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TARGETS & MECHANISMS

The tumor microbiome emerges as a new source of translational opportunities

BY KAREN TKACH TUZMAN, ASSOCIATE EDITOR

While the gut microbiome has attracted wide attention for its influence on anti-tumor immunity, a growing body of research suggests microbes living in the tumor itself may play even broader roles in cancer. At least two companies are acting on the data, but an early challenge will be sorting out true tumor microbiome signatures from sample contamination.

Since 2015, several high profile studies have linked gut microbiome composition to checkpoint inhibitor response, both as a means of patient stratification and as an opportunity for therapeutic intervention. The connection between gut microbes and anti-tumor immunity lies in the vast numbers of immune cells in lymphoid tissues surrounding the gut, whose activity can be influenced by the bugs (see "Checking Out the Microbiome").

But it turns out that tumors have their own microbiomes, and early findings suggest they play roles in tumor growth and chemotherapy response, as well as in anti-tumor immunity.

"A tumor is an immune compromised environment, and anaerobic. It makes sense bacteria can survive and thrive there," said Jonathan Solomon, CEO of BiomX Ltd, which is developing a preclinical phage therapy to treat colorectal cancer.

Research in the nascent field is starting to pick up steam, fueled by improvements in sequencing, cell culture and analysis tools, plus a growing interest in the tumor microenvironment, said Solomon. "There has been work over the last 15-20 years, but in the last year or so, there's been an explosion."

The bulk of the work still falls within the realm of the gut, but focuses on the microbes residing within gastrointestinal cancers. Investigators are also beginning to characterize the microbes that populate tumors in other barrier tissues, such as the skin and lung, and even supposedly sterile tissues like the pancreas.

A key study came from the lab of Ravid Straussman, a professor of molecular and cell biology at the Weizmann Institute of Science, who demonstrated the presence of bacteria in tumor samples from patients with pancreatic ductal adenocarcinoma (PDAC) in a 2017 paper in *Science*. Straussman is collaborating with Merck KGgA to look for predictive markers of response to PD-1 inhibition in a range of indications.

"Depending on what we find, we will determine if something is druggable," said Brian Rabinovich, a director at Merck KGgA.

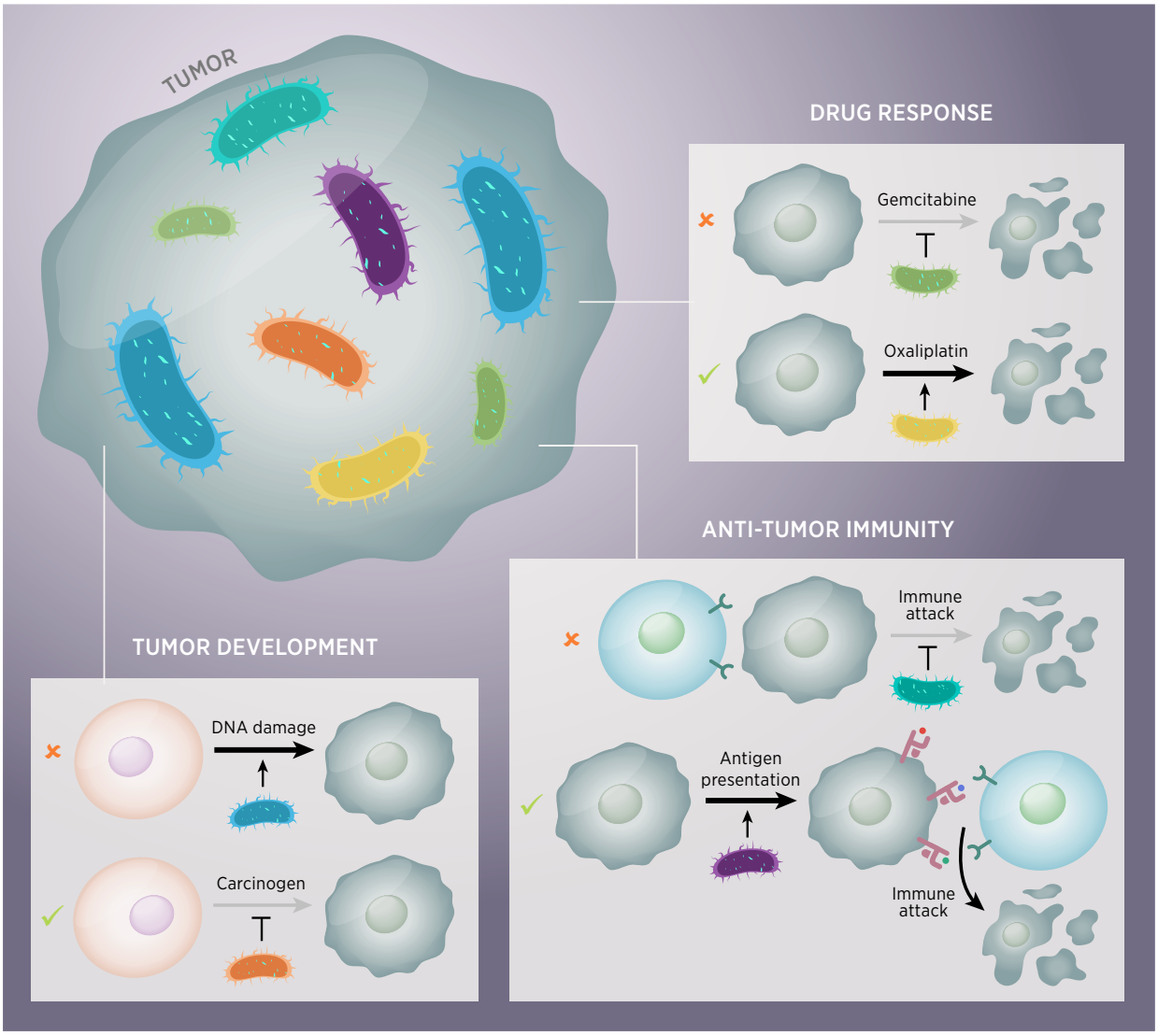
Two sides to the tumor microbiome

Emerging research on the tumor microbiome suggests bacterial strains inside tumors may have detrimental (**red X**) or beneficial (**green check**) effects on patient outcomes via their roles in tumor development, drug response or anti-tumor immunity.

Tumor development: Bacteria can promote tumor formation in surrounding tissues by instigating DNA damage; a pair of 2015 biochemical and mouse studies showed specific strains of *E. coli* induce DNA damage via the bacterial toxin colibactin. But bacteria can also oppose tumor formation via metabolic processes that inactivate carcinogens; a 2018 epidemiological study showed certain types of oral bacteria reduced smokers' risk of head and neck cancers by breaking down tobacco carcinogens.

Drug response: The tumor microbiome can contribute to chemotherapy resistance by metabolically inactivating gemcitabine, as shown in a a 2017 paper. But a 2013 paper showed some bacteria increase the efficacy of oxaliplatin chemotherapy by promoting formation of reactive oxygen species.

Anti-tumor immunity: Bacteria can protect tumors from immune attack by triggering immunosuppressive pathways, as was the case in a 2015 study. In a review article, a team from **University of Texas MD Anderson Cancer Center** hypothesized the tumor microbiome could also promote anti-tumor immunity by generating DNA damage that results in the generation and presentation of tumor neoantigens.



The proliferation of tumor microbiome studies was reflected at this year's American Association for Cancer Research (AACR) meeting, with presentations on the microbiomes of pancreatic, breast and colorectal cancers (see "AACR Moves in a Myeloid Direction").

Better understanding of the microbes in tumors could open up new classes of biomarkers, targets or bacteria-derived therapeutics.

The picture that is emerging is complicated, however, and it will take some time to unravel which bacteria are harmful and which are beneficial in particular tumor environments. The field also still faces technical challenges related to the small numbers of the microbes in tumors and the ubiquitous contamination of samples with bacterial DNA from the environment. The fear is that contamination will lead drug developers down wrong roads.

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Friends or foes

There is evidence that the tumor microbiome could be helpful or harmful through effects on tumor development, drug responses and anti-tumor immunity (see Figure: "Two Sides to the Tumor Microbiome").

Straussman thinks perturbing the tumor microbiome is a "double-edged sword," and researchers should learn more about its biology before developing therapies that augment or kill parts of it.

In tumor development, bacteria can cause DNA damage, leading to cancer, according to preclinical studies. On the other hand, clinical epidemiology studies have shown the presence of oral microbes can reduce the risk of head and neck cancer because the organisms break down tobacco carcinogens.

New York University's Jiyoung Ahn, who led the epidemiology studies, told BioCentury her team is in discussions with an undisclosed biotech about developing bacterial cell therapies to replicate this protective effect. Ahn is an associate professor of population health and environmental medicine at NYU's Langone Medical Center.

In drug treatment studies, bacteria have been shown to either increase or decrease response to chemotherapy. Straussman's 2017 paper showed mice with colon cancer became resistant to gemcitabine when the tumors contained a certain type of bacteria, and the effect could be reversed with antibiotic treatment. However, other researchers have shown antibiotics weakened the effects of oxaliplatin in mice by preventing microbial bacteria from generating reactive oxygen species that help kill cancer cells.

Fewer studies have yet explored the tumor microbiome's effect on anti-tumor immunity, but contrasting hints are emerging there as well. In one paper, a commensal strain prevented NK cells from attacking colorectal tumors by binding the inhibitory receptor TIGIT. Other studies have implicated the DNA damage caused by bacteria in the promotion of immune responses.

Wheat from chaff

Another complication is that the tumor microbiome may largely be made up of bystander strains, which could make it more difficult to pinpoint translationally relevant strains.

Solomon said BiomX is focusing on *Fusobacterium nucleatum*, a species enriched in colon cancer, "because of the amount of data there, and the fact that this specific bacteria is found in a majority of tumors."

But he said the therapeutic program has so far avoided the need to define *F. nucleatum*'s precise role in colon cancer because its goal is to use the bacteria as a delivery address.

"We've shown that we can get phage to the bacteria inside tumors in animal models," said Solomon. "That opens up the possibility of eradicating the bacteria and taking out whatever activity they have to protect the tumor, but it also offers us the chance to deliver a payload."

A 2017 *Science* study from Matthew Myerson at the Dana-Farber Cancer Institute found *F. nucleatum* in both primary colorectal tumors and their metastases. Based on those data, BiomX thinks its program could be effective against disseminated tumors.

"It seems like the tumor and the bacteria are progressing together and spreading together," Solomon said.

Meyerson, a professor of pathology at Dana-Farber and director of its Center for Cancer Genome Discovery, is part of a team awarded a five year £20 million ($26 million) Challenge Grant from Cancer Research UK to characterize and manipulate the tumor microbiome in colorectal cancer. Among its goals are developing tumor microbiome-based therapies, including engineered microbial cells, small molecules and vaccines.

Meyerson was unable to comment in time for publication.

Clean up your act

Researchers studying the microbiomes of tumors inside the gut have an easier time than those looking outside the gut, because GI tumors tend to be more densely populated with bacteria.

This means analyses of microbes in non-intestinal tumors requires more stringent protocols and tools than typical gut microbiome studies.

"It's a really low biomass microbiome, which is very different from the gut microbiome," said Straussman. "With the gut microbiome, it's

much easier for us to say, I sequence it, I see it, it's there. In the tumor microbiome it's much more difficult.""

The work requires procedures to monitor the contamination introduced by even supposedly sterile lab techniques. "You can find bacterial DNA in almost every kit that we're using," said Straussman.

One way to address the challenge is through vigorous use of controls. "If we contaminate the sample, then we should be able to read this contamination in our negative controls. In our lab, 20% of the samples are negative controls," he said.

Another way to stay on top of contamination is to use multiple bacterial detection methods in parallel, including live bacterial cultures, DNA sequencing, flow cytometry and imaging-based techniques, said Marlies Meisel, an assistant professor in the department of immunology at University of Pittsburgh who is studying the tumor microbiome in liver cancers.

To account for the contamination introduced into patient samples as they transit through the operating room and pathology department on their way to the lab, Straussman triangulates samples from different hospitals, and obtains tumor and control tissues from the same centers.

Results should still be taken with a grain of salt, he said. "Even when you apply all these precautions, it's still risky to say, every bacteria that I report on is really there in the tumor."

Straussman thinks early work on the tumor microbiome should be scrutinized. "When you look back at some of the papers published in the last few years, sometimes you see there are small numbers of samples, and lack of good enough controls."

And while showing a role for bacteria from the tumor microbiome in mice can provide some measure of validation, Straussman said it's important to keep in mind that mouse experiments typically supply bacteria in much larger numbers than are found in tumors.

He thinks understanding the tumor microbiome will require multiple labs at different centers working in parallel, and improvements in metagenomic techniques to better evaluate faint bacterial signals.

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"In the tumor microbiome, there's so much more human DNA than bacterial DNA," he said. "There's been some work looking at reads from whole exome or whole genome DNA, but I'm not sure it really covers all the microbiome that is present in these tumors, because usually you don't sequence deep enough to get all the bacterial DNA."

Solomon said Straussman's cautious approach will be key to avoiding the microbiome field's early mistakes.

"The microbiome for years had been dogged by these false observations," Solomon said. "It's people like him that are paving the road on how to do it properly."

COMPANIES AND INSTITUTIONS MENTIONED

American Association for Cancer Research (AACR), Philadelphia, Pa.
BiomX Ltd., Ness Ziona, Israel
Cancer Research UK, London, U.K.
Dana-Farber Cancer Institute, Boston, Mass.
Merck KGaA (Xetra:MRK), Darmstadt, Germany
New York University Langone Medical Center, New York, N.Y.
University of Pittsburgh, Pittsburgh, Pa.
Weizmann Institute of Science, Rehovot, Israel

TARGETS

TIGIT - T cell immunoreceptor with Ig and ITIM domains

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