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Q&A

Microbial metabolites in cancer: An interview with Wendy S. Garrett and Eran Elinav

We talk to Eran Elinav and Wendy S. Garrett about the evolving research landscape of the cancer-microbiome field, their motivation to delve into this area of research, and the biggest and most exciting challenges facing the field.

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To start with, tell us a bit about yourself, what motivated you to become a scientist, and your journey to your current position

E.E.: I have been interested in science and medicine from a young age. I graduated from medical school in 2000 and continued my clinical training as an intern, resident, and fellow in internal medicine and gastroenterology. In parallel to my clinical work, I've conducted research but became increasingly frustrated by the fact that I was not able to engage in it deeply and comprehensively enough. Already a senior physician-scientist at a gastroenterology institute in one of Israel's largest tertiary medical centers, I took a rather bold step to pursue a PhD in immunology at the Weizmann Institute of Science. These four busy years dedicated to basic research, in the lab of Zelig Eshhar, among the pioneers of the CAR-T therapy approach, were transformative to me. In my graduate studies, I was first to develop "CAR-Tregs," regulatory T cells whose antigen specificity was redirected by expression of a chimeric antibody receptor, enabling homing and activation at inflammatory sites. We showed that this new approach could be utilized as a powerful new therapy in inflammatory bowel diseases (IBDs). This approach is now being further developed as human treatment of inflammatory and transplant-related disorders. Being increasingly fascinated by immunology, I then engaged in postdoctoral studies with Prof. Richard Flavell at Yale University. With Richard, I was among the first to study the gut microbiome, a totally new frontier at the time, and its impacts on the immune system. We discovered the NLRP6 inflammasome, the first innate immune "microbiome sensor," and demonstrated that it regulates microbiome composition and function in maintaining homeostasis and preventing diseases such as IBD, cardiometabolic disease, and colorectal cancer. In 2012, I was recruited to establish my independent microbiome-focused lab at the Weizmann Institute of Science, Israel. The lab, now totaling over 40 students, postdocs, and staff from around the world, utilizes a variety of experimental and computational technologies to study aspects related to host-microbiome interactions, their regulation by nutritional and environmental facets, and downstream impacts on human health and "multi-factorial" disorders. In 2019, I established, as a second affiliation, the microbiome and cancer "bridging division," at the German Cancer Research Center (DKFZ) in Heidelberg, Germany. Together with a highly talented group of junior PI's, students, postdocs, and staff we harness our vast experience in studying how the microbiome may impact cancer development, progression, and response to treatment.

W.S.G.: I am a curious person and have been so since I was little. I think science is a welcoming discipline for people that enjoy nature and people that gravitate to "what," "why," and "how" questions. I was extremely fortunate to have incredibly supportive STEM teachers that nurtured my interest in science with exposure to computer terminals in elementary school, building devices in electronics club, and supporting participation in science fair projects from middle school onward. In college, I was incredibly lucky to have been welcomed into labs full of graduate students, postdocs, and PIs that were very excited about science and willing to take on students and train and teach me.

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I am a physician-scientist and a super fan of the big, broad perspectives and deep dives that the physician-scientist track enables. While I have interests outside of science that keep me motivated and inspired, I have not had a meandering path on the physician-scientist track. I also have had mentors that helped make the next steps clear and their unwavering support to take on steep climbs and big leaps when the situations presented themselves.

I never imagined having the lab that I have now or having a lab where I have one. Most challenges or projects that we have taken on in the lab, I never would have envisioned if you asked me five or ten or twenty years ago. Although my path may look linear, in the moment (past or present) it has not always seemed that way to me. For example, I can understand why I gravitated toward microbiome science ultimately but have been very interested in other fields of biology along the way.

Tell us about the research in your lab and how has it evolved over the years

E.E.: Research in my lab(s) is performed by a very talented and ambitious group of scientists coming from diverse backgrounds such as immunology, microbiology, metabolism, cancer, and computational biology. Together, we attempt to decode fundamental "rules of engagement" dictating host-microbiome interactions, their regulation by diet, environment, and host factors, and their impacts on downstream physiology and disease risk. While our research is driven by serendipity, our discoveries in many cases are potentially relevant to human health and may be further translated into human treatment. Given my background as a clinician, we involve in our research multiple genomic, immune, and molecular tools, including animal modeling, yet do not hesitate to further test the concepts we unravel in human trials. In general, our interests are wide and span immune, infectious, metabolic, neoplastic, and even neurodegenerative diseases. Our common aim in these diverse projects is to contribute to a shift from studying correlation to causation, overall gaining a better molecular-level mechanistic understanding of host-microbiome interactions and how they may impact human health and disease.

W.S.G.: One of the shifts in my lab, that is reflective of the field overall, is a shift from studies that focus on linking a taxon or taxa to a biological effect to microbial bioactives; often these bioactives are microbial metabolites. I think this shift over time is driven by a few factors, such as what labs can measure reproducibly, and that is, of course, tied to accessibility of data and increasing ways to analyze different types of data. For my lab, that drive for mechanism leads us to questions about how does the presence or absence of a micro-organism or features within a microbial community lead to X, Y, or Z in the host. The desire for how or why frequently leads us on a quest to define a bioactivity.

Sometimes I have decided to study something (e.g., a microbial metabolite or class of metabolites in my lab), because it intrigues me and even bothers me, like a kind of nagging itch. Other times, it has been driven by the fact that we can study it, that is, that we can measure it reliably.

One of the methods that I learned to identify and distinguish different culturable bacterial isolates was to measure their volatile and non-volatile fatty acid profiles. Short chain fatty acids, for example, are highly abundant in mouse intestinal contents and human stool. Because I knew how to measure these and they were relatively easy to reproducibly measure, they are one of the metabolites that I have studied in my lab since we started and that we continue to study. The multiple effects of short chain fatty acids across host cell types and how they culminate in shaping immune responses and affecting other aspects of physiology still intrigues me. Their presence and amounts within the lumen of course are telling us a lot about the gut microbiota that are there and their inputs, not only ingested food stuffs but also perturbations exerted by host biology.

What motivated you to focus on the microbiome, microbial metabolites, and cancer?

W.S.G.: First, why cancer? I trained as a medical oncologist. Why: the patients, the biology, the diagnostic, and therapeutic opportunities captivated me from before medical school and throughout my training in internal medicine. Basic scientific research had, has, and will continue to have an impact on cancer care. My answer might seem generic; undoubtedly,

it was the individual patients, physicians, and scientists that I encountered that shaped my affinity for the field. I can remember some advice from one of my mentors, Dr. Ralph Steinman. During my qualifying exam preparation, he said to me, "Wendy, you need to pick *a disease*." In my memory, there was a strong emphasis on the last two words.

I can remember thinking, just one, just one. Cancer seemed so broad and complex and so fraught with unmet medical need, it still is, that it seemed like the right answer for me.

Clinically, I am a medical oncologist with sub-specialty training in gastrointestinal malignancies. In my lab, I have not successfully chosen one disease. I am curious about many aspects of human physiology and many diseases interest me, although I try to maintain focus.

Why gut microbiota? I worked with salmonella as a graduate student, or rather salmonella taught me how dendritic cells might regulate membrane ruffling and antigen internalization. Learning about salmonella, led me to become very interested in enteric bacteria, mucosal immunology, and the gut overall. Developing those interests in graduate school was a springboard for studying the gut microbiota, especially having read some of the classical work of Metchnikoff and Pasteur along the way. Scientists have been thinking about the gut microbiota for a long time.

Why gut metabolites? I have a keen sense of smell and I think that may be a key reason. At some point, I came to realize, or was told, that many of the things that I could smell were related to volatile gut microbial metabolites. I was a biophysics and biochemistry major in college and that gave me a healthy respect for metabolic pathways, many of which were elucidated using bacteria. I think I was also really interested in understanding what factors beyond toll-like receptors, NOD-like receptor, and C-type receptor ligands influenced immune cells.

E.E.: We've been mechanistically studying the interactions between commensals and host cells for more than a decade. During this time, we became intrigued by the fact that microbiome communities could not only impact local cells, organs, and processes but also remotely located environments from the microbial niche. We (and others in the field) increasingly appreciate that metabolites, the thousands of small bioactive molecules produced or modulated by the microbiome, may explain some of these remote effects as they are able to influx into the host and reach distant cells and bodies. In a way, these metabolites may be regarded as the letters of the "alphabet" used in host-microbiome interactions. We try to tease their functions in different disease contexts and are even able to show that supplementation of depleted metabolites (termed postbiotic therapy), or blocking of their downstream effects, could be used as treatment interventions in some clinical contexts. This becomes especially important in our quest to unravel causative connections between the microbiome and cancer; such bioactive small molecules could impact remote primary tumors, metastasis, and even body sites impacted by cancer treatment. We are actively trying to investigate how commensals and their secreted products may impact these cancer-related processes.

Wendy, what are some of the key questions that interest you in this field?

W.S.G.: Overall, I am really excited to think about how the gut microbiota affects response to therapies for different cancer and inflammatory bowel diseases. I am interested in how gut microbiota features (different metabolites, peptides, enzymes) shape immune responses that inform susceptibility to disease and response to different therapeutics such as immunotherapies. I am hoping that we can learn enough about the gut microbiota and its metabolites to identify ways to tune the immune system to respond and heal from not only cancer therapeutics and immunomodulatory drugs for other diseases but also to afford more effective and durable responses to a panoply of infectious diseases.

Eran, which recent advances have you found exciting?

E.E.: The cancer-microbiome field has seen an explosion of advances in the past five years. The microbiomes at mucosal surfaces (such as the gut, oral cavity, skin, and genitourinary tract), and also low biomass microbial communities found within cancer niches (the "tumor microbiome") are increasingly suggested to impact cancer-related processes in these niches and also systemically. Mechanisms for these effects include contact-dependent and -independent influences, including on immune responses, as well as emerging impacts mediated by commensals and their products on cancer therapy responsiveness, in particular cancer immunotherapy. While causality and the mechanisms of many of these intriguing observations still merit investigation, these advances constitute a solid framework for the emerging cancer-microbiome field. Within the scope of these findings, secreted microbiome compounds, such as metabolites, microbial-associated molecular patterns and even outer membrane vesicles (OMVs) are increasingly suggested to participate in these cancer-related processes, while impacting tumor growth, metastasis and treatment responsiveness. Equally exciting are preliminary microbiome-based interventions aiming to exploit these discoveries in cancer. These include microbiome transplantation potentially improving cancer immune therapy, precision probiotics, personalized dietary interventions, metabolite supplementation, cancer-promoting microbe elimination by antibiotics and phage cocktails, and even microbial targeting into tumors. These will constitute exciting avenues of basic and translational research in the coming decade.

And speaking about correlative and causative effects, how do we parse out the difference and dig deeper into the mechanisms associated with these observations?

W.S.G.: That is the big question: the crux is to determine what is causal in what correlates. I think preclinical models remain key and to me that really means mice, especially gnotobiotic mice in which we can model not only relevant tumors but human microbiomes along with human diets when we test metabolites of interest.

E.E.: Advancing from correlation/association/prediction to causation and mechanistic insight constitutes, in my view, the biggest and most exciting challenge of our field. Achieving such level of understanding will enable us to tease apart the many microbiome changes that

are secondary to disease processes ("passenger changes") from the fewer, more biologically interesting microbiome changes that contribute to physiological and disease-related processes ("driver changes"). The latter changes will likely emerge to become potential druggable therapeutic targets. To achieve this level of knowledge, we apply a variety of methodologies. These include *in vitro* pipelines designed to test a large number of microbiome-related metabolites for effects mediated on cell lines relevant to cancer research. While such systems are usually reduced in complexity and do not fully recapitulate the *in vivo* scenario, they allow for high-throughput assessment of large numbers of candidates that are then validated by more complex and real-life-simulating modalities. The next level of causative microbiome metabolite research involves organoids and organ-on-chip systems that more accurately reflect human physiology. While limited in their throughputs, such systems enable us to validate *in vitro* generated findings for their impacts on differentiated cellular structures. Recent advances have included the addition of components such as microbes and immune cells into these modalities, further enhancing their physiological relevance. Ultimately, our most robust modality enabling mechanistic microbiome research involves small animal studies, including genetically-modified models (in studying human genes involved in host-microbiome interactions), and germ-free and antibiotics depleted mouse models that enable the testing of effects of transferred microbial consortia and/or their secreted metabolites on cancer-related phenotypes. As each of the above modalities has inherent limitations, we try to integrate multiple methods and readouts in addressing microbiome-related topics, in striving to reach a comprehensive understanding of how microbes and their products impact cancer-related processes.

You mention how different methodologies could be useful in answering questions on causality and mechanisms. What other tools and approaches are currently used and what are the associated challenges?

W.S.G.: We often use targeted and untargeted mass spectrometry-based metabolomics and proteomics to identify features from a bacterial monoculture or a complex microbial community (e.g., stool sample). However, figuring out the unknowns when it comes to the gut microbial metabolome and proteome is a gigantic challenge. Determining bioactivity in gut microbiota sample fractions and particular bioactive metabolites is an enormous task. Although biologists have many cell-based interrogative assays and solid preclinical models in organoid cultures and gnotobiotic mouse models, to thoroughly interrogate for biological effects is a time-consuming process. Genetically tractable micro-organisms or effective methods to generate bacterial mutants of interest as well as unbiased libraries of mutants are extremely helpful tools. However, even when one has a tractable organism perturbing a particular gene or gene cluster and having the desired effect on a metabolite of interest can be far from straightforward.

E.E.: We measure microbial metabolites by a combination of targeted and non-targeted mass spectrometry and NMR-based technologies, looking into polar, semi-polar, non-polar metabolites, and more recently, volatile metabolites. These are coupled with advanced computational pipelines that are aimed at identifying candidate bioactive metabolites within these extensive datasets and also integrate them with other metagenomic datasets to reach more comprehensive conclusions on the small molecules and their biosynthetic pathways. Importantly, in striving to go beyond correlations and predictions, candidate metabolites are then experimentally assessed (using a variety of *in vitro*, *ex vivo*, and *in vivo* approaches) for their potential biological impacts at the gene, pathway, cellular and organismal levels. Our overarching goal, once sufficient knowhow is reached, is to also test candidate bioactive molecules for their impacts on human disease. With these advances notwithstanding, we are also facing many challenges in "maturing" as a field-improving reproducibility and rigor; harmonizing sample collection, processing, sequencing and analysis; developing new computational methods enabling the integration of datasets; and developing more human-relevant models allowing us to better study causal impacts of discrete microbiome members and associated molecules on disease pathogenesis.

How has our view of cancer changed with the evolving understanding of the role of microbial metabolites?

W.S.G.: I hope it has opened our eyes to new opportunities and the increased complexity of these biological systems (micro-organism and tumor microenvironment and the importance of microbial metabolites). I see tremendous opportunities in microbial metabolites of ingested foodstuff and drugs and co-metabolites (metabolites that are produced as a result of host and microbe) for a variety of health and disease states.

E.E.: While immense strides have been made in the past few decades in understanding the biology of cancer, we remain limited in our current understanding of how environmental, nutritional, and microbial factors impact cancer development, progression, and response to treatment. We regard the microbiome as a "signaling hub" that may integrate these signals, together with immune and metabolic signals coming from the host, to impact cancer-related processes. Understanding these interactions will enable us to better direct cancer treatment to the individual and exploit the microbiome and its metabolites toward new avenues of preventive and curative interventions as part of precision medicine.

Finally, what do you think are the questions that will shape the future directions of the field?

W.S.G.: At the basic level, the questions have not changed—the "how," "where," "when," "why" questions—whether it pertains to an exposure increasing risk or susceptibility to cancer or a metabolite that shapes an immune response that is beneficial to the host in terms of anti-tumor immunity. Like many people, I am interested in how microbial metabolites (often generated from ingested foodstuffs) influence a variety of physiological parameters from healthy human development to propensity for side effects in response to a treatment, e.g., radiotherapy, chemotherapy, immunotherapy. For sure the COVID-19 pandemic, brings questions of infectious disease preparedness and

understanding if and how our microbiomes influence our immune response to infectious diseases to the fore; but cancer remains a pressing issue at the forefront of my mind where I think and hope understanding more about microbial metabolites hold opportunity for cancer patients.

E.E.: While major conceptual advances in microbiome studies have been made in the last years, understanding how microbial signals may impact cancer remains in its infancy. Tremendous questions remain open: how do microbiome-modulated small molecules reach the tumor microenvironment and which processes do they modulate? How do intra-tumoral microbes reach the tumor site and what are their functions once there? Do microbial antigens presented on tumor and immune cells impact tumorigenesis? Can we better understand how nutrition impacts cancer through its interactions with the microbiome? How do the non-bacterial microbiomes impact cancer? Are there niche-specific signals generated by local microbiomes, such as the female reproductive tract, that impact cancer development in those particular sites? Addressing these questions will constitute exciting avenues of microbiome research in the coming decade and hopefully enable the advancement of data-driven interventions targeting the microbiome in preventing or treating cancer and its complications.

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