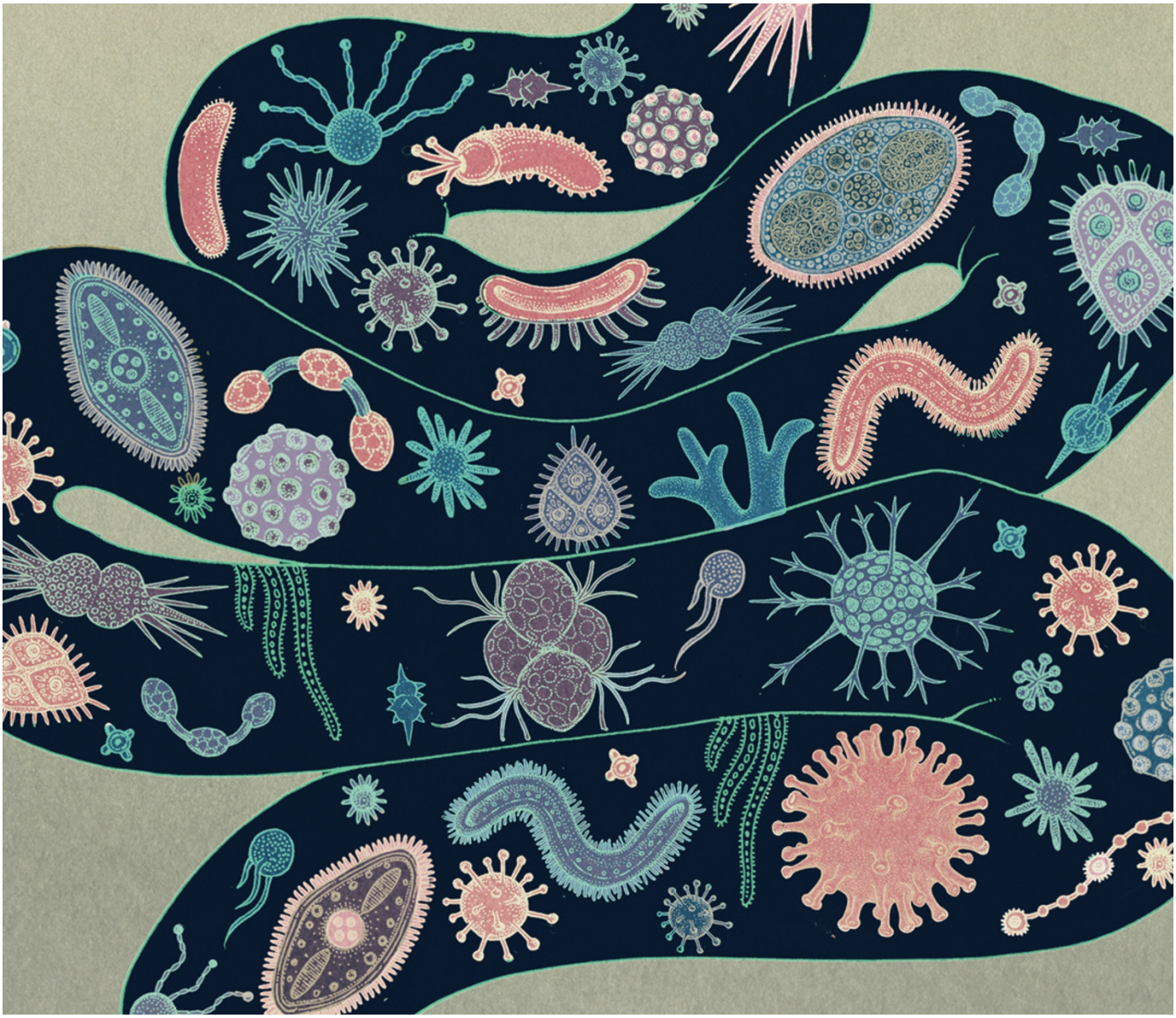


KATIE SCOTT

MICROBIOME

The bacterial tightrope

Imbalances in gut bacteria have been implicated in the progression from liver disease to cancer. This insight opens the way to preventive treatments.

BY KATHERINE BOURZAC

In 2012, Eiji Hara was studying the effects of a high-fat diet on cancer risk in mice. Researchers had known for many years that obesity was associated with liver cancer; Hara wanted to find out why. After deep study of the connection, he found something unanticipated: a link to bacteria living in the gut[1].

"We never expected a connection with the microbiome," says Hara, who is a biologist at the Japanese Foundation for Cancer Research in Tokyo. Yet when he and his colleagues exposed obese mice to a carcinogen that normally causes liver cancer and then gave them antibiotics, they found that killing the bacteria had a protective effect: the animals did not develop the disease.

The team's research, published last year, suggests that gut bacteria — which are part of the microbiome of bacteria and other microorganisms that live in and on the body — can play a crucial part in liver-cancer progression. If further studies bear out Hara's results, they might help to answer some fundamental questions about the disease itself, and potentially lead to new ways to prevent liver cancer.

Liver cancer almost always develops in the wake of preceding problems, such as fatty liver disease (see page S8) or viral hepatitis (see page S12). But it is not clear why some people with such problems go on to develop cancer, whereas others do not — only about 3% of cases become malignant each year. Still, "liver cancer without

liver disease is very rare", says Robert Schwabe, a gastroenterologist and liver researcher at Columbia University in New York. Schwabe, Hara and others think that certain elements in the microbiome might tip the scales towards cancer. If they are right, it could be possible to stop liver disease from turning malignant by targeting microbes in the gut.

GUT FEELING

There are trillions of microorganisms in the human microbiome — they outnumber their host's cells by around ten to one — and their exact role in health and disease is only now starting to be explored. Studies have found that people with non-alcoholic fatty liver disease have a different composition of bacteria in their gut from healthy individuals[2,3]. "We're not far along enough with this research to say why this is," says Gyongyi Szabo, who investigates the immune system and inflammation in the liver at the University of Massachusetts Medical School in Worcester.

Whatever the reason, changes in the microbiome are not sufficient to cause disease, she says. Instead, she sees an emerging picture of liver disease and cancer as a process in which various factors — including a high-fat diet, alcoholism, genetic susceptibility and the microbiome — can each contribute to the progression from minor to severe liver damage, and from severe liver damage to cancer.

It makes intuitive sense that the gut microbiome would be implicated in liver cancer, says Yu-Jui Yvonne Wan, who studies liver disease at the University of California, Davis. "Whatever comes from the gut enters the liver," she says. As blood leaves the intestines it passes through the liver, carrying nutrients extracted from our food. It also carries our own digestive chemicals as well as fragments of the bacteria that live in the digestive system (such as bits of DNA and cell walls) and their metabolic by-products. Some of these by-products help us to access energy and nutrients in food, but some are toxic. And a few might provoke an inflammatory immune response in the liver — if the inflammation becomes chronic, cancer can be the consequence.

The liver has several crucial functions, and it does more than just filter toxins and produce digestive chemicals. "The liver is an immune organ," says Richard Flavell, an immunobiologist at the Yale School of Medicine in New Haven, Connecticut.

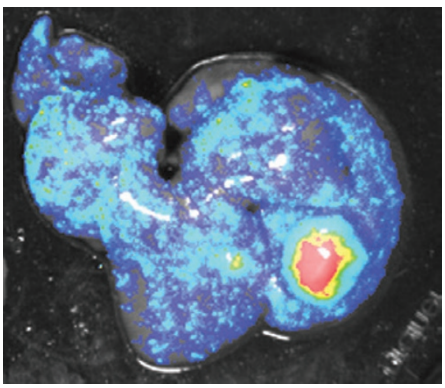
Flavell's research suggests that the liver has an important role in immune surveillance and helps to maintain bacterial balance in the gut. Specialized cells in the liver and intestines monitor the microbiome by keeping tabs on bacterial by-products as they pass through. These cells can detect infections and help to fight them.

But they can also pick up on subtler changes in the bacterial populations in the gut. When certain types of bacteria become too numerous — a state called dysbiosis — the immune system becomes activated and triggers inflammation, although at a lower level than it would for an infection. This usually goes undetected because it is not painful in the same way as arthritis or other kinds of inflammation, says Michael Karin, a molecular biologist at the University of California, San Diego, who studies the connection between inflammation and cancer. "Liver inflammation is an unappreciated, silent disease," he says.

Immune reactions aimed at fighting infectious bacteria and other foreign invaders can have off-target effects that lead to disease. Flavell's work suggests that dysbiosis causes inflammation that contributes to the worsening of non-alcoholic fatty liver disease in mice[4]. And certain cell-signalling molecules that are characteristic of inflammation can also encourage the growth of cancer cells. Now, research is emerging that suggests that dysbiosis and the immune reaction it provokes can even contribute to cancer.

Some of the earliest evidence of this comes from Schwabe, who studies the connection between liver injury and cancer. His work indicates that the immune response to the microbiome drives the progression of cancer in mice. "We discovered that cells in the liver express [immune] receptors that bind to bacterial products," Schwabe says, and the main culprits are lipopolysaccharides, large molecules that are found in the cell walls of many bacteria. When his group genetically engineered mice to lack Toll-like receptor 4, an immune receptor that lipopolysaccharides often bind to, the animals did not develop liver cancer.

Like Hara, Schwabe then went on to connect cancer progression to the microbiome by testing two groups of mice: one in which the animals had been isolated from birth so that they were completely bacteria-free, and another in which they had been dosed with strong antibiotics. All of the animals were treated with the same liver-damaging carcinogen as in the first experiment, but both groups remained cancer-free. For the disease to occur, the mice needed all three factors: an active immune pathway, the harmful bacteria and the carcinogen[5].



EIJI HARA (JAPANESE FOUNDATION FOR CANCER RESEARCH)

Following provocation from gut-bacteria metabolites, an obese mouse liver expresses cancer-associated proteins (red fluorescence).

Targeting these newly discovered pathways therapeutically is a difficult proposition, however. Knocking out Toll-like receptors, which are a vital part of the immune system, is not practical. Nor is a broad attack on all bacteria that make lipopolysaccharides, because both harmful and helpful bacteria (such as *Lactobacillus* strains found in yogurt) have these molecules in their cell walls.

Because the most harmful strains of bacteria have not yet been pinpointed, and because the specific details of the inflammatory process are still unclear, researchers need more information before they can target this process therapeutically. "Right now we're working on finding the exact mechanism of cancer progression and the exact cell type in the liver that is targeted by lipopolysaccharides," Schwabe says.

It makes intuitive sense that the gut microbiome would be implicated in liver cancer.

He thinks that at least part of this mechanism involves disruption in the balance of the various species of bacteria in the gut. An out-of-balance microbiome promotes a constant state of inflammation, which can contribute to cancer progression, Schwabe says. This aligns with the picture that is emerging of cancer, in general, as an inflammatory process: the same immune reactions that help the body to fight infection and disease can also promote unchecked cell growth.

OUT OF BALANCE

Another common bacterial product might also serve as a connection between the microbiome and liver cancer. The liver generates bile acids, which are stored in the gall bladder and are released into the intestines to digest fatty foods. They act as a kind of digestive detergent, helping the body to take up dietary fat along with fat-soluble vitamins. The acids then get absorbed by the intestines and passed back to the liver, which sends them back to the gall bladder.

Some gut bacteria chemically alter these bile acids to produce secondary bile acids, which are toxic to humans and even to certain bacteria. The most common of these secondary bile acids is deoxycholic acid (DCA). A certain amount of DCA is present in everyone's digestive system, and at low levels it does not seem to have ill effects. It carries fat in the same way as our own bile acids and follows the same path from gut to liver to gall bladder. But it can cause DNA damage, which in turn can lead to cancer.

An abundance of DCA can also lead to dysbiosis, which not only is a link in the cancer-progression chain, but also has been connected to obesity. Some of the earliest research on the human microbiome, published in 2006, demonstrated that the balance of gut bacteria in obese people is different from that in people of healthy weight. In particular, obese people tend

to have greater numbers of the bacteria that produce DCA and other secondary bile acids. The result is a positive-feedback loop, says Wei Jia, a pharmaceutical chemist at the University of Hawaii in Honolulu. DCA kills certain bacteria, which leads to a greater preponderance of the DCA-producing strains. "It really changes the composition of the microbiota in the intestine and can cause dysbiosis," Jia says.

Hara's work in Tokyo, which connected liver cancer to the microbiome of mice on a high-fat diet, also points to DCA[1]. Hara originally intended to study the connection between obesity and liver cancer, so he treated his mice with a carcinogen at birth and fed half of them a high-fat diet. None of the animals on a normal diet developed liver cancer, whereas all of those on a high-fat diet developed the disease. When Hara treated the overweight mice to decrease DCA production, the cancer incidence fell, too.

This line of research points to the microbiome as one potential link between obesity and liver-cancer risk (see 'Bacterial links'). And, much like Schwabe's work, Hara's results indicate that several factors converge to promote cancer: in this case, bacteria, diet and carcinogen exposure. Here, too, the ability to stave off the disease seems to depend on maintaining the appropriate microbial balance. Overweight mice and people have a different composition of gut microbiota from their lighter counterparts, and they have higher levels of DCA, too.

THE ROAD TO PREVENTION

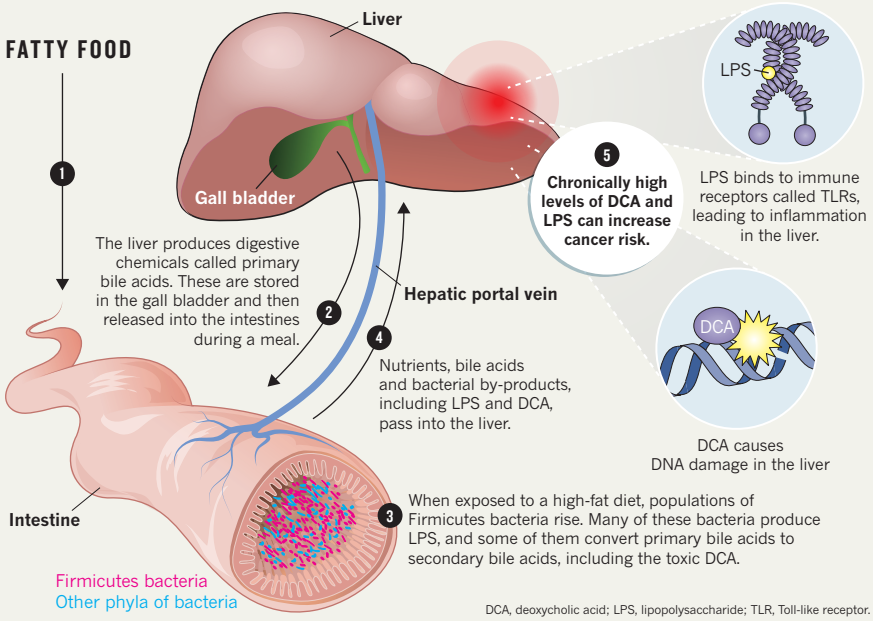
The complexity of the human microbiome presents considerable challenges for researchers. Improvements in genetic technologies have spurred progress in this area, and the US National Institutes of Health has invested US$200 million so far in the Human Microbiome Project, a large-scale genomics and analysis project that aims to characterize our microbial inhabitants. However, most work on the microbiome focuses on entire ecosystems of bacteria, and genomic sequencing is done on entire populations, making it difficult to identify individual species.

Hara hopes to identify specific bacteria that might be implicated in cancer progression, but it is a difficult proposition. Most of the bacteria living in the gut are anaerobic and cannot be grown using conventional laboratory methods. "We're trying hundreds of culture conditions but we can't isolate the bacteria yet," he says.

To prove that the microorganism–cancer link exists, and to develop preventive therapies, researchers must first demonstrate that the microbial effects seen in mouse studies also occur in humans. Hara's group is now collecting clinical samples of blood and stool from 1,000 healthy volunteers, 1,000 people with colon cancer and 50 people who developed liver cancer after having non-alcoholic fatty liver disease. He plans to analyse bacterial genes in stool and DCA concentration in the blood, and expects results in about two years.

BACTERIAL LINKS

The gut and liver are intimately connected. Bacterial populations living in the gut change their composition in response to diet, and such bacterial activity might contribute to liver-cancer risk and progression.



If Hara and his colleagues can identify the bacteria that contribute to liver cancer, tests could be developed to identify people whose microbiomes put them at risk. "Then we can screen people using PCR or antibodies," he says.

However, not everyone is convinced that individual bacterial species are to blame. Some researchers point out that dysbiosis, and therefore cancer risk, involves multiple strains of bacteria. And the bacterial mix can vary from person to person, meaning it is unlikely that scientists can pin all responsibility on a single species. They instead advocate targeting specific genes, rather than specific bugs. Phillip Hylemon, a microbiologist and immunologist at Virginia Commonwealth University in Richmond, is working on ways to block bacterial secondary-bile-acid synthesis pathways. "We've isolated some of the enzymes involved, and we have three-dimensional structures of some of them," he says. Pharmaceutical chemists can use these structures to develop molecules that are shaped specifically to bind to and inactivate these enzymes, thereby preventing secondary-bile-acid formation and reducing liver-cancer risk.

> *"We're trying hundreds of culture conditions but we can't isolate the bacteria yet."*

Others are looking for ways to promote the growth of healthy bacterial strains rather than target the bad ones. Jia, who is analysing the composition of people's bile acids, thinks it might be possible to get microbiomes back in balance by treating them with 'good' bacteria that have been genetically engineered to produce an enzyme that converts secondary bile acids back to their primary form.

There is also some early clinical evidence that specially formulated probiotics — cocktails of good bacteria — can bump the microbiome back into balance. Hylemon and his colleagues gave people with cirrhosis a probiotic containing *Lactobacillus* bacteria and found that their blood markers of inflammation decreased along with their cognitive dysfunction (a common symptom of cirrhosis)[6]. Although the study was not designed to evaluate cancer risk, it does show that delivering bacteria to the gut can have positive therapeutic effects on the liver.

It is still early days for this research. "One-third of the metabolites in the blood are coming from gut bacteria, and we've learned this only in the past few years," Hylemon says. But although the field is still in its infancy, it is already providing researchers with a whole new way to think about cancer. If physicians can identify people who are at the greatest risk of developing liver cancer — those with underlying liver damage and a genetic predisposition to disease — they might be able to harness the microbiome and stop the cancer before it starts. ■

Katherine Bourzac *is a freelance science writer in San Francisco, California.*

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