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## In vitro functional correction of Hermansky-Pudlak Syndrome type-1 by lentiviral-mediated gene transfer

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### Abstract

Hermansky-Pudlak syndrome (HPS) is a genetic disorder characterized by oculocutaneous albinism, bleeding tendency and susceptibility to pulmonary fibrosis. No curative therapy is available. Genetic correction directed to the lungs, bone marrow and/or gastro-intestinal tract might provide alternative forms of treatment for the diseases multi-systemic complications. We demonstrate that lentiviral-mediated gene transfer corrects the expression and function of the *HPS1* gene in patient dermal melanocytes, which opens the way to development of gene therapy for HPS.

### 1. Introduction

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by defective biogenesis of lysosome-related organelles (LROs) [1,2]. Nine genetic HPS subtypes have been defined with clinical manifestations that include hypopigmentation, bleeding diathesis, pulmonary fibrosis, granulomatous colitis and neutropenia. HPS-associated pulmonary fibrosis (HPS-PF) remains the most serious complication for patients affected with the HPS-1, -2 and -4 types of the disease [3]. Several lines of evidence suggest that alveolar type II pneumocytes (ATII) play a significant role in the susceptibility of HPS patients to PF [4,5]. The prognosis of HPS-PF is extremely poor and, currently, the only treatment is lung transplantation. Thus, the development of alternative forms of treatment for HPS-PF remains a high priority.

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#### Conflict of Interest

The authors have no conflicts of interest to declare.

Genes mutated in HPS encode subunits of the biogenesis of lysosome-related organelles complexes (BLOCs) which participate in intracellular protein trafficking and vesicle biosynthesis. The HPS1/HPS4 protein complex, BLOC-3, functions as a Rab32 and Rab38 (Rab32/38) guanine nucleotide exchange factor [6]. In melanocytes, activated Rab32/38 is important for efficient transport of tyrosinase and tyrosinase related protein 1 (TYRP1) from early endosomes to melanosomes and likely provides the pathophysiologic basis of HPS-1 complications. In ATII cells, Rab38 contributes to maintaining lamellar body morphology and surfactant homeostasis [7,8].

Thus, correcting BLOC-3 function in ATII cells by *HPS1* gene transfer may be an effective therapy for treating HPS1-PF. To begin to explore gene therapy strategies for HPS, we studied the effects of lentiviral (LV)-mediated gene correction in HPS-1 human dermal melanocytes (HDM) as an initial feasibility model.

## 2. Materials and methods

### 2.1. Cells

All HPS patient cells used in this study were homozygous for a 16-bp duplication in exon 15 of the *HPS1* gene (c.1472\_1487dup16) and cultured as previously described [9]. Patients were enrolled in protocol NCT00001456, “Clinical and Basic Investigations Into Hermansky-Pudlak Syndrome”, approved by the NHGRI Institutional Review Board, and gave written informed consent. All assays described below were performed in triplicate.

### 2.2. LV construction, production and transduction

We prepared two LV constructs: the LV-*HPS1* therapeutic vector, expressing both the human *HPS1* cDNA (GeneCopoeia, Germantown, MD) and the green fluorescent protein (*GFP*) reporter gene (Addgene Inc., Cambridge, MA) under the control of ubiquitously acting chromatin opening element (UCOE), and the LV-*GFP* control vector that expressed only GFP (Fig. 1A). Viral particles were produced as previously described [10]. HPS-1 HDM were exposed to LV-*HPS1* or LV-*GFP* viral particles for two hours at a multiplicity of infection of 10.

### 2.3. Quantitative real-time PCR

Genome-integrated vector sequences were detected using previously described methods [11] and by quantitative PCR analysis (using universal probe #67: 5'-TGCTGGAG-3' (Roche, Indianapolis, IN,) and primers: 5'-CCATGCCGAGAGTGATCC-3' (forward) and 5'-GAAGCGGATCACATGGT-3' (reverse)).

### 2.4. Western blotting

Protein cell lysates were immunoblotted using anti-HPS1 and anti-HPS4 polyclonal antibodies (Proteintech Group, Inc., Chicago, IL) and anti- $\beta$ -actin monoclonal antibody as loading control. Western blots were quantified using the ImageJ 1.32 software (National Institutes of Health, MD) after densitometric scanning of the films. The intensity of HPS1 and HPS4 protein was normalized to that of  $\beta$ -actin.

## 2.5. Immunofluorescence microscopy

Immunofluorescence microscopy was performed as described [9], using a mouse monoclonal antibody against TYRP1 obtained from BioLegend (Dedham, MA, USA), a sheep polyclonal antibody against TGN46 from AbD Serotec (Raleigh, NC, USA), and the DAPI nuclear stain (Vector Laboratories, Burlingame, CA, USA). All imaging was performed on a Zeiss 510 META confocal laser-scanning microscope (Carl Zeiss, Thornwood, NY, USA).

## 3. Results and discussion

### 3.1. Efficacy of HPS1 expression

Fluorescence-activated cell sorting (FACS) analysis and quantitative real-time PCR were performed to evaluate efficiency of expression of the lentiviral constructs 15 days after transduction. FACS analysis showed that lentiviral gene transfer in HPS-1 HDM resulted in 98.8% (*LV-GFP*) and 50.0% (*LV-HPS1*) GFP-positive cell populations (data not shown). Quantitative PCR analysis showed that cells transduced with the *LV-HPS1* and *LV-GFP* vectors carried 4.3-13.3 and 28.9-30.5 vector copies per cell, respectively (data not shown).

By western blot analysis, the HPS1 protein (~79 kDa) was detected in lanes containing extracts from non-transduced (NT) HDM from a healthy donor (Ctr) and in *LV-HPS1*-transduced HPS-1 HDM, while it was barely detectable in extracts from non-transduced (NT) and *LV-GFP*-transduced HPS-1 HDM (Fig. 1B). Quantification of HPS1 protein expression by ImageJ 1.32 software showed that HPS1 protein expression in *LV-HPS1* transduced HPS-1 HDM was 52.2% of the level expressed in HDM from a healthy donor. Because *LV-HPS1* transduction did not restore HPS1 protein expression to normal levels in HPS-1 HDM, it was important to obtain evidence of the functional effects of the gene correction. HPS1 and HPS4 proteins are stable in BLOC-3, but when either is absent the other is either absent or greatly reduced [12]. We therefore tested the stability of the BLOC-3 complex by evaluating HPS4 protein expression, which was significantly reduced in NT and *LV-GFP*-transduced HPS-1 HDM. Importantly, however, *LV-HPS1*-transduced HPS-1 HDM showed HPS4 protein increased to 89.3% of the level expressed in control HDM (Fig. 1B). Therefore, in addition to restoring HPS1 expression, *LV*-mediated *HPS1* gene transfer resulted in the correction of BLOC-3 formation, as indicated by regeneration of HPS4 expression in HPS-1 HDM.

### 3.3. Assessing the restoration of BLOC-3 function

To determine whether BLOC-3 function was restored in *LV-HPS1*-transduced HPS-1 HDM, we assessed melanocyte pigmentation. Fifteen days after transduction, pelleted *LV-HPS1*-transduced HPS-1 HDM showed significantly increased pigmentation, compared to NT or *LV-GFP*-transduced HPS1 HDM (Fig. 1C). Similarly, confocal microscopy showed increased pigmentation in *LV-HPS1*-transduced HPS-1 HDM (arrows), while *LV-GFP* transduced HPS-1 HDM (arrow heads) lacked pigmentation (Fig. 1D).

Lastly, we assessed intracellular localization of the melanogenic enzyme TYRP1 by immunofluorescence in HPS-1 HDM transduced with *LV-GFP* or *LV-HPS1* (Fig. 1E). In

control cells (Ctr NT), TYRP1 was localized to the Golgi region as well as to endosomes (likely early stage melanosomes) outside the Golgi region (arrow heads). In HPS-1 HDM (HPS-1 NT), however, TYRP1 remained mostly in the trans-Golgi region and was markedly decreased in the endosomes in the dendritic regions (arrow heads). In contrast, in *LV-HPS1*-transduced HPS-1 HDM (HPS-1 *LV-HPS1*), TYRP1 appeared to regain dendritic endosomal localization, similar to control cells (Fig. 1E).

These data indicate that restoration of HPS1 protein expression by *LV-HPS1* transduction in HPS-1 HDM corrected BLOC-3 function, likely through activation of Rab32/38 and correction of endosomal transport of TYRP1, leading to restored melanin production.

## 4. Conclusion

Our results show that lentiviral-mediated gene transfer resulted in effective *HPS1* gene expression in HPS-1 HDM, restoration of BLOC-3 function, and correction of pigmentation in these cells.

Because viral vector-mediated gene transfer has resulted in serious adverse events of insertional oncogenesis in clinical trials that used constructs based on murine gamma-retroviruses [13], we decided to use a lentiviral-based gene transfer vector in the current study, as a safer alternative for potential future clinical use [14]. Envisionable gene therapy approaches that would benefit HPS will have to target the lung, a task that has proven challenging. However, significant progress has been made in recent years with the development of lentiviral vectors that efficiently transduce lung tissue [15].

In this context, our *in vitro* studies represent a promising first step toward the development of *in vivo* gene therapy strategies for Hermansky-Pudlak syndrome.

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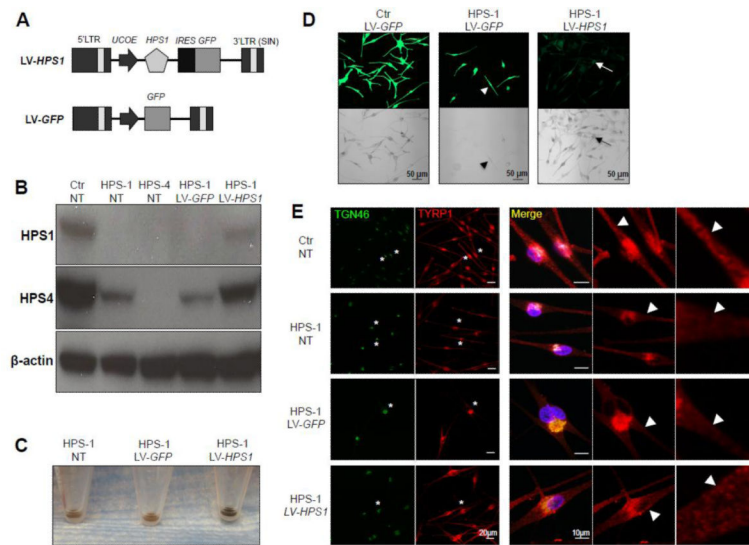
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### Highlights

- A lentiviral-mediated gene transfer system was developed for Hermansky-Pudlak syndrome (HPS).
- HPS1 protein expression was restored in human dermal melanocytes from HPS patients after lentiviral-mediated gene HPS1 gene transfer.
- Expression of HPS4 protein was also reconstituted in HPS dermal melanocytes indicating the restored stability of BLOC-3 in gene-corrected cells.
- The results are promising regarding the feasibility of gene therapy approaches for HPS.



**Figure 1. LV-HPS1 transduction corrects BLOC-3 function in HPS-1 human dermal melanocytes**

(A) Schematic representation of the lentiviral vectors. LTR, long terminal repeat; *UCOE*, ubiquitously acting chromatin opening element; *HPS1*, Hermansky-Pudlak syndrome 1 open reading frame cDNA (GenBank accession number NM\_000195); *IRES*, internal ribosome entry site; *GFP*, enhanced green fluorescent protein open reading frame cDNA; SIN, self-inactivating.

(B) Immunoblots of cell lysates from healthy donor (Ctr) and HPS-1 human dermal melanocytes (HPS-1) and HPS-4 fibroblasts (HPS-4). The cells were either non-transduced (NT) or transduced with LV-*GFP* or LV-*HPS1*. The results show the restoration of both HPS1 and HPS4 proteins in HPS-1 melanocytes transduced with LV-*HPS1*.

(C) Effects of gene transfer on HPS-1 melanocyte macroscopic pigmentation. HPS-1 melanocytes were either non-transduced (NT) or transduced with LV-*GFP* or LV-*HPS1* and cultured for 15 days. Cell pellets after 15 days of culturing showed significantly increased pigmentation in LV-*HPS1*-transduced cells, indicating correction of BLOC-3 function.

(D) Fluorescent (to visualize GFP expression in transduced cells, green) and brightfield (to visualize pigmentation, black) images of healthy control (Ctr) and HPS-1 patient melanocytes transduced with LV-*GFP* or LV-*HPS1*. LV-*GFP* transduced HPS-1 cells lacked pigmentation (arrowheads), while HPS-1 cells transduced with LV-*HPS1* showed increased pigmentation (arrows) with a similar intensity and distribution as in control cells.

(E) Corrected BLOC-3 function results in trafficking of the melanogenic enzyme TYRP1 to early endosomes/melanosomes. BLOC-3 function is evaluated by immunofluorescence analysis of TYRP1 expression and localization (red, visualized by a MEL5 mouse anti-TYRP1 antibody; BioLegend). Trans-Golgi network (TGN, green) is visualized by a sheep anti-TGN antibody (AbD Serotec). TYRP1 in Ctr melanocytes (Ctr NT) is localized in the TGN area, as well as in an endosomal, punctate distribution into the dendrites (arrowheads). HPS-1 melanocytes (HPS-1 NT) showed TYRP1 localization to the TGN, but the dendritic endosomal TYRP1 localization appeared markedly decreased (arrowheads), suggesting a BLOC-3 defect. Transduction of HPS-1 cells with LV-*HPS1* increased dendritic endosomal expression of TYRP1 (arrowheads), indicating improvement/correction of BLOC-3

function. Transduction of HPS-1 cells with *LV-GFP* did not correct TYRP1 distribution and/or BLOC-3 function. The perinuclear regions of selected cells (asterisks) were cropped and enlarged (right panels) to better visualize the perinuclear dendritic regions of the cells. All cell images were taken at the same intensity and magnification settings per experiment on a Zeiss LSM510 META confocal microscope using 20X (Figure D) or 40X (Figure E) objective.