Specialized blood vessels jumpstart and sustain liver regeneration

November 12 2010

The liver's unique ability among organs to regenerate itself has been little understood. Now Weill Cornell Medical College scientists have shed light on how the liver restores itself by demonstrating that endothelial cells -- the cells that form the lining of blood vessels -- play a key role.

The results of their study are published today in the online edition of the journal *Nature*, with a companion study in the Oct. 24 issue of Nature Cell Biology describing how endothelial cells are activated to initiate organ regeneration.

It has long been known that endothelial cells passively conduct blood, passing oxygen, nutrients and metabolic waste to and from tissues through capillary walls. However, in studies published in recent years, the Weill Cornell researchers have demonstrated that endothelial cells actively influence the self-renewal of certain stem cell populations and the regeneration of tissue. Now, these scientists have uncovered the endothelial cells' "instructive role" in liver regeneration. Further, the researchers believe that in the coming years it will be possible to facilitate healing damaged livers by transplanting certain types of endothelial cells with liver cells.

"We have found that specialized blood vessel cells in the liver -- a specific type of sinusoidal endothelial cell -- initiate and sustain liver regeneration by producing growth factors that we have identified. This finding will open the door for designing new therapies to treat damaged livers," says the study's senior author, Dr. Shahin Rafii, who is the

Arthur B. Belfer Professor in Genetic Medicine and co-director of the Ansary Stem Cell Institute at Weill Cornell Medical College and a Howard Hughes Medical Institute investigator.

The liver performs many physiological functions, including converting nutrients into essential blood components; storing vitamins and minerals; producing bile for digesting fats; regulating blood clotting; and metabolizing and detoxifying substances that would otherwise be harmful. When the liver malfunctions, the consequences can be grave. Liver failure, due to cirrhosis, various forms of hepatitis, and other diseases, kills some 60,000 Americans per year. But the liver's capacity for regeneration is amazing.

"Until our study, the molecular and cellular pathways that would initiate and maintain liver regeneration were not known," says Dr. Bi-Sen Ding, the study's first author and a senior postdoctoral fellow in Dr. Rafii's lab. "Attempts to transplant hepatocytes [liver cells] directly into the liver led to very limited success. But now we have identified liver sinusoidal endothelial cells (LSECs) -- that, when activated, are critical to liver regeneration and may enable proper engraftment when hepatocytes are implanted into the injured liver."

Dr. Rafii's team determined the mechanism by which LSECs regulate liver regeneration by studying this process in genetically engineered mice whose livers were 70 percent removed. Through a series of experiments involving strategic endothelial cell implantation, the team found that only those LSECs whose genes were producing the angiocrine growth factors Id1 or Wnt2 and "hepatocyte growth factor" (HGF) would initiate and sustain liver regeneration. It is thought that Wnt2 and HGF work together in initiating regeneration, and that the LSECs and the liver cells must be next to each other for successful regeneration were key findings.

"Therefore, to regenerate a long-lasting liver, we may need to co-

transplant hepatocytes with the properly activated endothelium, which produces the right growth factors for the hepatocytes to attach, grow and connect with other parts of the liver. Co-transplantation of primed activated endothelium with liver cells may be an important step to design future therapies to regenerate the liver," says Dr. Ding.

Despite these new insights, Dr. Rafii points to an unsolved enigma: How do endothelial cells sense the loss of liver tissue and initiate the regeneration process? "Change of blood flow might be one of the possibilities," suggests Dr. Sina Rabbany, study co-senior author, who is an adjunct professor at Weill Cornell and professor of bioengineering at Hofstra University. "It is well known that endothelial cells can sense subtle changes in the flow of blood because they are located at the interface between the blood flow and vessel wall. The loss of a liver lobe will inevitably alter the local blood flow patterns and resulting shear stresses that are redirected into the remaining lobes. This alteration in the biomechanical transduction process is part of a complex system likely to 'activate' endothelial cells to produce hepatocyte-active growth factors."

Dr. David Lyden, a co-author on the paper and the Stavros Niarchos Associate Professor in Pediatric Cardiology at Weill Cornell Medical College, says, "This is an important study. By targeting endothelial-specific genes such as Id1, as identified in this research, I hope that it will facilitate the design of new therapies to treat people with liver disease, whether due to infection, cancer, or acute or long-term damage."

Earlier this year, Rafii's team developed a new technique and described a novel mechanism for turning human embryonic and pluripotent stem cells into plentiful, functional endothelial cells, which are critical to the formation of blood vessels. The new approach allows scientists to generate virtually unlimited quantities of durable endothelial cells -- more than 40-fold the quantity possible with previous approaches.

"These embryonic-derived endothelial cells may provide a useful platform to expand liver and blood stem cells for therapeutic transplantation," states 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.

"One of the most remarkable findings of our studies is the realization that endothelial cells within each organ are functionally different, and once activated produce a unique set of growth factors," states Dr. Rafii. "The challenge that lies ahead is to discover the organ-specific growth factors produced by the endothelial cells that initiate the regeneration of that particular organ. Then, these factors could be exploited therapeutically to induce selective regeneration of one organ without affecting others."

Additional co-authors include Daniel J. Nolan, Jason M. Butler, Daylon James, Alexander O. Babazadeh and Koji Shido, all of the Ansary Stem Cell Institute in the Department of Genetic Medicine, Weill Cornell Medical College, and the Howard Hughes Medical Institute; Dr. Vivek Mittal, Department of Surgery, Weill Cornell Medical College; and Dr. Thomas N. Sato, Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan.

How Vascular Endothelial Cells Renew Blood Stem Cells and Control Stem Cells' Differentiation

Another study by the same group, published in the Oct. 24 issue of Nature Cell Biology, examines how a similar type of sinusoidal endothelial cells that promote liver regeneration also are activated to renew blood stem cells and control their differentiation into various

types of blood cells within the bone marrow. The findings may be used to create mass quantities of stem cells following trauma to the bone marrow's microenvironment.

Following injury from therapeutic radiation or chemotherapy, stem cells in the bone marrow are injured, hampering blood cell production. Some patients experience severe and potentially irreversible trauma to their ability to produce blood cells. Until now it has been unclear how the body signals these stem cells to regenerate and to differentiate into cells that form blood cells.

Dr. Rafii and his lab have shown that endothelial cells release specific angiocrine growth factors into the environment of the bone marrow, telling the body to produce more stem cells. The researchers showed that the Akt-pathway is activated in the endothelial cells, which turns on expression of a group of growth factors that induces the bone marrow to produce more stem cells. Following the activation of the Akt-pathway, the MAP kinase pathway is activated, which stimulates the production of angiocrine factors that control the differentiation of the stem cells into various cells needed to make up blood.

Results from the study show a 10-fold increase of stem cell production in mouse models that express higher levels of Akt selectively in endothelial cells, when compared with control mice. If proven applicable in humans, the findings may lead to a new way of treating patients suffering from bone marrow deficiencies.

"You are essentially creating a cell culture bioreactor capable of producing large numbers of stem cells as well as mature blood cells, which can restore bone marrow following trauma," says Dr. Jason Butler, who along with Dr. Hideki Kobayashi is the study's co-first author and senior postdoctoral fellows in Dr. Rafii's lab.

"Using properly activated organ-specific endothelial cells to propagate enough stem and progenitor cells, such as those of bone marrow and liver, so they can be used clinically has broad therapeutic implications not only for regenerative medicine, but also for the study of genetic diseases," concludes Dr. Rafii.

Additional co-authors include Mariko Kobayashi, Bi-Sen Ding, Bryant Bonner, Vi Chiu, Daniel Nolan, Koji Shido, all from Weill Cornell; and Laura Benjamin and Rebekah O'Donnell, from the Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass.

Provided by New York- Presbyterian Hospital