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Blood Vessel Cells Promote Self-Renewal of Blood Stem Cells

Howard Hughes Medical Institute (HHMI) scientists have discovered that endothelial cells, the building blocks of the vascular system, keep blood stem cells dividing healthily in a lab dish much longer and more effectively than previous methods of growing the cells. The new advance dramatically improves scientists' ability to manufacture large quantities of authentic adult blood stem cells, which may help revolutionize the field of bone marrow transplantation.

Shahin Rafii, an HHMI investigator at Weill Cornell Medical College in New York City, and his colleagues report on the development of an endothelial cell platform that supports self-renewal of the blood stem cells, known as long-term hematopoietic stem cells (LT-HSCs), in the March 2010 issue of the journal *Cell Stem Cell*. Their study also describes a novel mechanism by which endothelial cells support propagation of LT-HSCs in adult mice.

There are few naturally occurring stem cells in adult organs, so using them for organ regeneration is impractical. Adult stem cells can be grown in the laboratory, but until now, strategies to expand these cultures, which invariably used animal-based growth factors, serum, and genetically manipulated feeder cells, have not sustained the stem cells' ability to self-renew for more than a few days.

"Our findings highlight the potential of vascular cells for generating sufficient stem cells for therapeutic organ regeneration, tumor targeting, and gene therapy applications."

- Shahin Rafii

The new study employs endothelial cells, to propagate stem cells in the laboratory without added growth factors or serum. In the body, these endothelial cells establish a "vascular niche" that is essential for regenerating blood stem cells in the adult bone marrow.

The endothelial cell platform developed by Rafii's team supports adult blood stem cell self-renewal for more than 21 days, with true stem cells expanding by hundreds-fold within those three weeks. Such prolific expansion may help scientists grow functional blood stem cells abundantly for biologic research, and therapeutic bone marrow transplantation. Rafii expects his team's approach will help in growing other types of organ-specific or cancer stem cells, as well.

In this report the researchers also show that endothelial cells are not only endowed with the capacity to support long-term expansion of stem cells, but also instruct stem cells to differentiate into all of the mature components of blood, including immune cells, platelets, red and white blood cells --both in *in vitro* endothelial-stem cell cocultures and in the adult marrow

The research, spearheaded by Jason Butler, an investigator in Rafii's lab, began with inserting a gene called *E4ORF1* from an adenovirus into the endothelial cells to enhance their survival. This permits these metabolically demanding endothelial cells to propagate on command without the growth factors they usually need. This is an advantage when the endothelial cells are grown with stem cells, since adding growth factors and serum to the mix can bias stem cell behavior and inhibit their expansion. Using *E4ORF1* overcomes concerns about introducing cancer-promoting genes into the endothelial cells – an earlier strategy for eliminating the need for growth factors in the culture medium.

Next, the researchers isolated blood stem cells from mice, and grew them in the same dish as the engineered endothelial cells, without adding growth factors or serum. This resulted in massive expansion of hematopoietic cells, Butler said.

The research team next looked at how well the newly generated stem cells functioned by transplanting them into mice that could no longer produce blood cells due to radiation treatment. The transplanted cells gave rise to blood cells of all types for more than a year, suggesting that the expanded cells were indeed bona fide stem cells, Butler says. Rafii adds that although there was some concern that forced expansion of the stem cells might induce cancerous mutations, when the expanded stem cells were transplanted back into mice, there was no indication of tumor formation, even after a year. Thus, he says, endothelial cells provide a safe milieu that proliferates stem cells without creating cancer risk.

The researchers also showed, both in cocultures and in the marrow of the adult mice, that endothelial cells express specific stem-cell active growth factors, such as Notch-ligands, that support the expansion of stem cells. "Traditionally endothelial cells are perceived as passive conduits for supplying oxygen and nutrients," Butler says. "Now we have discovered that endothelial cells can serve an instructive role, by producing growth factors that promote stem cell self-renewal and differentiation."

Prior to this study, it was speculated that bone-forming cells called osteoblasts were the main feeder cells in the bone marrow that sustained stem cells. But using molecular imaging, the team showed that blood stem cells are in intimate contact with the marrow's endothelial cells that express Notch-ligands. "This finding indicates that endothelial cells could directly, independent of osteoblasts, stimulate expansion of stem cells," stated Butler.

The findings offer therapeutic potential. For instance, stem cells grown in the new system could be used for bone marrow transplants that might allow lifetime repopulation of needed blood cells. The newly developed research platform might also be useful for generating and studying other types of adult stem cells, Rafii says, noting that previous research has shown that other organ-specific stem cells typically reside near endothelial cells.

Rafii notes that the new vascular-based approach might also help researchers generate large quantities of cancer stem cells for research purposes. With a sufficient supply of these stem cells, which many scientists believe drive tumor growth, screens of potential therapies could be scaled up so researchers can test large libraries of chemicals to see which ones might help overcome the disease.

Rafii predicts that as researchers identify other factors with which endothelial cells influence stem cell behavior, this will establish a new arena in stem cell biology: "We will be able to selectively activate endothelial cells not only to induce organ regeneration, but also to inhibit the production of specific endothelial cell-derived factors in order to block tumor growth," he says. "Our findings highlight the potential of vascular cells for generating sufficient stem cells for therapeutic organ regeneration, tumor targeting, and gene therapy applications."