Cancer

Microbial signatures in tumours and blood

Nadim J. Ajami & Jennifer A. Wargo

Analysis of nucleic-acid sequences from human cancers, along with samples from adjacent tissue and blood, reveals the presence of microorganisms in tumours and blood across cancers. **See p.567**

The world is filled with microorganisms, which have a profound impact on many facets of life. Do these microbial communities influence cancer[1]? Many studies of microbes and their genomes (collectively called the microbiome) have focused on the gut, where most of the body's microbes reside. This work has revealed a role for the gut microbiome in several types of cancer that arise in the intestinal lining itself[2–4], and indicated that the gut microbiome might influence cancers at distant sites through its impact on the immune system[1]. In addition, emerging evidence indicates that microbial signatures (such as nucleic acids) can be found in tumours at other sites in the body[5,6] and in the tissues and blood of individuals who don't have cancer[7,8]. On page 567, Poore *et al.*[9] build on this evidence, identifying signatures of microbial DNA and RNA, both in tumours and in the blood, across multiple human cancers. The authors further suggest that these signatures might augment existing clinical diagnostic tools, although further work is needed in this area.

Poore *et al.* used The Cancer Genome Atlas (TCGA) — an online resource that includes DNA and RNA sequences — to analyse data for 33 cancer types, totalling more than 17,000 samples from some 10,000 patients. They analysed data sets derived from bulk tumour samples (primary tumours as well as recurrent ones, and tumours that had spread through metastasis), normal adjacent tissue and blood samples. The authors used multiple computational approaches, including independently trained artificial-intelligence (AI) models, to filter, normalize and classify microbial sequences in these samples. After stringent filtering approaches to address potential contamination and other variables, the group classified 7.2% of the total sequencing reads as non-human. Approximately one-third of those mapped to known sequences of bacterial, archaeal or viral origin, and 12.6% of these resolved to a particular genus from one of these groups.

The authors next trained machine-learning models to use these sequences to distinguish between cancer types and between different stages of the same cancer type, as well as between tumours and normal tissue. Overall, the models performed well in discriminating between cancer types and between cancer and normal tissue, but showed some variability in their ability to discriminate between various stages of cancer.

The researchers also tested the biological relevance of the microbial profiles against known microbial associations with cancer. In line with previous reports, they found *Fusobacterium* in gastrointestinal tumours, and viruses such as Alphapapillomavirus and Hepacivirus in cervical cancer, head and neck cancer and hepatocellular cancer.

Poore *et al.* next explored microbial signatures in the blood of people with cancer, using AI models that analysed whole-genome sequences from the TCGA cohort of individuals. Their findings suggest that blood-borne microbial DNA (mbDNA) could be used to discriminate between cancer types. The group sought to validate its mbDNA models against existing cell-free tumour DNA (ctDNA) assays in a separate cohort, which included 69 individuals without cancer and 100 who had prostate cancer, lung cancer or a skin cancer called melanoma (Fig. 1). The authors' models were generally good at discriminating between cancer types, although there were some limitations. Further validation of these results is needed in larger cohorts across cancer types using dedicated methods.

These results, along with another study of microbes in tumours that used TCGA data[6], are provocative. However, the studies had some limitations, suggesting that there is a tremendous opportunity to build on this work.

One limitation is that TCGA samples were not collected in a manner that controlled for contamination by microbes or mbDNA. This contamination could have been introduced at any time between sample collection and sequencing. Poore *et al.* and others[6] tried to control for this through stringent filtering of potential contaminants; however, such approaches might limit our ability to detect the full complement of microbes present in tumours. In addition, DNA and RNA sequencing for human studies might not be performed in a way that enables microbes to be characterized completely. Future studies that build on the current work should involve analysis of carefully curated tissues and blood using appropriate sequencing techniques to allow for characterization of microbial signatures.

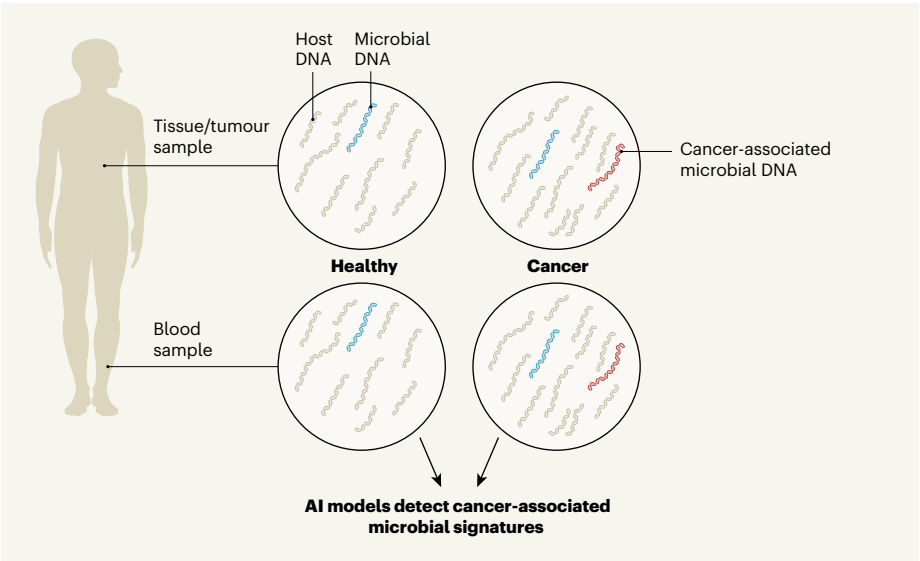

Figure 1 | Microbial signatures of cancer. Microorganisms can inhabit various tissues. Traces of these microbes' DNA and RNA can be found in various tissues, including the blood (here, only DNA is shown, for simplicity). Poore *et al.*[9] built on previous findings[6] to show that microbial DNA and RNA can also be found in tumours and act as a signature of cancer. Artificial-intelligence programs can use these nucleic-acid signatures from tissue or blood samples to discriminate between types of cancer, and between healthy individuals and those who have certain cancers.

In addition to validating the presence of these microbes in tumours and blood in cancer, it will be important to gain insights into their distribution and function. Poore *et al.* and others[6] identified microbial signatures in tumours on the basis of nucleic-acid sequences; however it is not known where these microbes are located (within or around tumour cells, immune cells or in connective tissue known as the stroma) and whether or not they are alive. And more work will be needed to determine whether the microbes are driving cancer or are merely passengers in an altered tumour microenvironment. There are clear examples of how microbes in tumours might contribute to cancer development and to resistance to cancer therapy[3,10]. However, other data suggest that the presence of microbes in tumours is associated with better long-term outcomes[11].

Finally, further mechanistic insights into how microbes enter and persist in cancerous tissue are needed, as well as research into how best to target them for treatment and even cancer prevention. Such strategies will need to be nuanced, and must take into account the potential effect on all microbial niches, because many of the body's resident microbes have a crucial role in overall physiology. Although some preclinical studies suggest that co-targeting microbes and tumour cells with antibiotics and chemotherapy is associated with delayed tumour outgrowth[10,12], other work suggests that treatment with broad-spectrum antibiotics[13] can worsen the outcomes of people receiving immunotherapy, probably owing to disruption of the gut microbiome. Thus, there is context dependence, which must be taken into consideration. Nonetheless, the opportunities for both clinical advances and basic insights that are presented by an ability to monitor and manipulate the microbiome are tantalizing.

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Molecular biology

A reaction chamber for chromatin modification

Nick Gilbert & Fred van Leeuwen

Chromatin, the complex of DNA and protein in cell nuclei, can be modified by ubiquitin molecules. It emerges that this modification occurs in a molecular reaction chamber formed from an enzyme and a scaffold protein. **See p.592**

DNA is packaged around histone proteins to form a macromolecular structure called chromatin. Histones are often modified by the attachment of small molecules, to guide gene activity and genome stability[1,2]. For example, ubiquitin modifications on histone H2B (termed H2Bub) are associated with domains of active gene expression[3,4]. But how these modified domains arise is far from understood. Previous work[4] has indicated a simple model for the formation of H2Bub domains: an enzymatic complex that is needed to add ubiquitin to H2B is recruited when the enzyme that catalyses gene transcription, RNA polymerase II, passes along chromatin. On page 592, Gallego *et al.*[5] provide an alternative model, in which the enzymatic complex forms a liquid-like, 'phase-separated' reaction chamber that adds ubiquitin to H2B, independently of RNA polymerase II.

In yeast, ubiquitin attachment to H2B (a process called ubiquitination) is executed by the enzymes Bre1 and Rad6. A third protein, Lge1, is also required for H2B ubiquitination in yeast ; Lge1 physically binds to Bre1, but its molecular role in ubiquitination has been a mystery[6]. Gallego *et al.* revisited Lge1 because its amino-acid composition indicates that it has an intrinsically disordered region (IDR) containing a 'sticker' sequence at the amino terminus, enriched in arginine, tyrosine and glycine amino-acid residues. Other proteins that contain IDRs have been shown to weakly interact and undergo liquid–liquid phase separation (LLPS) — a process in which proteins self-associate into liquid-like condensates or droplets, similar to membraneless organelles[7]. LLPS is gaining prominence as a concept that can explain key aspects of chromatin structure and function[8–12].

Gallego *et al.* showed that, in a reaction carried out in a test tube, Lge1 undergoes LLPS to form condensates. This process is driven by the protein's IDR — specifically, by tyrosine residues in the sticker region. The authors observed an odd phenomenon when they added Bre1 to the test tube: Lge1 acted as a scaffold around which Bre1 formed a shell, limiting growth of the condensate. The presence of Bre1 also led to the transient accumulation of Rad6 in the shell, along with arrays of nucleosomes (structural units of chromatin consisting of DNA coiled around eight histones). Rad6 and the nucleosomes subsequently spread evenly throughout the condensate. Thus, Lge1 and Bre1 form 'core–shell condensates' that act as reaction chambers, capturing the ubiquitination machinery and its substrate, H2B in nucleosomes.

"The authors' work provides an exciting model to describe the environment in which nucleosomes are modified."

One of the challenges of studying LLPS is testing ideas generated *in vitro* and through modelling, in living cells[13,14]. This is mainly because proteins that have IDRs are not amenable to structural studies, and condensates might be too small and dynamic to be visualized easily under physiological conditions. Using complementary approaches *in vitro* and in yeast cells, Gallego and colleagues provided evidence for the existence of the reaction chamber *in vivo*.

First, by analysing protein sedimentation in cell extracts, the group showed that Lge1 forms a large complex that can capture Bre1. Subsequently, they tagged Lge1 and Bre1 with fluorescent protein fragments to visualize interactions between the two proteins in