Microbes and colorectal cancer: is there a relationship?

J.M. Uronis PhD* and C. Jobin PhD*†

KEY WORDS

Colitis, colorectal cancer, inflammatory bowel disease, Nod-like receptor, Toll-like receptor, bacteria

The human colon plays host to as many as 15,000–36,000 bacterial species, amounting to more than 100 trillion bacteria. The microbiota and their associated prokaryotic genome is an integral part of the host and uniquely contributes to various biologic processes such as maturation and development of the mucosal immune system, metabolic capacity, and intestinal epithelial cell proliferation and differentiation. An international effort is currently underway to catalogue the repertoire of microorganisms present in the intestines of healthy humans and of those with pathologic conditions. The human microbiome project—for which the U.S. National Institutes of Health has contributed more than $110 million—is aiming to determine the structure of the microbial community associated with the human body and the functions thereby served in health and disease.

Arguably, mapping and comparing the composition of the microbial community harboured by healthy and disease-affected humans represents the next frontier in microbiology and medicine. Although this field of research is still in its infancy, the link between the microbiota and development of inflammatory bowel diseases (IBDs) has been established in animal models and human patients alike. Countless studies have established that an improper innate or adaptive host response to microbial constituents leads to unrestricted activation of immune cells—including T-lymphocyte effector cells—and development of chronic inflammation.

One of the greatest risks assumed by individuals with IBD is a heightened susceptibility to colorectal cancer (CRC). Epidemiology studies indicate that the duration and severity of chronic colitis are important risk factors for CRC. Because the microbiota play an essential role in the development of ulcerative colitis and Crohn disease (the two main manifestations of IBD), a close look at the relationship between bacteria and CRC is a must.

EVIDENCE THAT MICROBES ARE INVOLVED IN CRC

A tantalizing observation linking bacteria with the development of CRC was made more than a decade ago through germ-free technology, in which mice are born and maintained in the absence of microorganisms. Dove et al. observed that tumour development in germ-free ApcMin (multiple intestinal neoplasia allele of the adenomatous polyposis coli gene) mice, which serve as a model for human familial adenomatous polyposis, was halved as compared with that in mice housed under specific pathogen-free conditions.

More recent findings implicating the microbiota as key players in development of CRC came with the discovery of an important communication system between humans and bacteria. Innate bacteria-sensing receptors such as the Toll-like receptor (TLR) and the Nod-like receptor alert the host to the presence of bacteria. Interestingly, deletion of the myeloid differentiation factor 88 (Myd88) adapter protein, a key mediator of the TLR/interleukin 1 (IL-1) signalling pathway, attenuated polyt tumour development in the ApcMin mouse model. These findings support the idea that bacteria use TLR-mediated signalling components such as Myd88 to promote the development of CRC. However, because the pro-inflammatory cytokine IL-1β uses Myd88 to signal downstream of its receptor, a formal demonstration that bacterial signalling factors participate in tumour progression is still needed in this model.
EVIDENCE THAT MICROBES ARE INVOLVED IN COLITIS-ASSOCIATED COLON CANCER

A significant risk associated with IBD is the development of CRC, a collective pathophysiologic event called colitis-associated colorectal cancer (CAC). The risk of CAC developing in individuals affected with ulcerative colitis for 30 years or more has been evaluated at 7.6%–18%.

Mouse models of CAC have been integral to advancing an understanding of the effect of the gut microbiota on chronic colitis and cancer development.

In the most widely used mouse model of CAC, administration of the colon-specific carcinogen azoxymethane (AOM) induces initiating genetic mutations in the Wnt/β-catenin pathway; subsequently, administration of dextran sodium sulfate (DSS) disrupts the colonic epithelium, inducing chronic colitis. Work with this model showed that deletion of Tlr4 decreased tumour formation, suggesting that bacteria use the receptor to promote colorectal carcinogenesis. In contrast, deletion of Nod1 predisposes to AOM-DSS-induced CAC. Because Tlr4 and Nod1 recognize different microbial spectra (extracellular and intracellular respectively), these apparently opposite functions could highlight specific roles for microorganisms in protecting against or promoting cancer development. Clearly, establishing the composition of the microbial community present in a healthy as compared with a cancer-prone intestine may provide insight into the role of bacteria in tumorigenesis.

An intriguing observation from studies using AOM-DSS-induced CAC in Tlr4−/− mice is that attenuation of colorectal tumorigenesis occurs without a clear concomitant reduction in the severity of inflammation. Interestingly, a similar dissociation between inflammation and tumour development was recently observed in AOM-DSS-exposed Il6−/− mice. In that study, the authors demonstrated a strong reduction in tumour development in Il6−/− mice despite inflammation being significantly augmented in those mice as compared with wild-type mice. These findings contrast with the epidemiology data, which indicate that severity and duration of chronic colitis directly correlates with CRC risk.

The inherent difficulty encountered with the AOM-DSS model in the investigation of inflammation-driven tumorigenesis lies in its inability to uncouple processes elicited by acute wound-healing from those of a chronic inflammatory response. This difficulty may highlight a limitation of the AOM–DSS model in the study of bacterial/TLR signalling in CAC, because this pathway protects against intestinal injury induced by DSS. Indeed, although DSS-induced intestinal injury is worsened in Myd88−/− mice, spontaneous colitis observed in Il10−/− mice is attenuated in Il10−/−; Myd88−/− mice.

To avoid use of a chronic injury model, researchers have substituted the spontaneous Il10−/− mouse model of intestinal inflammation for the AOM–DSS model. Work using a combination of this AOM–Il10−/− model of CAC and gnotobiotic techniques recently identified a clear role for the microbiota in the development and progression of CAC. Whereas wild-type mice treated with AOM develop rare, low-grade colonic adenomas, Il10−/− mice with normal intestinal microbiota show a dramatic increase in CRC susceptibility, which directly correlates with the severity of intestinal inflammation. Conversely, AOM-treated Il10−/−; Myd88−/− mice and AOM-treated Il10−/− mice housed under germfree conditions fail to develop CAC. These findings highlight the essential role that the intestinal microbiota play in the development of CAC.

From the foregoing studies, it has become clear that the microbiota affect colorectal tumour development and progression. Certain innate sensors, such as Nod1, appear to prevent tumour development; others, such as TLR4 and Myd88, promote carcinogenesis. Further investigation will be required to elucidate the nature of these bacterial-mediated tumour-suppressing and -promoting signals. Identification of the microbial communities associated with these carcinogenic events will be equally important. Finally, key observations made using the AOM–Il10−/− model have to be validated in other animal models of CAC before novel paradigms can be established.

Although identification of the microbiota as an essential factor in colorectal carcinogenesis adds complexity to the pathophysiology of this disease, this initiative may represent a novel opportunity for therapeutic intervention.

ACKNOWLEDGMENTS

This work was supported by U.S. National Institutes of Health grants ROI DK 47700 and RO1 DK 73338 to C. Jobin and by Gastroenterology Research Training grant NIH S T32 DK007737 to J.M. Uronis.

REFERENCES

1. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular–phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 2007;104:13780–5.
2. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 2009;136:65–80.
3. Turnbaugh PJ, Ley RE, Hamady M, Fraser–Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804–10.
4. Mullard A. Microbiology: the inside story. Nature 2008;453:578–80.
5. Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003;3:521–33.
6. Itzkowitz SH, Yio X. Inflammation and cancer iv. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 2004;287:G7–17.
7. Rutter M, Saunders B, Wilkinson K, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 2004;126:451–9.
8. Xie J, Itzkowitz SH. Cancer in inflammatory bowel disease. *World J Gastroenterol* 2008;14:378–89.
9. Dove WF, Clipson L, Gould KA, et al. Intestinal neoplasia in the Apc(Min) mouse: independence from the microbial and natural killer (beige locus) status. *Cancer Res* 1997;57:812–14.
10. Beutler BA. TLRs and innate immunity. *Blood* 2009;113:1399–407.
11. Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein myd88. *Science* 2007;317:124–7.
12. Neufert C, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc* 2007;2:1998–2004.
13. Fukata M, Chen A, Vamadevan AS, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. *Gastroenterology* 2007;133:1869–81.
14. Chen GY, Shaw MH, Redondo G, Nunez G. The innate immune receptor NOD1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Res* 2008;68:10060–7.
15. Grivennikov S, Karin E, Terzic J, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 2009;15:103–13.
16. Karrasch T, Jobin C. NF-κB and the intestine: friend or foe? *Inflamm Bowel Dis* 2008;14:114–24.
17. Rakoff-Nahoum S, Paglino J, Esclam–Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004;118:229–41.
18. Rakoff-Nahoum S, Hao L, Medzhitov R. Role of Toll-like receptors in spontaneous commensal-dependent colitis. *Immunity* 2006;25:319–29.
19. Uronis JM, Muehlbauer M, Herfarth HH, Jones GS, Rubinas TC, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS ONE* 2009;4:e6026.

Correspondence to: Christian Jobin, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599 U.S.A.

E-mail: Job@med.unc.edu

* Department of Medicine and Center for GI Biology and Disease, University of North Carolina at Chapel Hill, Chapel Hill, NC, U.S.A.
† Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC, U.S.A.