Cancer Research

# The Gut Microbiome: A New Player in Breast Cancer Metastasis

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There is increasing interest in the role of the gut microbiome in health and disease, and a number of observational and *in vitro* studies have suggested it may play a role in breast cancer development and progression. Buchta Rosean and colleagues present the first functional evidence that a preexisting disturbance in the gut microbiome leads to increased breast cancer cell metastasis in a mouse model. This discovery places the gut microbiome as a new player in breast cancer metastasis; however, further studies are required to determine the relevance of the findings in this mouse model to human disease. A better understanding of the relationship between the bacterial ecosystem of the gut and progression of breast cancer has enormous potential for improving treatment outcomes for patients with breast cancer.

*See related article by Buchta Rosean et al., p. 3662*

The gut microbiome refers to the ecosystem of microorganisms that inhabit the gut, and predominantly consists of over a thousand bacterial species, as well as to a lesser extent fungi, viruses, archaea, and protists (1). This diverse array of microbes has a significant symbiotic relationship with the host and can exert profound effects on our health. Perturbations in the diversity of the microbiome and altered abundance of specific bacterial species can increase the risk of disease states such as gastric cancer and inflammatory bowel disease. Emerging evidence over the past 10 years, enabled by new technologies such as next-generation sequencing, has also suggested the influence of the gut microbiome on health and disease extends beyond the gastrointestinal tract (2). Of significance, a number of observational and *in vitro* studies suggest relationships between the gut microbiome and breast cancer (3). While these studies are intriguing, a functional relationship between the gut microbiome and breast cancer has yet to be demonstrated until recently. Buchta Rosean and colleagues now present the first evidence that disturbance in the gut microbiome can promote breast cancer metastasis in a mouse model (4). This research is in its early stages and there is still much to be done. Nevertheless, the impact of gut microbiome disturbance on tumor metastasis described by the authors opens the door for a new player in breast cancer metastasis, with enormous potential for how we might reduce the health burden of this disease.

Buchta Rosean and colleagues used a mouse model of hormone receptor–positive breast cancer to explore the effect of antibiotic-induced changes in the gut microbiome on cancer metastasis (4). Prior to establishment of mammary tumors, disturbance of the gut microbiome was induced using two different formulations of antibiotics by oral gavage, one being a broad spectrum of antibiotics that are absorbable, and another formulation that is nonabsorbable. The antibiotic administration regime was conducted over two weeks and resulted in increased cecum weight, reduced bacterial community diversity, and a shift in bacterial phyla from *Fermicutes* to *Verrucomicrobia* and *Proteobacteria*. Shifts in bacterial genera included reduced relative abundance of *Lachnospiraceae*, *Bacteroides*, and *Lactobaccillus*, and increased relative abundance of *Akkermansia*, *Alistipes*, *Escherichia*, and *Shigella*. These disturbances in the diversity and species of bacteria present in the cecum were termed "dysbiosis."

The effect of preexisting dysbiosis on development and metastasis of hormone receptor–positive breast cancer was investigated. Following a 4-day rest period after cessation of antibiotics, poorly metastatic BRPKp110 cells of mammary epithelial cell lineage, originally derived from *Mmtv-Pymt* transgenic mice, were injected into the mammary fat pad of syngeneic C57Bl6 mice. Induction of dysbiosis in the mice did not affect growth of the primary mammary tumor over a period of one month (4). However, dysbiosis had a profound effect on immune cell activity, with increased abundance of macrophages and inflammatory chemokines in the mammary gland, mammary tumor, and blood. The increased inflammation was accompanied by increased dissemination of tumor cells into the blood, lymph nodes, and to the lung. Increased tumor metastasis in mice with preexisting dysbiosis was not due to off-target effects of the antibiotic treatment. Transplantation of cecal contents from mice with dysbiosis elevated tumor dissemination in the blood, lymph nodes, and lungs of mice given a 1-week treatment with antibiotics, and this effect was not observed in mice transplanted with cecal contents from mice without dysbiosis.

The finding that perturbation in the gut microbiome can affect tumor dissemination at a distant site supports the notion that the gut microbiome can be considered as an endocrine gland (3, 5). A number of metabolites associated with activity of gut bacterial species can travel through the blood and have been shown *in vitro*

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Cancer Res 2019;79:3539–41

**doi:** 10.1158/0008-5472.CAN-19-1698

to affect breast cancer cell and immune cell function. Short-chain fatty acids, such as acetate, propionate, and butyrate, are produced by bacterial, species during fermentation of nondigestible carbohydrates. Short-chain fatty acids, as well as other metabolites of gut bacteria such as lithocholic acid and cadaverine, can affect mitochondrial metabolism in breast cancer cells, apoptosis, epithelial–mesenchymal transition, invasion, and immune cell signaling (3). The gut microbiome can also affect bioavailability of estrogen and estrogen metabolites. Bacterial β-glucuronidase activity can deconjugate estrogen and its metabolites, enabling reuptake and subsequent circulation to distant sites (5). Buchta Rosean and colleagues reported no substantial differences in estrous cycling in mice with dysbiosis, suggesting that fluctuations in circulating estrogen and progesterone occurred normally (4). However, further research is required to investigate whether dysbiosis-induced tumor dissemination is the result of altered estrogen or estrogen metabolite deconjugation and reuptake, or due to altered abundance of specific signaling factors such as short-chain fatty acids.

The composition of the gut microbiome is highly variable between individuals and is dependent on many factors including diet, early microbial exposure, and genetic background. The study by Buchta Rosean and colleagues raises questions on how modifiable an individual's microbiome is, and whether there might be interventions, such as a specific diet or targeted antibiotics, that could reduce the risk of tumor dissemination and improve breast cancer patient outcomes. Rather than focusing on the specific bacterial species that populate the gut, Moya and Ferrer suggest considering microbiome stability and disturbance in terms of the specific processes bacteria carry out within the niche environment (6). There is significant functional redundancy between bacterial species, where changes in relative abundance of specific species can result in the same overall outcome for the host in terms of protein or metabolite profiles. After a change is induced that disrupts the microbiome, such as a different diet, a disease, or medication, the microbiome may return to a similar functional state or adapt to a new state, and this will depend on the degree and nature of the change and other factors unique to the particular host and gut ecosystem. Thus, the composition of the gut microbiome exists in a dynamic equilibrium that becomes more complex over time and is influenced by early-life exposures, intrinsic and extrinsic perturbations, as well as random events.

Microbiome disruption has the potential to either harm or benefit breast cancer outcomes. Antibiotics are often prescribed to women with breast cancer during surgery or adjuvant therapy to treat or prevent infection. For example, prophylactic oral antibiotics are commonly prescribed to reduce the risk of febrile neutropenia during docetaxel–cyclophosphamide chemotherapy, which is considered safe during the course of chemotherapy (7). However, antibiotic-induced disruption to the microbiome might affect risk of breast cancer metastasis. Further studies on the long-term impact of administration of antibiotics during chemotherapy on the risk of breast cancer recurrence are required, particularly when antibiotics are given with neoadjuvant chemotherapy. Interventions that affect the gut microbiome could also be employed to improve the efficacy of current cancer treatments. Increased host inflammatory responses are associated with specific bacterial species in the gut microbiome and improved efficacy of anti-programmed cell death protein 1 therapy (2). A more nuanced understanding of the effect of the microbiome on cancer treatment could result in identification of biomarkers of treatment response and improved outcomes.

There is a growing industry for over-the-counter probiotic formulations to ameliorate a number of health conditions such as antibiotic-induced diarrhea, irritable bowel syndrome, and obesity despite concerns over clinical evidence (8, 9). Probiotic formulations, when taken following antibiotic use, can lead to persistent disturbance of the gut microbiome of human subjects (10). Following antibiotic use, diversity of the indigenous microbiome spontaneously recovers within three weeks. Probiotics can interfere with this, with a marked delay in indigenous gut microbiome recovery in terms of composition, function, and bacterial load, with persistent perturbation observed five months following the cessation of probiotic exposure (10). A prolonged disturbance in the gut microbiome, such as that induced by probiotics, might have implications for increased risk of breast cancer metastasis and this requires further investigation.

Human breast cancer is a highly heterogeneous disease, and even among specific subtypes of cancer, there exists significant variability in development, progression, and metastasis. Furthermore, the diversity and specific species comprising the human gut microbiome is complex, highly variable between individuals, and exists in a dynamic equilibrium. While the study by Buchta Rosean and colleagues offers exciting new insights into the role of the gut microbiome in breast cancer metastasis, caution is needed in extrapolating these findings into human health. Further studies are required to demonstrate these effects beyond this one mouse model of disease. Future research should address the physiologic role of specific bacterial proteins and metabolites, the mechanism of action on breast cancer cell dissemination, and the impact of current practices around antibiotic and probiotic use on risk of breast cancer recurrence. These studies will assist in determining how relevant the gut microbiome is to human breast cancer and what interventional strategies could be employed to improve patient outcomes.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

W.V. Ingman is supported by The Hospital Research Foundation Breast Cancer Research Fellowship.

Received May 30, 2019; accepted May 31, 2019; published online July 15, 2019.

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