Role of Heat Shock Protein-60 (HSP-60) in Trafficking of Tyrosinase Proteins

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Introduction: The biogenesis of melanosomes includes trafficking of several enzymes and regulatory cargo proteins from the Golgi through the endosomal system to the premelanosome. The trafficking of these cargo proteins is regulated by chaperone molecules. Mutations in some of these chaperone molecules, called BLOCs, result in a form of oculocutaneous albinism called Hermansky-Pudlak syndrome (HPS). Three BLOCs have been identified; BLOC-3 may be required for transfer of cargo proteins to the premelanosome through recognition or docking mechanisms. BLOC-3 is made up of proteins HPS1 and HPS4, as well as (an)other currently unidentified protein(s). We hypothesized that HSP-60 is part of BLOC-3 due to its involvement with trafficking in other cell model systems and preliminary proteomic in our laboratory.

Methods: Neonatal foreskins obtained from University Hospital Nursery were used to establish cell lines of melanocytes and fibroblasts. To visually demonstrate HSP-60 localization in the melanocyte, indirect immunofluorescence was performed with antibodies to HSP-60, HPS1 (BLOC-3 representative), HPS5 (BLOC-2 representative), GM130 (Golgi representative), EEA1 (early endosome representative), and NKI/Beteb (premelanosome representative). To molecularly demonstrate HSP-60 involvement with BLOC-3, Western blotting was used to visualize endogenous protein levels in different cell lysates. Immunoprecipitation was used to assess interactions between HSP-60 and proteins in different cell lysates.

Results: Metamorph analysis of indirect immunofluorescence showed that HSP-60 colocalized 67.5% with EEA1, 56.8% with NKI/Beteb, and 12.8% with GM130. HPS5 showed more colocalization in GM130 and EEA1 and less in NKI/Beteb. Western blotting showed existence of HSP-60 protein in a variety of cell lysates, with the largest amount in melanoma melanocytes (SKMel) and the least amount in melanocytes from patients with HPS. Immunoprecipitation analysis was inconclusive.

Conclusions: Indirect immunofluorescence showed that HSP-60 resided more toward the end of melanosome biogenesis; HPS5 resided more toward the beginning. Western blotting showed that HSP-60 was present in melanocytes from HPS1 patients. Conclusions cannot be drawn with immunoprecipitation analysis; more research is needed.

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