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The root of all evil?

Cancer may be caused by stem cells gone bad. If that proves to be correct, it should revolutionise treatment.

Much of medical research is a hard slog for small reward. But, just occasionally, a finding revolutionises the field and cracks open a whole range of diseases. The discovery in the 19th century that many illnesses are caused by bacteria was one such. The unravelling of Mendelian genetics was another. It now seems likely that medical science is on the brink of a finding of equal significance. The underlying biology of that scourge of modern

humanity, cancer, looks as though it is about to yield its main secret. If it does, it is possible that the headline-writer's cliché, "a cure for cancer", will come true over the years, just as the antibiotics that followed from the discovery of bacteria swept away previously lethal infectious diseases.

The discovery – or, rather, the hypothesis that is now being tested – is that cancers grow from stem cells in the way that healthy organs do. A stem cell

is one that, when it divides, produces two unequal daughters. One remains a stem cell while the other multiplies into the sorts of cells required by its organ. This matters for cancer because, at the moment, all the cells of a tumour are seen as more or less equivalent. Therapies designed to kill them do not distinguish between them. Success is defined as eliminating as many of them as possible, so those therapies have been refined to do just that. However, if all

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that the therapies are doing is killing the descendants of the non-stem-cell daughters, the problem has not been eliminated. Instead of attacking the many, you have to attack the few. That means aiming at the stem cells themselves.

Not all investigators support the cancer stem-cell hypothesis, but the share who do so is growing rapidly. A mere five years ago, few research papers on the subject were presented at big academic meetings. This year there were hundreds at one such meeting alone. Moreover, data from clinical trials based on the hypothesis suggest that it has real value for patients. As a result, drug companies have taken notice and are trying to develop substances that will kill cancer stem cells.

THE VIRTUES OF SELF-RESTRAINT

The root cause of both cancer and stem cells is multicellularity. In the distant past, when all living things had only one cell, that cell's reproduction was at a premium. In the body of an animal, however, most cells have taken a vow of self-denial. Reproduction is delegated to the sex cells. The rest, called somatic cells, are merely supporting actors, specialised for the tasks needed to give the sex cells a chance to get into the next generation. For this to happen required the evolution of genes that were able to curb several billion years' worth of

instinct to proliferate without killing that instinct entirely. Only then could somatic cells do their job, and be present in appropriate numbers.

The standard model of tumour formation was based on the fact that somatic cells slowly accumulate mutations. Sometimes these disable the anti-proliferation genes. If enough of the brakes come off in a somatic cell, so the theory went, it will recover its ancestral vigour and start growing into a tumour. Cancer, then, is an inevitable cost of being multicellular.

The discovery of stem cells changed this picture subtly, but importantly. Blood stem cells were found a long time ago, but only recently has it become apparent that all tissues have stem cells. The instincts of stem cells lie halfway between those of sex cells and ordinary body cells. They never stop reproducing, but they cannot look forward to making the generational leap. When the body dies, so do they. However, they are few in number, and because at cell division only one daughter continues to be a stem cell, that number does not grow.

This division of labour may even be another type of anti-cancer mechanism. It allows stringent locks to be put on somatic cells (which, for example, strictly limit the number of times they can divide), yet it permits tissue to be renewed. Without stem cells, such tissue-renewal would be the province of

any and every somatic cell—a recipe, as the traditional model observes, for tumorous disaster. The obverse of this, however, is that if a stem cell does mutate into something bad, it is likely to be very bad indeed. That, in essence, is the stem-cell hypothesis of cancer.

One obvious prediction of this hypothesis is that tumours will have at least two sorts of cell in them: a dominant population of daughter cells and a minority one of stem cells. The first person to show that to be true was John Dick, a molecular biologist at the University of Toronto. In 1997 he isolated what looked like stem cells from a blood cancer called acute myeloid leukaemia (AML). Blood cancers are easier to deal with in this context than solid tumours because their cells do not have to be separated from one another before they are examined. One characteristic of AML cells is that they have two sorts of a protein, called CD34 and CD38, on their surfaces. Dr Dick thus used two sets of special antibodies for his experiment. One sort stuck only to the CD34 molecule, the other only to CD38. Each sort was also attached to a fluorescent tag.

By mixing the AML cells from his patients with the two antibodies and running them through a machine that sorted them according to how they fluoresced, he showed that most were positive for both proteins. However, a small fraction (as low as 0.2%) were positive

The tumour stem cells suffered the same damage from the radiation, but were better able to repair it

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only for CD34. These, he suspected, were the stem cells.

He was able to confirm this by injecting the minority cells into mice. The resulting tumours had the same mix of cells as those from human patients. However, when he injected mice with samples from the majority cells, with both sorts of the protein, no tumours resulted. The CD34-only cells thus acted as cancer stem cells.

Moreover, this phenomenon was not confined to leukaemia. In 2003 a group of researchers at the University of Michigan in Ann Arbor, led by Max Wicha and Michael Clarke, used a similar trick on breast cancer cells. In this case the surface proteins were known as CD24 and

CD44, and the minority were those positive only for CD44. As with AML, these minority cells produced cancers in mice, whereas the majority cells did not.

Since these two pieces of work, the list of cancer stem cells has multiplied. It now includes tumours of the breast, brain, prostate, colon, pancreas, ovary, lung, bladder, head and neck, as well as melanoma, sarcoma, AML, chronic myelogenous leukaemia, Hodgkin's lymphoma and myeloma.

That is quite a list. The question is, what can be done with it? Jeremy Rich, a neurologist at Duke University in Durham, North Carolina, has one idea. He created mice that had human glioblastoma tumours, a form of brain

cancer, growing in them. Then he treated these mice with radiation (the standard therapy for such cancer in people). He found that the cancer stem cells were more likely to survive this treatment than the other cells in the tumour. That turned out to be because, although all the tumour cells suffered equal amounts of DNA damage from the radiation, the stem cells were better able to repair this damage. When he treated the mice simultaneously with radiation and with a drug that interferes with DNA repair, however, the stem cells no longer had an advantage. They were killed by the radiation along with the other cells.

If that result applies to people as well as rodents, it opens up a whole avenue of possibility. In fact, Dr Rich is now in negotiations with several companies, with a view to testing the idea in humans. That 'if' is a real one, though. A mouse is not a human being.

Indeed, the stem-cell hypothesis is often criticised for its reliance on animal models of disease. Some researchers worry that the experiments used to identify putative cancer stem cells are too far removed from reality – human tumour cells do not naturally need to survive in mice – and thus may not reflect human cancer biology at all.

Proponents of the hypothesis are alive to that concern, but they think that the same pattern has been seen so often in so many different cancers that it is unlikely to be completely wrong. The practical test, though, will be whether the hypothesis and ideas that emanate from it, such as Dr Rich's combination therapy, actually help patients survive.

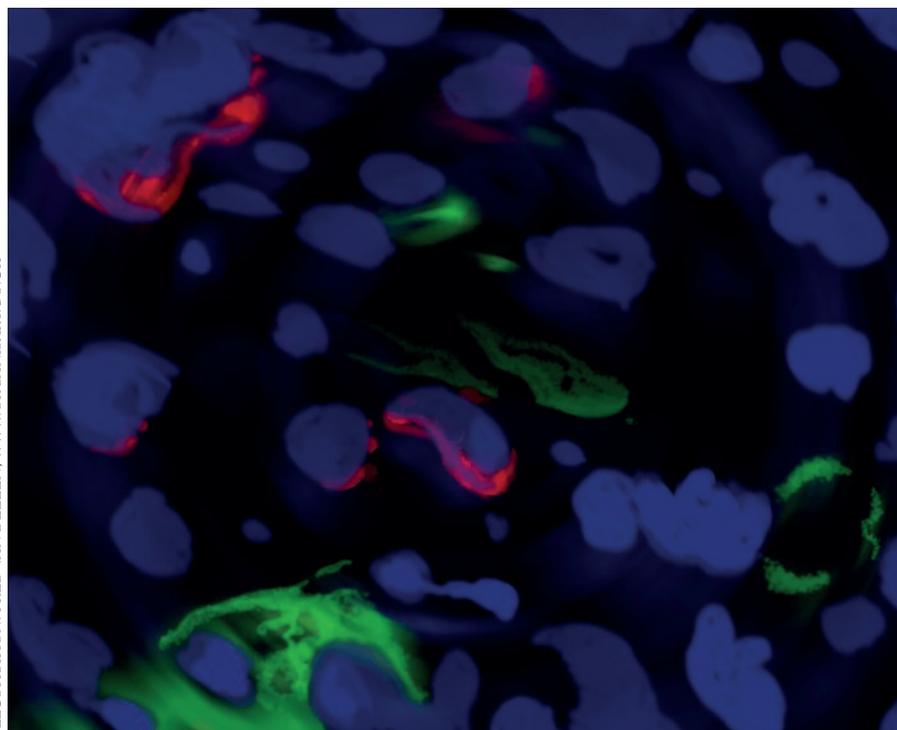


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SEARCHING FOR THE SUSPECTS

As a step towards discovering whether they do, William Matsui, an oncologist at Johns Hopkins University School of Medicine in Baltimore, looked for cancer stem cells in pancreatic-tumour samples taken from nearly 300 patients. His team found that patients whose tumours did contain such stem cells survived for an average of 14 months. Those whose tumours lacked them survived for 18 months.

That finding is consistent with the idea that cancer stem cells contribute to the most aggressive forms of the

behind. When the group repeated the experiment using a modern drug called Tykerb that blocks what is known as the HER2 pathway, they got a different result. HER2 is a gene which encodes a protein that acts as a receptor for molecules called growth factors which, as their name suggests, encourage cell growth and proliferation. After the HER2-blocking treatment, cancer stem cells formed the same proportion of the residual tumour as beforehand. That suggests they, too, were being clobbered by the new treatment. It is probably no coincidence that another

make up the bulk of such a tumour. Epithelial cells are immobile, grow in 'cobblestone' patterns and produce proteins that help them stick together. The gene expression of the putative stem cells, however, resembled that of a mesenchymal cell. Mesenchymal cells rarely stick together. Indeed, they are mobile and are able to slip through the matrix of proteins that holds epithelial cells together.

That finding is important because mobile cells are more likely to escape from a tumour and form secondary cancers elsewhere in the body. Once such secondaries are established, successful treatment is much harder. And the CD44-positive cells also expressed genes that are important for stem-cell self-renewal, particularly one called Notch that controls the flow of chemical signals within a cell.

Researchers at OSI Pharmaceuticals, a firm that makes a drug called Tarceva, found a similar pattern in lung cancer. Several years ago, they started looking for gene-expression patterns that correlated with response to Tarceva. They found that tumours with a pattern that resembled epithelial cells were sensitive to the drug. By contrast, those that had a mesenchymal pattern were not. They hypothesised that as tumours develop, some of their cells actually switch from a sticky, epithelial state to a mobile, mesenchymal one. This epithelial-to-mesenchymal transition, or EMT, is well known to biologists who study embryonic development, but OSI's results, and those of other researchers, suggest that cancers may have hijacked it for their own use.

Robert Weinberg, a molecular biologist at the Massachusetts Institute of Technology, and his colleagues have come to the same conclusion but they have taken the hypothesis one step further. They think that tumour cells which have undergone EMT have acquired many of the characteristics of cancer

It may be that stem cells are a result of a cancer, rather than its cause

disease, though it does not prove they cause tumours in the first place. And although the absence of detectable stem cells in some tumours may be seen as casting doubt on the whole idea, it may instead be that they are too rare to be easily detected. If the stem cell idea is confirmed, it may help doctors and patients choose how to treat different tumours. Those with detectable stem cells might be candidates for aggressive chemical and radiation therapies, while those without might best be treated with the surgeon's knife alone.

Breast cancer researchers are also testing the stem-cell hypothesis in the clinic. Jenny Chang's group at Baylor College of Medicine, in Texas, took samples of tumours from women before and after standard chemotherapy. When they counted the cells in the tissue they found that the proportion of stem cells in a tumour increased after treatment. That suggests the chemotherapy was killing the non-stem tumour cells and leaving the stem cells

drug, Herceptin, which also goes after HER2, is one of the few medicines that is able to prolong the lives of people with advanced cancer.

The stem-cell hypothesis has also changed the way people do basic research. For example, over the past few years cancer researchers have been grinding up pieces of tumour and using what are known as gene-expression microarrays to work out which genes are active in them. However, if the hypothesis is correct, this approach will probably yield the wrong result, because the crucial cells make up but a small part of a tumour's bulk and the activity of their genes will be swamped by that of the genes of the more common non-stem cells. The answer is to isolate the stem cells before the grinding starts.

This approach has already yielded one important finding. When Dr Chang used microarrays to study gene expression in the CD44-positive cells from breast tumours, she noticed that they did not look like those of the epithelial cells that

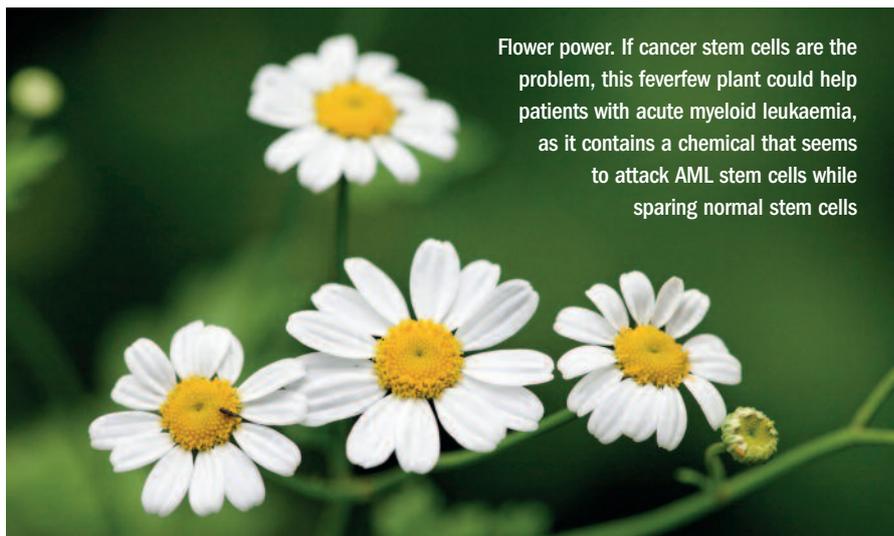
stem cells. Experiments in his laboratory, employing a variety of animal models of breast cancer, suggest that communication between tumour cells and surrounding non-cancerous support cells can lead some of the cancer cells to undergo EMT.

That is intriguing. If this transition really can be induced in tumour cells, then any of them might be able to become a cancer stem cell. So it may be that the fundamentalist version of the stem-cell hypothesis is wrong, and the stem cells are a result of a cancer, rather than its cause. That could be another reason why Dr Matsui found that pancreatic cancers do not always seem to contain stem cells.

Dr Weinberg is sensitive to this point, and is cautious when talking about these experiments. He refers to the cells that have undergone EMT as “having the qualities of stem cells” but avoids actually calling them cancer stem cells. If his idea is correct, though, it means that finding drugs which block the signals that induce EMT could reduce the stem-cell population and prolong the survival of the patient. It also means that both the epithelial cells and the mesenchymal ones will have to be attacked. And OSI is now testing a drug that does just that.

NOTCH UP A VICTORY?

Breast cancer researchers, too, are testing drugs that hit molecular targets highlighted by cancer stem cell studies. Merck, for example, has turned to a drug it originally developed to treat Alzheimer's disease. Although this drug, code-named MK0752, did not slow that disease, it does block activity of Notch, the stem-



Flower power. If cancer stem cells are the problem, this feverfew plant could help patients with acute myeloid leukaemia, as it contains a chemical that seems to attack AML stem cells while sparing normal stem cells

cell self-renewal gene, and might thus be an appropriate weapon against breast cancer stem cells. Dr Chang and Dr Wicha have started a clinical trial which uses MK0752 in combination with standard chemotherapy. By the end of the year they hope to have some idea of whether the combination kills cancer cells in human tumours.

Attacking Notch is a high-risk approach, because this gene is used by healthy stem cells as well as cancerous ones; healthy organs as well as tumours could be damaged. Some researchers are therefore taking a different tack and looking for drugs that hit only the unhealthy stem cells. Craig Jordan, a biologist at the University of Rochester Medical Centre, in New York state, is one such. He has discovered that a chemical called parthenolide, found in feverfew, a medicinal plant, kills AML stem cells. Normal stem cells, however, seem to be able to tolerate the drug without difficulty. The reason is that the

leukaemia cells are reliant on a biochemical pathway that parthenolide blocks, whereas normal stem cells are not. If all goes well, a trial to test the safety of a modified form of parthenolide will start in a few months.

If the safety issues can be dealt with – and most researchers think they can – then attacking cancer stem cells really could help patients survive. If, that is, the stem-cell hypothesis is correct.

At the moment, scientists being scientists, few are willing to be anything other than cautious. They have seen too many past cures for cancer vanish in a puff of smoke. The proof needs to come from patients – preferably with them living longer. But if the stem-cell hypothesis is indeed shown to be correct, it will have the great virtue of unifying and simplifying the understanding of what cancer is. And that alone is reason for hope.

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