Melanin-regulating ion channels discovery

A new study by **Dr Elena Oancea** and her laboratory at Brown University reveals the mechanism that regulates pigmentation. The discovery could aid development of future treatments for skin, eye and hair disorders caused by defective melanin production.

Ion channels that regulate melanin were discovered using patch clamp recordings of melanosomes, the cellular organelles that produce and store melanin. A glass patch pipette (shown in blue) was used to cut a small slit into the plasma membrane of a skin melanocyte in order to expose and patch a melanosome (small circle filled with blue) that is 1-3 microns in size.

Q&A

What are the implications of your new understanding of ionic signalling in melanosomes?
Beginning to understand ionic signalling in melanosomes is an important step towards understanding the complex mechanisms that regulate pigment generation and storage in the eye, skin and hair. Because melanosomes are the best-studied model for lysosomal-related organelles, understanding melanosome function will be highly relevant for other organelles, such as platelet dense granules and lung alveolar type II lamellar bodies.

Could you speculate what new treatments for pigmentation disorders and skin cancers may arise from your research?
It is too early to think about treatments, as we are only starting to understand how these molecules work. If I were to speculate based on our data, at least for some forms of albinism, decreasing melanosomal acidity could restore pigmentation levels. Altering melanosomal pH, however, without changing the acidity of other cellular organelles (like lysosomes or endosomes) is not a trivial task. We discovered that TPC2 is a negative regulator for pigmentation and thus blocking its ion channel activity could lead to more pigment being produced. This would also be very difficult, as TPC2 functions in the lysosomes of most cells in our body and is important for their function.

How did it feel to answer some of the key questions that arose from your previous research?
The TPC2 study was developed almost in parallel with the first OCA2 study that we published. Once we were able to measure ionic fluxes across melanosomal membranes, we found the anionic conductance mediated by OCA2 and the cationic one mediated by TPC2. There are certainly many more ion channels and/or transporters in melanosomes, but these were the first two that we could measure under our experimental conditions.

What direction do you think your future research will take?
We would first like to understand how the currents mediated by these two channels are regulated by different cellular signals, from the cytosolic side or from the melanosomal lumen side. In other words, are these channels always open or are there specific molecules that, when produced or activated in the cell, bind to the channels and allow them to open? We would also like to get a more complete picture of melanosomal physiology: What other channels are present in melanosomes and how do they function? How do the proteins encoded by genes mutated in other forms of oculocutaneous albinism contribute to pigmentation? What allows proteins like TPC2 to function only in melanosomes in melanocytes and in lysosomes in all the other cells?

Melanin is our body's natural pigment, responsible for the colour of our hair, eyes and skin. Skin disorders such as albinism, vitiligo and hyperpigmentation, occur when our cells abnormally synthesise or over/under produce melanin. As in the commonly known albino symptoms of pale skin, eyes and hair, the underproduction of melanin affects the development and function of the visual system and reduces an individual's protection against ultraviolet radiation. Worryingly, individuals who underproduce melanin are at increased risk of skin and eye cancers as their DNA is more exposed to harmful ultraviolet radiation. On the opposite side of the spectrum, the overproduction of melanin is usually a benign condition; with many choosing to embrace their unique

hyperpigmentation. However, in some cases, the appearance of blotchy, asymmetrical dark patches can have a negative psychological impact. To avoid defective melanin synthesis, a delicate balance of pigment production is required.

FINDING THE BALANCE

Melanosomes are specialised organelles within skin melanocytes and ocular pigment cells that regulate the production and storage of melanin. Melanosomes are surrounded by a membrane that contains transmembrane proteins that regulate the flux of ions into or out of the melanosomes, a process critical for pigment production in these organelles.

Dr Elena Oancea and her team at Brown University, have been studying ionic signalling in melanosomes, or how exactly ion channels regulate melanin synthesis, storage, and transfer. In 2014, the team made their first breakthrough, identifying an ion channel called OCA2 that is defective in people with oculocutaneous albinism type 2 (OCA2) and that functions to increase the production of melanin. More recently, the team found the counterpart to OCA2 - an ion channel with the reverse effect on cellular pigment production.

In their new study, Dr Oancea's team have discovered a new regulatory protein for the pigmentation process: two-pore channel 2 (TPC2). The team had a clue that TPC2 relates to pigmentation as two mutations in the gene encoding the ion channel were linked to light hair colour and fair skin in a 2008 study of northern Europeans. Using direct patch-clamp recordings, a laboratory technique that allows the study of ion channels, of skin and eye melanosomes from mouse melanocytes and frog retinal pigment cells, the researchers were able to identify the first reported melanosomal cation conductance mediated by TPC2. Mouse skin cells and frog eye cells have the same proteins and mechanisms as in humans. However, the melanosomes are larger making the experiments possible.

In the laboratory, Dr Oancea and the co-lead authors Nicholas Bellono and Illiana Escobar, measured the flux of ions across the membranes that surround the melanosomes found inside melanocytes. They found an electrical current that corresponds to positive ions flowing out of the organelles, into the cytoplasm. Notably, this current was found to be independent of the already known OCA2 ion channel, which the same group showed transports negative ions out of the melanosomes. The new current was consistent with a typical two-pore channel and appeared to depend on a lipid (fat) named PI(3,5)P2 that exists in the membrane around the melanosome and other cellular organelles. Investigating further, the researchers added verapamil to the cell culture, a chemical that blocks the activity of both TPC channels. This stopped the electrical current, as they had expected, and further experiments identified that the ion channels are of the TPC2 variety, not TPC1.

The team deleted the TPC2 gene using a gene editing tool called CRISPR-Cas9, confirming that the outflow of positive charges from melanosomes is indeed mediated by the TPC2 ion channel. What's more, they could reestablish the flow of current by adding the TPC2 gene back into the cells. During this process, they observed that the melanosomes of cells with fewer TPC2 channels are less acidic and produce more melanin, suggesting TPC2 is a negative regulator of pigmentation. They discovered that TPC2 counterbalances OCA2, a positive regulator of pigmentation, by increasing the melanosomal membrane potential and acidity to decrease melanin content. In simple terms, if TCP2 lets too many positive ions escape the melanosome, the production of melanin is turned off.

Defective Melanin Synthesis Disorder	Albinism	Melasma
Type	Underproduction and storage of melanin	Overproduction and storage of melanin
Prevalence	Affects 1 in 17,000 people worldwide Male and females equally affected, except for sub-type ocular albinism that occurs more often in males Can affect individuals of all ethnicities	Global prevalence unknown - relatively understudied area of dermatology Between 75-90% affected are female More common in people with naturally darker skin, or who tan easily
Characteristic Appearance	Very pale skin, eyes and hair	Symmetrical, blotchy, brownish facial pigmentation
Prognosis	Causes problems with eyesight Increased risk of skin and eye cancers	Benign condition, but can cause significant psychological distress and embarrassment

PROGRESS FOR PIGMENT TREATMENT

"We now know how TPC2 functions in melanosomes and can use this information to understand how melanosomes function under normal conditions, and how their function can be perturbed by mutations," says Dr Oancea. Providing vital knowledge required to construct a comprehensive model of ionic signalling in melanosomes, the researchers have provided a significant step towards a better understanding of human pigmentation. The research team are hopeful that their research will provide other scientists with the basis for uncovering "novel therapeutic targets" for pigmentation disorders, as well as skin and eye cancers. Perhaps as a result of the study, future research will be able to find a way to treat albinism and hyperpigmentation. Should that prediction prove correct, a significant proportion of skin and eye cancer could be pro-actively prevented, and much psychological distress eliminated.

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However, there is much research still to do, particularly due to the existence of TPC2 channels in other cells. Dr Oancea explains: "Unfortunately, this is not simple. TPC2 channels also have important functions in the lysosomes of non-pigment cells. Blocking TPC2 would not only increase pigmentation, but interfere with other functions mediated by the ion channels. Local delivery of specific TPC2 blockers to melanocytes might be a way to circumvent this problem."

• *Find out more about Dr Oancea's research: www.nature.com/articles/srep26570 elifesciences.org/interviews/early-career-researchers/elena-oancea*

Detail

RESEARCH OBJECTIVES

The focus of Dr. Oancea's laboratory is understanding signal transduction events primarily in pigment cells. Her research team investigates the molecular mechanisms mediating pigmentation in skin melanocytes and retinal pigmented epithelium, as well as the physiological and pathological effects of ultraviolet radiation on human skin.

FUNDING

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) branch of the National Institutes of Health (NIH)

COLLABORATORS

Nick Bellono was a PhD student in Dr Oancea's laboratory, who did all the patch-clamp recordings from melanosomes after learning how to patch lysosomes in Dr Dejian Ren's laboratory at University of Pennsylvania. Bellono then adapted the technique to melanosomes. A key collaborator on the OCA2 paper, adviser, and friend of Dr Oancea is Dr Michael Marks from University of Pennsylvania.

BIO

Associate Professor Elena Oancea earned her PhD at Duke University with Dr Tobias Meyer then worked with Dr David Clapham at Harvard University / Boston Children's Hospital. In 2008 Dr Oancea moved to Brown University to start her own lab and also shifted the focus of her research to pigment cells, how these cells respond to different physiological stimuli and the complex mechanisms that govern pigment production in melanosomes.

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