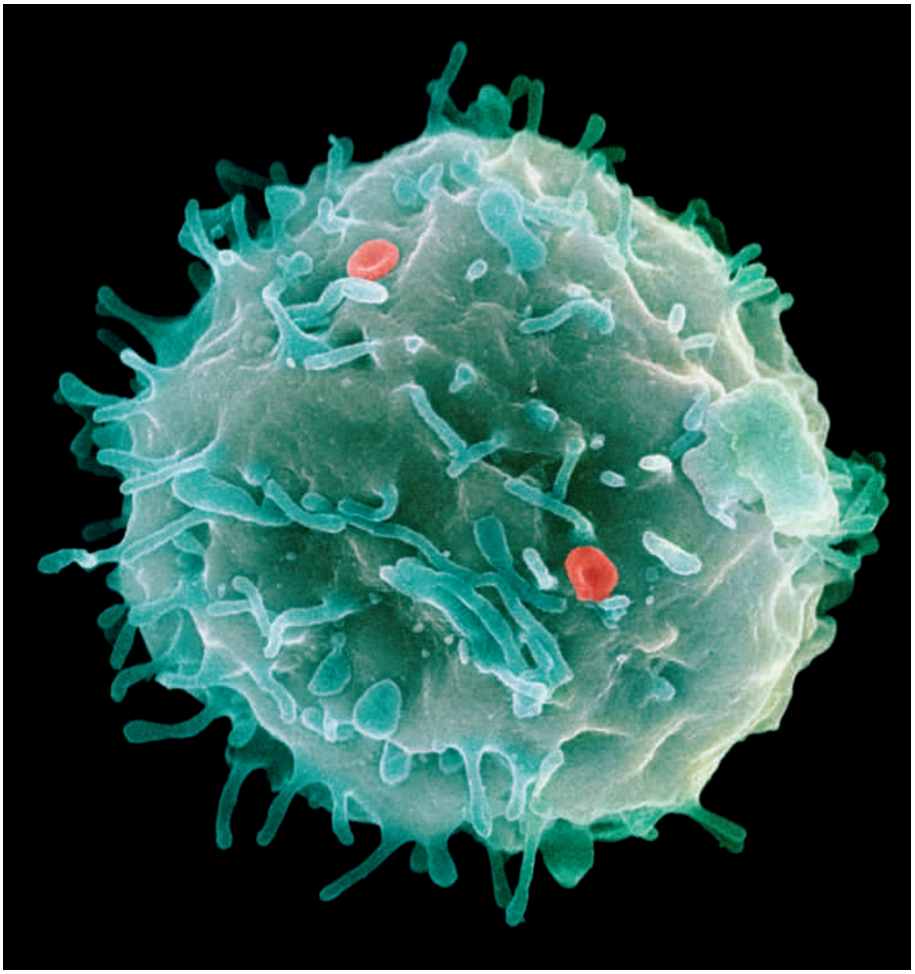

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Could mutations in haematopoietic stem cells, which give rise to blood cells, lead to leukaemia?

STEM CELLS

Bad seeds

Leukaemia treatments must eliminate the versatile cells that can bring the cancer back to life years later.

BY CASSANDRA WILLYARD

Deep within the spongy marrow that fills large bones lie cells that give rise to all the body's blood cells. These primitive cells, called haematopoietic stem cells, have the unique ability to divide indefinitely, making them essentially immortal.

Mounting evidence suggests that similarly immortal cells drive some types of leukaemia and other cancers. Just as haematopoietic stem cells can produce a vast array of blood cells, leukaemia stem cells generate the diversity of malignant cells seen in cancer.

John Dick, a cancer researcher at the University of Toronto in Canada, first isolated leukaemia stem cells in the 1990s. At the time, many cancer researchers believed that all cancer cells are equally capable of seeding new cancers.

In contrast, the cancer-stem-cell model proposes that only a rare subset of cells has the ability to give rise to a new tumour (see 'Tumour hierarchy'). These cells can self-renew, are long-lived and can lie dormant for years.

"Those three things are properties that we generally don't target with our standard chemotherapeutic agents," says Catriona Jamieson, director of stem-cell research at the Moores Cancer Center at the University of California, San Diego. Conventional therapies may kill most cancer cells and induce remission, Jamieson says, but cancer stem cells persist, allowing the disease to come roaring back even in individuals who seem healthy.

Over the past decade, this model has sparked heated controversy. Some types of cancer, such as acute myeloid leukaemia (AML), a disease that affects the cells that give rise to red blood cells, platelets and some immune cells, seem to follow the model. But in solid tumours and some other forms of leukaemia, the existence of cancer stem cells is uncertain.

Even for diseases that conform to the cancer-stem-cell model, researchers disagree over the cells' abundance, attributes and origin. One central question is whether cancer stem cells are normal stem cells that have turned malignant, or rather more mature cells that have regained some stem-cell-like properties.

"There is endless debate and confusion and contentiousness about their characteristics," says Mel Greaves, a cancer researcher at the Institute for Cancer Research in London. "They are slippery and diverse," he says. "That's why it's so tough to target them therapeutically."

SLIPPERY CELLS

Tough, perhaps, but not impossible. Researchers are developing drugs that can rouse leukaemia stem cells out of dormancy, making them more vulnerable to therapy, and target the pathways they need, such as those that promote self-renewal.

To isolate cancer stem cells for analysis, researchers use mice with compromised immune systems that allow human cancer cells to grow. They first sort cancer cells based on their surface markers — proteins found on the cell membrane that can be tagged with fluorescent molecules. For example, haematopoietic stem cells share an immune marker called CD34 and lack a marker called CD38.

The researchers then inject each group of cells into mice to see which ones cause disease. By taking the cancer cells from one mouse and transplanting them into another, they also examine the cells' ability to self-renew.

Dick, who developed the assay, first showed that it could be used to identify leukaemia stem cells in his landmark 1994 *Nature* paper[1]. His team reported that a subset of leukaemia cells that both carry CD34 and lack CD38 — the same markers that characterize haematopoietic stem cells — can seed leukaemia in mice. Moreover, cells with the opposite characteristics — those that lack CD34 or have CD38 — do not trigger new cancers. The researchers interpreted these results as confirmation that those with CD34 and without CD38 are leukaemia stem cells.

Leukaemia researchers largely accepted Dick's findings. "It proved what had been predicted since the 1960s and '70s," Dick says.

But the cancer-stem-cell connection to leukaemia turns out to be more complicated than that. Over the past 15 years, researchers have tried to identify and characterize leukaemia stem cells. They have found that the cells are vastly more diverse than previously thought and, as a result, are more difficult to eradicate. "Our original thoughts about cancer stem cells were a gross oversimplification," says Brian

Huntly, a leukaemia researcher at the Cambridge Institute for Medical Research, UK, who was not involved in Dick's work.

In 2011, Dick and his colleagues took leukaemia cells from 16 people with AML, once again sorted those cells based on their surface markers, and injected them into mice with even more defective immune systems than those in the original study. This time, however, the researchers found that the leukaemia stem cells were not confined to a particular subset[2]. In particular, the presence or absence of CD34 and CD38 did not dictate whether the cells could seed new tumours.

Another group, led by researchers from the University of Pennsylvania in Philadelphia, used a different mouse model but got similar results. The Pennsylvania team was likewise unable to isolate leukaemia stem cells using CD34 and CD38. What's more, they tried two other markers, CD45RA and lineage, which also showed no correlation to leukaemia cells.

Such results leave leukaemia researchers in a frustrating position. "Is there any marker or will there ever be a marker that is inextricably linked to the property of self-renewal?" asks Scott Armstrong, an oncologist at the Memorial Sloan-Kettering Cancer Center in New York. "I'd say we don't have such a marker."

Even so, scrutiny of leukaemia stem cells could lead to a better way to predict the course of the disease. In his 2011 study, Dick compared gene expression in leukaemia stem cells with other leukaemia cells. He identified 42 genes that were highly expressed only in the leukaemia stem cells. "Lo and behold," says Dick. "We came up with a signature." And this signature correlates with prognosis.

The researchers then divided 160 individuals with AML into two groups, based on their expression levels of the 42 genes. Those with high expression fared the worst: at any given time point, they were nearly two-and-a-half times more likely to die than members of the low-expression group.

Dick and his team now have data from 100 individuals that tie the signature even more closely to prognosis. "It tells us that not only are leukaemia stem cells real, but they must also be relevant," he says.

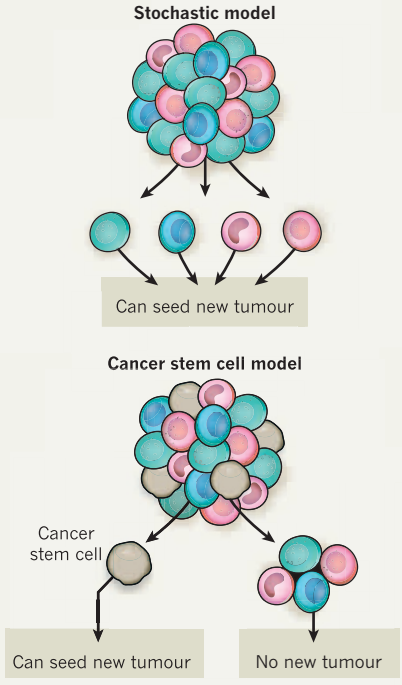
The fact that stem cells are difficult to pin down is not surprising. "Leukaemia is an evolutionary process," Greaves says. The disease originates with a single cell, but its descendants may acquire new mutations, making them genetically distinct. As a result, a drug that targets just one of these mutations may not eliminate all of the cancer cells, leaving some behind that can regenerate the tumour.

THINK LOCAL

One characteristic common to all leukaemia stem cells is that they require the particular microenvironment of the bone marrow, known as the stroma. Leukaemia cells grown in a flask divide rapidly. As a result, they are "exquisitely sensitive" to chemotherapy, which targets rapidly dividing cells, says oncologist John DiPersio at Washington University School of Medicine in St Louis, Missouri. But the stroma bathes the cells in growth factors and chemical signals that keep them in a dormant phase resistant to chemotherapy.

TUMOUR HIERARCHY

In the stochastic model, all tumour cells have an equal ability to seed new tumours. In the cancer-stem-cell model, only a few 'stem cells' can self-renew and seed new tumours.



Part of what keeps these cells entrenched in the bone marrow is a chemical signal sent by the stroma called stromal cell-derived factor (SDF-1). This signal binds to a protein called CXCR4 on the stem cells' surfaces. "It tells the stem cells to stay put," says Washington University haematologist Daniel Link.

DiPersio and his colleagues speculated that if they could interrupt this signal, they might be able to make the cells more sensitive to chemotherapy. They tested the idea in 52 people with AML who were set to begin chemotherapy. Before starting treatment, each patient was administered a small-molecule drug called plerixafor, which the researchers believed would block the CXCR4 receptor[3].

The results were encouraging. "Remission rates were close to 50%, which is about twice as high as historic controls for patients with relapsed AML," DiPersio says.

The group has launched a second study, this time combining plerixafor with granulocyte-colony stimulating factor (G-CSF), a growth hormone that dampens SDF-1 expression. They hope that the drugs — one affecting the signalling molecule, the other the receptor — will act synergistically and lead to an even higher rate of remission. In a separate pilot study, Link is testing whether G-CSF can increase the effectiveness of chemotherapy in individuals with relapsed acute lymphoblastic leukaemia, a type of the disease in which immature white blood cells take over the bone marrow and spill out into the bloodstream.

Other researchers are looking for proteins present in leukaemia stem cells but not in normal haematopoietic stem cells. In 2010, a team of researchers from the RIKEN Center for Integrative Medical Sciences in Japan identified haematopoietic cell kinase (HCK), an enzyme involved in cell signalling, in dormant leukaemia stem cells taken from the bone marrow of people with AML.

In April 2013, the RIKEN researchers took the next step towards a therapy by identifying a compound that inhibits HCKs and seems to eliminate leukaemia stem cells. In mice, this compound abolished nearly all AML cells from blood and from the mice's bone marrow and spleen after three weeks of treatment[4].

"The efficacy data in this paper are impressive, but it is way too early to get too excited," says Ravindra Majeti, a haematologist at Stanford University in Palo Alto, California. Majeti's research suggests that researchers may need to target not only leukaemia stem cells, but also seemingly normal haematopoietic stem cells.

Last year, Majeti and his team examined haematopoietic stem cells and leukaemia cells from six people with AML. In five of these individuals, haematopoietic stem cells in the bone marrow contained some of the same mutations present in the cancer cells[5]. They hypothesize that these cells could be precursors to fully fledged leukaemia. That's important, Majeti says, because it suggests that even if a drug eradicates the leukaemia, it may not provide a cure. "The preleukaemic stem cells could give rise to a related but distinct relapse through the acquisition of new mutations," Majeti says.

Researchers are also developing medications that target the key property of all stem cells — self-renewal. One signalling pathway that seems to play an important role in self-renewal hinges on two proteins: Wnt and beta-catenin. In April 2012, Armstrong reported that a small molecule that inhibits beta-catenin, given in combination with the targeted therapy imatinib (Gleevec, marketed in some countries as Glivec), eliminates leukaemia stem cells in a mouse model of chronic myeloid leukaemia.

Even cancers that do not seem to strictly adhere to the cancer-stem-cell model harbour cells that can self-renew. "They're bad actors," says Dick. "What we need is to begin to figure out what their Achilles heel is." ■

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