

M. SMITH



METASTASIS

The rude awakening

If detected early, most cases of breast cancer seem to be curable. But the tumour's deadly offspring could be sleeping in the body.

BY JOCELYN RICE

Ann Chambers has a series of graphs tacked up in her office at the London Regional Cancer Program in Canada, where she is an oncologist. "I stare at them all the time," she says.

These charts are assembled from more than 60 years of data gathered at the MD Anderson Cancer Center in Houston, Texas, and show how the ten-year survival curves for local and metastatic breast cancer have changed over the decades (see 'The hard facts', page S50). The picture they paint of breast cancer, Chambers says, has regions of darkness and light.

On the one hand, the overall survival rate of patients with breast cancer has vastly improved. Sixty years ago, only a quarter of patients were alive ten years after being diagnosed; now, that figure exceeds three-quarters. On the other hand, for patients whose tumour has metastasized — spread to distant sites

outside the breast — at the time of diagnosis, the picture remains dismal. Even now, a patient with metastatic breast cancer has only a 22% chance of surviving more than ten years.

The graph that troubles Chambers most, however, is the one for patients with local breast cancer who show no evidence of metastasis at diagnosis. Decade after decade, the survival curves for these patient cohorts decline over time, with the current ten-year survival rate at 86%. The implication is that, despite appearing free of disease, "there are hidden tumour cells in these women", says Chambers. These cells are lurking in a state of suspended animation. It might be a rare event; however, even 25 years after diagnosis, these dormant cells can reawaken, growing into a full-blown metastasis and ultimately killing a patient who was once considered to be cured.

The cells that give rise to such late metastases remain mysterious. Where are they hiding? Why do they reawaken? "This is a very peculiar biology," says Klaus Pantel, an oncologist at the University Medical Center Hamburg-Eppendorf in Germany. "If we can understand

what the body is doing to control this cancer for 10 or 15 years, and what stops this control and eventually leads to metastatic relapse, we can foresee completely new strategies for controlling disseminated cancer."

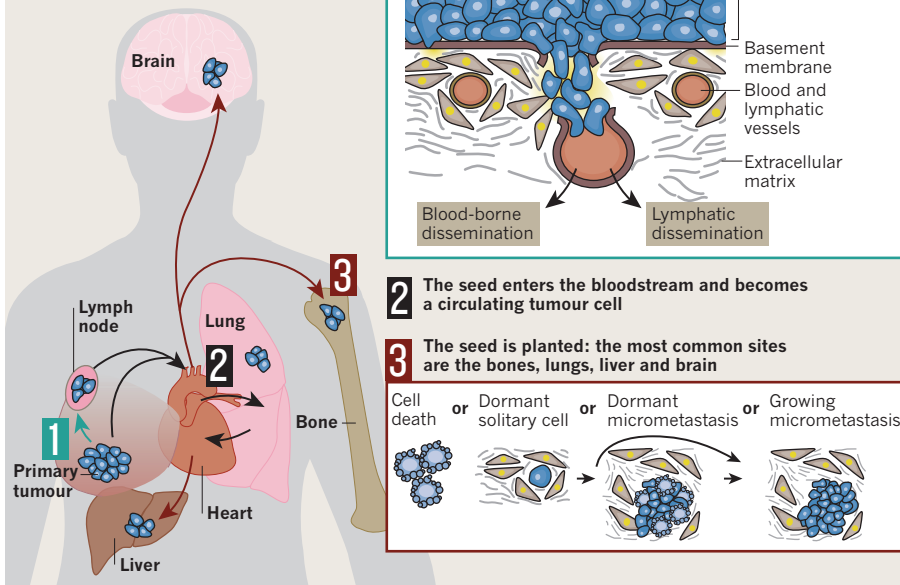
SEEDS AND SOIL

One thing we do know about these dormant cells is where they originally came from. In most patients who develop metastases, tumour cells have already undergone a cascade of transformations at the time of diagnosis, allowing many of them to escape from the primary lesion (see 'The right trials', page S58). These cells enter the bloodstream, either directly or by way of the lymph nodes, and circulate through the body. In cancer, "dissemination is an early event", says Christoph Klein, a cancer biologist at the University of Regensburg, Germany. Indeed, research by Klein and others shows^{1,2} that escaped tumour cells can sometimes be detected even in patients diagnosed with ductal carcinoma *in situ* (DCIS), an early-stage breast cancer in which the tumour appears to be confined to the lining of the milk ducts.

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THE GREAT ESCAPE

If a cell leaves the primary tumour, it can circulate and lie dormant in other tissues for decades.



The escaped cells in the bloodstream, called circulating tumour cells (CTCs), are the potential seeds of future metastases (see ‘The great escape’). After a patient has completed chemotherapy, the presence of CTCs is associated with a higher risk of recurrence. Monitoring CTCs during treatment might also provide a means to track a patient’s disease over time and adjust therapy accordingly, says Massimo Cristofanilli, a breast cancer clinician and researcher at Fox Chase Cancer Center in Philadelphia, Pennsylvania. CTCs, he says, provide “a real-time assessment of the disease”. For example, a patient might have a primary tumour that expresses a normal amount of the receptor HER2 (a HER2-negative tumour). If later testing reveals CTCs that overexpress HER2 (HER2-positive cells), this new information would expand treatment options — making the patient a candidate for HER2-targeted therapies, such as trastuzumab (Herceptin) and lapatinib. “Before, you wouldn’t have even considered that,” says Chambers. The expression of hormone receptors can also differ. Chambers says that monitoring the oestrogen- and progesterone-receptor (ER and PR) status of CTCs could offer another way to adapt a patient’s treatment over time.

CTCs are attractive candidates for prognosis and tracking disease status because they are accessible with a simple blood draw. A device for detecting CTCs in blood has been approved by the US Food and Drug Administration: Cell-Search (produced by the diagnostics company Veridex, based in Raritan, New Jersey) identifies tumour cells in a blood sample by using tiny magnetic particles coated with an antibody against a known epithelial-cell marker.

However, this marker is sometimes lost when cells leave the primary tumour, and researchers estimate that CTCs are missed in up to one-third of patients. Alternative approaches use different markers to distinguish the tumour cells or microfluidic technology to sort the larger tumour epithelial cells from the smaller blood cells. Either way, it’s a needle-in-a-haystack problem because white blood cells can outnumber CTCs by 500,000 to 1, says David Lyden, a cancer biologist and paediatric oncologist at Weill Cornell Medical College and Memorial Sloan-Kettering Cancer Center in New York.

CELLULAR RE-EDUCATION

Not all CTCs develop into a full-blown metastasis, says Chambers. They’re merely seeds. To develop, they must first find the appropriate soil. To some extent, she adds, the location of metastases is determined by the patterns of blood flow that carry cells away from the primary tumour. After entering the bloodstream, tumour cells first travel to the heart. From there, their next stop is the capillary beds of the lungs. Some cells may lodge there, says Chambers, whereas others “can weasel their way through, getting out into the circulation again to get access to other sites”.

Circulatory patterns don’t tell the whole story, however. Certain tissues are especially hospitable to CTCs, providing a microenvironment replete with the nutrients and growth factors that they need. “Cells can get to all sorts of places,” says Chambers, “but they’ll grow where they find a good match with what they need.” The best ‘soil’ for breast cancer cells, she says, can be found in the lungs, liver, brain and bones.

Complicating the seed-and-soil concept is the emerging realization that CTCs can send out signals — free-floating molecules such as growth factors and chemokines, as well as membrane-bound packets of protein and nucleic acid called exosomes — that can prime target sites to be more receptive to the cells’ arrival. In other words, the seed is fertilizing the soil in advance. Lyden and colleagues call these primed microenvironments ‘pre-metastatic niches’, and they suggest that metastases might be prevented by identifying and inhibiting these priming signals.

The process of site remodelling continues after CTCs have established themselves in a new destination, where they are now known as disseminated tumour cells (DTCs). Recent work suggests that this kind of communication might also happen in reverse. Tumour cells that make it to the bones will encounter quiescent stromal cells in the bone marrow. “It’s possible that tumour cells become educated by the surrounding cells,” says Lyden. *In vitro* studies have shown that in the bone-marrow stroma, microRNAs (short non-coding RNAs with a regulatory function) can be taken up by breast cancer cells, inducing them to become quiescent³. “Free-floating microRNA from the bone marrow can re-educate tumour cells to be more like bone-marrow stem cells, saying ‘be quiet, don’t proliferate, stay here in the bone marrow,’” says Lyden.

This process of re-education also raises another intriguing possibility, he says. Haematopoietic stem cells (blood-cell precursors) can leave the marrow and circulate through the body. If DTCs have been reprogrammed to behave like these cells, perhaps DTCs also take tours around the circulatory system. “If they’re in the bone marrow, are they just sleeping there,” asks Lyden, “or are they like bone-marrow stem cells, which can go out into circulation and come back?” If the latter is true, the bone marrow could be a long-term reservoir for metastatic disease. Patricia Steeg, a molecular biologist at the National Cancer Institute in Bethesda, Maryland, highlights the finding that bone-marrow-derived cells are recruited to pre-metastatic niches elsewhere in the body as evidence of this possibility. “It certainly suggests that these bone-marrow cells could provide a safe haven for dormant tumour cells,” she says.

Pantel has been analysing DTCs in the bone marrow of patients with breast cancer for more than 20 years. These cells are more difficult to study than CTCs, as aspirating bone marrow is a far more complicated and painful procedure than drawing blood. But, like their counterparts in the blood, DTCs can offer crucial information about a patient’s prognosis and can appear in bone-marrow aspirates even when the primary tumour is still very small. “These cells are not just innocent bystanders,” says Pantel. “Their presence is predictive of a poorer clinical outcome.” Yet the fate of these cells is wildly variable. Some will grow into metastases immediately; some will do so much later. And,

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he adds, if the cells do leave the bones for good, the microenvironment at their ultimate destination might yet block their outgrowth, leaving them in a state of lifelong dormancy.

This uncertainty is a problem for routinely testing patients for DTCs in the bone marrow, says Lyden. A negative result is great news: the patient is very unlikely to develop bone metastases. But a positive result is harder to interpret. “What do you tell the patient?” asks Lyden. It’s impossible to know the fate of a given DTC. When will it start proliferating? Will it proliferate at all? Researchers are searching for biomarkers (genetic or biochemical signatures) that could hold the answers to such questions. HER2 could be one such marker: as is the case for CTCs, tumour cells found in the bone marrow can have a different HER2 status from the primary tumour, and HER2-positive DTCs are more likely to grow into metastases than HER2-negative DTCs. A multicentre study is now under way in Germany to investigate whether monitoring the HER2 status of DTCs can guide treatment strategies for patients with HER2-negative primary breast cancer.

READING THE SIGNS

Some researchers think that bones are the most important site of dormancy and metastasis in breast cancer; a liver metastasis, for example, might grow from a cell that first spent a decade asleep in the bone marrow. Others think that liver metastases grow from CTCs that travelled directly to the liver and then became dormant.

Animal studies support the latter idea that dormancy is a systemic phenomenon. In one such study⁴, pathologist David Tarin and colleagues at the University of California, San Diego, injected human breast cancer cells tagged with green fluorescent protein into the mammary fat pads of mice. Not only did mammary tumours form but, in autopsy studies using fluorescence microscopy, green pinpoints were also evident in various other organs. When the fluorescent cells were retrieved, even from organs with no overt metastases, the individual — apparently dormant — cells were able to generate metastases in new mice, indicating that the cells had retained their metastatic potential.

In humans, however, researchers don’t have the option of tagging tumour cells for easier imaging. Analysis of liver and bone-marrow biopsies can hint at the presence of tumour-derived cells, but there is currently no imaging technique sensitive enough to locate individual DTCs inside the body. George Sledge, an oncologist at the Indiana University School of Medicine in Indianapolis, says: “Radiologists speak about the one-millimetre challenge” — the difficulty of imaging structures smaller than 1 mm. An isolated DTC falls well outside that range. Even in autopsy studies, in which a pathologist can assess tissues directly, identifying a solitary DTC is a tough problem.

Underlying the difficulty of locating dormant tumour cells is a more fundamental issue:

there’s still no consensus on what ‘dormancy’ entails. “I’m still stumped on the definition of dormancy,” says Steeg. In one interpretation, says Sledge, tumour cells are actively dividing, but a barrier prevents them from crossing the threshold to overt metastasis. That barrier could be blood supply: the micrometastasis is starved of nutrients until a genetic switch flips, allowing the cells to develop their own vasculature. Alternatively, it could be immune surveillance: the cells cannot proliferate beyond a micro-metastasis until they evolve a strategy to evade the constant policing of the immune system.

In a view championed by Chambers, dormancy in DTCs might be a truly quiescent state — a kind of long-term hibernation, where the cells might not be dividing at all. The mechanisms regulating this type of dormancy are unclear, but recent research by Julio Aguirre-Ghiso, an oncology researcher at the Mount Sinai School of Medicine in New York, indicates one possible scheme. Within cells, there is a balance between two signalling pathways: the growth-promoting ERK pathway, and the growth-restricting p38 pathway⁵. Some tumour cells lose p38 signalling, escaping quiescence and spurring their characteristic unchecked growth. Aguirre-Ghiso and his colleagues have proposed that once these cells leave the primary tumour, certain microenvironments might



disturb this balance: an environment that preserves ERK dominance might yield an immediate metastasis, whereas one that promotes p38 could lead to dormancy⁶. Aguirre-Ghiso calls this the target organ scenario: “The cells arrive, and they respond to that microenvironment.”

But Aguirre-Ghiso and colleagues found that the fate of disseminating cells might already have been at least partly determined in the primary tumour. By examining experimental dormancy gene-expression signatures associated with the ERK and p38 pathways, Aguirre-Ghiso’s team was able to predict with reasonable accuracy in patients whether an ER-positive primary tumour would produce early metastases or have a propensity for long-term dormancy⁷. “This quiescence-associated signature could be influencing the fate of the cells that left the primary tumour,” says Aguirre-Ghiso. That

would provide a mechanism to explain why some patients have an early recurrence and why some patients have a later recurrence after clinical dormancy, he says.

WAKE-UP CALL

The challenge of understanding dormancy pales, however, in comparison to uncovering why dormant DTCs reawaken. Researchers have plenty of ideas but little hard evidence. Lyden speculates that age-related changes in the microenvironment, such as a shifting ratio of myeloid and lymphoid cells or a decrease in physical space for haematopoietic stem cells, might stimulate DTCs to resume their growth. Infection or trauma — for example, a broken bone — might have the same effect, says Lyden. Chambers, meanwhile, sees a role for diet and exercise in regulating the recurrence of breast cancer (see ‘Powering up’, page S62). Prospective trials are needed to examine the effects of such lifestyle changes over decades, she says. But for now data are scarce.

One thing is clear though. If breast cancer survival rates are to continue to improve, we need to redefine breast cancer. Pantel foresees a future in which breast cancer is treated like a chronic disease, such as diabetes. With appropriate drugs and lifestyle modifications, he says, we could prevent recurrence by encouraging dormant cells to remain dormant or by destroying them before they reawaken.

On another level, Klein says, “we need a new pathology of systemic cancer because the characteristic of the disease is that it changes. It’s evolving. It’s progressing.” Right now, patients receive treatments that are tailored on the basis of information about the primary tumour. Tests can identify not only hormone-receptor and HER2 status, one of the standard tumour classifications, but also subtle genetic signatures that predict early recurrence.

“While we have lots of genomic assays that predict metastasis, most are better at predicting early recurrence rather than late recurrence,” says Sledge. “We clearly need better gene or protein signatures that predict late relapse — and people are working on this.” The search for better diagnostic and prognostic clues will probably have to shift away from the primary tumour towards the circulating and disseminated cells. Metastasis is a moving target, and researchers acknowledge that any effective treatment strategy will have to constantly recalibrate its aim. ■

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