CANCER

Probing the tumor micro(b)environment

Bacteria are widespread in tumors, are found within cells, and differ by cancer type

By **Chloe E. Atreya**[1] and **Peter J. Turnbaugh**[2,3]

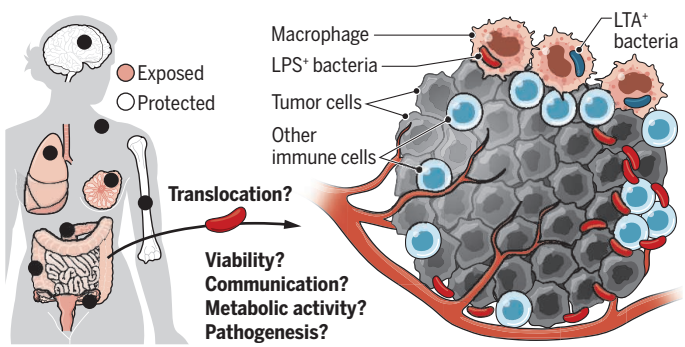
Bacteria have been implicated in the initiation and progression of cancers originating on mucosal surfaces that either harbor a diverse microbial community (microbiota) or are routinely exposed to microbes from the environment (*1*–*3*). Far less is known about the potential for bacteria to influence tumors in body sites that are typically considered sterile. One hypothesis is that the abundant and diverse microbiotas found on mucosal surfaces may exert "remote control" by releasing small molecules into circulation (*4*, *5*). An alternative, nonconflicting hypothesis is that the tumor microenvironment harbors microbes that exert local effects. This hypothesis is supported by the detection of bacteria in a growing number of tumor types (*6*, *7*), although the reliability of distinguishing low-abundance bacteria from contamination has been questioned (*8*). On page 973 of this issue, Nejman *et al*. (*9*) present the most rigorous and comprehensive survey of bacteria in human tumor samples to date.

Nejman *et al*. use a new five-region 16*S* ribosomal RNA gene sequencing method, microscopy, and cell culture to characterize tumor-residing bacteria at known and previously uncharacterized sites. They report that most cancers harbor bacteria, albeit at low diversity except in breast cancer. Surprisingly, these bacteria appear to be intracellular within both cancer and immune cells. Moreover, they report associations between specific bacteria and tumor type and subtype, smoking status, and immunotherapy response.

These results raise multiple important questions for future study (see the figure). For example, is the level of diversity and physiological status of these bacterial cells sufficient to constitute a "microbiota"? Although the defining characteristics of microbiotas remain in flux, two general themes are extensive microbe-microbe and host-microbe interactions, often over long time scales. Are the tumor-residing bacteria found within human cells able to communicate with each other? Prior work (*6*) suggests that bacteria found in tumors can metabolize drugs; however, the overall viability and metabolic activity of tumor-residing bacteria are unclear.

The hidden microbiota inside cancer

Intratumoral bacteria have been detected in both mucosal (exposed) body sites and protected sites. Nejman *et al*. examined bacterial occurrence in multiple primary tumor sites (black dots) and found that tumor cells and immune cells may harbor lipopolysaccharide-expressing (LPS$^+$) bacteria, whereas macrophages contain at least remnants of LPS$^+$ and lipoteichoic acid–expressing (LTA$^+$) bacteria. These findings raise numerous questions that require further study.



The stability of bacterial diversity in tumors remains to be determined. Are tumor-residing bacteria seeded early on in tumorigenesis, or does the tumor alter the microenvironment such that bacteria can continually invade? In mouse models of pancreatic cancer (*7*), the gut microbiota appears to determine which bacteria are found in tumors, suggesting that there is a potential for bacteria to migrate into tumors at later stages. Longitudinal studies in patients with paired analyses of microbial diversity in tumors and mucosal surfaces are an important next step.

Why are bacteria found in tumors? One possibility raised by Nejman *et al*. is that there are always low amounts of bacteria in human tissue, which is supported by analyses of matched normal adjacent tissue from the mammary glands of patients and even healthy controls (*9*). In precancerous conditions, the detection of enterotoxigenic bacteria may portend a generalized procarcinogenic inflammatory state (*10*). After tumorigenesis, disruptions to physical and molecular barriers, together with relative immunosuppression, may increase the potential for bacterial translocation to sites that are normally sterile. This "leakiness" of the tumor microenvironment has been extensively described in the context of vascular permeability; however, the degree to which leakiness enables bacterial invasiveness remains unclear. Additionally, the observation that tumor-associated bacteria are intracellular raises the possibility that the bacteria do not actually move freely into tumors or adjacent tissues—they may be transported there, intact or in fragments, through the migration of immune or cancerous cells.

A key barrier to progress is the lack of representative models for studying tumor-residing bacteria or other low-biomass microbial communities (*8*). Studies of colorectal cancer demonstrate the persistence of viable *Fusobacterium* over successive passages of human tumors in immunodeficient mice (*11*). However, laboratory mice harbor microbial communities (*12*) and immune profiles that are distinct from those found in humans. Development of "triple-humanized" models wherein cancer, immune, and microbial cells are transplanted from patients into germ-free mice may be necessary.

These models could help to untangle the relationships between tumor-residing bacteria and treatment response. By contrast, there is abundant evidence that gut bacteria modulate the immunotherapy responsiveness of cancers, even at distant sites (*4*). Immune cells in the tumor microenvironment also play major and actionable roles, but does this extend to tumor-associated microbes? For a given cancer type, it will be important to determine the contribution of microbial composition relative to other tumor cell intrinsic and

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extrinsic factors that drive malignancy.

The conceptual shift toward studying bacteria within tumors provides challenges and opportunities for translational research. Unlike the microbiotas found on mucosal surfaces, tumor-residing bacteria are not readily manipulatable. Current options for microbiota modulation rely on dietary, pharmaceutical, and microbiological perturbations (*13*). It remains unknown if tumor-residing bacteria depend at all on dietary substrates or if they subsist entirely on host-derived nutrients. Targeting intracellular tumor-residing bacteria also poses drug-delivery challenges; it may be possible to co-opt antibody-drug conjugates or other methods to specifically target bacteria. Although there is a long history of delivering viable bacteria to tumors (*14*), the risks and benefits of this approach need to be carefully considered.

Nejman *et al*. emphasize that diverse bacteria are found on and in the human body. Continued investigation may benefit from the rich history of research on intracellular bacteria in insects and plants. Intracellular bacterial pathogens harbor elaborate machinery to manipulate host cellular pathways (*15*); it will be interesting to see if tumor-residing bacteria encode similar effectors that enable their survival and dissemination. Achieving a comprehensive understanding of the tumor microenvironment is a daunting yet critical step toward an organism-wide mechanistic model of cancer progression and, if successful, may unlock the next wave of precision cancer diagnostics and therapeutics. ■

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GEOLOGY

Tracking the rapid pace of a retreating ice sheet

Seafloor mapping shows that Antarctic ice sheets retreated faster during the last deglaciation than today

By **Martin Jakobsson**[1,2]

Glaciers and ice sheets that extended from land into the ocean left traces behind on the seafloor called submarine glacial landforms. If mapped in sufficient detail and interpreted correctly, they can provide comprehensive information into past behaviors of glaciers and ice sheets. On page 1020 of this issue, Dowdeswell *et al.* (*1*) describe the mapping of glacial landforms in the seafloor created by a rapidly retreating ice sheet on the eastern Antarctic Peninsula. The high-resolution data suggest that the retreat rate was paced by ocean tides and at least an order of magnitude faster than modern rates observed in other sensitive areas, such as West Antarctica where the ice sheet drains into the ocean at several locations (*2*). The retreat on the eastern Antarctic Peninsula took place more than 10,000 years ago, pointing out the challenges in predicting the sea-level rise contribution from retreating glaciers and ice sheets in a warming climate.

Glacial landforms have long been used to reconstruct ice-sheet extent and, specifically, its retreat pace and dynamics. At a meeting in Stockholm 1889 (*3*), the Swedish geologist Gerard De Geer presented observations of moraines, a general term to describe glacial landforms that consist of a mixture of debris (mainly sediments and rock fragments) deposited and sometimes molded by glacier ice. The moraines were observed in Sweden northwest of Stockholm. They generally took the form of ridges, between 1 and 5 m high and a few to slightly more than 10 m wide, gently winding through the landscape for several kilometers. There were several parallel lines of the moraines mapped in the landscape 200 to 300 m apart. De Geer compared the distances between the parallel moraines with the notion prevailing at the time that Swiss glaciers could retreat up to 70 m during 1 year. He put forward a hypothesis that the moraines were deposited during winter along the margin of the Scandinavian Ice Sheet when it made a seasonal halt during its retreat over the landscape. The distance between the moraines of 200 to 300 m represented therefore a yearly retreat rate of the ice sheet. About 11,000 years ago, when the Scandinavian Ice Sheet's margin was located in the area northwest of Stockholm, land was depressed below the contemporary water level of the Baltic Sea (*4*). This meant that the moraines described by

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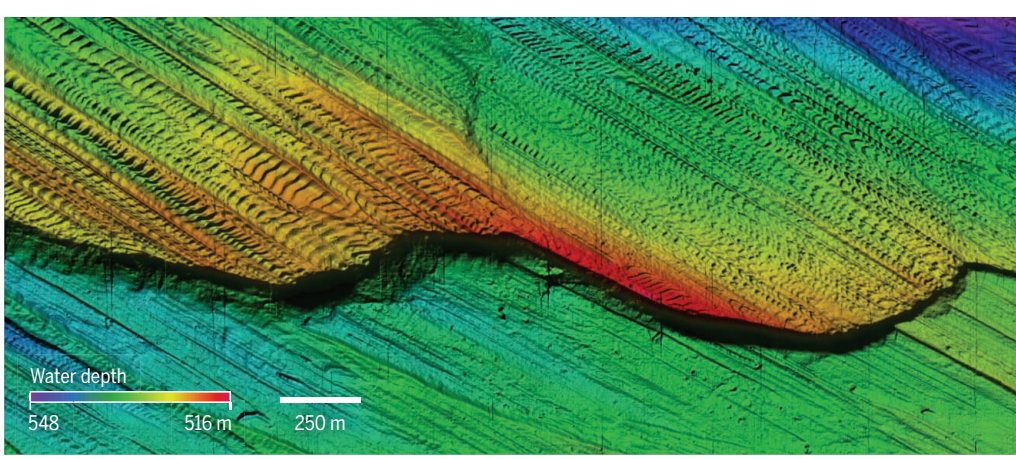

IMAGE: DOWDESWELL *ET AL.* (*1*)

Seafloor imagery of the shape and depth of a grounding-zone wedge complex (where ice transitions from a grounded ice sheet to a floating ice shelf) was derived from an autonomous underwater vehicle-deployed multibeam echo-sounder as it surveyed part of Larsen Inlet, Antarctica. Grid cell-size is 1 m.