Editorial on "Cancer and the microbiota" published in *Science*

Alison K. Hamm, Tiffany L. Weir

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523-1571, USA

Correspondence to: Dr. Tiffany L. Weir. Department of Food Science and Human Nutrition, Colorado State University, 1571 Campus Delivery, Fort Collins, CO 80523-1571, USA. Email: tiffany.weir@colostate.edu.

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Introduction

In recent years, improved sequencing technologies have resulted in a surge of information that has clarified the interactions between microorganisms and their environment. Most notably, examination of human-associated microbes has revealed their important role in the prevention and development of specific diseases. In particular, we now have a deeper understanding of how the microbiota can influence cancer development and progression as well as their potential application in cancer therapeutics. Dr. Wendy Garrett highlights these recent advances in her review "Cancer and the microbiota" which was published in April 2015 in *Science* (1). This is the most recent review of a relatively large number that cover this topic (2-6), but Dr. Garrett's review stands out in its coverage of the therapeutic potential of microorganisms for cancer prevention. Here we provide an overview of Dr. Garrett's manuscript in the context of the larger body of work on this topic.

The relationship between cancer and the microbiome is complex, with microbes being implicated in the initiation or progression of tumors in numerous types of cancer, particularly in the gastrointestinal (GI) tract (5-7). At present, only ten of the billions of human-associated microorganisms are recognized as carcinogenic by the International Agency for Cancer Research (IACR). These carcinogenic agents include *Helicobacter pylori*, hepatitis B and C viruses, and human papillomaviruses (HPV), and are responsible for 20% of all cancers (8). However, numerous comparative microbiota studies have shown differences between the bacterial populations of patients with certain types of cancer and healthy controls (9-11) and with tumor compared to healthy tissues (12,13), suggesting that there may be many more oncomicrobes. Although these studies provide support for a role of microbes in cancer they offer little insight into whether tumor-associated microbes directly initiate carcinogenesis, support tumor formation by increasing exposure to carcinogenic metabolites, or simply take advantage of the favorable environmental niche provided by cancer cells. Studies in germ free and other animal models of cancer development are beginning to shed light on this question (14,15). Host genetics and environment (i.e., diet, exposure to environmental toxins, etc.) are also important determinants in microbial influence on cancer development, adding to the complexity of unraveling the host-microbiota relationship in carcinogenesis. In her recent review, Dr. Garrett highlights three mechanisms by which microbes influence carcinogenesis: modulation of host cell proliferation and death, interference of immune system functions, and metabolism of food, pharmaceuticals, and host-produced chemicals.

Microbial role in host-cell proliferation and death

Oncomicrobes include microorganism that can directly damage DNA and alter host cellular processes. Many of the known oncomicrobes are viruses, such as HPV, which insert oncogenes into host genetic material and often preferentially target host genes involved in cancer (16). However, relatively few microorganisms are recognized as bona fide oncomicrobes. This may partially be due to challenges in identifying microbial species as the causal agents of carcinogenesis. The causal organism may be absent from the tumor site due to an environmentally-driven succession of organisms (17), or the microbe may have initiated host cellular damage through a "hit and run" mechanism after only brief contact with the host

tissue (2). Despite a lack of direct evidence linking cancer with certain bacteria, there are several direct mechanisms by which bacterial-induced carcinogenesis may occur. Many bacteria have developed competitive strategies which include the ability to damage DNA of competing organisms. Unfortunately, these same mechanisms can also alter host DNA, resulting in mutations potentially leading to carcinogenesis. Bacterial DNA can integrate into human cellular genomes, particularly the mitochondrial genome, via an RNA intermediate. This phenomenon happens more frequently in tumors than healthy tissues (18). DNA damage may also be induced by toxins produced by bacteria. *Escherichia coli* expressing colibactin, a recently characterized substituted spirobicyclic structure, promotes crosslinking of duplex DNA (19); and cytolethal distending toxin (CDT) produced by ε- and γ-proteobacteria, displays DNAse activity and can directly introduce breaks in double stranded DNA (20). Finally, bacterial proteins can initiate signaling events in host pathways that regulate cell stemness and growth. One such pathway, Wnt/β-catenin, is aberrantly modulated by proteins produced by several bacteria, including *Helicobacter pylori*, *Fusobacterium nucleatum*, and *Salmonella typhi* (7,21,22).

Influence on host immune system function

Inflammation is a central driver of the development of many chronic diseases, including cancer. Thus, bacterial regulation of host immune responses may be an important determinant in carcinogenesis. In the intestinal mucosa, commensal organisms help to maintain levels of T-regulatory cells that suppress inflammation through IL-10 production (23). Pathogen invasion or chemical/environmental insult can lead to intestinal microbial dysbiosis and incite a localized pro-inflammatory response, which can result in mucosal barrier degradation if persistent (5). As Dr. Garrett eloquently describes, cancer and inflammatory diseases can arise when a breach in mucosal barriers results in contact between microbes and immune components that have not coevolved (1). Microbes and microbial components such as lipopolysaccharide (LPS) can up-regulate Toll-like receptors (TLRs) (3,7), which can lead to activation of NF-κB, which is central to regulation of cancer-associated inflammation (24). Once initiation occurs, tumor progression and growth can be further influenced by microbially-driven immune modulation. Microbes can induce TLR or G-coupled protein receptor activation, which regulates JAK/STAT3; a well-characterized signaling pathway with roles in tumor cell proliferation, survival, invasion and immunosuppression (25). Bacterial LPS has also been shown to accelerate cell growth through c-Jun/JNK activation (26). Mouse models have provided evidence that immune system perturbations induced by microbiota in tissue mucosa, such as those involved in the pathology of colorectal cancer, can also influence the dynamics of the resident microbial population, reducing the ability of commensal microbes to repair tissue damage and reverse inflammatory processes (4,12,27,28).

Metabolism of foods, pharmaceuticals, and host-produced products

Metabolic interactions between resident microbiota and environmental, xenobiotic, and dietary components can indirectly influence cancer development. Metabolic end products may include pro-carcinogenic compounds that contribute to tumor evolution, or anti-inflammatory and anti-proliferative chemicals resulting from fermentative processes. The accumulated exposure to these compounds, referred to as the "exposome" can affect inflammation, oxidative stress and DNA stability in a host, influencing the risk of developing cancer and other chronic diseases (29). Many carcinogenic compounds can be generated by the cometabolism of xenobiotics by both liver enzymes and bacterial β-glucuronidases in the gut (2). Salient examples include the metabolism of azoxymethane, which induces colonic tumors in mice (4) and irinotecan, a chemotherapeutic drug that can cause severe diarrhea in a subset of individuals with high bacterial β-glucuronidase activity (30). Likewise, generation of detrimental metabolites is associated with microbial catabolism of dietary proteins. These putrefactive processes in the terminal bowel result in generation of pro-carcinogenic N-nitroso compounds that damage DNA through alkylation (31) and are positively associated with colorectal cancer incidence (32). Metabolism of aromatic amino acids also results in the generation of *p*-cresol, phenylacetic acid, phenols, and indoles to which chronic exposure could result in carcinogenesis (33). Polyamines are another class of toxic compound and catabolism of the major polyamines is associated with cancer and oxidative stress (34). The amino acid arginine can be used by gut bacteria to generate polyamines (35), and certain gut bacteria are known to enhance production of these compounds in host cells (34). High dietary fat consumption results in the increased production of bile acids in the gall bladder and deposition in the duodenum to assist with lipid emulsification and absorption. Modification of these

host derived bile acids by bacterial dehydrogenases and dehydroxylases form secondary bile acids, such as lithocholic and deoxycholic acid, which have been implicated in carcinogenesis in the GI tract and associated organs (36,37). Some intestinal bacteria are sensitive to bile acids and there is evidence to suggest that excessive bile secretion can remodel the gut microbial community, further influencing generation of detrimental metabolites and initiation of inflammatory processes (38). Interestingly, ursodeoxycholic acid, another secondary bile acid, has been suggested as a chemo-protective agent (39), highlighting the need for a better understanding of the mechanisms by which secondary bile acids modulate host cellular processes.

Metabolism of dietary components can also result in generation of bioactive molecules with chemo-protective properties. Carbohydrate fermentation results in production of the short chain fatty acids acetate, propionate, and butyrate. These compounds may have direct anti-inflammatory effects in intestinal cell populations and can interact with free fatty acid receptors in intestinal epithelia and adipose tissue to modulate immune processes, such as secretion of adipokines that can result in inflammation (40). Butyrate, in particular, is a preferential energy source for colonic epithelial cells (41) and butyrate metabolism by epithelial cells was demonstrated as critical in maintenance of the physiologic hypoxia necessary for HIF-1α-mediated regulation of the intestinal barrier (42). Depletion of butyrate has been noted in humans with colorectal cancer (10) and other inflammatory bowel conditions (43). There is also epidemiological evidence supporting the chemo-protective effects of fruit and vegetable consumption, which have been attributed to plant-specific secondary metabolites, such as glucosinolates and polyphenols (44). Polyphenolic compounds are potent anti-oxidants, but studies have shown they fail to reach physiologically bioactive concentrations in the blood (45); however, they are extensively modified by the intestinal bacteria (33). These metabolites have been shown to inhibit pro-inflammatory such as tumor necrosis factor (TNF-α) and NF-κB (46). Similar to the bile acids, these compounds can also influence immune responses and subsequent inflammation through alteration of the gut microbial composition (47). Thus, recent research has begun to focus on the biological effects of microbial metabolites of phytochemicals, both locally in the gut and systemically.

Microbes and modern cancer therapies

The microbiota's important role in metabolism and influence on host immunology poses the question: does the microbiota influence the efficacy of cancer therapies? Dr. Garrett's review highlights several mechanisms by which modulation of the microbiota or its products could be utilized in chemotherapy. The gut microbiota is known to directly metabolize dietary compounds and xenobiotics, including cancer drugs, through a variety of mechanisms that humans lack (48). Oral bacteria-derived β-glucuronidases have been shown to directly regulate the toxicity of irinotecan, a common chemotherapy used to treat several cancers, resulting in alleviation of the most common side effect of severe diarrhea (30). More often, microbiota indirectly affects therapy efficacy through the host immune system. The practice of cancer bacteriotherapy which utilized microbial stimulation of the immune system dates back to the late 1800's with Coley's toxins (49). Advancements in chemotherapies and immunotherapies since have mostly ignored microbes as an integral part of cancer treatments, until recently.

Cyclophosphamide, used in treatment of solid tumors and hematologic malignancies, injures the small intestine epithelium resulting in translocation of certain bacteria into lymph organs. This barrier breech results in T-helper cell mediated antitumor response and increased drug efficacy (50). Oxaliplatin, a platinum-based chemotherapy used to treat several GI cancers works in concert with the microbiota and immune system to prime myeloid cells to increase ROS production leading to oxidative stress within the tumor that enhances the drug's efficacy. Indeed, oral antibiotics impaired platinum chemotherapy and CpG-oligonucleotide immunotherapy response in mice with subcutaneous tumors. In absence of antibiotics, it is proposed that commensal Gram-negative bacterial LPS production modulates myeloid-derived cell functions within the tumor (51). In conclusion, when using the common practice of administering antibiotics alongside chemotherapy to improve white blood cell counts, the risks may outweigh benefits. More studies are needed on the specific interaction of chemotherapies and the specific microbes, either commensal in the host or used as a probiotic.

Conclusions/future outlook

Dr. Garrett's review offers a well-written and comprehensive look at the role of human associated microorganisms in the development, progression, and treatment of various types of cancer. She summarizes the relevant technological advances that have allowed us to gain a deeper understanding of

these interactions and acknowledges the current gaps in knowledge. The complexity of microbiome and host interactions makes it challenging for researchers to identify specific microbes and mechanisms related to carcinogenesis. It could take decades for cancers to develop, with varying microbiotas participating at different stages. However, the recent advent of next-generation sequencing and advanced culturing technologies are helping researchers characterize the host microbiota, elucidating the relationship between host microbiome and therapy.

This is definitely an exciting and evolving area of research. Synthetic biology approaches are focusing on engineering bacterial cells that can selectively target and invade cancer cells (52). The ability of microbes to target and proliferate within tumor sites opens up new possibilities not only for tumor detection, but delivery of therapeutic drugs (53). Very recently, a synthetic probiotic *Escherichia coli* Nissle 1917 was engineered to detect liver metastases through a quorum-sensing metabolite that is easily detected in the urine of mice (54). The next step will be pre-clinical research of such designer bacteria in humans.

The gut microbiome is now recognized as a separate organ with distinct metabolic capacities that exceed the liver's metabolism by a factor of 100 (55,56). It is not surprising that transplantation of the gut microbiome, most notably in the form of fecal transplants for *Clostridium difficile* infections, has become an accepted medical practice (57). Fecal transplants are also effective in treating intestinal inflammatory diseases that could lead to colorectal cancer, and could serve as a chemo-preventative treatment (58). Microbial transplantation shows promise for treating various cancers, just as organ transplants are commonly used to treat various other maladies. Stool "banks" for the collection and storage of donated stool for medical purposes are already being established. Future research will undoubtedly reveal specific organisms and mechanisms of cancer progression to advance newer therapies such as synthetic designer probiotics and microbial transplants and open novel microbiota-targeted chemotherapeutic avenues.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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