Gut Microbiome and Colon Cancer: A Plausible Explanation for Dietary Contributions to Cancer

Heidi Nelson, MD, FACS, Nicholas Chia, PhD

"With the wonderful development of our knowledge of germs … there has been a growing suspicion that cancer has microbic origin …" William J Mayo, 1892[1p432]

This is a review of how modern-day sequencing provides a fresh opportunity to consider environmental causes of cancer, specifically through our emerging understanding of the human microbiome. It is a story of how microbes shape the gut environment and impact human health, including the insidious transformation of nutrients into tumors.

It has rightly been assumed that sequencing technologies will facilitate the identification of new germline mutations, explaining hereditary conditions such as cancer. Less obvious at the outset has been the possibility that sequencing could bring clarity to what is now referred to as our second genome, that is, the genes of the microbes living in, on, and among us. The Human Microbiome Project sponsored by the National Institutes of Health accelerated the field of study of human microbiome and now we are coming to understand how the microbiome might be our most intimate environmental health determinant.[2] This article will review how new knowledge generated from sequencing of human microbiome challenges our previous concepts about the role of microbes in health and disease. The time has come for us to broaden our view on microbes and see them in the context of ecologic communities rather than as lone pathogens. We now have the computational and modeling tools to move beyond the concept of microbes as single pathogens. In addition, we can now see how ubiquitous and important the aggregate contributions of microbes are and how they just might be the missing link in how the foods we consume can turn into cancer.

Disclosure Information: Nothing to disclose.

Presented at the American College of Surgeons 103rd Annual Clinical Congress, Boston, MA, October 2018.

Received May 3, 2019; Accepted May 6, 2019.

From the Microbiome Program, Center for Individualized Medicine (Nelson, Chia), Colon and Rectal Surgery (Nelson), and Division of Surgical Research (Chia), Department of Surgery, Mayo Clinic, Rochester, MN.

Correspondence address: Heidi Nelson, MD, FACS, Microbiome Program, Center for Individualized Medicine, Mayo Clinic, 200 1st St SW, Rochester, MN 55905. email: nelsonh@mayo.edu

How the story of *Helicobacter pylori*, gastric ulcers, and gastric cancer disrupted traditional concepts of microbiology

It was not so long ago that we viewed the world of microbiology in simpler terms and considered bacteria to be little more than infectious disease-causing pathogens. Dating back to the 1600s, we understood the microbial world literally and figuratively through the lens of single-cell pathogens. The development of the microscope represented a major advancement that confirmed long-held suspicions that contagious diseases were transferred through some kind of intermediate. The causal relationship between a given microbe and a specific disease was further advanced by the ability to culture microorganisms and the postulates of causation put forth by Robert Koch and Friedrich Loeffler.[3] In Koch's 4 postulates, causation required that the microorganism must occur in every case of the disease and not in healthy unaffected subjects. In addition, the postulates put forth that the microorganism must be isolated and grown in pure culture and be able to cause disease when reintroduced into a healthy experimental subject. Lastly, the organism must be re-isolated from the experimental host and demonstrated to be the same as the original isolate. Koch's postulates fundamentally shaped the field of clinical microbiology for decades, and yet with time it has become evident that the postulates are not completely in keeping with our contemporary understanding of microbial pathogenesis. For example, universal and rigid adherence to the postulates would not accommodate our understanding of microbial carrier status or the role of the human immune system in explaining the inconsistent relationship between the presence of microbes and the manifestations of infection. Despite many inherent limitations, the postulates influenced our view of microbes to the extent that Marshall and colleagues[4] resorted to validating the exposure postulate to make the final argument forever linking pyloric *Campylobacter* with the condition of gastritis.

https://doi.org/10.1016/j.jamcollsurg.2019.05.003
ISSN 1072-7515/19

Despite strong evidence in support of the contribution of *H pylori* as a cause of chronic active gastritis, it took irrefutable self-inoculation for scientists and clinicians to believe that microbes could contribute to a human condition like ulcer disease, which is not considered a traditional infectious disease. Barry J Marshall and Robin Warren, who discovered *H pylori* and its role in gastritis and peptic ulcer disease, created a major paradigm shift in how we think of microbes and their role in chronic human conditions.[5] As the story evolved, it also became clear that *H pylori* plays a role in the development of gastric cancer. The cancer causation link is now sufficiently well established that the World Health Organization defines *H pylori* as a class I carcinogen.[6] It might be possible to reduce rates of gastric cancer going forward with medical forms of true primary prevention. Indeed, a meta-analysis of randomized controlled trials supports a 44% reduction of incidence of gastric cancer through eradication of *H pylori*. Whether or not eradication of *H pylori* can eliminate gastric cancer is yet to be demonstrated, but we now think differently about the role of microbes in cancer.

The relationship between *H pylori* and gastric cancer does not appear to be straightforward in that there is neither a one-to-one correlation between the organism and the disease nor an obvious pathogenic gene that causes the cancer.[6] In fact, it would seem that there is little correlation between rates of infection and rates of cancer, with some areas with high rates of *H pylori* infection and yet low prevalence of gastric cancer. Other risk factors for gastric cancer, such as older age, higher BMI, low socioeconomic status, smoking, alcohol consumption, and high-salt and low-fiber diet, as well as exposure to dietary n-nitroso compounds, likely explain the variance between exposures vs cancer development. The mechanistic basis for *H pylori* and gastric cancer might be indirect through a general inflammatory process that promotes a gradual accumulation of mitotic errors. Alternatively, *H pylori* could have a direct effect on gastric epithelial cells through virulence factors, including cytotoxin-associated gene A or vacuolating cytotoxin A. Such factors can disrupt cellular signaling pathways and have a downstream impact on tumor suppressor genes or disrupt the normal balance between proliferation and apoptosis. Even though our understanding of how *H pylori* causes cancer remains a bit murky, it has clearly broadened our understanding of how cancer can be caused by microbes. Just as we are broadening our perspective on how we imagine microbes might cause chronic human disorders, we also need to reformulate our thinking and language specific to the natural state of microbes in, on, and among the parts of the human body.

Human microbiome—a short primer on what it is and how we study it

Knowledge of the bacteria, fungi, viruses, protozoa, and bacteria phage, together referenced as *human microbiome*, has been accelerated by the availability and affordability of sequencing, as well as the formation of 2 early study consortiums. The National Institutes of Health sponsored the Human Microbiome Project and Europe sponsored MetaHIT.[2,7] These major coordinated efforts set the stage for launching this new field of study by funding the development of microbiome methodologies and conducting the first systematic characterization of the microbiome of hundreds of human subjects.

The terms and concepts that are used to communicate results from sequencing studies of the microbiome might be familiar to scientists, but deserve mention here, as they are likely new to most clinicians. The naming and classification of microbes still follows the familiar taxonomic description of phylum, class, order, family, genus, and species. Groups of organisms thought to have evolved from a shared ancestor are referred to as a *clade* and are depicted in cladograms, which allow community comparisons.[8] 16S ribosomal RNA gene sequencing can fingerprint the identity of microbes through the application of primers. A less familiar term for referencing microbes is *operational taxonomic unit*. Operational taxonomic units are determined using sequencing data and set similarity thresholds (typically 97%) for classifying microbes based on genetic similarities. The operational taxonomic unit system accommodates the unique gene-sharing properties of the microbiome, specifically horizontal gene transfer, and allows a more direct comparison of the genetic material found in microbes, beyond what might be known based on species classification.

The importance of measuring and comparing genetic material speaks precisely to the functional capacity of these microbes and microbial populations. Although the species-level identification of microbes is still widely pursued and provides valuable knowledge as part of microbiome studies, increasing attention is paid to the knowledge of functional genes. Metagenomic studies are sequencing studies that inform us as to what genes are present and what function the gene provides. For example, in clinical microbiology, resistance and pathogenic genes describe microbial resistance to antimicrobial agents and unique pathogenic capacity, respectively. For gut microbiome studies, metagenomics informs us about the metabolic capacity and capabilities of microbes, and are fundamental to the development of metabolic models. Although clinical work has historically focused on the impact of single microbes, the human microbiome studies

are teaching us to consider the microbiome as communities of organisms or ecologies. Borrowing from the field of microbial ecology, basic measures of diversity include α-diversity, which measures species richness within a site or subject; and β-diversity, which measures species differences between sites or subjects.

With these methodologies in mind, Human Microbiome Project and MetaHIT were able to characterize the human microbiome.[2,7] Key findings emerged from these coordinated efforts, including the fact that remarkable and unexplained differences in microbial populations occur within the skin, vagina, and gut microbiomes of an individual and major differences also exist among healthy individuals. Human Microbiome Project studies showed that the diversity and abundance signatures among anatomic sites or habitats varied widely. Strong niche specialization occurred both within and among healthy subjects. Patterns were identified and although oral and stool communities were highly diverse, vaginal sites represented more simple, low-diversity communities. As might be expected, over time the differences between subjects were greater than differences within a given subject, suggesting a certain base level of microbial community stability. There were no universally present taxa among all body habitats and individuals, but there were prevalent clades in each habitat.

This new-found awareness of just how densely populated the human body is with microbes forever reshaped our thinking about microbes. Shockingly, initial quantitative estimates asserted that humans were composed of 10 times more microbial cells than human cells.[9] This has been recalculated and newer estimates suggest a 1.3:1 ratio of microbe cells to human cells, but the point was made that microbial presence and contributions can no longer be ignored.[9,10] It soon became apparent from these early studies that we had a lot to learn about the gut by interrogating the vast population of microbial inhabitants, the presence of which was assumed, but poorly understood. Metagenomics sequencing was the key to unlocking the rich genetic information contained in the gut. We can now imagine the gut as a bioreactor full of microbes, human cells, and nutrients. With this in mind, we can now create models that will someday predict how a bioreactor will function under specific conditions.[11] Being able to model and predict how an individual will respond to dietary nutrients is the first critical step in our journey toward understanding how diet can play a role in colon cancer development.

Gut microbiome

What we now know about the gut microbiome is that the microbial gene set is about 150 times larger than the human gene complement. Although the microbiome is defined as representing all types of microbes, >99% of the genes in the human gut are bacterial. Each individual carries roughly 160 species; *Bacteroidetes* and *Firmicutes* constitute >90% of the phylogenetic categories. In early life, up until age 3 years, there can be dramatic differences in the diversity of microbes, but after infancy the microbiome converges to a more similar and stable phyla composition. The contributions of gut microbes cannot be disported or overestimated, as they have a profound impact on human physiology. Gut microbes contribute to energy harvest from food, extraction of unique nutrients, protection against enteropathogens, and contribute toward the normal functioning of the immune system.[12] Given what we now know about how the gut microbiome supports human health, it should come as no surprise that there is an ever-growing list of studies describing the relationship between gut microbiome and human disorders, including gastrointestinal disorders (eg inflammatory bowel disease; irritable bowel syndrome; *Clostridium difficile* infection; liver disease; and gastric, esophageal, and colon cancer), as well as obesity, diabetes, metabolic syndrome, allergic disorders, autism, rheumatoid arthritis, and cardiovascular disease, to name a few.[13-16] In some cases, the reports describe taxonomic differences or community structure differences between microbes, which is referred to as "dysbiosis." In other cases, mechanisms have been investigated through experimental models. For example, the metabolic products of gut microbes have been shown to drive gut motility. Although the field is still evolving, gut metabolites are also thought to play a key role in what is referred to as a gut-brain axis.

Going forward it would seem that relying on Koch's postulates will not be a useful construct for how to consider communities of organisms. In addition, descriptive associations alone will also not be sufficient for establishing causation. We are optimistic that with more data and analytics we will see critical patterns emerge among abnormal community structure, metabolic imbalances, and mechanistic explanations as to how metabolites contribute to abnormal health conditions. Although we assume normal will represent some form of neutral nutrient metabolic balance, this requires validation. In the meantime, we are investigating the possibility that an imbalance between production and consumption of toxic nutrients can cause damage to local colon epithelial cells, initiating mutagenesis.

Gut microbiome and colon cancer

The contribution of Western diet to the incidence of sporadic colon cancer has long been established and confirmed repeatedly.[17,18] Despite the large body of

knowledge linking diet and colon cancer, primary prevention strategies remain elusive. This is believed to be due, in large part, to the lack of understanding of how food mechanistically leads to mutagenesis. Thanks to the evolving study of the human microbiome, we are now pursuing the concept that the gut microbiome plays a pivotal role, perhaps the missing link, in the relationship between the Western diet and colon cancer. Our investigative efforts are focused on testing the hypothesis that microbial metabolites initiate colon epithelial damage at the DNA level.[8] The metabolite of interest in our research is hydrogen sulfide, based on what is already known about the ability of hydrogen sulfide to damage DNA. Specifically, we are exploring whether the relative imbalance between sulfate-reducing bacteria, which consume hydrogen and produce hydrogen sulfide, and methanogens, which restore hydrogen balance, might contribute to colon neoplasia. To test this hypothesis, we are building microbial metabolic models and conducting human studies. Fortunate to have access to a large bank of well-curated and preserved stool samples, we have been able to test for differences in the gut microbiome between patients with and without adenomatous polyps. The intent of such studies is to investigate early conditions leading to neoplasia, hoping to detect key metabolic drivers of DNA damage where they might exist. So far, microbial sequencing data show results uniquely different from the gastric cancer story. Unlike the relationship between *H pylori* and gastric ulcers and cancer, colon neoplasia does not appear to be correlated with a single dominant organism. At a taxa level, there are no significant differences between samples from polyp vs no polyp patients, and although there are some unique differences for a few specific microbes, these differences do not support a single microbe mechanism. There is evidence, however, for a significant correlation between community microbial composition and colon polyps.

Several taxa were more abundant in the stool samples from patients with polyps, including those that are hydrogen-sulfide-producing and pro-inflammatory.This shift in gut microbiome would predict increases in primary and secondary bile acid production, as well as starch, sucrose, lipid, and phenylpropanoid metabolism.These early findings could support a linkage between Western diet-based metabolism and colon cancer on the condition that increased bile acid production contributes to the growth of bile-tolerant microbes, which then produce high local concentrations of hydrogen sulfide and inflammatory metabolites. Another approach we are taking to test our hypothesis specific to microbial metabolites is to investigate colon tissue samples taken from colon cancers and adjacent normal colon tissue.[19] Although we cannot measure colonic levels of gaseous hydrogen sulfide directly, we are measuring levels of local amino acids for which there is obligate hydrogen sulfide co-production, which can offer a surrogate measure. In fact, when cystathionine and lanthionine are measured in colon cancer and adjacent normal tissue, differences in levels of these amino acids are apparent, and are striking for deficient mismatch repair tumors compared with proficient mismatch repair tumors. These findings lend credence to the concept of these 2 types of cancer being fundamentally unique from each other, a fact that bears out clinically. Although we are early in the discovery phase of study, we remain optimistic that new actionable knowledge will emerge and help inform dietary prevention strategies. In addition, we would expect these strategies to be effective at the level of the individual.

The promise of this line of investigation is the possibility of the development of personalized nutrition options for the prevention of polyp formation. It is our expectation that one day the complex metabolic models coupled with the experimental human studies together will provide us with predictive tools that advise people on optimal diets to support wellness. Evidence in support of personalized nutrition comes from work in the field of glycemic responses to food. Wearable continuous glucose monitors facilitate real-time gathering of knowledge correlating food ingestion with postprandial glycemic responses. Complex algorithms based on clinical, phenotypic, and microbial data can now predict glycemic response in ways that outperform traditional dietary approaches that historically relied on information specific to the caloric and carbohydrate content of food.[20-22] The success of this work provides proof of principle in support of the future benefits of personalized nutrition.[23]

Lastly, it should be clearly stated that we do not expect hydrogen sulfide to be the only story relevant to colon neoplasia formation. We fully expect that diverse mechanistic pathways involving microbes will emerge as the field matures. In fact, others have made the case for the contribution of biofilms and tumorigenic bacteria.[24] For example, the microbiome of patients with familial adenomatous polyposis compared with controls were likely to harbor bacterial biofilm, *Bacteroides fragilis* toxin, or both, and less likely to be negative for both. When studies were conducted in a murine model of colon cancer, it appeared that the presence of both the biofilm and toxin together increased tumor onset and tumor-related mortality significantly. Finally, it should be said that it is likely that the microbiome will be a key environmental factor linking diet, obesity, and colon cancer development.[15]

CONCLUSIONS

In closing, the application of modern sequencing technologies has surely opened a vital field of study, the human microbiome and this field of study is reshaping how we think about microbes and their role in human diseases. In the gut, where microbes are abundant and where they play a symbiotic role in metabolism and other vital functions, we see a viable path to cracking the code of how food can contribute to mutagenesis and other human disorders. Importantly, new solutions will follow where new knowledge takes us.

Author Contributions

Study conception and design: Nelson, Chia
Drafting of manuscript: Nelson
Critical revision: Chia

REFERENCES

1. Mayo WJ. Cancer and its surgical treatment. In: Mayo WJ, Mayo CH, eds. A Collection of Papers Published Previous to 1909, Vol II. Philadelphia, PA: WB Saunders; 1912:431–438.
2. The Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486[7402]:207–214.
3. Byrd AL, Segre JA. Infectious disease. Adapting Koch's postulates. Science 2016;351[6270]:224–226.
4. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric *Campylobacter*. Med J Aust 1985;142:436–439.
5. Marshall BJ, Warren RM. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;16:1311–1315.
6. Ishaq S, Nunn L. *Helicobacter pylori* and gastric cancer: a state of the art review. Gastroenterol Hepatol Bed Bench 2015;8 [Suppl 1]:S6–S14.
7. Qin J, Ruiqiang L, Raes J, et al. A human gut microbial gene catalog established by metagenomic sequencing. Nature 2010; 464[7285]:59–65.
8. Hale VL, Chen J, Johnson S, et al. Shifts in the fecal microbiota associated with adenomatous polyps. Cancer Epidemiol Biomarkers Prev 2017;26:85–94.
9. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. PLoS Biol 2016;14 [8]:e1002533.
10. Gilbert JA, Blaser MJ, Caporaso JG, et al. Current understanding of the human microbiome. Nat Med 2018;24: 392–400.
11. Sung J, Kim S, Cabatbat JJT, et al. Global metabolic interaction network of the human gut microbiota for context-specific community-scale analysis. Nat Commun 2017;8:15393.
12. Krajmalnik-Brown R, Ilhan Z, Kang DW, DiBaise JK. Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract 2012;27:201–214.
13. Lozupone CA, Stombaugh JI, Gordon JI, et al. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489[7415]:220–230.
14. Holmes E, Li JV, Athanasiou T, et al. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol 2011;19:349–359.
15. Rogers CJ, Prabhu KS, Vijay-Kumar M. The microbiome and obesity-an established risk for certain types of cancer. Cancer J 2014;20:176–180.
16. Lau K, Srivatsav V, Rizwan A, et al. Bridging the gap between gut microbial dysbiosis and cardiovascular disease. Nutrients 2017;9[8].
17. Nigro ND, Bull AW. Prospects for the prevention of colorectal cancer. Dis Colon Rectum 1987;30:751–754.
18. Hou N, Huo D, Dignam JJ. Prevention of colorectal cancer and dietary management. Chin Clin Oncol 2013;2[2]:13.
19. Hale VL, Jeraldo P, Chen J, et al. Distinct microbes, metabolites, and ecologies define the microbiome in deficient and proficient mismatch repair colorectal cancers. Genome Med 2018; 10:78.
20. Zeevi D, Korem T, Zmora N, et al. Personalized nutrition by prediction of glycemic responses. Cell 2015;163: 1079–1094.
21. Mendes-Soares H, Raveh-Sadka T, Azulay S, et al. Assessment of a personalized approach to predicting postprandial glycemic responses to food among individuals without diabetes. JAMA Network Open 2019;2[2]:e188102.
22. Mendes-Soares H, Raveh-Sadka T, Azulay S, et al. Model of personalized postprandial glycemic response to food developed for an Israeli cohort predicts responses in Midwestern American individuals. Am J Clin Nutr 2019 May 16 [Epub ahead of print].
23. Kashyap PC, Chia N, Nelson H, et al. Microbiome at the frontier of personalized medicine. Mayo Clin Proc 2017;92: 1855–1864.
24. Dejea CM, Fathi P, Craig JM, et al. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. Science 2018;359:592–597.