Mechanisms of evasive resistance in cancer (MERIC)

Understanding the adaptation strategies of tumors

Targeted therapies are being increasingly employed to fight tumors. In contrast to classical chemotherapy, the drugs used target only the cancer cells, without affecting the rest of the body. Unfortunately, many tumors soon become resistant to these drugs. Researchers are now trying to understand the molecular mechanisms behind this adaptation.

Niko Beerenwinkel and Christian Beisel looking at a flow cell, which will later be filled with DNA.

Communication within a cell can be thought of as a kind of relay race. The runners are the proteins. They dock on to other proteins, where they pass their message on. When the signal arrives at the nucleus, it might turn on a gene that, for example, causes the cell to divide.

With targeted treatment, the drug attaches itself to one of the proteins in the communications relay race and blocks it. "This means that signal transmission is interrupted and, as a result, the cell dies," says Niko Beerenwinkel of ETH Zurich, project leader of "Mechanisms of evasive resistance in cancer" (MERIC). However, cancer cells can evade a blocked signal by using an alternative signal pathway, whereby different proteins come into play that the administered drug cannot block. This is known as "evasive resistance". The MERIC project team is now concentrating on elucidating the underlying mechanisms. Understanding these could clear the way for the development of new drugs to which cancer cells can no longer, or only much more slowly, adapt.

Analysis on three levels

To understand the mechanisms behind this molecular adaptation, the researchers are working with real cases from the University Hospital Basel. Tissue samples are taken from patients suffering from liver carcinoma (HCC). "A liver cancer is often inoperable, as the liver could be severely damaged during such a procedure," says Beerenwinkel. "Drugs are the only treatment option in many cases."

Biopsies are carried out at regular intervals. Ideally, they are conducted before, during and after therapy. "This allows us to see exactly how the cancer cells adapt to a drug," he explains.

The DNA, RNA, protein and protein phosphorylation levels are examined in the removed cells. "In this way, we can see how mutations in the genome affect first the RNA and then the proteins and signaling pathways," says Beerenwinkel.

First the cancer cells' DNA is decoded. This work is being carried out in collaboration with Christian Beisel, head of the Genomics Facility Basel. He receives prepared DNA extracted from tumor samples at the University Hospital Basel. From this, his team compiles a sort of library. "We're not able to sequence a long DNA strand in one piece. Instead, we have to dissect it into smaller portions for our machines," says Beisel. Each of these strands is just 200 bases long. The sections are then put into flow cells. These resemble microscope slides, but their surface is subdivided into narrow channels, in which there are docking sites for the DNA segments. They remain bound to the sites for the duration of the analysis.

The letters of life

The DNA-filled flow cell is then put into a sequencing machine. This contains tanks holding the nucleotide bases adenine (A), cytosine (C), guanine (G) and thymine (T); the building blocks of DNA. They are flushed together through the flow cell's channels. When each of the four bases meets the DNA segments, they attempt to dock on to them. If, for example, the first base of a section is a guanine, then its counterpart, cytosine, docks there. If, on the next strand, the first base is thymine, an adenine will bind to it.

"The docking bases are modified in such a way that they fluoresce, and that the docking reaction stops once a connection is created," explains Beisel. A microscope in the sequencing machine registers each successful connection as a white pixel. The machine can distinguish between the signals from A, C, G or T, and so is able to discern the first letter of each DNA segment after the first round of measurements.

The fluorescent dyes are then cleaved and the DNA segments prepared for the next reaction step. The four bases are flushed through the flow cell once more, but now they dock on to the second location. This continues until all 200 bases of each section have been detected. In this way, the genotype of the cancer cell is sequenced within a few hours.

Evolution of resistance

The RNA is also sequenced in this way, while the proteins inside the cells are determined by means of mass spectrometry. The collected data are delivered to Beerenwinkel, who analyses them statistically. "I want to get an overview of the evolution processes," he says. Specifically, he is looking for areas in the genome that show a lot of variation and which might lead to alternative communication strategies in the cancer cell. This is where Jörg Stelling from ETH Zurich comes in, incorporating these results into a computational model of the signaling pathway. "This helps us understand how the resistance of cancer cells works at the molecular level, from gene to RNA to protein," concludes Beerenwinkel.

He and his colleagues have already analyzed cancer cell DNA and RNA from over a hundred samples. Furthermore, the cell samples are being cultivated in Petri dishes, enabling the team to create a living database.

Some of the cells are additionally implanted in mice. By means of these "xenografts", the cancer cells continue to grow in the living organism. The mice are then treated with different drugs. "This allows us to study the development of resistance in real time under controlled conditions that are as realistic as possible," says Beerenwinkel. "In the next step, we can use these findings to search for new drugs or better combinations of known treatments."
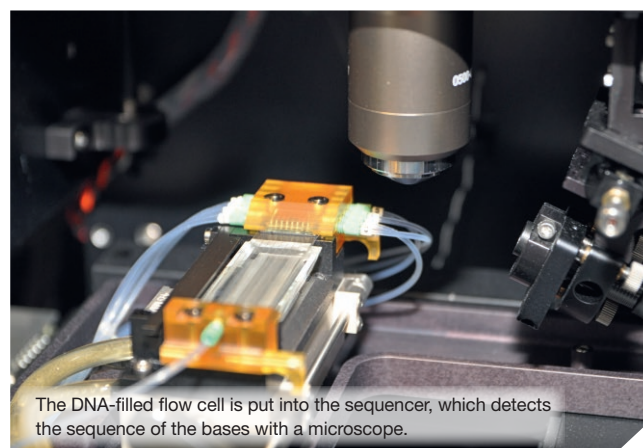
The DNA-filled flow cell is put into the sequencer, which detects the sequence of the bases with a microscope.
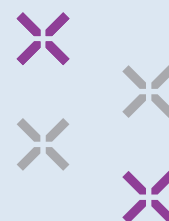
MERIC at a glance

Principal investigator: Prof. Niko Beerenwinkel

Research groups:

- Prof. Niko Beerenwinkel, Department of Biosystems Science and Engineering, ETH Zurich – Computational biology

- Prof. Gerhard Christofori, Department of Biomedicine, University of Basel – Tumor biology

- Prof. Michael N. Hall, Biozentrum, University of Basel – Signal transduction

- Prof. Markus Heim, Division of Gastroenterology and Hepatology, University Hospital Basel – Clinical hepatology

- Prof. Jörg Stelling, Department of Biosystems Science and Engineering, ETH Zurich – Computational systems biology

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