of these patients had carcinomas, which is significantly higher than their prevalences in the general population [12]. As such, this bacterium signals (often asymptomatic) colonic malignancy and *S. gallolyticus* infection should thus be considered as an indication for full bowel examination [24]. Interestingly, the idea that this passenger bacterium has a competitive advantage in the CRC microenvironment was recently confirmed in an experimental *in vitro* model [25]. These studies showed that in contrast to many other gut bacteria, *S. gallolyticus* had a significant growth advantage in the presence of excreted tumor cell metabolites, such as glucose derivatives and certain amino acids [22,25]. In addition to increased proliferation in the CRC microenvironment, *S. gallolyticus* seems to benefit from the distorted epithelial structure of colonic malignancies to access the previously unexposed collagen fibers in the basement membrane for which it has a high affinity. Together, these factors allow this bacterium to colonize and proliferate in the tumor environment and subsequently invade the human body through the intestinal lesions, resulting in clinical infections in a subset of susceptible CRC patients [12,23,26]. The fact that passenger bacteria, such as *Fusobacterium* and *Streptococcus*, are secondary colonizers of CRC tissue does not necessarily exclude their active involvement in CRC progression. For instance, *S. gallolyticus* may further promote tumor progression through induction of the proinflammatory cytokine IL-8 or the COX-2 pathway [27,28]. However, if present *in vivo*, such tumor-promoting effects would be more pronounced during the later stages of CRC.

Clinical utility of CRC-associated microbiota profiles

Based on the recent advancement in knowledge as summarized above, we can now start to address the question of which bacteria can give us relevant information about CRC in clinical practice. First, the indigenous gut microbiota composition may indicate whether or not we have an increased risk for development of CRC through a higher abundance of bacteria with CRC-driving potential. In addition, personal microbiota profiles may provide an estimate on the microbial potential to convert

procarcinogenic substances into carcinogens through increased abundance of specific bacterial metabolic pathways. Establishment of a CRC microbiome risk profile could aid the selection of individuals that require a more intensive surveillance protocol, which could become instrumental for the early detection of CRC [29]. In addition, diets may be formulated that, for instance, lack the substrates for bacterial conversion to carcinogens if such pathways are abundantly present in an individual's gut microbiota. It is tempting to think about strategies to replace driver bacteria by health-promoting bacteria to delay or prevent initiation of CRC. However, in view of the fact that an established microbiota tends to return to its original composition after antibiotic or probiotic treatment makes such endeavors highly challenging. Second, but not less important, the increased abundance of bacterial passengers, or associated bacterial infections, provides a novel class of biomarkers for the diagnosis of CRC itself. Proof-of-concept for blood tests that exploit the presence of CRC-associated antibody responses to bacterial antigens has already been established [24,30–32]. Together, these studies suggest that early disease stages (polyps and localized tumors) can be detected, which is crucial as early-stage CRC can still be effectively cured by polypectomy or surgery [29]. Future translational research should reveal to which extent microbiota-based feces and/or blood tests may tell us whether we are at increased risk for CRC or that we have early-stage disease that needs to be treated. This quest has only just begun.

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References

- Qin J, Li R, Raes J *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464(7285), 59–65 (2010).
- Arumugam M, Raes J, Pelletier E *et al.* Enterotypes of the human gut microbiome. *Nature* 473(7346), 174–180 (2011).
- Boleij A, Tjalsma H. Gut bacteria in health and disease: a survey on the interface between intestinal microbiology and colorectal cancer. *Biol. Rev. Camb. Philos. Soc.* 87(3), 701–730 (2012).
- Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2009. *CA Cancer J. Clin.* 59(4), 225–249 (2009).
- Rutter M, Saunders B, Wilkinson K *et al.* Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 126(2), 451–459 (2004).
- Thun MJ, Namboodiri MM, Heath CW. Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.* 325(23), 1593–1596 (1991).
- Sellon RK, Tonkonogy S, Schultz M *et al.* Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* 66(11), 5224–5231 (1998).
- Wu S, Rhee KJ, Albesiano E *et al.* A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat. Med.* 15(9), 1016–1022 (2009).
- Cuevas-Ramos G, Petit CR, Marcq I *et al.* *Escherichia coli* induces DNA damage *in vivo* and triggers genomic instability in mammalian cells. *Proc. Natl Acad. Sci. USA* 107 (25), 11537–11542 (2010).
- Huycke MM, Abrams V, Moore DR. Enterococcus faecalis produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. *Carcinogenesis* 23(3), 529–536 (2002).
- Knasmüller S, Steinkellner H, Hirschl AM *et al.* Impact of bacteria in dairy products and of the intestinal microflora on the genotoxic and carcinogenic effects of heterocyclic aromatic amines. *Mutat. Res.* 480–481, 129–138 (2001).
- Boleij A, van Gelder MM, Swinkels DW *et al.* Clinical importance of *Streptococcus gallolyticus* infection among colorectal cancer patients: systematic review and meta-analysis. *Clin. Infect. Dis.* 53(9), 870–878 (2011).
- Dethlefsen L, Eckburg PB, Bik EM *et al.* Assembly of the human intestinal microbiota. *Trends Ecol. Evol.* 21(9), 517–523 (2006).
- Claesson MJ, Cusack S, O'Sullivan O *et al.* Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc. Natl Acad. Sci. USA* 108(Suppl. 1), 4586–4591 (2011).
- Green GL, Brostoff J, Hudspeth B *et al.* Molecular characterization of the bacteria adherent to human colorectal mucosa. *J. Appl. Microbiol.* 100(3), 460–469 (2006).
- Marchesi JR, Dutilh BE, Hall N *et al.* Towards the human colorectal cancer microbiome. *PLoS One* 6(5), E20447 (2011).
- Kostic AD, Gevers D, Pedamallu CS *et al.* Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res.* 22(2), 292–298 (2011).
- Castellarin M, Warren RL, Freeman JD *et al.* *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Res.* 22(2), 299–306 (2011).
- Tjalsma H, Boleij A, Marchesi JR *et al.* A bacterial driver–passenger model for colorectal cancer: beyond the usual suspects. *Nat. Rev. Microbiol.* doi:10.1038/nrmicro2819 (2012) (Epub ahead of print).
- Sears CL, Pardoll DM. Perspective: alpha-bugs, their microbial partners, and the link to colon cancer. *J. Infect. Dis.* 203(3), 306–311 (2011).
- Newman JV, Kosaka T, Sheppard BJ *et al.* Bacterial infection promotes colon tumorigenesis in Apc(Min/+) mice. *J. Infect. Dis.* 184(2) 227–230 (2001).
- Hirayama A, Kami K, Sugimoto M *et al.* Quantitative metabolite profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. *Cancer Res.* 69(11), 4918–4925 (2009).
- Klein RS, Recco RA, Catalano MT *et al.* Association of *Streptococcus bovis* with carcinoma of the colon. *N. Engl. J. Med.* 297(15), 800–802 (1977).
- Boleij A, Roelofs R, Danne C *et al.* Selective antibody response to *Streptococcus gallolyticus* pilus proteins in colorectal cancer patients. *Cancer Prev. Res.* 5(2), 260–265 (2011).
- Boleij A, Dutilh BE, Kortman G *et al.* Bacterial responses to a simulated colon tumor microenvironment. *Mol. Cell. Proteomics* doi:10.1074/mcp.M112.019315 (2012) (Epub ahead of print).
- Boleij A, Muytjens CM, Bukhari SI *et al.* Novel clues on the specific association of *Streptococcus gallolyticus* subsp *gallolyticus* with colorectal cancer. *J. Infect. Dis.* 203(8), 1101–1109 (2011).
- Ellmerich S, Schöller M, Duranton B *et al.* Promotion of intestinal carcinogenesis by *Streptococcus bovis*. *Carcinogenesis* 21(4), 753–756 (2000).
- Abdulmir AS, Hafidh RR, Bakar FA. Molecular detection, quantification, and isolation of *Streptococcus gallolyticus* bacteria colonizing colorectal tumors: inflammation-driven potential of carcinogenesis via IL-1, COX-2, and IL-8. *Mol. Cancer* 9, e249 (2010).
- Lieberman D. Clinical practice. Screening for colorectal cancer. *N. Engl. J. Med.* 361(12), 1179–1187 (2009).
- Tjalsma H, Schöller-Guinard M, Lasonder E *et al.* Profiling the humoral immune response in colon cancer patients: diagnostic antigens from *Streptococcus bovis*. *Int. J. Cancer* 119(9), 2127–2135 (2006).
- Abdulmir AS, Hafidh RR, Mahdi LK *et al.* Investigation into the controversial association of *Streptococcus gallolyticus* with colorectal cancer and adenoma. *BMC Cancer* 9, e403 (2009).
- Garza-González E, Ríos M, Bosques-Padilla FJ *et al.* Immune response against *Streptococcus gallolyticus* in patients with adenomatous polyps in colon. *Int. J. Cancer* doi:10.1002/ijc.27511 (2012) (Epub ahead of print).