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Getting Skin Deep: Studies Show How Skin Forms Deep Layers

Howard Hughes Medical Institute researchers have new evidence that pushes aside old theories about how skin is able to create layers of different cell types while simultaneously forming a continuously self-renewing, protective barrier.

The discovery helps to explain how skin becomes “stratified” into different layers and may yield new insights into the basic processes by which stem cells can both self-replicate to produce more stem cells and also mature and differentiate to form a tissue.

HHMI investigator Elaine Fuchs and colleague Terry Lechler at The Rockefeller University published their findings on August 10, 2005, in an advance online publication in the journal *Nature*.

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- Elaine Fuchs

In their paper, the researchers show that skin stratifies into layers in an unusual process involving asymmetric cell division - a fundamental developmental mechanism in which a mother cell gives rise to two distinctly different daughter cells.

Lechler and Fuchs found that the cell's machinery orients spindles, which are the machines that orchestrate cell division, in a direction perpendicular to the skin plane. This orientation of the spindles permits one of the two daughter cells formed during cell division to remain attached to a growth-promoting structure called the basement membrane, while the other daughter is deposited above, closer to the skin surface.

The researchers found that the daughter cell remaining attached to the basement membrane retains the characteristics of a proliferative epidermal cell while the other daughter cell differentiates and matures into the cells that form the skin's protective outer epidermal cells. These cells eventually die and slough off, only to be replaced by new cells rising from the innermost layer of the epidermis.

Before the new discovery, the prevailing theory, based on studies of adult skin cells in culture, was that all the cells in the innermost layer divided laterally to produce two identical daughter cells. In this scenario, the differentiating layers of cells arose from spontaneous detachment or "delamination" of some daughter cells from the basement membrane. This gave little hint about the mechanism of how an epidermal cell transitions from a dividing, self-renewing state to a differentiating state.

In their initial experiments, Lechler and Fuchs fluorescently labeled spindle poles in embryonic mouse skin to monitor the orientation of the spindles during mitosis. "Since the mitosis happens over a relatively short period of time relative to the cell cycle, we focused on embryonic skin, where many more cell divisions are needed to keep up with the growth rate of the animal," said Fuchs. Lechler observed a dramatic increase in perpendicular divisions as the embryonic skin developed from a single to multi-layered structure. Nearly every time he observed a spindle oriented perpendicular to the skin surface, it was always in a region where the epidermis was beginning to stratify.

"Based upon the delamination theory, we expected that after the mice were born, the divisions would revert back to being primarily lateral," said Fuchs. "But to our surprise, the majority of divisions remained perpendicular, even during postnatal development. Perpendicular divisions appear to be an important mechanism of stratification not only in the embryo but also in the adult," she said.

In the fruit fly, the process of asymmetric cell division was known to involve a group of three genes: *Bazooka*, *Inscuteable* and *Partner of Inscuteable (PINS)*. Lechler cloned the mouse *Inscuteable* gene, and using fluorescent tracers, he showed that the three mouse versions of these proteins appear in a crescent directly over the mitotic spindles that are oriented perpendicular to the skin surface. Using additional mutant mice, he uncovered roles for intercellular adhesion, the basement membrane and integrins in the process of asymmetric cell division.

“Since integrins are known to associate with both the basement membrane and with growth factor receptors at the base of the epidermis, the asymmetric division provides a natural mechanism by which the growth-promoting molecules of the epidermal stem cell are partitioned favorably in the daughter cell that is left in the innermost layer, leaving the suprabasal daughter cell to differentiate in the stratified layers of the skin,” explains Fuchs. “It also suggests that the mechanism of asymmetric divisions found in primitive organisms has been evolutionarily conserved, and is operative in mammals.”

Further studies, she said, will aim at identifying other components of the regulatory mechanism that instructs the epidermal cell when to align its spindle perpendicularly and how to use the Inscuteable complex of proteins to do so.

While their discovery of the basic process of skin stratification does not have immediate clinical implications, said Fuchs, insights from these studies may be applicable to asymmetric cell division in human stem cells.

“Understanding the processes of asymmetric and symmetric divisions in stem cells are of central importance to the stem cell field,” she said. “It seems likely that the basic mechanistic process that we've documented in the embryonic skin stem cell will be utilized by other tissues and cell types, particularly stem cells.”