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Key Molecule Puts Brakes on Stem-Cell Differentiation

Howard Hughes Medical Institute researchers have identified a protein that could aid development of methods to grow new skin to treat patients with burns or skin ulcers. The protein maintains hair follicle stem cells in their immature, undifferentiated state.

The findings also offer a new perspective on how stem cells are regulated to keep them from differentiating prematurely. Howard Hughes Medical Institute investigator Elaine Fuchs and first author Horace Rhee, both at The Rockefeller University, published their findings June 30, 2006, in the journal *Science*.

"Because skin is so readily accessible, and there are so many stem cells in the skin, there is considerable interest in using them for clinical applications. And in order to develop such applications, one has to understand the fundamental properties of stem cells, particularly stem cell self-renewal and stem cell maintenance."

— Elaine Fuchs

Basic discoveries about hair follicle stem cells could have promising clinical applications, said Fuchs. There is considerable interest in the use of adult human epidermal cells for treating burns and chronic skin ulcers, she said. These studies could improve the quality and function of stem cells in such applications.

Stem cells are immature progenitor cells with the capability to differentiate into a variety of specialized cells that form tissues and organs. Scientists are working toward using stem cells to grow mature specialized cells that could regenerate damaged or diseased skin, brain, heart or other organs.

One of the unique properties of stem cells is that they self renew to provide a continuous source of mature cells. By dividing in a manner that allows one cell to mature, while another remains undifferentiated, a continuous supply of stem cells is ensured. The growth and development of stem cells is supported by a specialized cellular environment called a niche that nurtures stem cells and helps regulate the cell cycle, which drives cell division.

The hair follicle serves as a beautiful model for studying these processes, because we know exactly where the stem cell niche is, said Fuchs. We know how many cells are within the stem cell compartment, and we know that these cells typically cycle infrequently. This makes it possible to monitor when and how the stem cells of the skin are used.

Less understood is how the adult stem cells are established during development. To address this, Rhee and Fuchs developed a strategy to isolate early hair follicle progenitor cells from embryonic mice. Using a number of genetic and molecular markers, they were able to distinguish these early hair progenitors from the unspecified interfollicular epidermal cells.

They then used DNA microarrays to compare the genes that were active in the two kinds of cells. DNA microarrays, also called gene chips, can test the expression of thousands of genes present in a cell. Through this comparison, Rhee and Fuchs identified 1,394 genes that were preferentially expressed in one type of cell versus another. Importantly, said Fuchs, many of the genes they found activated in the embryonic hair progenitors were also activated in adult follicle stem cells, suggesting that the two types are functionally similar.

Their studies pinpointed one gene, called *Lhx2*, which was 18-fold more abundant in the developing hair follicles than in the adjacent epidermal cells. This finding is significant, said Fuchs, because *Lhx2* is a central regulatory protein known as a transcription factor. Prior research had already shown that *Lhx2* governs many developmental processes, but its possible role in stem cells and in the skin had not been explored. Further studies by the Fuchs' lab revealed that as development progressed, *Lhx2* expression became restricted to the stem cell compartment of the adult hair follicle.

The researchers next sought to understand the regulatory role of *Lhx2* by studying the effects in mice of both over-activating it and knocking out its expression. Overexpressing the gene tended to suppress cell differentiation, while knocking it out activated the stem cell compartment, they found. Thus, concluded the researchers, *Lhx2* acts as a brake on differentiation, maintaining cells in a quiescent, undifferentiated state.

Rhee and Fuchs also studied whether *Lhx2* was activated in genetic mutant embryos with various defects in pathways regulating hair follicle formation. Those studies indicated that *Lhx2* functions downstream of signals that trigger specification of hair follicle stem cells. However, the gene functions upstream of signals that drive stem cells to differentiate into hair cells. This tells us that *Lhx2* is a marker of the early stages of stem cell maintenance, but that it's not a requirement for the differentiation of the hair follicle per se, said

Fuchs.

Fuchs said that the discovery of *Lhx2* offers useful lessons for researchers studying stem cell biology. This finding raises the question of what it takes to activate a stem cell to commit it to one lineage or another, she said. It supports the idea that there are positive-and negative-acting signals that a stem cell must receive in order to coax it to follow one lineage or another.

The identification of *Lhx2* also enables us to get at the basic research questions of how the stem cell niche maintains a balance of a constant number of stem cells, so that every time one is utilized it is replenished, she said.

Fuchs noted that skin stem cells could play a role in scientists' efforts to reprogram the cells to produce neurons or other cell types to regenerate organs damaged by disease or trauma. Because skin is so readily accessible, and there are so many stem cells in the skin, there is considerable interest in using them for clinical applications, she said. And in order to develop such applications, one has to understand the fundamental properties of stem cells, particularly stem cell self-renewal and stem cell maintenance.