Scientists unable to find evidence of 'embryonic-like' cells in marrow of adult mice

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Research on human embryonic stem cells has been a political and religious lightning rod for more than a decade.

The cells long have been believed to be the only naturally occurring pluripotent cells. (Under the right conditions, pluripotent cells can become any other cell in the body.) But some people object to the fact that the embryo is destroyed during their isolation. Induced pluripotent stem cells, created by experimentally manipulating an adult cell such as a skin or nerve cell, are much more ethically palatable. But many researchers feel it is important to continue studying both types of cells.

In 2006, a group of researchers led by Mariusz Ratajczak, MD, PhD, at the University of Louisville, described another possible alternative: a special population of very small, pluripotent embryonic-like cells in adult bone marrow of mice and humans. These cells, called VSEL (very small embryonic-like) cells, presumably arise through the self-renewal of embryonic stem cells during the developmental process and, as described, could provide all the benefits of embryonic stem cell research with none of the ethical controversy. However, subsequent research from other labs has provided conflicting results as to the pluripotency —and even the existence—of VSEL cells in bone marrow.

A company, NeoStem, has proposed a human clinical trial of the cells for periodontitis to begin this year.

But scientists in the laboratory of Irving Weissman, MD, a professor of pathology at the Stanford University School of Medicine, say they have been unable to identify any very small, pluripotent cells in the bone marrow of mice, despite exhaustive efforts to duplicate the original experimental procedures.

"It has become important to know to what extent and where these VSEL cells exist to understand how they may affect the field of stem cell research," said Weissman, who directs Stanford's Institute for Stem Cell Biology and Regenerative Medicine and the Ludwig Center for Cancer Stem Cell Research and Medicine at Stanford. "We tried as hard as we could to replicate the original published results using the methods described and were unable to detect these cells in either the bone marrow or the blood of laboratory mice."

Although other groups have seemingly confirmed the existence of these cells as defined by size and the expression of key cell-surface molecules, Weissman's study is the first to evaluate the biological potency of the cells.

The research will be published online July 24 in *Stem Cell Reports*. Weissman, who is also the Virginia & D.K. Ludwig Professor for Clinical Investigation in Cancer Research and a member of the Stanford Cancer Institute, shares senior authorship of the study with instructor Jun Seita, MD, PhD. Postdoctoral scholars Masanori Miyanishi, PhD, and Yasuo Mori, MD, PhD, are the lead authors.

Using a variety of methods, the researchers found that most of the very small (less than 5 micrometers in diameter) particles in mouse-bone marrow were not cells, but were in fact cell debris or dead cells with a less-than-normal complement of DNA. Because it can be difficult to determine actual cell size, they opted to include even larger cells in their subsequent analysis.

Another way the cells are defined is by the expression, or lack of expression, of a combination of key molecules on their surface. Specifically, the cells are defined as CD45-/intLin-SCA-1+. Weissman and his colleague found that cells from the mouse-bone marrow meeting this criteria, or phenotype, failed to express the stem-cell-specific marker Oct-4, were unable to form spheres in culture (a hallmark of pluripotent cells) and could not differentiate into any cells of the hematopoietic, or blood-forming, system.

"A true pluripotent cell would be able to differentiate into any tissue type," said Weissman. "But we couldn't confirm that cells of that size or phenotype could generate hematopoietic cells with any reliability."

Weissman has previously proposed three key tenets necessary to prove the accuracy of a scientific result: The original finding must be published in a peer-reviewed journal; the experiment must be repeatable, as published, by many independent laboratories; and the finding as described should be detected by multiple experimental methods. Although the original research was peer-reviewed and published, the difficulty experienced by other labs attempting to replicate the findings has led the Weissman group to conclude in the paper that "the existence of adult mouse VSELs in the bone marrow remains dubious."

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