Movies of cancer cells in living mice show how streams of melanoma cells (green) invade skin tissue.

The cells are guided by structures such as muscle fibres (orange), nerve fibres (blue), collagen (grey) and blood vessels (red).

Cancer caught in the act

Methods for monitoring tumour cells in living animals are transforming our view of cancer.

BY CORIE LOK

Mikala Egeblad was blown away when she made her first action film of tumour cells inside live mice. Until then, she had studied samples on microscope slides, where the cells sat still, frozen in time. But seeing them in a living animal brought the cells to life. “You turn on the microscope and look in the live mouse and suddenly these same cells are running around like crazy,” says Egeblad, a cancer researcher at Cold Spring Harbor Laboratory in New York. “It really changed my thinking.”

Increasingly, cancer researchers are embracing the chance to spy on individual tumour cells in their native environment. In studies of static tissue cultures, investigators have to infer what cancer and other cells surrounding the tumour might be doing, and how they might be interacting. Tracking cancer in live animals over time — an approach called intravital imaging — puts those interactions on display, and allows biologists to zoom in on the small number of dangerous cells within a tumour that drive the disease or resist treatment.

The technique is young, and labs are still

working out how best to analyse the gigabytes of video data it generates. But the increasing use of intravital imaging over the past decade has already helped researchers to piece together timelines for key cellular and molecular events, such as the process by which tumour cells sneak into blood vessels. Such clues have yielded new hypotheses about how cancers grow, spread and resist treatment — information that could, for example, eventually enable drug developers to understand why some cancer cells do not succumb to therapy.

And in a video-obsessed culture, the imaging technique holds instant appeal. “When we show our movies, people fall out of their seats when they see how dynamic a tumour lesion can be,” says Peter Friedl at Radboud University Nijmegen in the Netherlands. “It’s a change in perception.”

DEEP DIVE

First used by cancer biologists in the late 1990s, intravital imaging involves focusing powerful microscopes directly onto exposed tissue in a live, anaesthetized mouse. More labs have adopted intravital imaging as technological improvements have made it possible to peer further into tissue — now as many as 20 cells deep — and to tease out fainter signals. A growing library of molecular markers has given researchers the ability to visualize up to eight different kinds of cells and structures,

BETTINA WEGELIN & PETER FRIEDL, RADBOUD UMC

including various immune-system cells and the endothelial cells that line blood vessels. “The markers and the microscopy technology make this a powerful combination,” says Frederic de Sauvage, vice-president of molecular oncology at the biotechnology company Genentech in South San Francisco, California, who has seen the technology in action.

Putting these components together creates a comprehensive picture of cancer as a complex ecosystem of cells that migrate, proliferate and interact. Although cancer researchers have long understood that cells in a tumour are genetically heterogeneous, intravital imaging is revealing how the behaviour of individual cells can also differ. For example, cancer cells may march in single file or collectively as a tight-knit group, depending on the type of tumour and its environment.

One mysterious cellular behaviour that has landed in the sights of these microscopes is that of the macrophage, a type of immune cell that normally engulfs pathogens, removes dead cells and stimulates immune responses. Macrophages can incite immune cells to fight cancer, but more often they boost a tumour’s growth and spread.

Intravital imaging studies showed that macrophages, along with tumour cells and endothelial cells, form a structure that pumps tumour cells into the bloodstream — a key step in metastasis. Working with rodents, researchers led by John Condeelis at Albert Einstein College of Medicine in New York found that when macrophages come into contact with mammary tumour cells, the tumour cells become more invasive, degrading the protein-rich matrix around blood vessels and squeezing between the endothelial cells. Macrophages cause the endothelial cells to lose contact with each other, opening a hole in the vessel wall and allowing tumour cells to stream out of the tissue and into the bloodstream^{1,2}.

Condeelis’s team has shown that this ‘pump’ is present in human breast cancer. The group has also identified three molecular markers, one for each cell type in the structure, that indicate its presence in tumours. In a study³ of 60 people with breast cancer, individuals with a higher density of these pumps in their tumours were more likely to develop metastases in other organs. A start-up company, MetaStat in Montclair, New Jersey, has licensed this prognostic technology and is developing a test that predicts metastatic risk in people with breast cancer. The company hopes to have the test in clinical trials by the end of this year. Condeelis’s group is also working on a probe to identify the pumps using magnetic resonance imaging, avoiding the need to take tissue biopsies from patients.

Others are using intravital imaging to track cancer drugs in the body, and to explore why some drug treatments fail. Cancer biologists typically test the effect of chemotherapies *in vivo* by measuring changes in tumour

growth and size in mice. Intravital imaging gives a more direct view, revealing which cells in a tumour take up the drugs, and whether those cells live or die.

Egeblad and her team have made films of doxorubicin, a naturally fluorescent cancer drug, as it infiltrated mammary tumours in mice. They were surprised by the degree of variability — even within small regions of the tumour — in the amount of the drug that got into the cells, and in the number of cells

“When we show our movies, people fall out of their seats when they see how dynamic a tumour lesion can be.”

that died. One important factor, they found, was the ‘leakiness’ of the blood vessels in the tumour⁴. Mid-stage tumours, which have more porous blood vessels than early- or late-stage tumours, were more sensitive to the drug. Compounds that boost the permeability of vessels could therefore improve the delivery of cancer drugs, suggests Egeblad.

VIEWING WINDOW

To capture films in live mice, researchers were initially restricted to a single imaging session. Ideally, they would like to watch tumours in the same animal over days or weeks to track longer-term changes. Many are adopting a technique that implants a glass coverslip in a frame in the mouse’s skin. These windows, which can provide views into areas including the brain, abdomen and mammary glands, allow investigators to image the same location in the same mouse many times over. The mice wake up after an imaging session and carry on as normal in their cages.

Using the windows, a team led by Jacco van Rheenen at the Hubrecht Institute in Utrecht, the Netherlands, watched colorectal-cancer cells colonize the livers of living mice over the course of two weeks. Newly arrived cancer cells moved within small areas of the organ during the first few days, but by day five they had stopped migrating and were becoming densely packed. The team found that in mice in the early stages of tumour spread, treatment with a molecule that inhibits cell migration decreased the number of metastatic liver tumours that developed later⁵.

As intravital imaging of cancer has matured, the field has moved beyond eye-catching films and has begun to generate quantitative data detailing, for example, the speed and direction of moving cells. Such data allow researchers to construct and refine mathematical models of cell behaviour. These could predict, for example, how tumour cells invade tissues, says Friedl.

But generating such quantitative data is difficult and time-consuming: analysing the movies

can take up to 15 times longer than making them, says Egeblad. Others note that software for quantitative-image analysis is limited, so many labs are writing their own programs.

And intravital imaging continues to pose technical challenges. The technique can access only tissues near the surface, which makes it applicable to just a few tumour types, says de Sauvage. It has also been difficult to integrate intravital imaging with classical tools of molecular biology, such as fluorescent biosensors used

by researchers to see when and where cell-signalling pathways are turned on. Many of those sensors work well *in vitro* — where cells can be manipulated to amplify changes in signalling — but are not sensitive enough to pick up the more subtle changes seen *in vivo*, says van Rheenen. This sort of information might provide clues to the molecular escape routes that allow some tumour cells to dodge the effects of cancer drugs, says Scott Powers, a cancer geneticist at Cold Spring Harbor. “It would be nice to know what’s happening biochemically inside the cells that’s making them do what they’re doing.”

Egeblad is now integrating biochemical and genetic tools in her imaging work. She will soon be launching a new project to trace the history of different subsets of cells in mouse mammary tumours as they grow over several weeks. At the end of the experiment, her team will remove the tumours and sequence the genomes of individual cells. The aim is to link genetic signatures to cellular behaviours, such as rapid growth or drug resistance, in different regions of the tumour. The team also plans to image the activity of key cancer genes in mice as tumours grow.

For Egeblad, the new project is a chance to return to the questions that first drew her to intravital imaging: how do different components of the tumour and its environment co-evolve? Powers says that working with Egeblad and watching her movies helped him to see how the tumour’s environment, not just its genetics, can influence cancer. “How could it not have an impact?” he says. “You’re recording things that haven’t been recorded before.” ■

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