Study finds that protein puts the brakes on melanin

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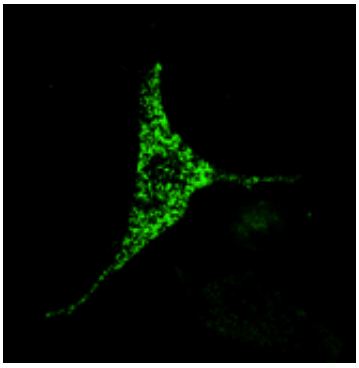

Green fluorescent protein marks where the protein TPC2 is located in a melaonsome. Credit: Oancea et. al./Brown University

A year and a half ago, researchers at Brown University found a molecular gas pedal for melanin production. Now they've found a brake. For scientists, the finding deepens not only the basic understanding of how eyes, skin and hair gain color, but also what perhaps can be done in disorders, such as albinism, when that doesn't happen.

The study in the Nature journal *Scientific Reports* shows that pigmentation is reduced by the activity of "TPC2," a protein that channels the flow of positive sodium ions out of the melanosomes, compartments that produce melanin in cells. When TPC2 lets those ions out, the inside of the melanosomes become more acidic, the researchers found, and that shuts down the enzyme that drives melanin production.

"We know now how TPC2 functions in these melanosome and can use this information to further understand how melanosomes function in normal conditions and how their function is perturbed by disease-causing mutations," said corresponding author Elena Oancea, an associate professor of molecular pharmacology, physiology and biotechnology at Brown.

A lack of melanin production can be associated with albinism, visual impairment and a greater susceptibility to skin and eye cancer. Melanin protects DNA from ultraviolet radiation. For years, however, scientists have had little insight into how pigmentation is governed. In late 2014, Oancea's team discovered that melanosomes employed an ion channel, "OCA2," whose activity increases melanin production by reducing their acidity. OCA2 is named for the disease caused by mutations in the protein, oculocutaneous albinism type II.

The new study, therefore, finds that TPC2 and OCA2 counterbalance.

"Having more than one ion channel regulating the pH allows for complex regulatory mechanisms that can be fine tuned to regulate the pH under diverse conditions," Oancea said.

Tracking down TPC2

Heading into the new study the team, including co-lead authors Nicholas Bellono and Illiana Escobar, only knew that the TPC2 gene had been generally associated with pigmentation. Two mutations in the gene, for instance, were linked in 2008 to fair skin and light hair color in a study of northern Europeans.

But in the lab at Brown, the team was able to prove exactly how TPC2 affects pigmentation. They worked in mouse skin cells and frog eye cells, which have larger melanosomes than human cells do. Otherwise all

the same proteins and mechanisms are in place as in humans.

By making direct electrical measurements on the melanosomes, the team spotted a large inward flow of current (negative electrical charge) corresponding to positive ions flowing out. They showed that the current was independent of that regulated by OCA2 and depended on a lipid called PI(3,5)P2 being in the membrane of the melanosome.

The current was consistent with what's typically produced by TPC ion channels. When the researchers blocked TPC channels with the appropriate chemical called verapamil, the current stopped. Further testing showed that TPC2, rather than TPC1, is found all over the melanosome membrane.

Then came the smoking gun. The team deleted the TPC2 gene using CRISPR-Cas9 gene editing and not only found that doing so abolished the current inflow, but that adding back human TPC2 protein restored it.

They observed that cells with reduced TPC2 levels have more melanin, suggesting that TPC2 is a negative regulator of pigmentation.

From there they showed that melanosomes with TPC2 were a bit more acidic than those without it and that it indeed directly competes with OCA2. Acidity matters because the main enzyme that mediates melanin synthesis, tyrosinase, is only active at around neutral acidity.

The pigment picture

Even after having added their new findings about TPC2 to what they had learned about OCA2, the team is not done studying how melanosomes work. There may be more ion channels or other mechanisms involved, Oancea speculated.

For people with albinism, one of the biggest questions remains how to turn the newfound knowledge into a viable drug strategy. TPC2 could be a target, but it doesn't just function in melanosomes, Oancea cautioned.

"Because TPC2 is a negative regulator of pigmentation, specific TPC2 blockers could be used to compensate for defects in pigmentation caused by acidic melanosomal pH," she said. "Unfortunately, this is not a simple task because TPC2 channels also have important cellular functions in the lysosomes of non-pigment cells, and blocking TPC2 would not only increase pigmentation, but also interfere with the other vital functions mediated by the ion channel. Local delivery of specific TPC2 blockers to melanocytes might be a way to circumvent this problem."

With each study, the pigmentation picture gets colored in a little more.

Provided by Brown University