Probing the cause of skin cell differentiation in mammals with new technique

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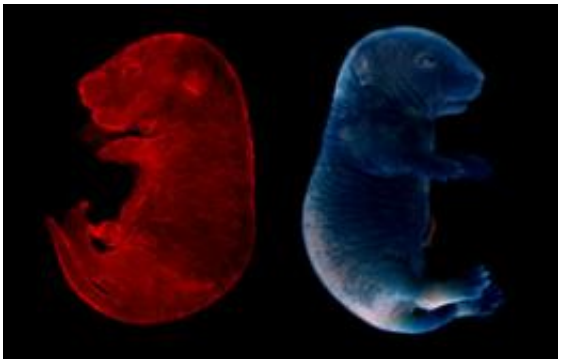

Thin-skinned. Researchers infected mouse embryos with a virus (left, red) which, through RNA interference, blocked the activity of key genes involved in skin cell differentiation and stratification. The result was that the skin failed to develop and thicken properly, allowing it to soak up a blue dye (right) that normal skin would exclude.

A tremendous amount of genetics research has been done in flies and tiny worms, in part because scientists have good tools for tweaking these creatures' DNA. Now, by adapting a powerful method of RNA interference for use in mice, researchers have identified key pathways that cause skin cells to differentiate, eventually forming the flexible but protective outer casing of the body. The work, published February 17 by *Nature*, illustrates the potential for performing relatively fast and complex genetic studies in a fellow mammal, and also provides a deeper understanding of cell differentiation in early development.

Led by Scott Williams, a postdoctoral fellow in Elaine Fuchs' Laboratory of Mammalian Cell Biology and Development, the researchers targeted the genes in a pathway for skin cell differentiation that had not been previously probed in vertebrates. Deploying a technique co-developed by Slobodan Beronja and Geulah Livshits in Fuch's lab and published last July in *Nature Medicine*, the scientists used RNA interference to systematically block the function of genes in developing mouse embryos. Williams and his colleagues in the lab were interested in the chain of events that leads to asymmetric cell division, a common developmental phenomenon by which stem cells balance self-renewal and differentiation, allowing them to generate the diversity of cell-types that create the panoply of an adult organism's tissues and organs.

Prior research in Fuchs' lab, published by Nature in 2005, showed that in early development skin begins as a single layer of symmetrically dividing epidermal progenitor cells. But at a certain point, cells begin dividing asymmetrically, or perpendicular to that layer. In asymmetric division, one daughter cell stays in the original layer, self-renews and maintains its progenitor potential; the overlying daughter cell differentiates in a process of stratification that produces an effective barrier. "As more asymmetric divisions occur, multiple layers of terminally differentiating cells are produced, so that by the time the mouse is born, its epidermis displays a self-renewing, protective skin barrier to keep harmful microbes out and bodily fluids in," says Fuchs, who is also a Howard Hughes Medical Institute investigator.

These findings pointed the way to the latest research. Drawing on previous experiments that identified genes involved in asymmetric cell division in the developing neurons of fruit flies, the Fuchs team targeted a pathway involving the mouse versions of these genes: LGN, NuMA and Dctn1. They used a method of RNA interference which is based on the fact that short pieces of RNA, called small hairpin RNAs, can destroy RNA messages from specific genes, thereby preventing the

genes from producing proteins. The researchers loaded a virus with short RNA bits that target the genes of interest, and guided by ultrasound, they injected the virus into the amniotic fluid surrounding the embryos in a pregnant mouse. The virus infected the outermost layer of the embryos, which shortly after gastrulation, is the single-layered skin. This effectively silenced the genes they were targeting at precisely the right time and blocked asymmetric cell divisions. The result was that the infected mice's skin failed to develop properly, in large part because there were now too few differentiating layers to provide a good skin barrier.

Looking more closely, the scientists also found that silencing the asymmetric cell division genes LGN, NuMA and Dctn1 effectively halted signaling by a molecule, Notch, which is known to regulate differentiation in many types of cells, including skin. When they added Notch signaling back into the genetically modified embryos, the skin developed normally, providing strong evidence that Notch is a key player in the normal differentiation of skin cells. "This technique allows us to do the kind of precise experiments that have been done in worms and flies in the much more complex system of the mouse," Williams says. "And we can do them fast, going from gene to function in about two months."

Existing methods for creating mice that lack certain genes — "knockout" mice — sometimes take years of intensive breeding. Fuchs' laboratory plans to use the new technique to examine in increasing detail the molecular pathways that govern the healthy differentiation and development of skin. The findings could help explain the possible role of stem cells in cancer, an area of research that is heating up.

"Cells that acquire characteristics of self-renewing stem cells but fail to respond to growth inhibitory signals from their environment are likely at the root of cancers," Fuchs says. "It will be interesting in the future to

see whether mutations in the pathways that govern asymmetric cell division might be responsible."

More information: Scott E. Williams, et al. Asymmetric cell divisions promote Notch-dependent epidermal differentiation, *Nature* 470: 353–358 (February 17, 2011)

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