

4. Unraveling lysosome-related organelle biogenesis through the cell biology of Hermansky–Pudlak syndrome. A. Helip-Wooley^a, H. Dorward, W. Westbroek^a, H. Stanescu^a, R. Hess^a, R. Boissy^b, M. Huizing^a, W.A. Gahl^a. ^aNHGRI, NIH, Bethesda MD, USA; ^bUniversity of Cincinnati, OH, USA.

Hermansky–Pudlak syndrome (HPS) is a disorder of lysosome-related organelle biogenesis characterized by albinism, a bleeding diathesis and occasional colitis or pulmonary fibrosis. Of the seven human HPS subtypes now identified, all are caused by genes coding for proteins of unknown function, except HPS-2, which is caused by a defective b3A subunit of adaptor complex-3 (AP3). The remaining HPS proteins interact with each other in BLOCs: biogenesis of lysosome-related organelle complexes. HPS7 is found in BLOC-1, HPS3, HPS5, and HPS6 interact in BLOC-2 and HPS1 and HPS4 are components of BLOC-3. We performed confocal microscopy on cells from patients with different subtypes of HPS to study the cell biology of the disease. First, we examined tracking of the melanosomal proteins tyrosinase and TYRP-1 in AP3-deficient HPS-2 melanocytes. We showed that AP3 recognizes tyrosinase, but not TYRP-1, and that these two proteins travel to melanosomes by different routes. Next, we studied fibroblasts of patients with homozygous missense changes in HPS5. We observed an abnormal LAMP3 distribution similar to that found in patients with severe HPS5 defects, supporting the diagnosis of HPS-5 in patients with milder (i.e., missense) mutations. A similar cellular phenotype was observed in fibroblasts with mutations in HPS3, another component of BLOC-2. In HPS-3 fibroblasts, early endosome and lysosome markers exhibited a clustered, perinuclear distribution compared to normal fibroblasts. This clustering was corrected by transfection with GFP-HPS3. Transfection with a GFP-HPS3 construct in which the putative clathrin-binding domain of HPS3 was mutated (GFP-HPS3delCBD) failed to correct this maldistribution. In normal melanocytes, GFP-HPS3 partially co-localized with clathrin, while GFP-HPS3delCBD did not. Immuno-EM confirmed the co-localization of GFP-HPS3 and clathrin on small, probably Golgi-derived vesicles. In addition, clathrin was co-immunoprecipitated by HPS3 antibodies when the source was a homogenate of normal but not HPS-3 null melanocytes. These studies demonstrate the strength of using patients' cultured cells to characterize the cellular phenotype of HPS and to study the role of HPS proteins in lysosome-related organelle biogenesis.