TECHNOLOGY FEATURE

TRACKING METASTASIS AND TRICKING CANCER

Physics – and engineering – based approaches are helping researchers stop the spread of cancer by anticipating tumour cells' moves and habits.

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Live fluorescence microscopy imaging of metastasis — the spread of cancer — showing melanoma cells (green) invading lymphatic vessels (thick red vessels).

BY VIVIEN MARX

In the 1880s, British surgeon Stephen Paget examined autopsy reports from more than 900 patients with advanced breast cancer. He was trying to understand metastasis — the process by which tumours spread through the body. Paget reasoned that if all the organs were equally susceptible, with the location of tumour fragments governed by chance alone, then all the organs should be equally affected.

Instead, he found that the breast cancers had spread mainly to the uterus and the bones. He suggested that when tumours shed cells that move through the bloodstream, the cells are like seeds that grow only where they find congenial soil. To understand metastasis, it might be useful, he wrote, to study not only the seeds, but the soil too¹.

Paget's 'seed and soil' hypothesis "still holds forth today", writes Isaiah Fidler of the University of Texas MD Anderson Cancer Center

in Houston². It informs present-day efforts to understand every detail of metastasis — including the genetic pathways that direct a cancer's growth, the factors that set some tumour cells free to travel through the bloodstream, and their interactions with the environments at the organs in which they settle³.

Only a few of those cells manage to seed new tumours. Metastasis is a "low-probability event", says Robert Gillies of the H. Lee Moffitt Cancer Center in Tampa, Florida. Even ►

► so, more than 90% of cancer deaths are the result of metastasis. Understanding the processes involved is therefore something of a priority.

Studying metastasis in patients would require unethical experimentation, so scientists are creating artificial conditions similar to those in the body. They are developing approaches based on physics, chemistry and engineering, and are increasingly using three-dimensional assays for experiments that are usually two-dimensional. For example, they have created mimics of the extracellular matrix (ECM) — the body's tissue scaffolding — in devices that are hooked up to imaging systems. This set-up allows them to track tumour cells over extended periods of time to see how they detach from one spot and attach at another, and how they creep through tissue that should be too dense to let them pass. Other approaches involve scooping migrating tumour cells out of the blood and locking them in highly engineered cages to discover how they seed secondary tumours.

Scientists are making rapid progress thanks to an array of approaches that consider networks of interacting genes and proteins, and breakthroughs in imaging and animal

modelling are yielding increasingly comprehensive data (see 'Technology timeline in metastasis research'). Such technological innovations are galvanizing efforts to overcome gaps in the scientific understanding of metastasis, as Yibin Kang, a cancer biologist at Princeton University in New Jersey, and Nilay Sethi, a former member of Kang's lab now at the University of California, San Francisco, have pointed out⁴.

READY TO SPREAD

Metastasis is thought to begin with the epithelial–mesenchymal transition (EMT), a cascade of events in which tumour cells lose many of their 'epithelial' characteristics and become more like mesenchymal cells with the ability to spread and invade tissue⁵. This process occurs naturally in healthy cells but is poorly understood in tumours. Researchers are not even sure whether it happens early in tumour development or only in advanced tumours. But they know that the transformation involves a chatter of signalling, both within a tumour cell and between cells. For example, both normal cells and tumour cells shed small vesicles, known as exosomes, into the bloodstream. Previously considered a cell's way of putting out the rubbish, exosomes are now known to contain proteins and signalling molecules — like messages in a bottle — that prepare the target tissues to accept metastatic cells.

Even before cells migrate, tumours create

their own microhabitat that promotes metastasis. "We believe there are components of the microenvironment that matter in the adaptive changes in tumour cells," Gillies says. Clinicians can identify genetic signatures in patients' cancers and prescribe drugs to attack the specific tumour type, but this treatment often fails because tumours activate alternative genetic pathways instead, he says. If clinicians can understand the adaptive landscape of the tumour and its environment, they will have more lines of attack and may even be able to create a habitat that inhibits metastasis.

Researchers have found that as tumours grow they create a unique microclimate with highly acidic areas that are low in oxygen — conditions that seem to favour metastasis. Combining imaging with technology that characterizes cancer metabolism in this microhabitat helps to reveal how tumour cells shift to a metastatic state, Gillies says. Rapid cancer growth is fuelled by a metabolic process called glycolysis. Gillies and his team are able to detect this — and thereby identify cancer cells — using an instrument from Seahorse Bioscience in Billerica, Massachusetts, that allows them to analyse both tumour metabolism and the microenvironment in an automated fashion. "Since we got the device, it has been running non-stop," he says.

The instrument, an XF Analyzer, measures the acid produced by glycolysis in cells, thereby identifying tumour cells, which have a high

TECHNOLOGY TIMELINE IN METASTASIS RESEARCH

Adapted from ref. 4.

1994 ►

Advances in mass spectrometry allow identification of individual cancer proteins.

1995 ►

DNA-microarray technology shows many patterns of gene expression.

1996–1998 ►

Advances in microscope technology and biomarker tagging allow live imaging of tumour metastasis.

1998 ►

Therapies emerge that use an antibody to target a specific tumour protein.

Tissue microarrays are developed to detect specific proteins in a large number of tumour samples.

1999–2000 ►

Assays pinpoint the target sites of DNA-binding proteins that shape gene expression.

2000–2002 ►

Gene-expression profiling helps to find metastasis genes and reveals cancer subtypes in patients.

Protein microarrays can screen for markers of cancer progression.

2002 ►

Gene-expression profiles show cancer progression signatures. Metastatic behaviour of single breast cancer cell is imaged.

2003 ►

Microarray profiling identifies factors that influence bone metastases.

2004 ►

Drug based on monoclonal antibody is approved for inhibiting angiogenesis in metastatic colon cancer.

2005 ►

Discovery of a niche that forms before the arrival of metastatic cancer cells in secondary organs to nurture their growth.

2006 ►

High-throughput sequencing increasingly used to sequence cancer genomes.

2007 ►

Mass spectrometry and new types of protein labels pinpoint changes in proteins co-opted by tumours.

First metastasis-related microRNA identified.

2008 ►

RNA sequencing applied to cancer genomes to quantify expression of cancer genes.

2009 ►

Experiments indicate that tumour self-seeding may even drive the growth of primary tumours.

2010 ►

Drugs targeting tumour oncogenes or supporting host cells approved to address some types of metastasizing cancers.

2011 ►

Sequencing reveals the natural history of metastatic progression in human cancer patients.

2012 ►

Recognition of tumour exosomes as means of long-distance communication in cancer metastasis.

glycolysis rate, says Min Wu, director of biology at Seahorse Bioscience. A sensor cartridge positioned above the cells creates a temporary microchamber and measures oxygen and pH levels. This arrangement lets scientists detect cell metabolism in real time, typically within 1–2 hours, says Wu.

Buffers that neutralize the tumour's acidity have been shown to make chemotherapies more effective and inhibit metastasis. To determine the right buffer mix, Gillies uses the analyser to type cells according to their metabolic profile. Several research groups are also exploring how drugs can be tailored to release a warhead loaded with anticancer drugs into tissue areas with a profile characteristic of cancer.

ON THE MOVE

The next step in metastasis is for cells to break away from the tumour so that they can enter the bloodstream. Several relatively low-tech but tried-and-tested assays can help scientists watch this migration. Companies such as EMD Millipore of Billerica, Massachusetts, and Cell Biolabs in San Diego, California, offer two-dimensional assays that help researchers track and quantify migration and chemotaxis (the movement of cells towards a chemical signal).

Scientists sometimes build these assays themselves, but the companies say that standardized products result in more comparable experimental results. One technique developed in the 1960s to study cell migration is the Boyden chamber, which is essentially a small cup containing cells placed inside a larger cup containing growth medium or chemoattractant to attract cancer cells. The two chambers are separated by a porous membrane through which cells can migrate. In this way, scientists can track chemotaxis and see which cells migrate through the membrane.

In a similar test known as a scratch assay, tumour cells are spread in a layer across growth medium in a plate. When the surface is scratched — by a researcher running a pipette tip across it, for example — cells move together to fill in the artificial gap, much as they would when closing a wound in the body. Richard Klemke, a cell biologist at the University of California, San Diego, and a consultant at EMD Millipore, developed a comb for the scratch assay, putting “a new spin on a very old technique”, says Jun Ma, a product manager at EMD Millipore. The comb makes uniform, high-density scratches that induce large numbers of cells to migrate synchronously for scientists to image, says Klemke.

A German company, Ibidi, based in Munich, offers what it believes is an improved migration assay. Most labs use the Boyden chamber to study chemotaxis, but Ibidi's president, Roman Zantl, is aware of its limitations. “I doubt that it gives valid results in case of adherent cancer and other cell types,” he says. The synthetic membrane between the two cups is unlike the material through which cells travel in the

Scientists have engineered an environment that lets them follow metastatic tumour cells while chemokines and growth factors flow in from the side to mimic the tumour microenvironment.

body and might alter the cells' behaviour. And although the assays work well enough for fast-migrating cells, such as leukocytes, they are less suited to the slower movement of tumour cells, which might not be counted if they take too long to complete their journey across the membrane from one cup to another.

To address these issues, Ibidi developed a migration assay based on the Zigmond chamber, an approach developed in the 1970s that uses two reservoirs, one containing cells and a second containing a chemoattractant. In Ibidi's version, cells move along a chemoattractant gradient across a small area that can be viewed under a microscope. Diffusion of the chemoattractant is slow, allowing researchers

“It’s a physics problem in some ways, it’s an integrated problem.”

Mingming Wu, a bioengineer at Cornell University in Ithaca, New York. Wu says she uses them because “cancer metastasis is a game of going somewhere”. But she hopes to be able to model migration in three dimensions.

SEEING 3D

“What you see in two dimensions has very little apparent correlation with what you see in three dimensions,” says Wu. In a two-dimensional assay, tumour cells use adhesion molecules to inch along. “If you disable the adhesion molecules, the cells cannot move,” she says. But in a three-dimensional assay, cancer cells switch to a different type of movement when their adhesion molecules are disabled. “A cancer cell is very smart in some ways,” Wu says.

Imaging experiments by several researchers, including John Condeelis at Albert Einstein

College of Medicine in New York, have provided a visual, three-dimensional view of metastasis in mice, says Wu. Now she wants to bring this observational power to an engineered environment that realistically mimics human physiology. “It’s a physics problem in some ways, it’s an integrated problem.”

Wu has developed a device in which tumour cells move through a synthetic version of an ECM. As they move, they generate force and migrate along the ECM fibrils to reach the bloodstream. “Some of them climb the ropes. They extend and cling onto fibres and pull themselves, which makes them more elongated, but some are more rounded,” she says. Where the matrix is too dense, tumour cells secrete chemicals to create a passageway.

The device has three tiny channels patterned into the surface of a growth medium, and Wu places tumour cells embedded in ECM in the middle channel. Chemoattractants known to affect cancer cells are added to another channel. Wu uses time-lapse microscopy to observe what happens. To explore why breast cancer cells first metastasize to lymph nodes, the researchers use chemoattractants that correspond to molecules present in lymph nodes. The device makes it possible to observe tumour cells interacting as naturally as possible with one another, with healthy tissue cells and with the body's immune cells.

Once again, commercial alternatives are available for scientists who do not want to build their own microfluidic platform. Last year, for example, EMD Millipore began selling one after acquiring CellASIC, which launched the platform in 2007.

The company's programmable perfusion system is hooked up to a microscope and lets scientists use software-based commands instead of manual ones to make cells or molecules flow in or out of a microenvironment.

They can investigate factors that might promote or thwart a metastasis-like state in cells and “get real-time movies of these cells as they’re behaving”, says bioengineer Alex Mok, who was in Luke Lee’s lab at the University of California, Berkeley, where the device was developed, and is now a product manager at EMD Millipore. Mok describes the device as “a semiconductor chip for biologists” that should allow more researchers to do this kind of experiment.

SCOOPING UP THE SEEDS

Once the malignant cells enter the bloodstream they are known as circulating tumour cells (CTCs). Clinicians can gauge how a breast, prostate or colon cancer patient is faring from the number of CTCs in the blood and from their molecular characteristics. However, CTCs are extremely difficult to isolate. Test instruments typically sample 5–10 millilitres of blood, a tiny fraction of the body’s total of 5 litres. And a cancer patient’s blood may contain only one metastatic cell for every 1 billion to 5 billion other cells, says Mehmet Toner, a bioengineer at Massachusetts General Hospital in Boston. “It’s a statistical nightmare.”

One device that can help to improve the odds of finding CTCs is the CellSearch, made by Veridex, a subsidiary of Johnson & Johnson based in Raritan, New Jersey. Approved by the US Food and Drug Administration in 2004, it tags tumour cells with magnetic beads coated with antibodies that adhere to proteins found on the surface of tumour cells. The CTCs are then separated using a magnet and are then stained and imaged.

Toner and other scientists want devices that capture CTCs to be more sensitive and to keep the cells viable for further analysis, which CellSearch does not. The technical challenge and its commercial promise have led to a flurry of device-building. “There are lots of good ideas out there,” Toner says.

His own team has been developing different types of bank-card-sized devices coated with antibodies that trap CTCs by attaching to proteins on their cell surface. In his most recent device, the herringbone-chip (HB-chip), blood flows in a see-through channel that lets scientists image the captured cells. The herringbone pattern of grooves on the inside of the channel’s roof creates a gentle vortex in the blood, increasing the number of tumour cells that stick to the antibody-coated surface, he says.

A LIQUID BIOPSY

Toner thinks that CTC chips such as this will help cancer researchers understand the separate steps in the transformation from tumour cells to the metastatic seeds of a new tumour. The devices make it possible to use extremely high-definition imaging, which can enhance the work of pathologists, he says.

In a test using blood from patients with metastatic prostate cancer, Toner’s team was

able to distinguish between circulating tumour cells before and after medical treatment, an ability that could help to guide therapy. In a separate test, the researchers found higher levels of a signalling molecule called WNT2 in circulating pancreatic tumour cells than in primary tumour cells⁶. This difference is a potential biomarker that could help drug developers find ways to inhibit metastasis.

The test also revealed metastatic cells’ survival strategies. Cells need to be anchored if they are to survive — once they are in the bloodstream, even tumour cells should die, Toner says. But turning on the *Wnt2* gene helps them avoid cell death. “Cells that express *Wnt2* can survive in a suspended state,” he says.

In contrast to CellSearch, Toner’s chip does not need blood samples to be preprocessed. It also keeps cells viable for further profiling. In 2011, his team landed a five-year, US\$30-million collaboration agreement with Johnson & Johnson to refine CellSearch technology with a new approach to characterize tumour cells. Toner says he cannot comment on the progress yet, but he challenges views that metastatic cells are too scarce to capture in sufficient numbers or that microfluidics cannot be used for large-scale blood analysis. Using

A cancer patient’s blood may harbour only one metastatic cell for every 1 billion to 5 billion other cells.

such a tiny device has engineering efficiencies. “Microfluidics gives us multiplexing, building redundancy into the system, and it gives us very uniform conditions,” he says.

Many other labs are also working on CTC chips, including the National University of Singapore, Stanford University in California, Louisiana State University and the University of California, Los Angeles (UCLA). Hsian-Rong Tseng, a chemist at UCLA, says CellSearch has raised awareness among scientists, physicians and patients that CTCs allow “a biopsy directly from the blood”. Tseng’s team has developed a microfluidic ‘Nano-Velcro’ assay, in collaboration with researchers at the RIKEN Advanced Science Institute in Saitama, Japan, and several institutions in China, including Wuhan University. Tseng’s group has now founded a company called CytoLumina Technologies to commercialize the instrument.

Tseng’s device can capture and release live CTCs from a blood sample. When the team used it to analyse CTCs from patients with metastatic melanoma, they sequenced the cells’ DNA and identified clinically relevant mutations. To ramp up the scale at which it can be used with genetic testing, Tseng teamed up with the Beijing Genomics Institute in China.

The device contains polymer-coated silicon nanowire brush hairs studded with antibodies that match proteins on the surface of metastatic cells. As the blood flows through the device, also aided by a herringbone pattern, the tumour cells stick to the surface — hence

A ‘Nano-Velcro’ device captures circulating tumour cells and keeps them viable for further analysis.

the Nano-Velcro name. “We can release them simply by changing the temperature,” he says. Lowering the temperature changes the polymer’s configuration, pulling the antibodies inwards, allowing the tumour cells to detach and flow out of the channel — viable, intact and ready for further study.

LESSONS FROM THE HEART

The final stage of metastasis is when the CTCs find fertile ‘soil’ in the body, to use Paget’s term, and begin to grow. It’s not clear what makes particular environments hospitable to certain tumour cells, but one place to look for answers is the heart — “the one organ in the body that has actually beaten cancer,” according to cardiology researcher Jay Schneider at the University of Texas Southwestern Medical Center in Dallas. Some cancers such as melanoma can metastasize to the heart, but it is “very, very rare”, he says. Attempts to achieve metastasis to the heart experimentally have worked only with one cancer cell line, he adds.

The heart’s defence mechanism remains unknown, but it seems to be shielded by either mechanical or physical barriers that make the microenvironment hostile to CTCs. It’s likely that some component of the heart’s microenvironment — maybe its tissue, cells or the scaffolding around the cells — is protecting it. One way to understand the mechanism is to identify what aspect of the ‘soil’ makes healthy tissue vulnerable to metastatic seeds.

Biomedical engineer Sangeeta Bhatia and her graduate student Nathan Reticker-Flynn at the Massachusetts Institute of Technology (MIT) in Cambridge have built a platform to help them study the interactions between tumour cells and various synthetic models of the ECM (the dense weave of fibrils that make up the scaffolding connecting cells in tissues). The pair want to learn which components of the ECM are most hospitable to metastatic seeds and favour cell migration and adhesion.

Far from being mere stabilizing mortar, the ECM has “its own universe of biology; it is a signalling hub”, she says. “And the cells are constantly modifying it, so it’s a dynamic and biologically active glue.” In a primary tumour, cancer cells stick to one another. To spread, metastatic cells must first detach from their mooring, then move within the ECM to the bloodstream, travel in the blood until they reach a suitable destination, and then attach to the ECM and grow into a tumour there. “The attachment at that metastatic site is what this experiment was about,” she says.

Bhatia’s team used robots to print arrays of ECM dots, each with a different composition. The researchers scoured catalogues to find all the synthetic ECM molecules on the market and developed 800 unique combinations. They coated glass slides with polyacrylamide, which swells to trap the ECM molecules in one spot, and then seeded metastatic lung cancer cells on the spots. “We developed a platform

This system uses software-based commands to flow cells in a controlled microenvironment.

to query these all at once,” Bhatia says, and it has attracted so much interest that she plans to distribute it commercially.

The researchers imaged the different ways the cells adhered to the ECM spots and compared the adhesion profiles. “We found they have gained the ability to stick to different things than in the primary tumour,” she says. As the cells grew over a period of time, the scientists used a clustering algorithm to find patterns in the data. They hope that understanding how tumour cells adhere to the ECM could open the way for a potential therapeutic to interfere with this ability.

THE GAZE OF PHYSICISTS

Metastasis research is benefiting from a multi-disciplinary approach that includes biologists, physicists, chemists and engineers. In 2009, for example, the US National Cancer Institute (NCI) started funding a network of 12 Physical Science–Oncology Centers (PSOCs). In 2010 it launched a Provocative Questions initiative requesting research proposals that address “perplexing” problems in cancer, such as devising engineering approaches to improve the study of metastasis.

Another NCI scheme, the Innovative Molecular Analysis Technologies (IMAT) programme, launched in 1998, also fosters these partnerships and aims to develop tools that could accelerate cancer research, says IMAT director Tony Dickherber. “Our understanding of how metastasis works, and the importance and complexity of the microenvironment, is significantly influenced by what tools we have to tell us about either of those things,” he says. Several IMAT-funded projects have become widespread research tools as well as commercial products. “I really appreciate the community they have brought together,” says Tseng, who received a grant from IMAT.

“We don’t prescribe what kinds of innovations we’re interested in, we want investigators to come to us and surprise us with their innovative ideas,” Dickherber says. The programme puts together interdisciplinary review panels to score prospective research tools according to their potential impact.

Tyler Jacks, who directs the MIT’s David H. Koch Institute for Integrative Cancer Research, says his institute is “expressly about bringing biologists and engineers together under one roof”. Collaborations are encouraged by ensuring that everyone circulates in the same sections. “We’re neighbours now and we interact much more extensively,” he says.

Physicist Jean-François Joanny at the Curie Institute in Paris is also using his expertise to benefit cancer biology. Building on work by the late Malcolm Steinberg, a Princeton University biologist, Joanny’s team has looked at how pressure affects the growth of clusters of cancer cells known as spheroids. Perhaps a physical property is what distinguishes cancer cells. “Could you characterize the degree of invasiveness by looking at properties like this?” he says. “That’s the idea at the back of our minds.”

One cancer biologist proposed a scientific challenge for Joanny’s group. He asked the physicists to make pressure measurements in a mouse with multiple tumours and determine, from those measurements alone, which cancer is most likely to metastasize. As the biologist knows the correct answer, his team will use biology to validate the physics-based results.

The Curie Institute has a tradition of cross-disciplinary collaboration, with cancer biologists approaching suggestions from physicists with open minds, Joanny says. “We are used to the idea that they might consider us crazy.” But if wild ideas from physicists can boost the fight against cancer — and their work to help biologists understand the various processes of metastasis suggests that they can — then maybe they’re not quite so crazy after all. ■

Vivien Marx is technology editor at *Nature* and *Nature Methods*.

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CORRECTION

The Technology Feature ‘Two microscopes are better than one’ (*Nature* **492**, 293–297; 2012) wrongly stated that the enzyme APEX is a singlet oxygen generator, it is a peroxidase. Also, Alice Ting was a postdoc in the lab of Roger Tsien, not Mark Ellisman.