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Researchers Create Insulin-Producing Cells from Adult Pancreatic Cells

Howard Hughes Medical Institute researchers have converted adult pancreatic cells into insulin-producing beta cells in living mice. This is a first because the researchers directly changed the functional identity of adult cells without using embryonic stem cells or relying on techniques that reverse a cell's genetic programming to its earliest stages.

Remarkably, the investigators repurposed the adult cells quickly by using viruses to shuttle just three regulatory genes that triggered the remarkable developmental changes. It took only a brief blip of activity by the regulatory genes to imbue the cells with their new job descriptions, which they have retained for as long as nine months.

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— Douglas A. Melton

The experiments, which are reported on August 27, 2008, in an advance online publication in the journal *Nature*, realize a longtime goal in regenerative medicine: To produce specialized repair cells directly from a pool of adult cells that are healthy, abundant and easily obtained. Until now, repair cells have been generated from embryonic stem cells or more recently from pluripotent stem cells created by fully reprogramming adult cells.

What this shows is that you can go directly from one type of adult cell to another, without going back to the beginning, said Douglas A. Melton, a Howard Hughes Medical Institute (HHMI) investigator at Harvard University and co-director of the Harvard Stem Cell Institute. You could say, for example, it's like turning a scientist into a lawyer without sending her all the way back to kindergarten.

In this case, the strategy was used in mice to convert exocrine cells, which compose 95 percent of the pancreas, to the relatively scarce beta cells that produce insulin. For more than a decade, Melton has studied how embryonic stem cells give rise to the pancreas and its insulin-producing beta cells, which are destroyed in patients with type 1 diabetes. Ultimately, his studies could

lead to ways to generate new pancreatic beta cells that could be used as a treatment for diabetes. However, Melton cautioned that the new results are a proof of principle and do not have immediate medical applications.

Exocrine cells are specialized to churn out an array of digestive enzymes. Although they, like all cells, carry the genes that enable insulin production, those genes have been silenced. Melton's experiments attempted to modify the genome of the exocrine cell to awaken certain genes and activate the insulin-producing features of beta cells.

The concept of adult cell-switching, or lineage switching as it is sometimes called, has been a major goal of regenerative medicine researchers. This approach has advantages because it avoids using stem cells derived from human embryos.

With the advent of newer techniques that obviate the need for human embryonic cells, researchers have been racing to incorporate those ideas into their own work. In a major breakthrough in 2006, Japanese researcher Shinya Yamanaka and his colleagues made stem cells from adult mouse skin cells (fibroblasts) by inserting four specific genes that were active in mouse embryonic stem cells. Those genes, which code for transcription factors, reprogrammed the skin cells so they became pluripotent and therefore had the capacity to develop into any type of tissue. These induced pluripotent stem cells or iPS cells, could in theory be guided in the laboratory to become specialized cells that might repair damaged nerves, hearts, or other organs.

Melton and postdoctoral fellow Qiao Joe Zhou, first author on the *Nature* paper, were encouraged by the revelation that a handful of transcription factor genes reactivated the embryonic program of adult skin cells. They wondered whether an equally small number of transcription factors could turn off the specialized functions of a given adult cell and turn on those needed to generate the target repair cell.

Starting from a list containing all 1,100 transcription factors in mice, the HHMI scientists selected 200 that were active in cells that form the pancreas. They later narrowed that list to just 28 transcription factors that were most active in the region of the pancreas that contains beta cells. The researchers next used retroviruses to ferry genes for nine of the 28 transcription factors into the exocrine cells of live mice.

Melton and Zhou were surprised to learn that, in fact, only three of the nine genes were necessary to turn exocrine cells into beta cells - an extreme makeover, as one of Melton's colleagues termed it. Those genes were *Ngn3*, *Pdx1*, and *Mafa*.

The maneuver converted about 20 percent of the exocrine cells to beta cells that produced insulin. This was enough to reduce blood sugar levels in diabetic mice. The expression of the three transcription factor genes disappeared less than two months after they were introduced with the virus - but the converted cells remained.

While they believe that it will be possible to convert a wide range of adult cells to other cell types using a small number of regulatory genes, the scientists say a number of questions need to be explored. Among them: How closely related to the desired target cell does the donor cell need to be? What other types of cells can be converted to beta cells? And - since using viruses to ferry genes into human patients poses unacceptable risks — can the same outcome be accomplished with chemicals or other drugs?

George Daley, an HHMI investigator and stem cell researcher at Children's Hospital Boston, commented that Melton's work is going to inspire an explosion of experiments in directing the fate of tissues in one way or another in ways that may be more practical than having to reprogram them back to pluripotency. Daley and colleagues reported recently they had converted cells from individuals with 10 degenerative diseases into stem cells with the same genetic errors. These newly created stem cells will allow researchers to reproduce human tissue formation in a Petri dish as it occurs in individuals with any of the diseases,

Both Melton and Daley emphasized that the apparent success of the shortcut method in no way eliminates the need for continued research on strategies that use iPS cells or stem cells obtained from human embryos.