Researchers find marker identifying most basic form of blood stem cell

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After a long series of experiments, researchers at the Stanford University School of Medicine have identified a unique cell marker that they say allows them to pick out the most fundamental form of the stem cell that gives rise to the blood and immune system.

If confirmed, their finding would help settle long-standing controversies about the identity of these stem cells and their support cells. It also may pave the way for understanding how these cells maintain themselves, and provide scientists with the necessary information to grow blood stem cells in the laboratory or clinic.

A paper describing the research was published Feb 11 in *Nature*. Irving Weissman, MD, a professor of pathology and of developmental biology at Stanford, is the senior author.

In 1988, Weissman and his colleagues isolated the hematopoietic stem cell, which goes on to become the body's blood and immune cells. Since that time, researchers have had only mixed success in their attempts to get a detailed picture of how these HSCs maintain themselves and grow in the body. Over the years, it became clear why. The hematopoietic stem cells they isolated came in two flavors: most are short-term HSCs that lose their powers of replication over time, while a small fraction are long-term HSCs that can replicate indefinitely and are critical to lifelong blood production. To understand how other cells nurture the HSC, researchers needed to study only the long-term HSC.

With the new study, the Stanford researchers believe they have now found a reliable way to tell the difference between long-term and short-term HSCs. "In this paper we have found a single marker that, in the entire bone marrow, is only found in these long-term stem cells," said Weissman, who is also the Virginia and D.K. Ludwig Professor in Clinical Investigation in Cancer Research and the director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine.

Now that the researchers can identify the long-term HSCs, they hope to be able to look at how those cells and nearby cells create a "niche"—a biological space where long-term stem cells are supported and maintained.

"For nearly 30 years, people have been trying to grow HSCs outside the body and have not been able to do it—it's arguably the 'holy grail' in this field," said James Chen, an MD/PhD candidate at Stanford and co-lead author of the paper. "Now that we have an anchor, a way to look at long-term HSCs, we can look at the cells around them to understand and, ideally, recreate the niche." If that niche can be created in a laboratory setting, people may be able to grow long-term HSCs in the lab.

A two-year search

In the last decades, many scientists have proposed various markers that they felt were unique to long-term HSCs, but the reliability of each proposed marker has been heatedly debated by other research groups, said postdoctoral scholar Masanori Miyanishi, MD, PhD, the other lead author.

To settle the issue, Chen and Miyanishi devised a method that was highly systematic, but also expensive and time-consuming. "Many times, we were about to quit," Chen said.

They started with a list of over 100 genes that are expressed in the bone marrow, where long-term HSCs are found, that seemed like good candidates to be unique markers of long-term HSCs. With the assistance of their colleagues, they eliminated genes that are turned on in areas of the bone that don't involve the creation of new blood and immune cells. That narrowed the field to 45 genes.

Then they performed a sophisticated, painstaking analysis to determine how much protein these genes were making in various cells. They found that only three proteins were produced at a high enough level to mark HSCs. Finally, they needed to find if one of these three was turned on in long-term HSCs and turned off in short-term HSCs. Although they couldn't yet identify which cells were long-term HSCs, they knew that any collection of HSCs should have both long-term and short-term HSCs, so they expected to find the candidate gene turned completely off in some cells and on in others. They found that only one gene fit that bill: a gene called Hoxb5.

The researchers point out that there may be other unique markers of long-term HSCs, such as genes that weren't among the initial group of the more than 100 they screened. But among the screened genes, only Hoxb5 was a unique identifier of the long-term stem cell.

Finding the niche

The researchers were also able to solve another key mystery by showing where in the bone marrow long-term HSCs reside. Satoshi Yamazaki, PhD, a member of the Tokyo lab of Stanford genetics professor Hiromitsu Nakauchi, MD, PhD, used technology recently developed in Japan to prepare bone marrow tissue and do computational analysis that validated the location and architecture of the HSC niche. "More than 90 percent of these cells reside on a particular type of blood vessel called venous sinusoids," said Nakauchi, a co-author of the paper.

The ability to identify long-term stem cells will give scientists a powerful tool for further study, the researchers said. "This opens the way to observe long-term HSCs and other cells in the niche as they exist in the body, without transplanting them," said Weissman, who is also director of the Ludwig Center for Cancer Stem Cell Research and Medicine. "This is how science works, by getting down to the purest irreducible element—in this case, blood stem cells—in order to develop new tools and understandings."

More information: James Y. Chen et al. Hoxb5 marks long-term haematopoietic stem cells and reveals a homogenous perivascular niche, *Nature* (2016). DOI: 10.1038/nature16943

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