Researchers find chemistry behind skin’s barrier

A group of researchers at Vanderbilt has discovered unusual chemical activity behind an inherited skin disorder that causes fish-like scaling and flaking. The findings give life to an enzyme thought to be inactive, and link its function to a second enzyme, both of which appear to be essential to creating the normal permeability barrier of the skin.

The group found that the two enzymes, both lipooxygenases, work together in a coordinated and unexpected way that provides a logical explanation for why disruption of one or other of the genes encoding the enzymes leads to skin disease. A description of the work appears in the Aug. 5 issue of Proceedings of the National Academy of Sciences.

There are six lipooxygenase (LOX) enzymes known to occur in people and mice, according to Alan R. Brash, Ph.D., professor of Pharmacology and principal investigator of the study. One of them — 5-LOX — is particularly well understood, because it’s involved in inflammation, activating substances called leukotrienes. The enzyme works in a similar manner to the way cyclooxygenase, or COX, enzymes activate prostaglandins.

“In the same way that you take aspirin and Celebrex, which are COX inhibitors, to knock down prostaglandins for an anti-inflammatory effect, you can take medications to combat these leukotrienes,” said Brash. “The drug Singular, for example, is an asthma treatment that blocks the receptor for products of 5-LOX.”

The function of the other LOX enzymes is less certain, but they are known to be specific to certain cell types and to produce distinct products.

“The two that are essential to this study — 12R-LOX and eLOX3 — are mainly confined to the skin,” Brash said.

The 12R-LOX enzyme, which Brash’s lab discovered in 1998, is highly expressed in psoriasis. “The thinking was that this enzyme might have an inflammatory role in psoriasis in the same way that 5-LOX has a role in inflammation of the lungs in asthma,” he said. “But when Diane Keeney, Ph.D., assistant professor of Biochemistry, looked to see where the enzyme was expressed, it wasn’t deep down in the skin where all the trouble was arising. It was expressed out near the edge, just where the cells are changing from the last living cells to the ones that help form a barrier.”

The other enzyme, eLOX3, was discovered at the German Cancer Institute in Heidelberg. The enzyme is expressed in the epidermis, but until this latest study, Brash said, no one knew what it did.

“We tried the human eLOX3, the mouse eLOX3, and we tried them under lots of different conditions to get the enzyme to make products,” he said, “and nothing. It didn’t do anything. So it sat in the freezer waiting for a better idea.”

A better idea came along, Brash said, when a paper came out linking mutations in these two LOX enzymes with a rare type of ichthyosis, or scaly skin disease. “The funny thing was,
Researchers find chemistry behind skin’s barrier

some of the families had mutations in 12R-LOX and some of them had mutations in eLOX3, which we thought had no function, yet both had the same skin problems.”

Up to this point, all LOX genes were considered to work independently, but these results suggested that the enzymes might be in the same pathway. “It was an enlightening thought,” said Brash. “Neither we nor anybody else had thought about that possibility, but it makes sense because you could block the pathway by one way or the other.”

Zheyong Yu, a third year graduate student in Brash’s lab, took on the task of exploring this idea. Yu added eLOX3 to every lipoxygenase product available to test for activity.

“It turned out that the products from 12R-LOX were the best substrate by far,” said Brash. “And they were being converted by eLOX3 by a previously undescribed enzymatic activity that appears to be unique.

“The reason this is such a nice finding is that the enzyme that seemed to be dead really did have activity when the right substrate was provided,” he said. “And it pointed to a very rational explanation for what was wrong in these patients with the inherited skin disease. Furthermore, it suggests that in normal people these two enzymes are helping in the final stages of sealing off the barrier of the skin.”

Brash theorizes that in the disease, the skin is making a lot of extra cells in an attempt to compensate for the imperfect process. The result is the scaling and flaking that gives ichthyosis its name, which stems from a Greek root meaning fish.

The lab’s next steps will include testing the product of eLOX3 in cultured skin cells to determine its biological activity; determining if the product is being made in normal skin or in normal cultured skin cells; and looking at where eLOX3 is expressed in skin and if it is expressed in the same cells as 12R-LOX. Other Vanderbilt researchers listed as authors on the PNAS paper include Claus Schneider, Ph.D., William E. Boeglin, and Lawrence J. Marnett, Ph.D. The work was supported by grants from the National Institutes of Health and the Core Laboratories of the Vanderbilt Skin Disease Research Center Grant.