A common gut microbe secretes a carcinogen

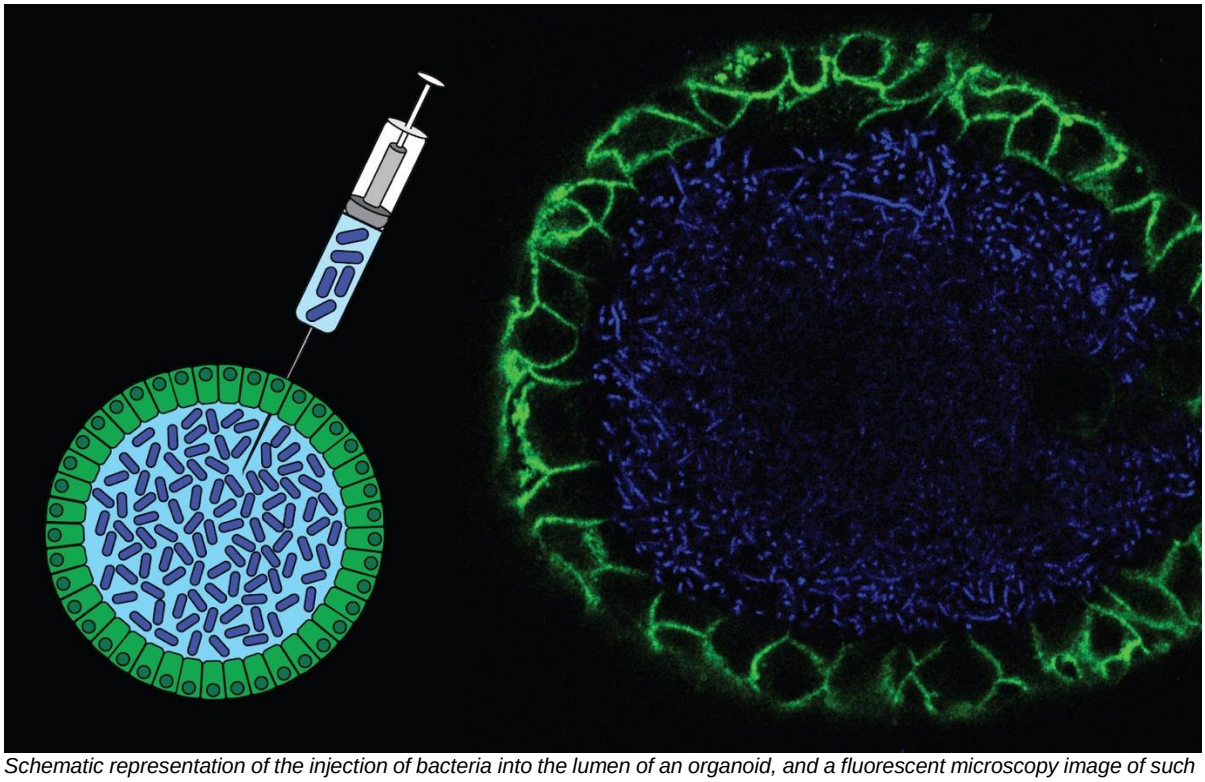
Cancer mutations can be caused by common gut bacteria carried by many people. This was demonstrated by researchers from the Hubrecht Institute (KNAW) and Princess Máxima Center in Utrecht, the Netherlands. By exposing cultured human mini-guts to a particular strain of *Escherichia coli* bacteria, they uncovered that these bacteria induce a unique pattern of mutations in the DNA of human cells. This mutation pattern was also found in the DNA of patients with colon cancer, implying that these mutations were induced by the 'bad' bacteria. It is the first time that researchers establish a direct link between the microbes inhabiting our bodies and the genetic alterations that drive cancer development. This finding may pave the way to prevention of colorectal cancer by pursuing the eradication of harmful bacteria. The results of this research were published in *Nature* on the 27th of February.

Our body contains at least as many bacterial as human cells. Most of these microbes contribute to a healthy life, while others may cause diseases. Among the bacteria with potentially harmful consequences is a strain of the best-known gut bacterium: *Escherichia coli* (*E. coli*). This particular *E. coli* strain is "genotoxic": it produces a small chemical, called "colibactin", which can damage the DNA of human cells. It has therefore long been suspected that the genotoxic *E. coli*, which live in the intestines of 1 out of 5 adults, could be harmful to their human hosts. "There are probiotics currently on the market that contain genotoxic strains of *E. coli*. Some of these probiotics are also used in clinical trials as we speak" explains Hans Clevers (Hubrecht Institute). "These *E. coli* strains should be critically re-evaluated in the lab. Though they may provide relief for some bodily discomfort in the short term, these probiotics could lead to cancer decades after the treatment".

Damage in the dish

Cancer cells are driven by specific DNA mutations, which allow these cells to grow into a tumor. Exposure to UV light or smoking can directly cause DNA damage, which induces mutations, and thus increase the chance that normal cells transform into cancers. But until now, it was unknown that the bacteria in our gut can similarly induce cancer mutations in cells through their DNA damaging effects.

A team of three PhD students from the groups of Hans Clevers (Hubrecht Institute) and Ruben van Boxtel (Princess Máxima Center for pediatric oncology) set out to identify the damaging effects of colibactin on human DNA. For this, they used tiny lab-grown human intestines, so called organoids, a model system that was previously developed in the group of Hans Clevers. The team developed a method to expose healthy human intestinal organoids to the genotoxic *E. coli* bacteria. After five months of bacterial exposure, they sequenced the DNA of the human cells and studied the number and types of mutations caused by the bacteria.



Schematic representation of the injection of bacteria into the lumen of an organoid, and a fluorescent microscopy image of such an organoid. Human intestinal organoid (green) filled with labeled bacteria (blue). Credit: Cayetano Pleguezuelos-Manzano, Jens Puschhof, Axel Rosendahl Huber, ©Hubrecht Institute.

A tell-tale footprint

Each process that can cause DNA damage leaves behind a specific mutation pattern, which is called a mutational footprint or signature. Such specific signatures have already been identified for various cancer-causing agents, including tobacco smoke and UV light. Presence of these specific footprints in the DNA of cancers can tell us about past exposures, which may underlie disease initiation. “These signatures can have great value in determining causes of cancer and may even direct treatment strategies”, explains Van Boxtel. “We can identify such mutational footprints in several forms of cancer, also in pediatric cancer. This time we wondered if the genotoxic bacteria also leave their unique distinguishing mark in the DNA.”

“I remember the excitement when the first signatures appeared on the computer screen” says Axel Rosendahl Huber, “we had hoped for some indication of a signature that we could follow up on in other experiments, but the patterns were more striking than any signature we had analyzed before.”

A puzzle falling into place

The genotoxic bacteria caused two co-occurring mutational patterns in the DNA of the organoids: the change of an A to any other of the four possible letters of the DNA code, and the loss of a single A in long stretches of A’s. In both cases, another A was present on the opposite strand of the DNA double helix, 3 to 4 bases away from the mutated site.

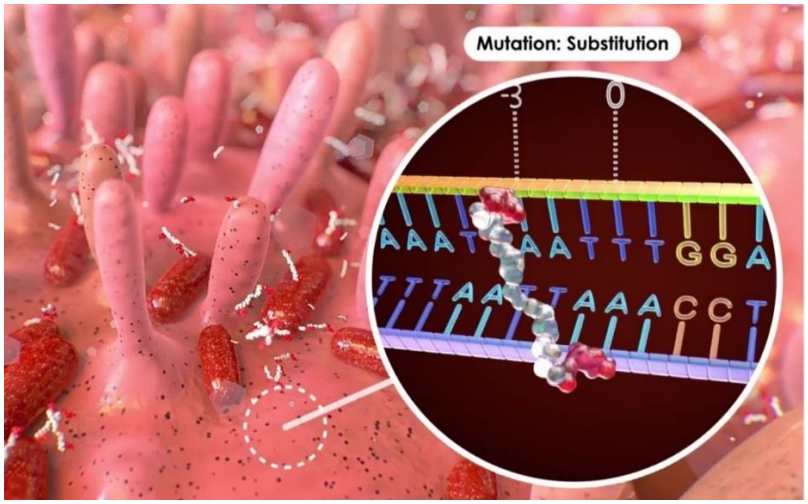


Illustration of colibactin binding to specific DNA sequence.
Credit: DEMCON | nymus3D, *©Hubrecht Institute.*

The team was wondering if they could learn something about the mechanism of colibactin-induced DNA damage from their data. “While we were at the final stage of the project, different research teams identified the structure of colibactin and how it interacts with the DNA”, says Cayetano Pleguezuelos-Manzano. Their research revealed colibactin’s ability to bind two A’s at the same time and cross-link these. “It was like a puzzle falling into place. The mutational patterns that we saw in our experiments could very well be explained by colibactin’s chemical structure”.

From organoid to patient

Once they established the footprint of genotoxic *E. coli*, the researchers set out to find traces of it in the DNA of cancer patients. They analyzed mutations in more than 5,000 tumors, covering dozens of different cancer types. Among these, one type stood out: “More than 5% of colorectal cancer had high levels of the footprint, while we only saw it in less than 0.1% of all other cancers,” recalls Jens Puschhof, “Imagine studying a gut bacterium’s footprint for months in a dish, and then finding back the same footprint in the DNA of patients.” Only a few other cancers, known to be exposed to the bacteria, such as cancers in the oral cavity and the bladder, also had the footprint. “It is known that *E. coli* can infect these organs, and we are keen to explore if its genotoxicity may act in other organs beyond the colon. The signature we defined experimentally helps us with this”.

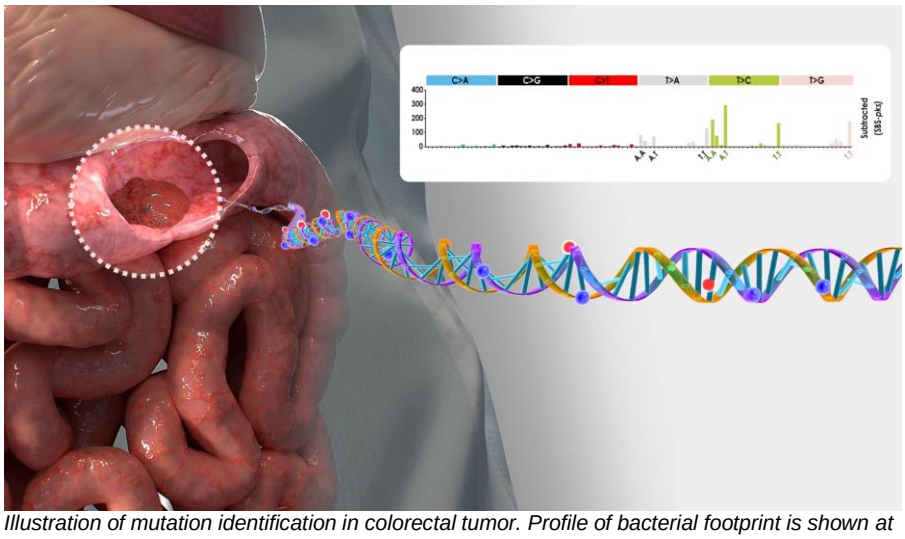


Illustration of mutation identification in colorectal tumor. Profile of bacterial footprint is shown at the top right, specific mutations are highlighted as red spheres in the DNA molecule. Credit: DEMCON | nymus3D, ©Hubrecht Institute.

An early warning
This study may have direct implications for human health. Individuals may be screened for the presence of these genotoxic bacteria; it is reported that 10-20 percent of people can harbor the 'bad' version of *E. coli* in their intestines. Antibiotic treatment could eradicate these bacteria early on. In the future it may be possible to catch colorectal cancer development very early or to even prevent tumors from developing.

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Publication
A mutational signature in human colorectal cancer induced by genotoxic *pks+ E. coli*. Cayetano Pleguezuelos-Manzano*, Jens Puschhof*, Axel Rosendahl Huber*, Arne van Hoeck, Henry Wood, Jason Nomburg, Carino Gurjao, Freek Manders, Guillaume Dalmasso, Paul Stege, Fernanda Paganelli, Maarten H. Geurts, Joep Beumer, Tomohiro Mizutani, Reinier van der Linden, Stefan van Elst, The Genomics England Consortium, Janetta Top, Rob Willems, Marios Giannakis, Richard Bonnet, Phil Quirke, Matthew Meyerson, Edwin Cuppen, Ruben van Boxtel, Hans Clevers. Nature 2020.

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About the Hubrecht Institute
The Hubrecht Institute is a research institute focused on developmental and stem cell biology. It encompasses 23 research groups that perform fundamental and multidisciplinary research, both in healthy systems and disease models. The Hubrecht Institute is a research institute of the Royal Netherlands Academy of Arts and Sciences (KNAW), situated on Utrecht Science Park. Since 2008, the institute is affiliated with the UMC Utrecht, advancing the translation of research to the clinic. The Hubrecht Institute has a partnership with the European Molecular Biology Laboratory (EMBL). For more information, visit www.hubrecht.eu.

About the Princess Máxima Center for pediatric oncology
In the Princess Máxima Center health care and research are integrated in one pediatric oncological center. Yearly, 600 children in the Netherlands are diagnosed with cancer; only 75 percent survives. Therefore, 32 research groups, consisting of over 470 scientists, focus their research on childhood cancer with the long term mission to be able to cure every child with cancer with an optimal quality of life. The Máxima is situated in the Utrecht Science Park and collaborates closely with other institutes in Utrecht, the Netherlands and worldwide. For more information, visit www.prinsesmaximacentrum.nl/eng