Assessment of Intracellular Vesicles Characteristic of Melanocytes Inflicted with Hermanksy-Pudlak Syndrome

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Little is currently known of the genes responsible for the biogenesis of lysosomal-related organelles, which include the melanosome and platelet dense body. However, a collection of genes responsible for Hermansky-Pudlak Syndrome (HPS) have been found to be of importance in the formation of these lysosomal-related vesicles from the Golgi apparatus. The six forms of HPS (i.e., HPS 1-6) result from a mutation in one of five *HPS* genes (*HPS1*, *HPS3*, *HPS4*, *HPS5*, *HPS6*) or in an adaptor complex gene (*ADTβ3A*). HPS presents with congenital albinism, a bleeding diathesis, and a pulmonary disorder resulting from deformed pigment granules, platelet dense bodies, and lysosomes, respectively. The *ADTβ3A* gene, defective in HPS-2 patients, encodes the β3A subunit of adaptor complex-3 (AP-3), which is thought to bind to clathrin and mediate trafficking of vesicles from the trans-Golgi to the lysosomal-related organelles. The roles of the other five HPS proteins are unknown. Due to the presence of readily observable morphological HPS alterations and the ease of which they can be cultured, human melanocytes were chosen as models to assess the mechanisms underlying HPS. HPS-1 melanocytes exhibit unusual tyrosinase-positive peri-nuclear complexes bounded by a double membrane hypothesized to represent autophagosomes. HPS-2 melanocytes exhibit numerous tyrosinase-positive multivesicular bodies hypothesized to represent late endosomes. To assess these hypotheses, localization of a panel of organelle markers at the light and electron microscopic level were performed on control, HPS-1, and HPS-2 cultured melanocytes. In HPS-1 melanocytes, an increase in co-localization of tyrosinase with cathepsin (lysosomal protease) and ribophorin II (integral ER protein) was observed. This data suggests an increase in ER-derived autophagosomy of tyrosinase-positive vesicles in HPS-1 melanocytes, supporting the first hypothesis. These findings were further corroborated by acid phosphatase and HRP-gold electron microscopy. In HPS-2 melanocytes, an increase in the co-localization of cathepsin and transferrin receptor (early/recycling endosomal marker) with tyrosinase was observed. Furthermore, both a striking increase in the amount of transferrin receptor and large transferrin receptor-positive vesicles were also found in HPS-2 melanocytes. Although an increase in co-localization of this marker with tyrosinase did exist in HPS-2 cells, the abnormal distribution of transferrin receptor may be due to a defective AP-3 dependent pathway that is independent of and overlapping with the AP-3 dependent tyrosinase trafficking pathway.