Researchers successfully reprogram keratinocytes attached to a single hair

October 17 2008

The first reports of the successful reprogramming of adult human cells back into so-called induced pluripotent stem (iPS) cells, which by all appearances looked and acted liked embryonic stem cells created a media stir. But the process was woefully inefficient: Only one out of 10,000 cells could be persuaded to turn back the clock.

Now, a team of researchers led by Juan Carlos Izpisúa Belmonte at the Salk Institute for Biological Studies, succeeded in boosting the reprogramming efficiency more than 100fold, while cutting the time it takes in half. In fact, they repeatedly generated iPS cells from the tiny number of keratinocytes attached to a single hair plucked from a human scalp.

Their method, published ahead of print in the Oct. 17, 2008 online edition of *Nature Biotechnology*, not only provides a practical and simple alternative for the generation of patient- and disease-specific stem cells, which had been hampered by the low efficiency of the reprogramming process, but also spares patients invasive procedures to collect suitable starting material, since the process only requires a single human hair.

"Having a very efficient and practical way of generating patient-specific stem cells, which unlike human embryonic stem cells, wouldn't be rejected by the patient's immune system after transplantation brings us a step closer to the clinical application of stem cell therapy," says Belmonte, PhD., a professor in the Gene Expression Laboratory and director of the Center of Regenerative Medicine in Barcelona, Spain.

Keratinocytes form the uppermost layer of skin and produce keratin, a tough protein that is the primary constituent of hair, nails and skin. They originate in the basal layer of the epidermis, from where they move up through the different layers of the epidermis and are eventually shed.

While scientists have successfully reprogrammed different types of mouse cells (fibroblasts, liver and intestinal cells), skin fibroblasts were the only human cell type they had ever tried their hands on. Fibroblasts help make the connective tissue in the body and are the primary cell type in the deeper layers of the skin, where they are responsible for wound healing and the secretion of proteins that form collagen.

For the first set of experiments, first author Trond Aasen, Ph.D., a postdoctoral researcher at the Center of Regenerative Medicine in Barcelona, used viral vectors to slip the genes for the master regulators Oct4, Sox2, as well as Klf4 and c-Myc into keratinocytes cultured from human skin explants. After only 10 days — instead of the more typical three to four weeks — one out of 100 hundred cells grew into a tiny colony with all the markings of a typical human embryonic stem cell colony.

The researchers then successfully prodded what they call keratinocyte-derived iPS cells or KiPS cells to distinguish them from fibroblast-derived iPS cells into becoming all the cell types in the human body, including heart muscle cells and dopamine-producing neurons, which are affected by Parkinson's disease.

Taking advantage of the high efficiency of the keratinocyte reprogramming process, Aasen decided to test whether he could establish KiPS cells from minute amounts of biological samples. "We plucked a single hair from a co-worker's scalp and cultured the keratinocytes, which are found in the outer root sheet area," recalls Aasen. He then successfully reprogrammed these cells into bona fide

KiPS cells.

Just why keratinocytes appear to be much more malleable than other cell types is still an open question. "We checked a whole rainbow of cells and found keratinocytes to be the easiest to be reprogrammed," says Belmonte. "It is still not clear exactly why that is and knowing it will be very important for the technology to develop fully," he speculates.

They researchers did find one hint, though. When they compared the expression profiles of genes related to stem cell identity, growth or differentiation between keratinocytes, fibroblasts, human embryonic stem cells (hESC) and KiPS cells, keratinocytes had more in common with hESCs and KiPS cells than with fibroblasts.

Source: Salk Institute