Background

Hermansky-Pudlak syndrome (HPS) is a rare disorder caused by mutations in genes that regulate the biogenesis and trafficking of lysosomes and lysosome-related organelles. To date, 10 genetic loci have been associated with HPS in humans, and pulmonary fibrosis has been reported in several subtypes, including HPS1 and HPS2. Naturally occurring mutations in HPS mice reliably model important features of the human disease, including susceptibility to profibrotic stimuli. Although the genetics of this syndrome are well-defined, the mechanisms by which individual mutations contribute to driving lung fibrosis remain unknown. In recent work, mitochondrial function and the proteasome system have been shown to be perturbed in fibroblasts of the IPF lung, however, it remains unclear whether similar changes are present in lung fibroblasts in HPS.

Objective

To determine whether mitochondrial function and/or the proteasome system is altered in fibroblasts from the HPS mouse lung.

HPS Proteins in Vesicular Trafficking

Methods

- Fibroblasts were obtained from the lungs of C57Bl/6J, HPS1 and HPS2 mice.
- Cells were grown to confluence and cell lysates were collected for protein and gene expression analysis.
- Transcript and protein levels were assessed for several key components of the 26S proteasome (20S, PSMB5, PSMD11) and for the major transcription factor of proteasome proteins, Nrf1.
- Chymotrypsin-, caspase- and trypsin-like activity of the 26S proteasome was measured using commercially available kit.
- Cellular oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were assessed by using the Seahorse Bioscience XFp instrument.

Results

Fig. 1: Oxidative phosphorylation is increased in HPS2 lung fibroblast. (A) OCR in C57B, HPS1 and HPS2 lung fibroblast. (B) ECAR values in control, HPS1 and HPS2 fibroblast. Seahorse data are representative of three separate experiments with control and senescent cells. Statistical significance was assessed by Student t-test *p<0.05, versus C57B group.

Fig. 2: Proteasome activity is increased in lung fibroblasts of HPS2 mice. (A) Chymotrypsin-like proteasome activity, (B) Caspase-like activity and (C) Trypsin-like activity in lysosomes of lung fibroblast obtained from C57B, HPS1 and HPS2 mice. Statistical significance was assessed by Student t-test ***p<0.001 versus C57B group.

Fig. 3: Nrf1-dependent 26S proteasome subunits are upregulated in HPS2 lung fibroblast. Western blot of proteasome transcription factor (Nrf1) and proteasome subunits (20S, PSMD11). Densitometry analysis was performed from n=6 independent experiment. Statistical significance was assessed with Student’s t-test ** p<0.01, ***p<0.001 versus C57B group.

Fig. 4: Expression of profibrotic markers in HPS lung fibroblast. mRNA levels of Tgf-β1, Cola1a1 and fibronectin from C57B, HPS1 and HPS2 lung fibroblast. Statistical significance was assessed by Student t-test *p<0.05, **p<0.01, ***p<0.001 versus C57B group.

Fig. 5: TGF-β1 treatment increase mitochondrial respiration and extracellular acidification rate in HPS lung fibroblast. (A) OCR and (B) ECAR values of naive (TGF-β1-untrated) and differentiated (TGF-β1-treated) lung fibroblast from C57, HPS1 and HPS2 cells measured using Seahorse.

Conclusions

- Our results highlight the fact that individual HPS mutations are likely to have different effects on the behavior of lung fibroblasts, at least in terms of mitochondrial oxygen consumption and activation of the 26S proteasome.
- These findings might have important implications for developing individualized therapeutic strategies for patients with specific HPS subtypes.

References


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