Treating vascular disorders with a cell-based strategy

October 18 2012

A research team at Weill Cornell Medical College has discovered a way to utilize diagnostic prenatal amniocentesis cells, reprogramming them into abundant and stable endothelial cells capable of regenerating damaged blood vessels and repairing injured organs.

Their study, published online today in *Cell*, paints a picture of a future therapy where amniotic fluid collected from thousands of amniocentesis procedures yearly, during mid-pregnancy to examine fetal chromosomes, would be collected with the permission of women undergoing the test. These cells, which are not embryonic, would then be treated with a trio of genes that reprogram them quickly into billions of endothelial cells—the cells that line the entire circulatory system. The new endothelial cells could be frozen and banked the same way blood is, and patients in need of blood vessel repair would be able to receive the cells through a simple injection.

If proven in future studies, this novel therapy could dramatically improve treatment for disorders linked to a damaged vascular system, including heart disease, stroke, lung diseases such as emphysema, diabetes, and trauma, says the study's senior investigator, Dr. Shahin Rafii, the Arthur B. Belfer Professor in Genetic Medicine at Weill Cornell Medical College and co-director of its Ansary Stem Cell Institute.

"Currently, there is no curative treatment available for patients with vascular diseases, and the common denominator to all these disorders is

dysfunction of blood vessels, specifically endothelial cells that are the building blocks of the vessels," says Dr. Rafii, who is also a Howard Hughes Medical Institute investigator.

But these cells do much more than just provide the plumbing to move blood. Dr. Rafii has recently led a series of transformative studies that show endothelial cells in blood vessels produce growth factors that actively participate in organ maintenance, repair and regeneration. So while damaged vessels cannot repair the organs they nurture with blood, he says an infusion of new endothelial cells could.

"Replacement of the dysfunctional endothelial cells with transplantation of normal, properly engineered cultured endothelial cells could potentially provide for a novel therapy for many patients," says study co-author Dr. Sina Rabbany, adjunct associate professor of bioengineering in genetic medicine at Weill Cornell. "In order to engineer tissues with clinically relevant dimensions, endothelial cells can be assembled into porous three-dimensional scaffolds that, once introduced into a patient's injured organ, could form true blood vessels."

Dr. Rafii says that this study will potentially create a new field of translational vascular medicine. He estimates that as few as four years are needed for the preclinical work to seek FDA approval to start human clinical trials to advance the potential of reprogrammed endothelial cells for treatment of vascular disorders.

As part of their study, the research team proved, in mice, that endothelial cells reprogrammed from human amniotic cells could engraft into an injured liver to form stable, normal and functional blood vessels. "We have shown that these engrafted endothelial cells have the capacity to produce unique growth factors to promote regeneration of the liver cells," says the study's lead investigator, Dr. Michael Ginsberg, a senior postdoctoral associate in Dr. Rafii's laboratory.

"The novelty of this technique is that, from 100,000 amniotic cells—a small amount—we grew more than six billion new authentic endothelial cells within a matter of weeks," Dr. Ginsberg says. "And when we injected these cells into mice, a substantial amount of them engrafted into regenerating vessels. It was remarkable to see that these cells went right to work building new blood vessels in the liver as well as producing the right growth factors that could potentially regenerate and repair injured organs."

The Goldilocks of Cellular Reprogramming

To date, there have been many failed attempts to clinically produce endothelial cells that can be used to treat patients. Isolation of endothelial cells from adult organs so they can be grown in the laboratory is not efficient, according to Dr. Daylon James, study co-author and an assistant professor of stem cell biology in reproductive medicine at Weill Cornell Medical College. Attempts to produce the cells from the body's master pluripotent stem cells have also not worked out. Experiments have shown that prototypical pluripotent stem cells, such as embryonic stem cells, which have the potential to become any cell in the body, produce endothelial cells but often grow poorly, and if not fully differentiated could potentially cause cancer. "Coaxing adult cells to revert to a stem-like state so they can then be pushed to form endothelial cells is, at this point, not clinically feasible, and ongoing studies in my lab are focused on achieving this goal," says Dr.

James, who is also assistant professor of stem cell biology in obstetrics and gynecology and genetic medicine at Weill Cornell. Therefore, Dr. Rafii's team searched for a new source of cells that they could turn into a vast supply of stable endothelial cells. They probed human amniotic fluid-derived cells, which some studies had suggested have the potential to become differentiated cell types, if stimulated in the right way—which no one had yet identified.

In their first experiments with these cells three years ago, Dr. Ginsberg used cells taken from an amniocentesis given at 16 weeks of gestation. Researchers found that amniotic cells are the "Goldilocks" of cellular programming. "They are not as plastic and unstable as endothelial cells derived from embryonic cells or as stubborn as those produced from reprogramming differentiated adult cells," Dr. Ginsberg says. Instead, he says amniotic cells provide conditions that are just right—the so-called "Goldilocks Principle"—for producing endothelial cells.

But in order to make that discovery, the researchers had to know how to reprogram the amniotic cells. To this end, they looked for the genes that embryonic stem cells use to differentiate into endothelial cells. Dr. Rafii's group identified three genes that are expressed during vascular development, all of which are members of the E-twenty six (ETS) family of transcription factors known to regulate cellular differentiation, especially blood vessel formation.

Next, they used gene transfer technology to insert the three genes into mature amniotic cells, and then shut one of them off after a brief and critical period of activity by using a special molecular inhibitor. Remarkably, 20 percent of the amniotic cells could efficiently be reprogrammed into endothelial cells. "This is quite an achievement since current strategies to reprogram adult cells result less than one percent of the time in successful reprogramming into endothelial cells," says Dr. Rafii.

"These transcription factors do not cause cancer, and the endothelial cells reprogrammed from human amniotic cells are not tumorigenic and could in the future be infused into patients with a large margin of safety," Dr. Ginsberg says.

The findings suggest that other transcription factors could be used to reprogram the amniotic cells into many other tissue-specific cells, such

as those that make up muscles, the brain, pancreatic islet cells and other parts of the body.

"While our work focused primarily on the reprogramming of amniotic cells into endothelial cells, we surmise that through the use of other transcription factors and growth conditions, our group and others will be able to reprogram mouse and human amniotic cells virtually into every organ cell type, such as hepatocytes in the liver, cardiomyocytes in heart muscle, neurons in the brain and even chondrocytes in cartilage, just to name a few," Dr. Ginsberg says.

"Obviously, the implications of these findings would be enormous in the field of translational regenerative medicine," emphasizes study co-author Dr. Zev Rosenwaks, the Revlon Distinguished Professor of Reproductive Medicine in Obstetrics and Gynecology at Weill Cornell Medical College and director and physician-in-chief of the Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine at NewYork-Presbyterian Hospital/Weill Cornell Medical Center. "The greatest obstacle to overcome in the pursuit to regenerate specific tissues and organs is the requirement for substantial levels of cells—in the billions—that are stable, safe and durable. Our approach will bring us closer to this milestone."

"Most importantly, these endothelial cells could be reprogrammed from amniotic cells from genetically diverse individuals," says co-author Dr. Venkat R. Pulijaal, director of the Cytogenetic Laboratory, associate professor of clinical pathology and laboratory medicine at Weill Cornell. What endothelial cells a patient receives would depend on their human leukocyte antigen (HLA) type, which is a set of self-recognition molecules that enable doctors to match a patient with potential donors of blood or tissue.

"Selecting the proper immunologically matched endothelial cells for

each patient would be akin to blood typing. There are only so many varieties, which are well represented across the amniotic fluid cells that could be obtained, frozen and banked from wide variety of ethnic groups around the world," Dr. Rafii says.

A patent has been filed on the discovery.

More information: Ginsberg et al.: "Efficient Direct Reprogramming of Mature Amniotic Cells into Endothelial Cells by ETS Factors and TGFβ Suppression." DOI: 10.1016/j.cell.2012.09.032

Provided by Cell Press