Mice cloned using skin cells for first time: study

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Using a technique called nuclear transfer, mice were cloned using adult skin stem cells (right) and a more differentiated type of skin cell (left). The mouse on the right is almost two years old and the mouse on the right is one and a half. Credit: Rockefeller University

Healthy and viable mice that survive until adulthood have, for the first time, been cloned from adult stem cells.

Scientists from Rockefeller University, including Howard Hughes Medical Institute investigator Elaine Fuchs, used cells called keratinocyte stem cells, which represent a new model system for cloning. Keratinocytes come from the skin, making them a particularly attractive stem cell source because of their ready accessibility. One day, they could

be used to tailor therapies, as well as to better understand and treat diseases.

Fuchs and her colleague Peter Mombaerts published their laboratories' findings online February 12, 2007, in the Proceedings of the National Academy of Sciences.

A number of laboratories have cloned mice using cumulus cells (cells that surround and support a developing egg) or fibroblasts (connective tissue cells). Others have used cultured neural cells that were generated from embryonic stem cells. While these efforts have been successful, they are also notoriously inefficient.

Because adult stem cells retain the ability to differentiate into multiple cell types, it has long been expected that they may be better sources of nuclear material for the cloning technique used by Fuchs and her colleagues, known as nuclear transfer. Fuchs, Mombaerts, and their coworkers are the first to successfully and reproducibly clone healthy mice from any type of adult stem cell.

The keratinocyte stem cells used by Fuchs are found in a part of the hair follicle called the bulge. They are involved in hair growth and in repairing skin wounds. "Researchers have known about these infrequently dividing cells for some time, but only recently have scientists revealed their potential to self-renew and produce multiple types of cells -- the hallmarks of stem cells," Fuchs explained. Keratinocyte stem cells in the bulge have been successfully cultured in the Fuchs' laboratory. When tested in mice, these cells can produce surface skin cells, hair follicles, and sebaceous (oil) glands.

Because they reside in the skin, the cells are easily accessible. But purifying and characterizing them has taken years of research. "We had to learn enough about the properties of the different cells within the skin

in order to come up with a strategy to isolate pure populations of these cells, and determine that these cells are in fact stem cells," said Fuchs.

In 2004, the group first published a strategy for labeling and monitoring the cells in the journal Science. "Since then, we and others have devised several different ways to isolate these cells, and this has accelerated the rate at which we are learning about the properties and potential of skin stem cells."

To clone the mice, researchers removed the nucleus from an unfertilized egg cell, called an oocyte and replaced it with the nucleus from an adult keratinocyte stem cell. They cultured these hybrid cells in the laboratory to grow them to the blastocyst stage, when the embryo is a tiny hollow ball of cells. At this point, the cultured blastocysts were implanted in a mouse's uterus and allowed to develop into a cloned fetus. This is the cloning technique known as nuclear transfer.

Typically, only about one to two percent of transferred mouse blastocysts result in a live birth. Furthermore, cloned mice that do survive to birth are often not healthy. In Fuchs' and Mombaerts' study, the success rates were 1.6 percent when using skin stem cells from female mice. When the stem cells came from male mice, however, 5.4 percent of the transferred blastocytes developed into mice. The oldest of these animals is now nearly two years, which is old age for a mouse. In addition, many of these mice were fertile and healthy.

The difference in cloning rates of male and female stem cells seems likely to involve epigenetics," Fuchs said. Epigenetic modifications are those that affect a gene's function without altering its DNA sequence. These changes are reversible, and to some extent can be stripped away from chromosomes during the nuclear transfer process. This epigenetic reprogramming removes the "memory" of the skin cell's chromosomes, allowing them to act like embryonic chromosomes instead. Because one

of a female's two X chromosomes is inactivated through epigenetic modifications, female cells have to undergo a more complicated epigenetic reprogramming than male ones.

While the new research shows that adult skin stem cells can be a promising starting point for cloning mice, Fuchs said she is more enthusiastic about these cells' potential for generating embryonic stem cells. Instead of implanting blastocysts and cloning mice, the blastocysts can be cultured in the laboratory to generate embryonic stem cells. In theory, these embryonic stem cells could be coaxed into producing any other type of cell, from neurons to muscle cells to skin cells. Fuchs said that is the use of nuclear transfer technology that researchers would like translate to human. All it would involve, she pointed out, is an unfertilized oocyte, a skin biopsy, and a tissue culture dish.

If embryonic stem cells can be generated from a patient's skin, and then used to create cells or tissues according to the patient's specific need, the problem of immune rejection might be circumvented. "As importantly, these cells would also allow scientists to study the disease," said Fuchs.

Fuchs cautioned that human applications are far in the future. "We don't have the capability of generating human embryonic stem cells from skin cells, and scientists are still learning how to differentiate embryonic stem cells into different cell types, such as particular types of neurons or pancreatic islet cells," she said.

Source: Howard Hughes Medical Institute