Final Report

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Project Title: The role of the novel NCKX5 protein in skin pigmentation

1.1 Project Summary

The project goal was to uncover the pigmentation-related function of the novel NCKX5 protein. We utilized CRISPR/Cas9 gene-editing to permanently silence NCKX5 in pigmented human melanoma cells. As a result of this approach, the depigmented melanoma cell line was created, confirming the role of NCKX5 in skin colour.

1.2 Project Outcomes and Impacts

The novel NCKX5 protein was suggested to play a role in pigmentation. NCKX5 belongs to the family of K+-dependent Ca2+/Na+ membrane transporters and is found exclusively in pigmented skin and the retinal epithelium. We hypothesized that the NCKX5-knockout cells must become depigmented, so to test our hypothesis we aimed to create a stable cell line with nonfunctional NCKX5. Outcomes: By utilizing the CRISPR/Cas9 gene-editing approach to permanently knock out NCKX5 in pigmented human melanoma cells (MNT1 cell line), we created a stable depigmented melanoma cell line. The fact that pigmented cells became depigmented when NCKX5 was silenced confirmed the role of NCKX5 in skin colour. Additionally, this stable depigmented cell line can be used for further research on pigmentation-related functions of the NCKX5 protein. Further, to determine if NCKX5 transport function is required for pigmentation, we reintroduced wild-type functional NCKX5 with membrane transport activities back to depigmented cells (deficient of NCKX5). Reinsertions of functional NCKX5 resulted in pigmentation recovery, demonstrating a direct involvement of this protein in skin colour. This project is instrumental in understanding the role that the novel NCKX5 protein plays in skin colour. Additionally, I started collaborating with the US National Institute of Health on a discovery project aiming to explore if and how mitochondrial membrane lipids may change nuclear DNA makeup. The data we collected will be published in Nature Communications. For the context: As an expert in the field of Membrane Lipids and Lipidomics, I was contacted in summer 2024 by the group leader of the NIH National Toxicology Program Dr. Santos and the director of the MitoCare Center for Mitochrondrial Research & Diagnostics Dr. Hajnoczky asking for a research collaboration on the involvement of mitochondrial membrane lipids in DNA methylation.