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A Bountiful Harvest Offers Promise for Regenerating Blood Vessels

Howard Hughes Medical Institute (HHMI) researchers have devised a new method that increases the number of blood vessel-forming cells they can make from human embryonic stem cells. The advance could improve the odds for successful cell-based therapies to treat heart disease or stroke, and might also aid engineering of artificial organs.

The movie shows the three-dimensional structure of blood vessels formed from endothelial cells (green) derived from human embryonic stem cell cultured in the presence of TGF inhibitor. Cell nuclei appear in blue.

Video: James, D., Hyung-song, N., Seandel, M., Nolan, D., Janovitz, T., Tomishima, M., Studer, L., Lee, G., Lyden, D., Benezra, R., Zaninovic, N., Rosenwaks, Z., Rabbany, S.Y., & Rafii, S. *Nature Biotechnology* Published online: 17 January 2010.

Embryonic stem cells hold the potential to become any cell type in the body and thus to regenerate any damaged organ or tissue. But in order to fulfill that potential, stem cells must receive the right instructions that direct them to turn into a particular kind of cell. Moreover, once they take on the characteristics of a particular cell type, their fate is usually set and cannot be changed easily. Although scientists have identified some of the cues that direct cells toward different fates, they've had a hard time making the large numbers of cells needed for a robust therapy.

Now, HHMI investigator Shahin Rafii at the Weill Cornell Medical College in New York City and his colleagues report a better method for generating human blood vessels in a study published online in *Nature Biotechnology* on January 17, 2010. They conducted a targeted search for factors that turn stem cells into vascular endothelial cells, which form blood vessels. Their findings pointed them toward a strategy that boosts the efficiency of producing vascular endothelial cells 40-fold.

Daylon James, a scientist in Rafii's laboratory, engineered a new line of human embryonic stem cells that produce green fluorescent protein when they become vascular endothelial cells. The label allowed the scientists to rapidly and easily track when the stem cells morphed into this cell type. The researchers bathed the stem cells in a variety of different small molecules and looked for those that resulted in more green cells. They found the most green—and hence vascular endothelial—cells when the stem cells were exposed to a compound that blocks TGF-beta, a growth factor that helps control cell specialization.

Blocking TGF-beta at just the right time during cell culturing dramatically increased the number of vascular endothelial cells produced. Previously, the researchers needed to start with five stem cells for every endothelial cell they hoped to generate. But with the new method, starting with five stem cells gave Rafii and his colleagues 40 endothelial cells. "We've turned the ratio around," says James.

Most importantly, the cells work. The researchers grew human vascular endothelial cells and injected them into mice. After a week, the new "humanized" cells had assimilated into the mouse circulatory system. The cells still glowed green, allowing the team to pick out the injected cells. In addition, a molecule that sticks to the walls of working blood vessels also stuck to the green cells, suggesting that the new cells functioned normally.

The finding represents substantial progress in making endothelial cells from embryonic stem cells, but "the ultimate proof will come from testing in humans," says Rafii. Several steps remain before such testing can proceed to humans. First, the scientists must be sure that the cells don't form tumors—always a concern with embryonic stem cells. So far Rafii's team has not seen any sign of tumors in mice.

Using a small molecule inhibitor of TGF-beta circumvents some problems of testing in humans. Other methods for generating endothelial cells from embryonic stem cells have required factors derived from animals, and because of safety concerns, cells produced in this way are not suited for clinical application. Rafii's approach, however, avoids the use of animal-derived factors, making it appealing for therapeutic blood vessel formation in patients.

Rafii says researchers will also need to determine the best way to deliver lab-generated blood vessels to injured organs so that they can assemble into a network of vessels that will restore a healthy blood supply to the organ. According to Sina Rabbany, a bioengineer who is a coauthor of the study, as blood flows through the circulatory system, the forces it exerts on the cells that line the blood vessels can influence the structures' durability. If newly formed blood vessels are unable to withstand these biomechanical forces, they will regress. Rafii's team is working to build microscopic biological scaffolds that mimic the body's biomechanical microenvironment, so that they can ensure that the vessels they generate will be long-lasting in patients.

The major obstacle, however, is generating cells that are compatible with a patient's immune system. It's impossible to create a bank of different stem cell lines that represents the spectrum of all immune system variants in the human population. Scientists have explored making stem cells from a patient's own cells by reprogramming genes in skin cells or other adult cells. This method has seen some success, but isn't yet ready for testing in people. Rafii's own lab has developed an improved method of generating and isolating stem cells from testes, although such an approach would only benefit men. Considerable work remains to develop cells that don't cause an immune response. But when the problem is solved, Rafii's team will be ready and waiting with the new method to make vascular endothelial cells.

Eventually, Rafii wants to inject the vascular endothelial cells into the hearts of patients with late-stage heart failure and see if the cells boost the heart's function. It could take three to four years to get there, he says, but with the current study, "we've taken the first major step to get to that stage." The impact could extend beyond heart disease, as well. Patients with many conditions, such as stroke, clogged vessels in the lower limbs, or diabetes, could benefit from repairing blood vessels. And engineering organs requires blood vessels too, so Rafii's method of making blood vessel cells could aid efforts to construct organs in culture.