A biofilm comprising three species of bacterium (green, cyan and magenta) on a colon tumour.

MICROBIOME

Microbial mystery

Gut bacteria have an important but elusive role in the formation of colorectal cancer.

BY SARAH DEWEERDT

Five years ago, four women and two men donated samples of their gut bacteria to science. Three women had healthy guts, but the other donors had colorectal cancer. Researchers implanted the human gut microorganisms into 'germ-free' mice, which were delivered by Caesarean section and reared in sterile cages, and so lacked gut microbes of their own.

This is a common technique for studying the health effects of the microbiome — the roughly 100-trillion bacteria that inhabit the gut. But the results were unexpected. When the scientists exposed the mice to a chemical mutagen that causes colorectal cancer, mice given microbes from colorectal-cancer patients developed fewer tumours than did mice that had received bacteria from healthy human donors¹. "It was a very weird result," says study leader Patrick Schloss, a microbial ecologist at the University of Michigan in Ann Arbor.

The findings are emblematic of the puzzle researchers face when trying to understand the microbiome's contribution to colorectal

cancer. "There's growing evidence for a role of bacteria in colon cancer, but it's not definitive," says Alberto Martin, an immunologist at the University of Toronto, Canada, who has studied microbial interactions with a biochemical pathway involved in an inherited form of colorectal cancer.

It makes intuitive sense that the gut microbiome has some role in colorectal cancer, whether for good or for ill. The idea is in line with evidence linking the microbiome to liver cancer and to other aspects of health and disease, such as obesity and the regulation of metabolism. Microbes are also known to be involved in the initiation and progression of inflammatory bowel disease, which is a risk factor for colorectal cancer.

What is more, most of the bacteria in the human gut make their home in the colon, or large intestine. "You almost never see cancer in the small bowel," says Jun Sun, a biochemist at Rush University in Chicago, Illinois. "Most bowel cancer originates in the colon." In other words, the cancer occurs where the bacteria reside.

There is more to the connection between the microbiome and colorectal cancer than theory and logic. Over the past decade or so, experimental evidence from animal studies, comparisons of gut bacteria from people with and without colorectal cancer, and analyses of the biochemical workings of individual bacterial strains all point to a link. But a full picture of how the microbial community in the gut acts and reacts during the development and progression of colorectal cancer remains elusive.

Many studies have documented differences between the microbiomes of people with colorectal cancer and people with healthy guts. The patterns vary widely from study to study, however, so scientists cannot say much about the microbiome abnormalities, or dysbiosis, in colorectal cancer. The most consistent finding is a high level of *Fusobacterium*, which is commonly found in the mouth but rarely in the healthy gut. Several studies have also shown that people with colorectal cancer have higher than normal levels of *Escherichia coli* bacteria².

But colorectal cancer can take decades to develop, so such differences may reveal little about the origin of the disease, says Christian Jobin, a microbiologist at the University of Florida in Gainesville. "What was cooking in their gut before the cancer diagnosis?" he asks.

Researchers have tracked changes in the microbiome throughout the course of colorectal-cancer development in mice, but there have been no such longitudinal studies in people. In 2014, however, Schloss and his colleagues reported differences between the microbiomes of three groups of people: those with healthy colons, those who had precancerous lesions called adenomas, and those with colorectal cancer. Adding microbiome data to conventional risk factors, such as age, race and body mass index, improved the ability to predict which of the three clinical categories a person belongs to — hinting at the potential to use microbiome analysis in colorectal-cancer screening.

But the cause-and-effect relationships still remain unclear. "What comes first — is it the cancer, the disease process, or the dysbiosis?" asks Shahid Umar, who studies gut bacteria at the University of Kansas in Kansas City. "That's the million-dollar question."

OF MICE AND BACTERIA

Direct evidence that gut-dwelling microbes help to cause colorectal cancer comes mainly from studies on mice. For example, Schloss and his colleagues transferred the gut microbiome from mice with colorectal cancer into germ-free mice, and then exposed these mice to a chemical mutagen. As long as this technique is used to transfer gut microbiota within a species, the results are straightforward: mice that received their microbiome from donors with cancer developed more and bigger tumours than mice that received their microbiome from healthy donor mice.

The researchers also showed that mice

exposed to the chemical mutagen do not develop tumours if they receive a dose of antibiotic that kills off some of the microbiome, suggesting that the microbiome plays a part in the onset of cancer. And they found that mice that receive a cancer-causing microbiome do not develop tumours if they are not exposed to the mutagen — so the mutagen is essential too. Other research groups have also found that cancer does not develop without the presence of certain bacteria, but that these bacteria do not, on their own, trigger tumours.

To unravel the biochemical mechanisms underlying these patterns, scientists have simplified matters by investigating individual bacterial species. This research has focused mainly on three types of bacterium: *Fusobacterium*; strains of *E. coli* that produce colibactin toxin; and toxin-producing strains of *Bacteroides fragilis* (a common cause of diarrhoeal illness). All three of these bacteria increase tumour formation in mouse models that are susceptible to colorectal cancer. For example, cancer-prone mice that are raised in a sterile environment and then colonized with *Fusobacterium* develop more tumours than mice not exposed to the bacterium.

Not all species of bacteria have this carcinogenic effect, however. Jobin's team has shown that immune-compromised mice colonized with either *E. coli* or *Enterococcus faecalis* (another common gut species) develop colon inflammation, but only the mice that receive *E. coli* develop tumours. Inflammation is clearly important: transfer *E. coli* into a mouse that cannot produce an inflammatory response, and no tumours appear. It seems that an inflammatory environment in the gut changes the pattern of *E. coli* gene activity, Jobin says. To confirm this hypothesis, he has shown that deleting these inflammation-induced genes from *E. coli* removes the microbe's ability to cause cancer³. The carcinogenic effects of *B. fragilis* also depend on inflammation, according to Cynthia Sears and her colleagues at Johns Hopkins University in Baltimore, Maryland, who showed that the bacterium does not produce intestinal tumours when interleukin (IL)-17, an inflammatory molecule, is blocked⁴. Sears says that IL-17 is important in tumorigenesis in a variety of other body sites as well.

These animal studies paint a clear picture. "All this tells us that it's the microbiome," says Schloss. "And also importantly, it tells us that it's not just the microbiome."

HUMAN VARIATION

Demonstrating that similar processes occur in humans has been problematic, as illustrated by Schloss's study that found fewer tumours, not more, in mice that received microbiomes from human colorectal-cancer patients. But

transferring microbiomes between species is an unnatural process and might be expected to throw up murky results.

Even so, there is some evidence that links *Fusobacterium*, *B. fragilis* and *E. coli* to colorectal cancer in humans. For example, *Fusobacterium* is more abundant in precancerous adenomas than in other sites in the colon. And the gene encoding *B. fragilis* toxin is present in the colons of 90% of people with colorectal cancer but in about half of the colons of healthy people. Sears points out that this is similar to

Transmission electron micrograph of bacteriophages, which could be used to kill strains of bacteria implicated in colorectal cancer.

observations for stomach cancer, which is associated with the bacterium *Helicobacter pylori*.

But it would be an oversimplification to think of the microbiome's role in colorectal cancer primarily in terms of a pathogenic infection by one or two troublesome microbes. "There are cases where individual bacteria seem to be associated with tumours," Schloss says. But far more often, he says, there is a "community effect" — a more generalized dysbiosis that may enable an individual cancer-promoting bacterium to gain a foothold, or to alter the microbiome's overall function in a way that leads to tumour formation.

Dysbiosis can have many different causes, but a common one is diet. High-fat Western diets have been linked to the development of colorectal cancer. Studies into the role of diet and fibre intake on colorectal cancer have yielded inconsistent results. Some researchers say this is because these studies have not taken into account people's different microbiota.

For example, individuals differ in their microbiome's capacity to produce butyrate, a fatty acid made by certain microbes when they break down dietary fibre. Butyrate is important for the health of the intestinal epithelium, which is one of the body's most rapidly renewing tissues — Umar points out that all the cells in the epithelium are replaced every 3–5 days.

Butyrate fuels this energy-intensive process and acts as a potent anti-inflammatory agent. It also feeds into important biochemical pathways, such as the Wnt and Notch pathways, which regulate epithelial-cell proliferation

and have been implicated in colorectal cancer. "You need very controlled and regulated activity of these pathways," Umar says. Changes in microbial products — either the absence of butyrate or the presence of other metabolites — can affect these pathways and increase the proliferation of gut epithelial cells, a key step towards cancer.

Researchers should also consider the genetic background of individuals who have a particular community of microbes. Studies of mice with a genetic defect linked to a familial colorectal-cancer syndrome suggest that in individuals with this genetic background, butyrate can actually promote the development of colorectal cancer.

About 20% of colorectal cancers have similar genetic defects, which affect DNA-mismatch repair⁵. This means that it is crucial to carefully select the patients or subjects used in studies if we are to understand the effects of the microbiome on colorectal cancer, says Martin, who led the mismatch-repair work.

Unravelling all these effects is only the first step to understanding what causes colorectal cancer. "What we are after is not so much the nature of this dysbiosis, but whether we can do something about it," Jobin says. Some scientists have tentatively wondered

about using antibiotics to knock out cancer-promoting gut microbes but worry about the potential effects on the microbiome as a whole. Jobin has a different idea: develop bacterium-killing viruses known as bacteriophages to target specific strains of bacteria that are implicated in cancer while leaving the rest of the microbiome to rumble along as usual.

Other scientists suggest that diet or probiotics (microorganisms thought to provide health benefits) could potentially manipulate the composition of the microbiome. However, this strategy needs a lot of work because researchers have had difficulty using probiotics to induce stable, long-term changes in the microbiome.

But microbiome interventions have shown promise for treating other gastrointestinal conditions. For instance, persistent infection with the bacterium *Clostridium difficile* responds to the unlikely intervention of faecal transplantation. Such examples offer hope that one day a microbial solution might also be found for colorectal cancer. ■

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