Claus Nerlov

Waxing cutaneous

When we're in the bath, our skin prevents both water from moving into our bodies and essential nutrients from leaching into the tub. But because most of us don't spend our entire lives submerged underwater, our skin's chief role is to control how much water evaporates from our bodies. In fact, the skin's role as a semi-impermeable barrier to fluid loss is so important that people suffering from serious burns often die, not as a direct consequence of their injuries, but from dehydration.

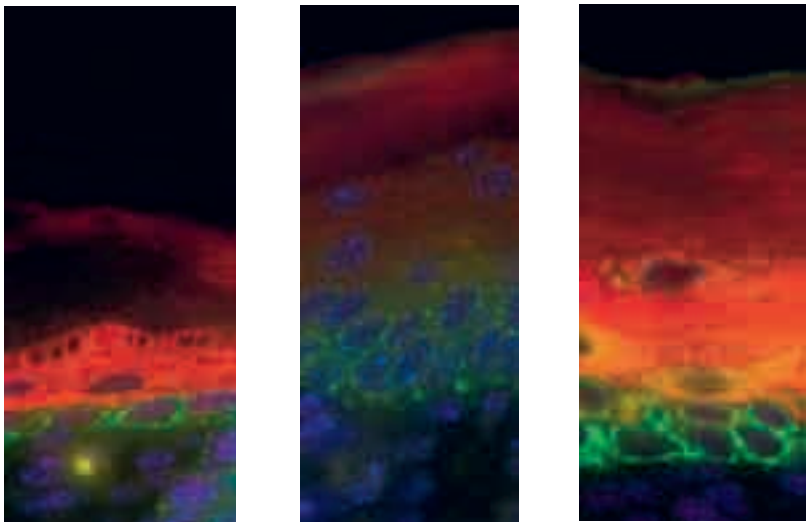
Each of us is covered by about 2 square meters of skin – about the area of a queen-size bed. For this waterproof suit to do its job, stem cells at the base of the skin replenish the layers above by producing a continuous stream of new cells initially like themselves and then a variety of specialised cell types. As a result of this continuous production, the specialised cells – which are destined to become the different layers of skin – move outwards until they are finally shed.

The reason why stem cells stop producing more of themselves and start producing other types of cells had flummoxed researchers ever since the existence of skin stem cells was uncovered in the 1970s. So cell biologist Claus Nerlov and his colleagues at EMBL Monterotondo set about identifying the proteins responsible for this switch. As the processes of cell proliferation and differentiation are so tightly coupled, the researchers suspected that a single protein acted as the toggle between them. From the start, Claus' team looked to a group of proteins called C/EBPs, because they are known to regulate this shift in other parts of the body.

To examine whether C/EBPs form the potential stem cell switch in skin, Claus and his group, in collaboration with colleagues at the Centro de Investigaciones Energéticas, Medioambientales y Tecnologicas (CIEMAT) in Madrid, looked at how skin forms in mice in their absence. Eliminating C/EBPs from all cells prevented mouse embryo development at an early stage – too early for the researchers to observe the proteins' role in skin formation. And so Rodolphe Lopez, a postdoc working in Claus' lab, created a mouse strain in which these proteins could be deactivated in skin cell tissue while continuing to function in all other tissues. This allowed the mouse embryo to develop more or less normally.

The researchers found that mice without C/EBPs had taut, shiny skin that didn't act as a barrier to water, and that they died of dehydration shortly after birth. When they looked more closely at the skin of these young mice, they saw that the cells at the base of the skin were not differentiating into mature skin cells; instead, they remained in their immature state and continued to multiply.

"We saw what was happening at the cellular level, but we didn't know what was happening at the molecular level," explains Claus. To investigate, he and his team introduced mutations into different stretches of fully working copies of the genes that encode the C/EBP proteins. They found that some mutations prevented the C/EBPs from stifling the activity of proteins that halt cell proliferation, whereas other mutations blocked C/EBPs from binding



In normal skin (left), the stem cells at the base (green) differentiate into skin cells (red). In mice whose skin has neither C/EBPα nor C/EBPβ (middle), stem cells appear in upper layers of skin, and there are no differentiated skin cells. In skin where C/EBPα is present but has lost its capacity to interact with E2F (right), skin cells start differentiating abnormally, before they have properly exited the stem cell programme (yellow/orange).

to DNA and activating genes that signal to the cells to start differentiating. "Essentially, we found that C/EBPs are doing two things – stopping proliferation and starting differentiation," says Claus. "C/EBPs tie together these two processes by doing both of them simultaneously, which makes a lot of sense when you consider how closely coupled these processes are in the cell."

In addition to confirming their suspicions, this experiment hinted at something more unexpected, admits Claus. In adult mice with no working copies of the C/EBP proteins, his team discovered a slew of genes that are usually expressed only in embryonic stem cells but were still active in the fully mature skin. These same genes are expressed in highly aggressive skin cancers, suggesting that C/EBPs are actively suppressing genes usually associated with malignancy – an important find for anyone interested in understanding how epithelial cancers like skin, breast, and oral cancers develop.

But the role of C/EBPs in cancer goes more than skin deep. Since 2001, Claus has been studying a form of blood cancer called acute myeloid leukaemia, and his pursuit has taken him deep within our bones. He explains that in the bone marrow of a healthy individual, blood stem cells churn out more cells like themselves and generate others that are destined to become the medley of cells that form our blood – for example, red blood cells, white blood cells and platelets.

Such cellular differentiation is hierarchical, with blood stem cells at the top giving rise to immature progenitor cells, which then become more and more differentiated to form many types of specialist cell. But when a person develops leukaemia, there is an explosion of one type of immature progenitor cell that overtakes the production of red blood cells in the bone marrow and eventually results in anaemia and death. "Fifty years ago, before chemotherapy, this type of leukaemia killed you within weeks," says Claus.

When doctors looked at the DNA of patients suffering from this cancer they found that 90% of the tumours harbour two types of mutations in the C/EBP gene. Claus wondered how these mutations affect the functioning of the resulting C/EBP protein so Oxana Bereshchenko, a postdoc in Claus' lab, created mice with each mutation individually, and then another strain with both mutations. From these mice they extracted blood, which they injected into the bone marrow of a healthy mouse, and watched to see how the cancers developed.

They saw that mice with both mutations became sick and died more rapidly than the mice with only one. They therefore concluded that these mutations have complementary roles: one of them allows the expansion of the malignant population of stem cells, whereas the other helps the stem cells to differentiate into the progenitors, which ultimately grow to such high densities that they kill the mouse.

"This has important implications for the treatment of these cancers," Claus says. If clinicians use drugs to kill the population of progenitors – which seem to be the problem when a patient's blood is examined – then they are aiming at the wrong target, he explains. This is because the mutations are occurring in the stem cells that seed the cancer and not the progenitor cells that maintain it, and so even if the progenitors are eliminated, the stem cells will just give rise to more.

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Bereshchenko O, Mancini E, Moore S, Bilbao D, Mansson R, Luc S, Grover A, Jacobsen S, Bryder D & Nerlov C (2009) Hematopoietic Stem Cell Expansion Precedes the Generation of Committed Myeloid Leukemia-Initiating Cells in C/EBPα Mutant AML. *Cancer Cell* **16**: 390-400