Breakthrough reveals blood vessel cells are key to growing unlimited amounts of adult stem cells

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In a leap toward making stem cell therapy widely available, researchers at the Ansary Stem Cell Institute at Weill Cornell Medical College have discovered that endothelial cells, the most basic building blocks of the vascular system, produce growth factors that can grow copious amounts of adult stem cells and their progeny over the course of weeks. Until now, adult stem cell cultures would die within four or five days despite best efforts to grow them.

"This is groundbreaking research with potential application for regeneration of organs and inhibition of cancer cell growth," said Dr. Antonio M. Gotto Jr., the Stephen and Suzanne Weiss Dean of Weill Cornell Medical College and Provost for Medical Affairs of Cornell University. "We are indebted to Shahla and Hushang Ansary for founding this Institute and to the Starr Foundation Tri-Institutional Stem Cell Initiative for ongoing support."

This new finding sets forth the innovative concept that blood vessels are not just passive conduits for delivery of oxygen and nutrients, but are also programmed to maintain and proliferate stem cells and their mature forms in adult organs. Using a novel approach to harness the potential of endothelial cells by "co-culturing" them with stem cells, the researchers discovered the means to manufacture an unlimited supply of blood-related stem cells that may eventually ensure that anyone who needs a bone marrow transplant can get one.

The vascular-cell model established in this study could also be used to grow abundant functional stem cells from other organs such as the brain, heart, skin and lungs. An article detailing these findings appears in the March 5 issue of the journal *Cell Stem Cell*.

In adult organs, there are few naturally occurring stem cells, so using them for organ regeneration is impractical. Until now, strategies to expand cultures of adult stem cells, which invariably used animal-based growth factors, serum, and genetically manipulated feeder cells, have only been marginally successful. This study, which employs endothelial cells to propagate stem cells without added growth factors and serum, will likely revolutionize the use of adult stem cells for organ regeneration, as well as decipher the complex physiology of the adult stem cells.

"This study will have a major impact on the treatment of any blood-related disorder that requires a stem cell transplant," says the study's senior author, Dr. Shahin Rafii, the Arthur B. Belfer Professor in Genetic Medicine, co-director of the Ansary Stem Cell Institute and a Howard Hughes Medical Institute Investigator, at Weill Cornell Medical College. Currently, stem cells derived from bone marrow or umbilical cord blood are used to treat patients who require bone marrow transplants. Most stem cell transplants are successful, but because of the shortage of genetically matched bone marrow and umbilical cord blood cells, many patients cannot benefit from the procedure.

"Over the last few decades, substantial funding has been spent to develop platforms to expand adult stem cell cultures, but these efforts have never been able to coax an authentic adult stem cell to self-renew beyond a few days," continues Dr. Rafii. "Most stem cells, even in the presence of multiple growth factors, serum, and support from generic non-endothelial stromal cells, die after a few days. Now, employing our endothelial stem cell co-cultures, we can propagate bona fide adult stem

cells in the absence of external factors and serum beyond 21 days with an expansion index of more than 400-fold."

If this vascular-based stem cell expansion strategy continues to be validated, physicians could use any source of hematopoietic (blood-producing) stem cells, propagate them exponentially, and bank the cells for transplantation into patients.

In a true first, the study demonstrates how this novel vascular cell platform or "vascular niche" can self-renew adult hematopoietic stem cells for weeks, both in vitro and in vivo, by co-culturing them on a bed of endothelial cells. The researchers chose endothelial cells because they are in close contact with blood stem cells, and previous work from Dr. Rafii's lab had demonstrated that endothelial cells produce novel stem-cell-active growth factors. However, maintenance of the endothelial cells is cumbersome and if they are not "fed" specific substances, such as growth factors known as "angiogenic factors," they immediately die. To get around this problem, the researchers genetically engineered the endothelial cells to stay in a long-term survival state by inserting a recently discovered gene cloned from adenoviruses, which does not promote oncogenic transformation of the human cells. This earlier discovery, using a single gene to put endothelial cells into a long-lasting "suspended animation" state without harming their ability to produce blood vessels, was also discovered in Dr. Rafii's lab and published in the journal Proceedings of National Academy Sciences in 2008.

Endothelial Cells Could Generate Stem Cells and Their Differentiated Progeny

In this study, the researchers also discovered that endothelial cells not only could expand stem cells, but also instruct stem cells to generate mature differentiated progeny that could form immune cells, platelets,

and red and white blood cells, all of which constitute functioning blood.

"We are the first group to demonstrate that endothelial cells elaborate a repertoire of stem-cell-active growth factors that not only stimulate stem cell expansion but also orchestrate differentiation of these stem cells into their mature progeny," says Dr. Jason Butler, a senior investigator at Weill Cornell Medical College and first author of the study. "For example, we have found that expression of specific stem-cell-active factors, namely Notch-ligands, by the endothelial cells lining the wall of working blood vessels promote proliferation of the blood-forming stem cells. Inhibition of these specific factors on the endothelial cells resulted in the failure of the regeneration of the blood-forming stem cells. These findings suggest that endothelial cells directly, through expression of stem-cell-active cytokines, promote stem cell reconstitution."

Further describing this innovative concept, in a high-impact article published in the January 2010 issue of Nature Reviews Cancer, Drs. Rafii and Butler, and Dr. Hideki Kobayashi, who is also a co-author of the current study, have elaborated on specific endothelial cell-produced growth factors that promote the growth of tumor cells besides stem cells.

Development of the vascular-cell technology that supports long-lasting growth of stem cells will also allow scientists to generate abundant sources of functional and malignant stem cells for genetic and basic studies. This study has also resolved a long-standing controversy in which several groups had claimed that bone-forming cells (osteoblasts) exclusively support the expansion of blood-forming stem cells. "However, using a highly sophisticated molecular imaging approach, we show that regenerating blood-forming stem cells in the bone marrow are in intimate contact with the blood vessels, indicating that endothelial cells are the predominant regulator of stem cell repopulation in the adult bone marrow," states Dr. Daniel Nolan, a senior scientist in Dr. Rafii's lab and a co-author of the new study.

One other important concern addressed in this study was whether forced expansion of the stem cells over a long period of time would induce cancerous mutations in the stem cells. However, the authors of this study show that, even after one year, there was no indication of tumor formation, such as leukemias, when the expanded stem cells were transplanted back into mice. This suggests that the endothelial cells provide a milieu that proliferates stem cells without creating cancer risk.

The current breakthrough represents the culmination of many years of work by Dr. Rafii and his lab, including their research in converting adult mouse spermatogonial stem cells to endothelial cells (Nature, September 2007) and in deriving stable, copious endothelial cells from human embryonic stem cells (*Nature Biotechnology*, Jan. 17, 2010).

The ability to generate many stable endothelial cells from human embryonic stem cells leads to new research opportunities, according to Dr. Zev Rosenwaks, who is a co-author in this study and director and physician-in-chief of the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, as well as the director of the Tri-Institutional Stem Cell Initiative Derivation Unit at Weill Cornell Medical College.

Dr. Rosenwaks says, "Generation of endothelial cells derived from diseased embryonic stem cells that are being propagated in our Derivation Unit will open up new avenues of research to molecularly eavesdrop on the communication between vascular cells and stem cells. This innovative line of investigation -- to determine how normal and abnormal human vascular cells induce the formation of organs during development of embryos and how dysfunction of endothelial cells results in developmental defects -- will lay the foundation for novel platforms for therapeutic organ regeneration."

Dr. Rafii sees even more opportunities. "Identification of as yet

unrecognized growth factors produced by human embryonic cell-derived endothelium and adult endothelial cells that support stem cell expansion and differentiation 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 specifically the production of endothelial cell-derived factors in order to block the growth of tumors. Our findings are the first steps toward such goals and they highlight the potential of vascular cells for generating sufficient stem cells for therapeutic organ regeneration, tumor targeting, and gene therapy applications," concludes Dr. Rafii.

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