The authors took two X-ray polarimetry measurements and, by comparing them with radio and optical polarimetric data, were able to deduce that the initial kick was given to the particles by a shock wave that propagated out along the jet (Fig. 1). Such shock waves occur naturally when particles travelling close to the speed of light encounter slower-moving material along their path[7,8]. Particles travelling through this shock wave lose radiation rapidly and efficiently – and, in doing so, they produce polarized X-rays. As the particles move away from the shock, the light they emit radiates with progressively lower frequencies, and becomes less polarized.

Blazar jets are some of the most powerful particle accelerators in the Universe. Their conditions could never be reproduced on Earth, so they provide excellent 'laboratories' in which to study particle physics. Thousands of blazars have now been detected, and at every accessible wavelength, but the mechanisms by which the particles are emitted and accelerated remain elusive. Liodakis and colleagues' multi-wavelength polarimetric data provide clear evidence of the particle-acceleration mechanism in Markarian 501, making the authors' results a turning point in our understanding of blazars.

This huge leap forward brings us yet another step closer to understanding these extreme particle accelerators, the nature of which has been the focus of much research since their discovery. X-ray polarimetry will now enable us to study several of these jets to understand whether these shocks are common to all sources. The next big unknown is whether electrons alone produce all the light that we see coming from blazars – up to the highest γ-ray frequencies – or whether other charged particles (such as protons) also make them shine[9]. Solving this puzzle will be a milestone for the field, and X-ray polarimetry will no doubt have a crucial part to play in finding the answer.

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Cancer

A spatial perspective on bacteria in tumours

Ilana Livyatan & Ravid Straussman

Bacteria are frequently present in human cancers. The use of state-of-the-art methods for tumour analysis that capture spatial information and single-cell molecular profiles paves the way to clarifying the roles of these microorganisms. **See p.810**

By gathering highly detailed 'portraits' of tumours, on page 810, Galeano Niño *et al.*[1] identify the locations of tumour-associated bacteria. The findings reveal how these bacteria interact with various cell types in the tumours.

A cancer and the associated components that surround it, termed the tumour microenvironment (TME), form a tissue ecosystem consisting of a variety of cells, including immune cells, along with structures such as blood vessels and the extracellular matrix[2]. Many of these constituents affect tumour growth and the tumour's response to treatment, and are thus the focus of a rapidly growing field of study[3,4]. However, these studies have typically been host-centric, even though

"The presence or absence of bacteria correlated with certain characteristics of cancer cells and immune cells."

organisms such as bacteria, viruses and fungi (the microorganisms that constitute the human microbiome) have been detected in the TME of a wide variety of tumours[5–9]. These previous studies[5–9] of the intratumoral microbiome have characterized its microbial profiles, uncovered their tissue-specific nature and indicated that microbes are located mostly intracellularly[5] in both cancer cells and immune cells.

Over the past decade, discoveries about how the human microbiome affects tumour biology[10,11] have resulted in the presence of intratumoral bacteria being designated a hallmark of cancer[12]. Yet our understanding of these bacteria is still rudimentary. This is partly because of a lack of tumour-microbiome studies that pinpoint precise bacterial locations in the tissue and reveal cellular contexts. Galeano Niño and colleagues used cutting-edge spatial and single-cell research methods to advance such investigations.

The authors focused on two types of tumour – oral squamous cell carcinoma and colorectal cancer – for which some cancer-promoting mechanisms mediated by microbes have been described[13]. Galeano Niño and colleagues split each of 11 human colorectal tumour samples into 4 pieces of tissue, and then subjected the tissues to an approach called 16S ribosomal DNA (rDNA) analysis. This revealed that, for seven of the tumours, the microbiome was heterogeneous across the four different pieces. The authors used various methods to explore this bacterial variation and to examine how it correlated with factors in the host.

Applying a technique called RNAscope, which allows visualization of RNA molecules in individual cells, the authors assessed the distribution of specific bacteria, such as *Fusobacterium* (a microbe thought to have a tumour-promoting role), and of bacteria in general, in tumour slices. This information guided the selection of areas of tissue that contained or lacked bacteria, and the selected areas were the focus of subsequent analysis. These regions were further subdivided using microscopy analysis into compartments that consisted of mostly tumour cells or mostly immune cells. The selected regions were also analysed using a technique called GeoMX digital spatial profiling. In this approach, 77 antibodies were used to detect immune-cell-related proteins, proteins characteristic of particular cell types and proteins involved in major cancer-associated signalling pathways. To capture a genome-wide picture of the tissue gene-expression repertoire, the authors used 10X Visium spatial transcriptomic technology, which profiles RNA transcripts while retaining information about their spatial location.

Although both GeoMX and 10X Visium technologies can characterize minute sections of tissue (microniches), they cannot achieve resolution at the single-cell level. As a step in this direction, the authors developed an RNA-sequencing method that they called INVADEseq, which enables human transcripts to be sequenced alongside bacterial rDNA

transcripts in the same cell. However, this method requires the tumours to be dissociated into single cells, and so the spatial context of gene expression in the tissue is lost. Nevertheless, the technique provides a strong cellular context by enabling the detection of cell types that harbour bacteria, identifying the bacteria and sampling the transcriptional profiles of host cells with and without bacteria. Reassuringly, INVADEseq identified *Fusobacterium* as one of the most abundant types of bacterium in oral squamous cell carcinoma and colorectal cancer, a finding consistent with previous work demonstrating the presence of *Fusobacterium* in many tumours of the gastrointestinal tract[14].

The authors report that the presence or absence of bacteria correlated with certain characteristics of cancer cells and immune cells (Fig. 1). Crucially, many of the findings were supported by multiple lines of evidence, strengthening their validity. The researchers found that tumour areas that contained bacteria were generally more immunosuppressed than were areas that did not contain bacteria. Human proteins associated with immunosuppression, such as PD-1 and CTLA4, were highly expressed in bacterium-rich areas of tumours, whereas hallmarks of cell proliferation, such as the protein Ki67, were depleted, providing another indication of a reduced immune response. Immune cells such as myeloid cells (specifically, macrophages and neutrophils) were preferentially found in these areas, but T cells, which can have anticancer activity, were sparse. The presence of bacteria in macrophages was associated with increased expression of genes encoding inflammatory and immune-signalling proteins, such as CXCL8, which is a potent neutrophil attractant.

Bacteria were more common in tumour cells that had an abnormal number of chromosomes than in cells with a normal chromosome composition, suggesting a probable bacterial preference for cancer cells over normal cells. Although the gene-expression profiles of cancer cells that harbour bacteria were specific to the bacterium, some general patterns emerged. These included inhibition of genes associated with proliferation, reduced expression of genes for DNA repair and a rise in the expression of genes associated with cell migration. This finding is reminiscent of a phenomenon called the proliferation–migration trade-off[15] of cancer-cell states, which is another hallmark of tumour heterogeneity.

Some of these observations were validated when the authors performed functional laboratory tests, thus supporting the view that bacteria had a causal effect on the phenomena studied. The authors examined colorectal cancer cells grown using a 3D system (generating groups of cells called cancer spheroids) in which neutrophils were present in the microenvironment. Infection of the cancer cells with *Fusobacterium* resulted in the cancer cells gaining a higher capacity for migration and showing reduced activity in signalling pathways related to proliferation and DNA-damage repair. Furthermore, activated neutrophils were recruited into the infected cancer spheroids.

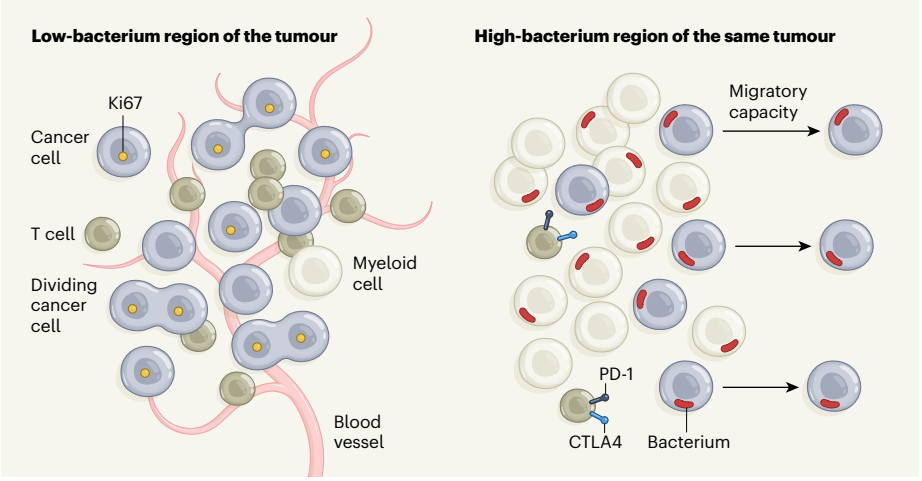

Figure 1 | Characterizing the hallmarks of bacterial presence in a tumour. Certain bacteria can promote tumour growth, but many details are unknown about their location and effects in human tumours. Galeano Niño *et al.*[1] used various techniques to gain spatial insight into the composition of bacterium-rich areas of tumours compared with bacterium-poor areas. The authors report that tumour areas low in bacteria were associated with the presence of more blood vessels, and had high levels of immune cells called T cells and low levels of immune cells called myeloid cells. Cancer cells in such regions showed higher levels of proliferation, as indicated by expression of the protein Ki67. A different picture emerged for the tumour regions associated with bacteria (in which microbes resided inside tumour and myeloid cells). In these regions, myeloid cells were more common and T cells were rare and showed signs of immunosuppression — expression of the proteins PD-1 and CTLA4. Cancer cells in such regions also had a higher capacity for migration.

Galeano Niño and colleagues' research provides a glimpse of how tumour bacteria affect their microniches and the cells in which they reside. The findings indicate a crucial role for intratumoral bacteria, reinforcing the need for more research in this area and demonstrating the technical feasibility of such work. Future studies should investigate a larger number of samples than did the present study, explore further tumour types and possibly harness other technological advances to allow more-detailed profiling of intratumoral bacteria, rather than relying on 16S rDNA profiling. It would also be worth studying microscopic entities other than bacteria in the microbiome, such as fungi and viruses.

The past two decades have seen efforts to advance 'precision therapy' in cancer treatment, because it is thought that a personalized approach might lead to an improved clinical response. Yet, despite huge strides in characterizing various tumour landscapes (such as the genetic underpinnings and immune-cell populations), uncertainty about how this information could feed into treatment decision-making is the rule rather than the exception. This is reflected, for example, in the limited success in using molecular biomarkers to predict treatment outcome, and in our failure to overcome most mechanisms of drug resistance or to control tumour spread. Shifting the focus from host-centric studies to a more holistic assessment of the tumour microenvironment might advance our understanding of cancer biology and open up promising avenues of clinical research. Galeano Niño and colleagues' work shows the potential importance of intratumoral bacteria, and how new technologies can aid the study of this fascinating and still mostly under-studied aspect of cancer biology.

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