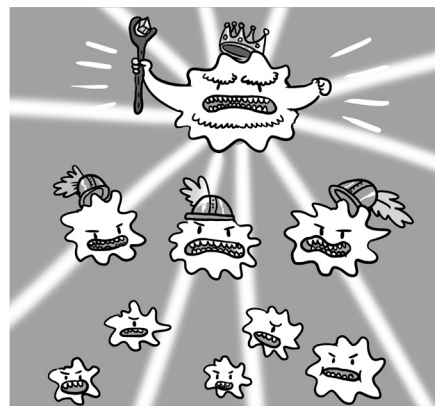


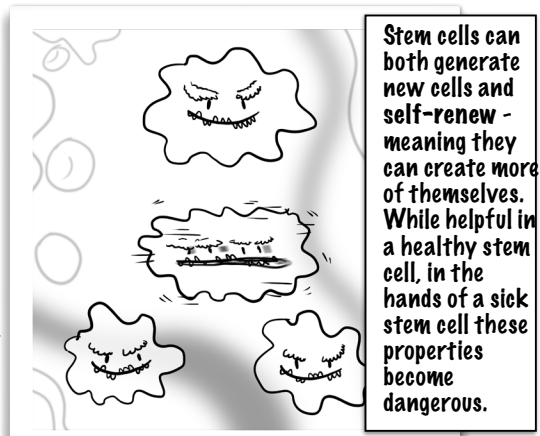
Unlocking the Mystery of Leukemic Stem Cells

1



We think of cancer as a blob-like tumor, full of generic 'bad' cells. Dr. John Dick is a scientist who discovered that not all cancer cells are equal. They are organized in a hierarchy, with a rare and powerful cell at the top - cancerous stem cells.

2



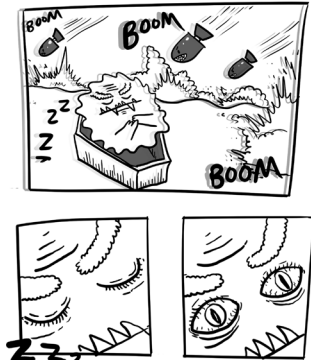
Stem cells can both generate new cells and self-renew - meaning they can create more of themselves. While helpful in a healthy stem cell, in the hands of a sick stem cell these properties become dangerous.

3



Dr. Dick studies acute myeloid leukemia (AML), an aggressive blood cancer. While patients with AML seem to respond well to chemotherapy, over half of patients relapse. But how does AML develop again so quickly?

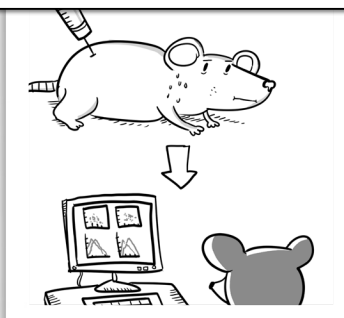
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Dr. Dick discovered the culprit: leukemic stem cells (LSCs). LSCs can remain dormant for long periods of time and survive chemotherapy. After treatment, LSCs can begin generating new cancer cells and re-start the cancer growth.

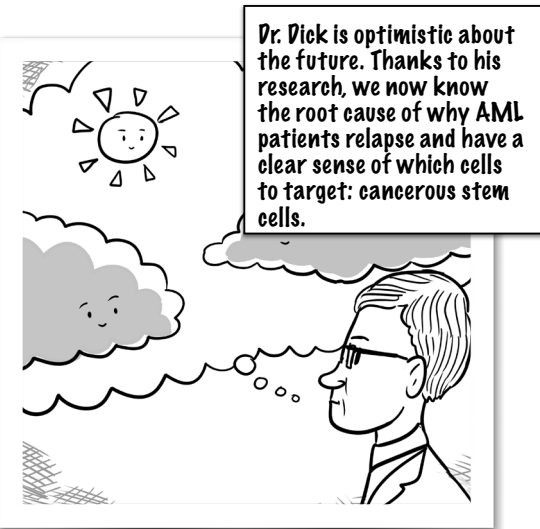
LSCs were not easy to find. Dr. Dick developed two experiments to locate this rare cell type. He first transplanted human cancer cells using a xenograft assay into immuno-deficient mice, to show that only a small subset of cancer cells are able to propagate leukemia.

5



Second, he used flow cytometry to isolate LSCs based on their unique cellular characteristics.

6



Dr. Dick is optimistic about the future. Thanks to his research, we now know the root cause of why AML patients relapse and have a clear sense of which cells to target: cancerous stem cells.



Unlocking the Mystery of Leukemic Stem Cells



In acute myeloid leukemia, not all cells are created equal

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October 2022

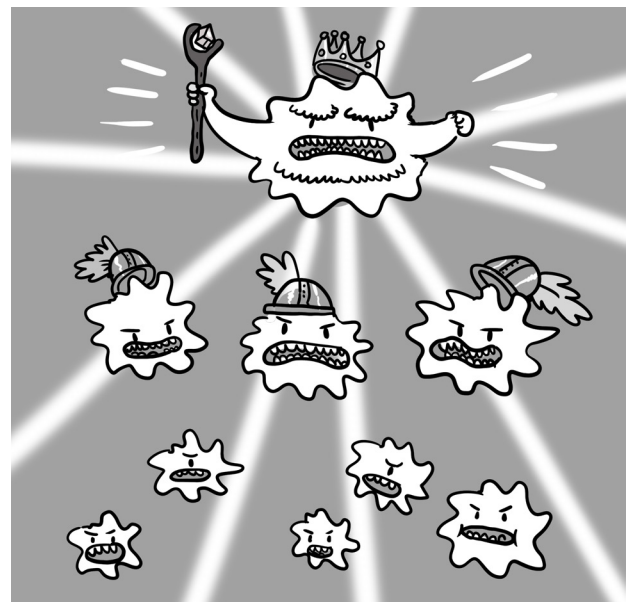
Not all cancer cells are created equal

Cancer is often thought of as a homogenous lump. A blob-like tumour full of generic ‘bad’ cells that expands and grows in an uncontrolled way, getting noticed and making a person sick when it begins to affect the healthy cells and tissue surrounding it. The distinction between tumour and tissue is supposed to be simple: you’re either a cancer cell, or you’re not. A healthy cell, or a sick cell. A good cell, or a bad cell.

For a long time, researchers thought about cancer in this way. Assuming all cancer cells within a tumour were essentially copies of the same bad cell, scientists would break down a tumour and combine the tumour cells together to study the properties of the cancer cells relative to healthy cells.

This approach to cancer research was important and led to many valuable insights into cancer genetics, metabolism, and biochemistry. A notable example is the discovery of **oncogenes** - genes capable of triggering a healthy cell to transform into a cancer cell under certain circumstances.

But there is one large oversight to this perspective. “Previous approaches to studying cancer assumed that all the cells in cancer are equal,” explains Dr. John Dick, a scientist at the Princess Margaret Cancer Centre in Toronto, Ontario who has studied cancer for decades. “But what it missed was



the individual cells of that tumour, and it sort of assumed that all cells are the same.”

Dr. Dick’s award winning research pushed back against the assumption that cancer cells are uniform within a tumour or type of cancer. His work demonstrated that cancer cells actually organize themselves within hierarchies, with cells containing different, advantageous properties enabling them to drive cancer growth residing at the top. “Our work [...] was one of the pieces of information that really drove down the idea that only certain cells have the ability to keep that cancer going, in a way that regular cancer cells cannot” says Dr. Dick. These powerful cells are a cancerous form of stem cells, or **cancer stem cells**.

Stem cells: small but mighty

In order to understand what a cancer stem cell is, we first have to take a step back and understand the role of a healthy stem cell.

Healthy stem cells are a critical part of maintaining the body's cellular ecosystem. A small but mighty subset of cells, stem cells contain the powerful ability to generate all other types of cells. Stem cells themselves are **undifferentiated**, meaning they do not have a niche function like a skin cell or a nerve cell. Instead, stem cells spend their life producing all manner of **differentiated**, or specialized cells, needed to maintain the body's regular functioning.

After development, stem cells live in small populations throughout the body and generate new cells in an organ-specific way. For example, stem cells in the colon will continuously produce the specific cells needed to maintain the intestines. In the bone marrow, stem cells produce blood cells at a rapid rate in order to meet the high demand for new oxygen-carrying blood cells. To maintain a healthy blood supply, adult humans produce over 100 million new blood cells *per minute*. This extraordinary task is possible only by your stem cells.

Importantly, stem cells also have an enormous capacity for **self-renewal**. They possess the powerful ability to replicate and generate more of themselves. Stem cells can divide while maintaining their special undifferentiated state and therefore maintain their own population indefinitely. This property of self-renewal is an important characteristic which enables stem cells to exist in independent, self-sustaining populations.

However, imagine if a stem cell began to collect mutations over time that slowly caused them to start generating sick cells. Now the incredible ability of a stem cell to pump out the new cells needed to maintain a healthy system has turned into a factory for creating massive quantities of dysfunctional cells.

Importantly, generic cancer cells are also able to divide and replicate, and they do so rapidly. This is

why chemotherapy and radiation treatments target rapidly dividing cells – their intense growth means that they take up more from their surroundings. This is also why some healthy cells that divide frequently, such as hair cells, getting caught in the crossfire. However, the rapidly dividing cells that make up the bulk of a cancer have a limited capacity for self-renew and will exhaust their ability to replicate after a finite number of divisions. It is only cancer stem cells that can continuously produce new cells and fuel long-term cancer growth.

Acute myeloid leukemia: an aggressive blood cancer

Dr. Dick's area of expertise is cancer of the blood. In Canada, over 140,000 people are currently living with blood cancer, with leukemia as one of the most common diagnoses. With leukemia, the bone marrow produces a large number of abnormal and dysfunctional blood cells - aka cancer cells. These cancerous blood cells multiply more rapidly than regular blood cells and are also less likely to die naturally, creating a recipe for the rampant spread of diseased blood cells throughout the blood system.

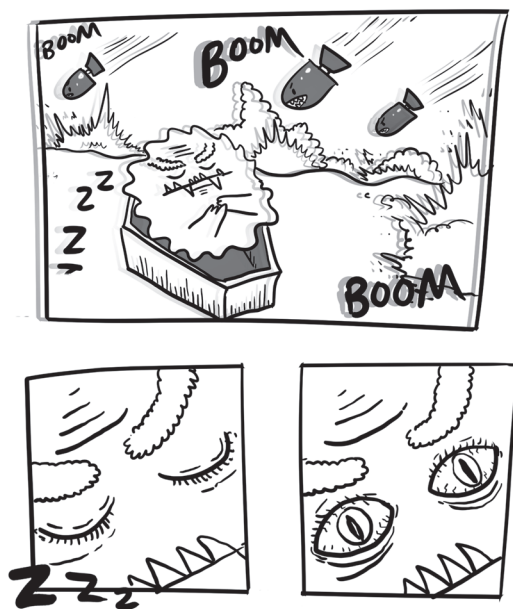
In particular, Dr. Dick studies **acute myeloid leukemia** (AML), a serious and aggressive form of leukemia which can cause a rapid decline in patients. While patients with AML often respond well to chemotherapy and the cancer seems to disappear, AML has a high rate of relapse. "80% of patients get put into remission and the disease goes away. But for the vast majority of these people, within two years the disease is back," says Dr. Dick. The five-year survival rate for patients over the age of 60 is less than 15%.

But how does a cancer which has virtually disappeared manage to regrow? Dr. Dick's laboratory

Although there are many types of stem cells, the basic idea is that they tend to be rare and special in that they have the unlimited ability to make more of themselves (self renewal), and that they are not committed to one cell type, so have the potential to become lots of different things.

was the first to solve this mystery. In AML, the culprit turned out to be a small population of dysfunctional stem cells, called **leukemic stem cells** (LSCs).

LSCs contain the perfect storm of characteristics to kickstart cancer relapse. LSCs have a slower rate of cell division than regular cancer cells and go through long periods of ‘dormancy’, making them resistant to traditional therapeutics like chemotherapy. In fact, not only can LSCs survive chemotherapy, they can actually become *activated* by chemotherapy. This means that while chemotherapy destroys regular cancer cells, it can cause LSCs to come out of hibernation and start producing more cancer cells again, thus triggering cancer relapse.



Finding the needle in a haystack: locating the rare LSC

Pinpointing LSCs as the culprit of AML relapse was not easy. Dr Dick’s team had to develop two experiments to locate this rare type of cell and confirm that LSCs were responsible for cancer growth. The first of these experiments was a **xenograft assay**, in which human cancer cells were transplanted into immuno-deficient mice. Some of these mice would go on to develop human leukemia, while others would not. This provided a clue that it wasn’t simply the presence of any cancer cell that could cause cancer to develop.

A specific type of cancer cell had to be present that possessed the ability to drive cancer growth.

But these mysterious cells appeared to be quite a rare subset of the cancer cell population, vastly outnumbered by the regular cancer cells they were producing. To further isolate the culprit, Dr. Dick’s lab investigated using a cell-sorting method called **flow cytometry** to differentiate LSCs from non-LSC leukemia cells based on their unique cellular characteristics. The results of these experiments showed that LSCs are extremely rare, with a frequency of approximately one out of every million leukemia cells.

These experiments, combined with further genetic analysis, enabled Dr. Dick’s team to map out the complex evolutionary pathway of LSCs. “You need a sequence of events to happen before you actually get a full cancer,” says Dr. Dick, “Cancer doesn’t happen overnight. One normal cell picks up a mutation that makes it just a little bit more advantageous. That cell expands a little bit more, picks up another mutation, and so forth.” His team tracked how normal blood stem cells develop into pre-leukemic stem cells, and then eventually become mature LSCs. The point at which LSCs become capable of generating AML can occur up to a decade after the first healthy stem cell begins mutating. Further genetic analysis enabled Dr. Dick to develop a ‘stemness score’ that uses 17-genetic markers to predict therapy outcome in AML patients.

Cancer and optimism: the future of cancer treatment

Despite the difficulties of cancer research, Dr. Dick finds the future of AML to be filled with hope. Thanks to Dr. Dick’s team, we now understand why patients with AML are so prone to relapse and have a better idea of which cells to target moving forward.

This key piece of information also comes at a time when rapid advances in scientific technology and gene therapy are changing the odds for the better when we face cancer. “Technology is just coming together on a massive scale,” says Dr. Dick, “The

idea that what used to require 100 billion cells to get insight into genetics, we can now do with a single cell is remarkable. So we can quickly sift through single cells of a normal tissue or leukemia or any cancer and begin to ask, ‘what are that particular cancer’s vulnerabilities?’”



The stemness score that Dr. Dick developed for AML also helps guide therapeutic choice by determining the particular genetic makeup of a patient and their cancer. “Now we can begin to tailor therapies based on not just an individual patient, but the individual cells that have particular vulnerabilities,” says Dr. Dick, “So that gives me huge optimism for the pace of advances that we’re going to undertake.”

Dr. Dick’s optimism provides confidence that the outcome for patients with AML is improving. With a clear idea of what we’re facing, we know which cells to target for future cancer treatments: leukemic stem cells.