ORIGINAL RESEARCH

Dysregulation of Galectin-3
Implications for Hermansky-Pudlak Syndrome Pulmonary Fibrosis

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Abstract

The etiology of Hermansky-Pudlak syndrome (HPS) pulmonary fibrosis (HPSPF), a progressive interstitial lung disease with high mortality, is unknown. Galectin-3 is a β-galactoside–binding lectin with profibrotic effects. The objective of this study was to investigate the involvement of galectin-3 in HPSPF. Galectin-3 was measured by ELISA, immunohistochemistry, and immunoblotting in human specimens from subjects with HPS and control subjects. Mechanisms of galectin-3 accumulation were studied by quantitative RT-PCR, Northern blot analysis, membrane biotinylation assays, and rescue of HPS1-deficient cells by transfection. Bronchoalveolar lavage galectin-3 concentrations were significantly higher in HPSPF compared with idiopathic pulmonary fibrosis or that from normal volunteers, and correlated with disease severity. Galectin-3 immunostaining was increased in HPSPF compared with idiopathic pulmonary fibrosis or normal lung tissue. Fibroblasts from subjects with HPS subtypes associated with pulmonary fibrosis had increased galectin-3 protein expression compared with cells from nonfibrotic HPS subtypes. Galectin-3 protein accumulation was associated with reduced *Galectin-3* mRNA, normal Mucin 1 levels, and up-regulated microRNA-322 in HPSPF cells. Membrane biotinylation assays showed reduced galectin-3 and normal Mucin 1 expression at the plasma membrane in HPSPF cells compared with control cells, which suggests that galectin-3 is mistrafficked in these cells. Reconstitution of *HPS1* cDNA into HPS1-deficient cells normalized galectin-3 protein and mRNA levels, as well as corrected galectin-3 trafficking to the membrane. Intracellular galectin-3 levels are regulated by HPS1 protein. Abnormal accumulation of galectin-3 may contribute to the pathogenesis of HPSPF.

Keywords: biogenesis of lysosome-related organelles complex; fibroblast; Hermansky-Pudlak syndrome; type 2 cell

Clinical Relevance

We demonstrate that galectin-3 accumulation is found in lung cells from patients with Hermansky-Pudlak syndrome (HPS) type 1, and may be a consequence of abnormal intracellular trafficking of galectin-3. Our studies using dermal fibroblasts derived from patients with different HPS subtypes show that high levels of intracellular galectin-3 are found only in those that are associated with pulmonary fibrosis. Reconstitution of HPS1 cDNA into HPS1-deficient cells normalized galectin-3 levels. These results demonstrate that intracellular galectin-3 is regulated in part by HPS1 protein, and they identify galectin-3 as a potential pathogenic factor in HPS pulmonary fibrosis.

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Hermansky-Pudlak syndrome (HPS) is a genetic disorder characterized by abnormal biogenesis of lysosome-related organelles (1, 2). People with HPS can experience oculocutaneous albinism, a bleeding diathesis secondary to a platelet storage pool defect, granulomatous colitis, and pulmonary fibrosis. These clinical manifestations are variable, and are generally dependent on HPS subtype. The nine human subtypes of HPS, known as HPS-1 through HPS-9, are associated with defects in the adaptor protein-3 (AP3) complex or biogenesis of lysosome-related organelles complex (BLOC)-1, BLOC-2, or BLOC-3 (2–7). Adults with HPS-1 or HPS-4, who have an absence of BLOC-3, develop pulmonary fibrosis, and children and young adults with HPS-2, who have a defect in the AP3 complex, can develop interstitial lung disease, including pulmonary fibrosis (8, 9). Pulmonary fibrosis is the leading cause of mortality in HPS-1. Clinical trials investigating treatment for HPS pulmonary fibrosis (HPSPF) have been conducted, but no approved medical therapies are available (10, 11). However, lung transplantation has been performed in individuals with advanced HPSPF, and is a therapeutic option for eligible patients (12, 13).

Abnormal biogenesis of lysosome-related organelles, including melanosomes and platelet delta granules, causes oculocutaneous albinism and a bleeding diathesis in HPS, but the pathogenesis of HPSPF is unknown. Pneumocytes of interest in HPSPF are alveolar macrophages, type 2 cells, and fibroblasts, which are effector cells in pulmonary fibrosis. In HPS-1, high concentrations of activated alveolar macrophages that are capable of secreting proinflammatory proteins are found in the lung (14). Absence of HPS-1 protein is associated with pulmonary fibrosis and hyperplastic type 2 cells that are engorged with giant lamellar bodies, which are lysosome-related organelles containing surfactant (15). In addition, type 2 cell stress and apoptosis have been reported in HPSPF (16). In a murine model of HPS, surfactant protein B and C accumulate in the lung, and type 2 cell phospholipid secretion is impaired (17). These data suggest that cellular dysregulation may contribute to the pathogenesis of HPSPF.

Galectin-3, a β-galactoside–binding lectin, is expressed in the nucleus or cytoplasm or within the extracellular milieu of various tissues, including the lung (18). Galectin-3 is involved in diverse physiological and pathological conditions, including fibrosis (19–28). Stimulation of fibroblasts with galectin-3 induced cell migration and collagen synthesis *in vitro*, and absence of galectin-3 ameliorated the fibrotic response in murine models of renal, hepatic, and lung fibrosis *in vivo* (23–26). Lung tissue from mice deficient in galectin-3 with bleomycin-induced pulmonary fibrosis showed reduced transforming growth factor-β1–induced epithelial-to-mesenchymal transition, myofibroblast activation, and extracellular matrix production compared with that from wild-type mice (26). Furthermore, galectin-3 is increased in human fibrotic disease, including cirrhosis and idiopathic pulmonary fibrosis (IPF) (23, 25, 26). Taken together, these data indicate that galectin-3 promotes fibrosis in multiple organs. The objective of this study was to investigate the potential involvement of galectin-3 in the pathogenesis of HPSPF. Some of the results of these studies have been previously reported in the form of an abstract (29).

Materials and Methods

Subject Selection

Written informed consent was obtained from subjects with HPS-1 or IPF and healthy research volunteers without lung disease who were enrolled in protocol 04-HG-0211 or 95-HG-0193; these protocols were approved by the institutional review board of the National Human Genome Research Institute. HPS-1 was diagnosed on the basis of oculocutaneous albinism and a storage-pool deficiency, characterized by an absence of dense bodies on electron microscopic examination of platelets (1). A mutation of *HPS1* was identified in all subjects with HPS-1. Pulmonary fibrosis was diagnosed in subjects with HPS-1 by characteristic findings on high-resolution computed tomography (HRCT) scans of the chest (3, 14). IPF was diagnosed using published guidelines (30).

Pulmonary Function Testing

Forced vital capacity (FVC) was measured as previously described (14). Mild disease was defined as an abnormal HRCT scan with an FVC of at least 80% predicted; moderate disease was an abnormal HRCT scan with an FVC between 70 and 80% predicted; and severe disease was an abnormal HRCT scan with an FVC below 70% predicted.

Isolation of Human Bronchoalveolar Lavage Fluid and ELISA Analysis

Bronchoscopy with bronchoalveolar lavage (BAL) and isolation of BAL fluid were performed as previously described (31). Concentrations of human galectin-3 in BAL fluid were measured using an ELISA kit in accordance with manufacturer's instructions (Bender MedSystems, Burlingame, CA). Protein concentrations of BAL fluid were measured using a NanoDrop ND-1000 apparatus (Nanodrop Technologies, Wilmington, DE), and concentrations of galectin-3 were normalized for total BAL fluid protein content.

Immunohistochemistry

HPSPF (n = 3) or IPF (n = 10) lung specimens were procured from subjects undergoing open lung biopsy or lung transplantation. Normal lung specimens were collected from three anonymous donors without known lung disease who expired within 6 hours of tissue procurement (National Disease Research Interchange, Philadelphia, PA). Immunohistochemistry was performed as described in the online supplement.

Tissue Culture

Three subjects with HPSPF underwent lung transplantation, and primary lung fibroblasts were isolated from explanted lung tissue. Dermal fibroblasts were isolated from skin punch biopsies performed on subjects with HPS. Cells were cultured as described in the online supplement.

The following methods can be found in the online supplement: Protein Extraction and Immunoblotting; Plasmids and Transfection; Plasma-Membrane Protein Biotinylation; RNA Analysis: Extraction, cDNA Synthesis, and qRT-PCR; Northern Blot Analysis of miR-322 Expression.

Statistical Analysis

Data are expressed as mean values (± SEM). Significance of difference between means was evaluated using a paired or unpaired Student's t test. Analyses were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA).

Results

Correlation of Concentrations of Galectin-3 in Alveolar Fluid with Disease Severity in HPSPF

To determine the concentrations of galectin-3 in BAL fluid, bronchoscopy was performed in subjects with HPSPF, IPF, or no known lung disease. We found that concentrations of galectin-3 in BAL fluid were significantly higher in subjects with severe HPSPF (n = 8) compared with severe IPF (n = 19) or normal volunteers (n = 40) (P = 0.014 and 0.024, respectively; Figure 1A).

To determine whether concentrations of galectin-3 in BAL fluid correlate with severity of lung disease, subjects were divided into those with mild, moderate, or severe pulmonary fibrosis based on pulmonary function tests. Subjects with severe HPSPF had significantly higher concentrations of galectin-3 in BAL fluid compared with those with mild disease (n = 13; P = 0.017; Figure 1B). In contrast, although concentrations of galectin-3 tended to be lower with increasing severity of IPF, there were no significant differences in concentrations of galectin-3 in BAL fluid among subjects with mild (n = 12), moderate (n = 11), or severe IPF (n = 19) (Figure 1C).

Expression of Galectin-3 in Multiple Lung Cells in HPSPF

To localize galectin-3 expression in the lung, immunohistochemistry was performed using lung tissue procured from subjects with HPSPF (n = 3), IPF (n = 10), or no known lung disease (n = 3). Galectin-3 was expressed strongly in alveolar macrophages, hyperplastic type 2 cells, and interstitial cells in lung tissue from subjects with mild and severe HPSPF, including serial specimens from a subject with progressive HPSPF (Figure 2). Galectin-3 immunostaining was less intense in alveolar macrophages and hyperplastic type 2 cells in IPF lung samples compared with HPSPF. In normal lung tissue, type 2 cells expressed galectin-3. Control sections without primary anti–galectin-3 antibody showed no colorimetric signal (*see* Figure E1 in the online supplement).

Localization of Galectin-3 in Type 2 Cells in HPSPF

Galectin-3 is expressed in epithelial cells, but little is known about intracellular localization of galectin-3 in lung cells. Type 2 cells, which are alveolar epithelial cells with lamellar bodies containing surfactant protein C, become hyperplastic in pulmonary fibrosis. In HPSPF, type 2 cells are also engorged and accumulate enlarged lamellar bodies. To localize galectin-3 in type 2 cells in lung tissue sections, immunofluorescence was performed using anti–galectin-3 and anti–pro–surfactant protein C antibodies. Our data demonstrated that many hyperplastic type 2 cells in HPSPF expressed galectin-3 (Figure 3). Consistent with our colorimetric immunostaining, the intensity of galectin-3 immunofluorescence in hyperplastic type 2 cells in HPSPF was greater than that in IPF or normal lung tissue. No colocalization of galectin-3 and pro–surfactant protein C was found in type 2 cells in HPSPF, IPF, or normal lung. Control sections without primary antibody showed some autofluorescence, but no signal above background in type II cells (Figure E1).

Intracellular Accumulation of Galectin-3 in HPSPF

In HPSPF, galectin-3 was expressed strongly in several lung cell types, including interstitial cells. To determine whether lung fibroblasts derived from subjects with HPSPF express galectin-3, immunoblots of whole-cell lysates from fibroblasts were performed. Immunoblots generated using a primary anti–galectin-3 antibody demonstrated that lung fibroblasts from three subjects with HPSPF had increased galectin-3 protein levels compared with normal lung fibroblasts (Figure 4A). Analyses using an anti-HPS1 antibody confirmed that cells from normal donors, and not HPS-1 subjects, express HPS1 protein.

Dermal fibroblasts may be cultured from skin biopsies and, thus, are more readily obtained than lung fibroblasts. To analyze galectin-3 expression in multiple HPS subtypes, whole-cell lysates were prepared from dermal fibroblasts of subjects with different HPS subtypes associated with defects in either a BLOC or the AP3 complex. Immunoblots showed that galectin-3 levels in HPS-1 (BLOC-3), HPS-4 (BLOC-3), and HPS-2 (AP3), which are subtypes associated with HPSPF, were greater than those in normal or five HPS subtypes that are not associated with HPSPF (Figure 4B). Immunoblots performed using

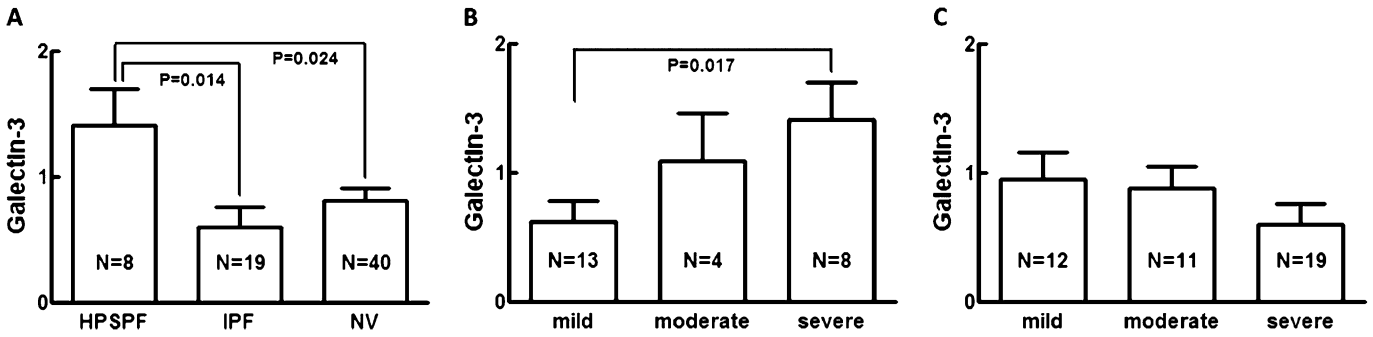

Figure 1. Correlation of galectin-3 bronchoalveolar lavage (BAL) fluid concentrations with severity of Hermansky-Pudlak syndrome (HPS) pulmonary fibrosis (HPSPF), but not idiopathic pulmonary fibrosis (IPF). (*A*) Concentrations of galectin-3 in BAL fluid. Concentrations of galectin-3 (ng/ml/μg protein) in BAL fluid from subjects with severe HPSPF are significantly higher than those from subjects with severe IPF or normal volunteers (NV). (*B*) Concentrations of galectin-3 in BAL fluid in HPSPF. Significantly higher concentrations of galectin-3 in BAL fluid are found in subjects with severe HPSPF compared with those with mild disease. (*C*) Concentrations of galectin-3 in BAL fluid in IPF. No significant differences in concentrations of galectin-3 in BAL fluid are found among subjects with mild, moderate, or severe IPF. *Error bars*, ± 1 SEM.

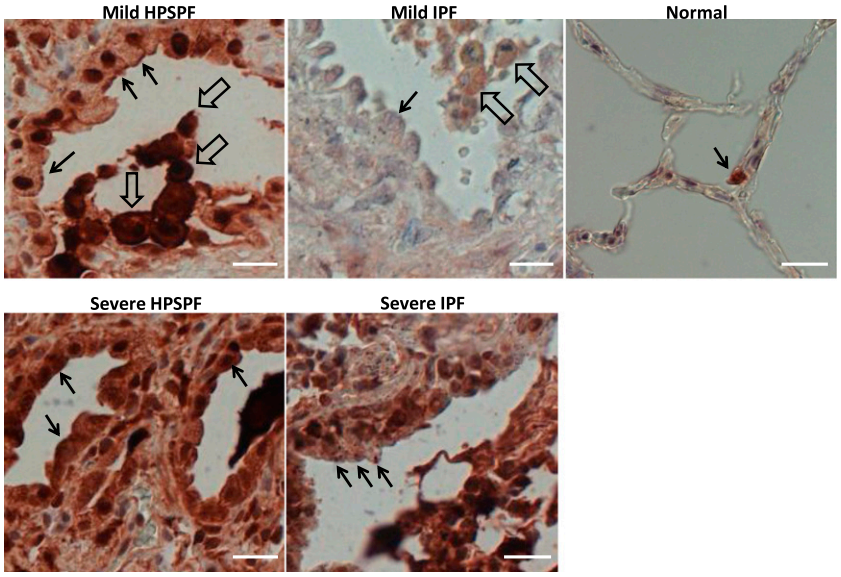

Figure 2. Galectin-3 up-regulation in HPSPF. Immunohistochemistry was performed using a primary anti–galectin-3 antibody. Images shown are representative of tissue sections of open-lung biopsies from subjects with mild HPSPF or mild IPF, postmortem lung from donors without lung disease (normal), and explanted lung from subjects with severe HPSPF or severe IPF. The images of HPSPF lung tissue are from the same subject with HPSPF who progressed from mild to severe disease. Galectin-3 immunostaining in HPSPF (*brown*) is intense in alveolar macrophages (*open arrows*), and nuclear and cytoplasmic expression of galectin-3 is found in interstitial cells and hyperplastic type 2 cells (*solid arrows*). Intensity of galectin-3 immunostaining in alveolar macrophages, interstitial cells, and type 2 cells is less in IPF compared with HPSPF. Type 2 cells in normal lung express galectin-3. *Scale bars*, 20 μm.

anti-HPS1 antibody showed absent or reduced HPS1 protein in subjects with HPS-1 and HPS-4, respectively, and normal expression in other HPS subtypes. We also found that whole-cell lysates from HPS-1 and HPS-4 dermal fibroblasts with either a missense or null allele express higher levels of galectin-3 compared with normal control dermal fibroblasts (Figure 4C). In addition, normal lung fibroblasts express higher levels of galectin-3 compared with normal dermal fibroblasts (Figure 4C).

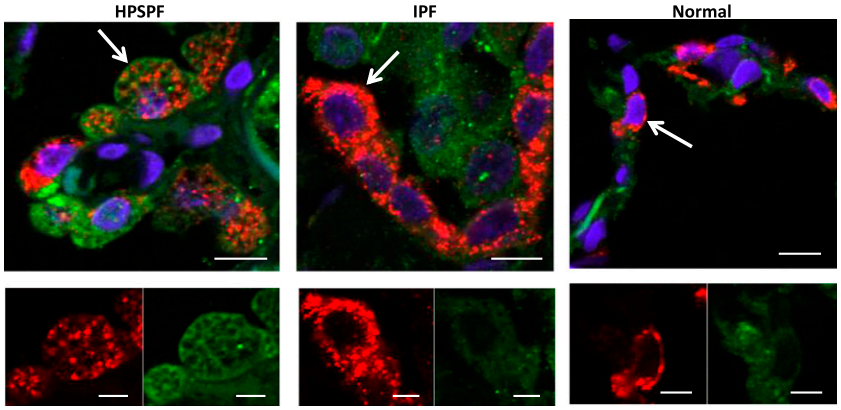

Figure 3. Galectin-3 localization in HPSPF type 2 cells. Merged immunofluorescence images demonstrate intracytoplasmic expression of galectin-3 (*green*) and pro–surfactant protein C (*red*) within type 2 cells (*arrow* and *bottom photomicrographs*) in HPSPF, IPF, and normal lung. No colocalization of galectin-3 and pro–surfactant protein C is found. *Bottom photomicrographs* show cropped and enlarged nonmerged immunofluorescence images of type 2 cells in lung tissue sections from the *top panels*. *Scale bars*, 10 μm (*top images*) and 5 μm (*bottom images*).

To study the mechanism contributing to accumulation of galectin-3 protein in HPS, we measured *Galectin-3* (*LGALS3*) mRNA by quantitative real-time PCR. Our data demonstrated that *Galectin-3* mRNA levels in lung fibroblasts from three subjects with HPS-1 were significantly less than those in normal lung fibroblasts ($P < 0.001$; Figure 4D). Thus, accumulation of galectin-3 protein in HPS-1 cells is not associated with high *Galectin-3* mRNA levels.

Reduction of Galectin-3 Plasma Membrane Levels in HPSPF

In fibrotic lung disease, it is possible that high levels of Krebs von der Lungen 6 antigen, which is a MUC1 glycoprotein that is highly expressed by type 2 cells in pulmonary fibrosis, cause up-regulation of galectin-3 through an microRNA-322–dependent regulatory loop (32, 33). MUC1 undergoes autoproteolysis in the endoplasmic reticulum to produce two peptides, MUC1-N and MUC1-C (34). MUC1-C has a transmembrane domain, and MUC1-N noncovalently binds to MUC1-C in the extracellular space. To investigate whether this mechanism contributes to accumulation of galectin-3 in HPS-1, we examined MUC1 and miR-322 expression. To determine whether lung fibroblasts could be used to study MUC1, immunoblots were performed using whole-cell lysates from normal lung fibroblasts; lysate from H441 lung epithelial cells was used as a positive MUC1-expressing control. Immunoblots showed expression of MUC1-C at different glycosylation states (ranging from 15 to 25 kD) in H441 lung epithelial cells and normal lung fibroblasts (Figure 5A). Thus, lung fibroblasts can be used to study MUC1 regulation of galectin-3. We found that levels of MUC1-C protein and MUC1 mRNA in lung fibroblasts from three subjects with HPSPF were similar to those in normal subjects (Figures 5B and 5C). Consistent with reduced *Galectin-3* mRNA, levels of miR-322 were up-regulated in subjects with HPSPF compared with normal subjects (Figure 5D). Thus, increased galectin-3 protein in subjects with HPS-1 is not due to transcriptional dysregulation, and may be secondary to a trafficking defect where lysosomal degradation of galectin-3 is impaired.

Membrane protein biotinylation showed a reduction in galectin-3 and

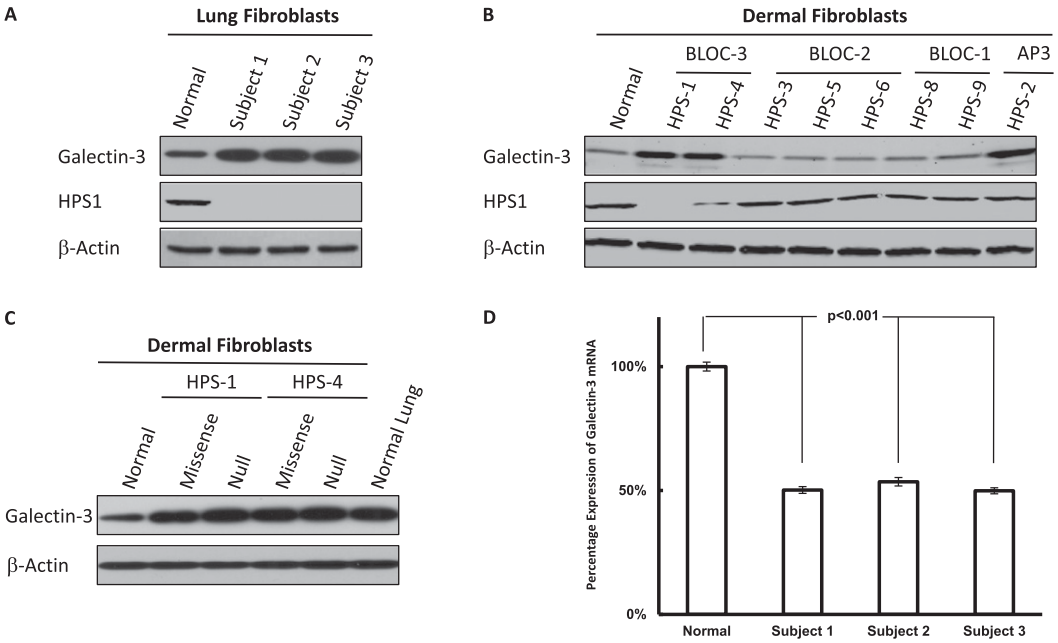

Figure 4. Intracellular galectin-3 protein is elevated in HPSPF. (*A*) Immunoblots of lung fibroblasts. Whole-cell lysates from lung fibroblasts derived from subjects with HPS-1 or normal volunteers were immunoblotted using a primary anti–galectin-3 antibody. All three subjects with HPS-1 had increased galectin-3 protein expression compared with normal subjects. The same lung fibroblast lysates were analyzed with primary antibodies against HPS1. HPS1 was expressed in normal subjects, and not in subjects with HPS-1. (*B*) Immunoblots of dermal fibroblasts. Whole-cell lysates isolated from dermal fibroblasts of normal volunteers or subjects with different HPS subtypes that are associated with defects in a biogenesis of lysosome-related organelle complex (BLOC) or the adaptor protein-3 (AP3) complex were immunoblotted using a primary anti–galectin-3 antibody. Protein from HPS-1, HPS-4, and HPS-2 showed an increase in galectin-3 levels compared with normal or five HPS subtypes not associated with HPSPF. HPS1 protein is absent or reduced in subjects with HPS-1 and HPS-4, respectively, and normal in other HPS subtypes. (*C*) Immunoblots of HPS-1 and HPS-4 dermal fibroblasts. Immunoblot of whole-cell lysates from HPS-1 and HPS-4 dermal fibroblasts with either a missense or null allele, as well as normal control dermal fibroblasts and normal lung fibroblasts. Four subjects with either HPS-1 or HPS-4 had increased galectin-3 levels compared with normal control subjects. Normal lung fibroblasts express more galectin-3 compared with normal dermal fibroblasts. All membranes were stripped and reprobed with β-actin antibodies to demonstrate equal loading of lysates. Densitometry measurements of galectin-3 expression were normalized to β-actin expression; normalized values for galectin-3 are shown as percentages of normalized control galectin-3 expression. (*D*) *Galectin-3* mRNA levels in lung fibroblasts. Quantitative RT-PCR (qRT-PCR) results of *Galectin-3* mRNA show significantly reduced expression in lung fibroblasts from three subjects with HPS-1 compared with normal cells. Values shown are mean percentage expression of *Galectin-3* mRNA normalized to *ACTB* and relative to normal ($n = 3$ in three independent assays; *error bars*, ± 1 SEM; $P < 0.001$ by two-tailed *t* test).

normal MUC1-C on the plasma membrane in subjects with HPSPF compared with normal subjects (Figure 5E). In the same experiment, immunoblots of lung fibroblast lysates confirmed increased expression of galectin-3 and normal expression of MUC1-C in subjects with HPSPF compared with normal subjects. Taken together, these findings indicate that accumulation of galectin-3 in HPS-1 is associated with a defect in intracellular galectin-3 trafficking to the plasma membrane.

Normalization of Galectin-3 Expression in HPS-1 Lung Fibroblasts Reconstituted with *HPS1*

To determine whether galectin-3 expression would normalize in HPS-1 cells that are reconstituted with *HPS1*, HPS-1 lung fibroblasts were transfected with either a myc-HPS1 or myc-empty construct. HPS-1 lung fibroblasts that were transfected with a myc-HPS1, and not a myc-empty, construct reduced the expression of galectin-3 to near-normal levels (Figure 6A). In addition, levels of *Galectin-3* mRNA in HPS-1 lung fibroblasts reconstituted with a myc-HPS1, and not with a myc-empty, vector significantly increased with return to near-normal levels (Figure 6B). Furthermore, a plasma membrane biotinylation assay showed an increase of galectin-3 in the membrane of HPS-1 cells after transfection with myc-HPS1; MUC1-C showed no change (Figure 6C). These results show that restitution of *HPS1* within deficient cells is associated with normalization of galectin-3 mRNA, protein expression, and trafficking to the plasma membrane.

Discussion

The etiology of HPSPF remains enigmatic. It is believed that BLOC-3 and the AP3 complex contribute to the pathogenesis of disease, because people with defects in these complexes exhibit pulmonary fibrosis (1, 3, 4, 8, 9). Pulmonary fibrosis in HPS-1 and HPS-4, which are BLOC-3 diseases, typically develops in adults in their fourth or fifth decade of life, and patients with HPS-2, which is an AP3 complex disease, can develop fibrotic lung disease. The development of lung disease at an early age may indicate that defects in BLOC-3 and the AP3 complex are associated with an

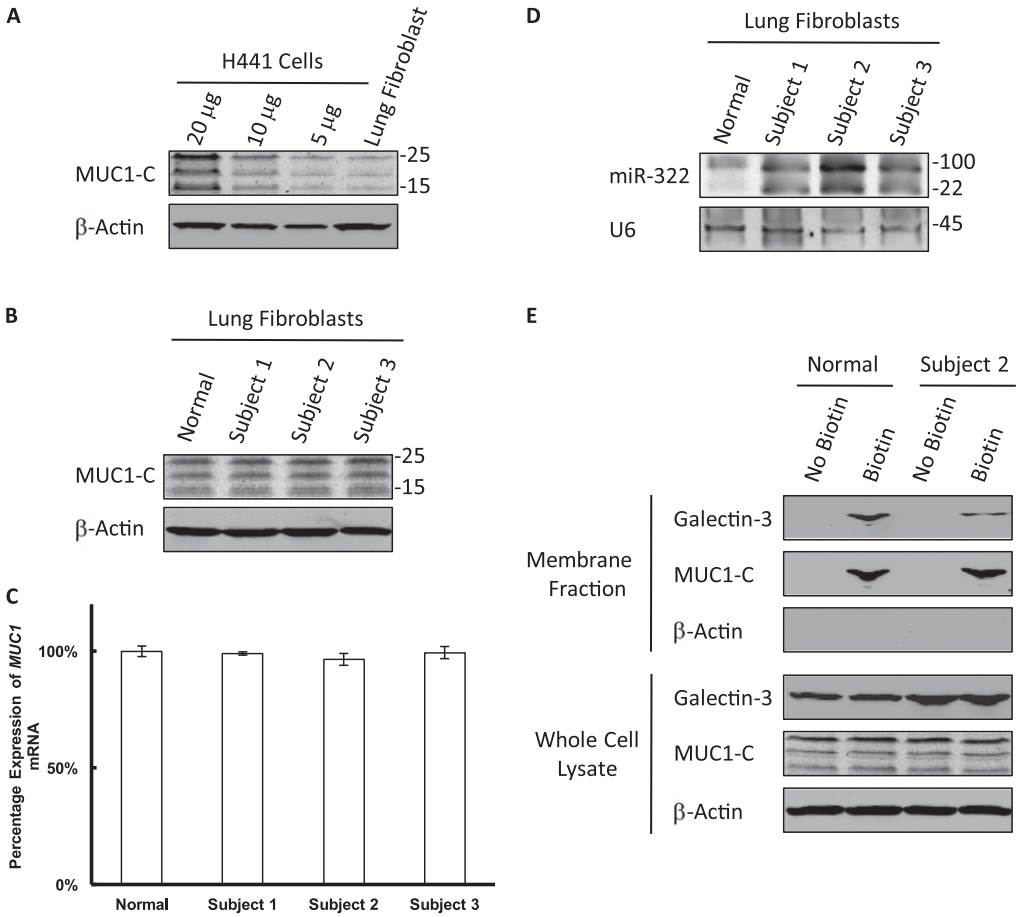

Figure 5. Galectin-3 accumulation in HPSPF is associated with reduced plasma membrane levels of galectin-3 and normal MUC1 expression. (*A*) MUC1-C expression in lung cells. Immunoblots of whole-cell lysates from H441 lung epithelial cells and normal lung fibroblasts show expression of MUC1-C at different glycosylation states (ranging from 15 to 25 kD) in both cell types. (*B*) MUC1-C expression in lung fibroblasts. Expression of MUC1-C in lung fibroblasts from three subjects with HPSPF is similar to normal. All membranes were stripped and reprobed with β-actin antibodies to demonstrate equal loading of protein. (*C*) *MUC1* mRNA levels in lung fibroblasts. qRT-PCR results of *MUC1* mRNA shows no significant difference in expression in lung fibroblasts from three subjects with HPS-1 compared with normal cells. Values shown are mean percent expression of *MUC1* mRNA normalized to *POLRA2* and relative to normal (n = 3 in three independent assays; *error bars*, ± 1 SEM). (*D*) MicroRNA-322 expression in lung fibroblasts. Northern blot analysis of microRNA-322 expression in lung fibroblasts reveals an up-regulation of miR-322 in three subjects with HPS-1 compared with normal. U6 mRNA shows equal loading of RNA. (*E*) Plasma membrane galectin-3 and MUC1-C expression in lung fibroblasts. Plasma membrane protein biotinylation assay of lung fibroblasts shows reduced galectin-3 and normal MUC1-C protein on the plasma membrane in Subject 2 with HPSPF compared with normal subjects. Purity of membrane fractions is shown by absence of β-actin. Immunoblot of whole-cell lysates of lung fibroblasts shows increased expression of galectin-3 in HPSPF compared with normal; expression of MUC1-C in HPSPF is similar to normal. The membrane was stripped and reprobed with β-actin antibody.

aggressive form of pulmonary fibrosis. Consistent with these data, the murine models for HPS-1 (*pale ear*) and HPS-2 (*pearl*) are highly susceptible to bleomycin-induced pulmonary fibrosis, and mice with homozygous mutations of both *HPS1* and *Ap3B1* (HPS-2) develop spontaneous fibrotic lung disease (16, 35).

Our data suggest that galectin-3 may contribute to the development of HPSPF. We found that HPS-1 type 2 cells, alveolar macrophages, and lung fibroblasts express high levels of galectin-3. Using primary human dermal fibroblasts derived from subjects with different HPS subtypes, we showed that high levels of intracellular galectin-3 are found only in HPS subtypes that are associated with HPSPF. Given the known profibrotic effects of galectin-3, these results strongly suggest a role for galectin-3 in the pathogenesis of HPSPF.

Galectin-3, a β-galactoside–binding lectin that promotes fibrosis, is regulated by different mechanisms. Levels of intracellular galectin-3 depend on a feedback loop involving MUC1 and miR-322 (33). In this post-transcriptional regulatory mechanism, suppression of miR-322 by MUC1 stabilizes *Galectin-3* transcripts and leads to an increase in their levels. Other mechanisms that contribute to regulation of galectin-3 include synexin-mediated translocation of galectin-3 to mitochondrial membranes, as well as calpain-4 modulation of galectin-3 phosphorylation and secretion (36, 37). Hormones, including 17β-estradiol, progesterone, human chorionic gonadotropin, and glucocorticoids, also regulate galectin-3 expression and/or secretion (38, 39). Our data show that galectin-3 accumulates in cells with defects in AP3 or BLOC-3, but not BLOC-1 or BLOC-2. BLOC-3 functions in vesicle trafficking from the trans-Golgi network to the early endosomes, and it may also be involved in lysosome or

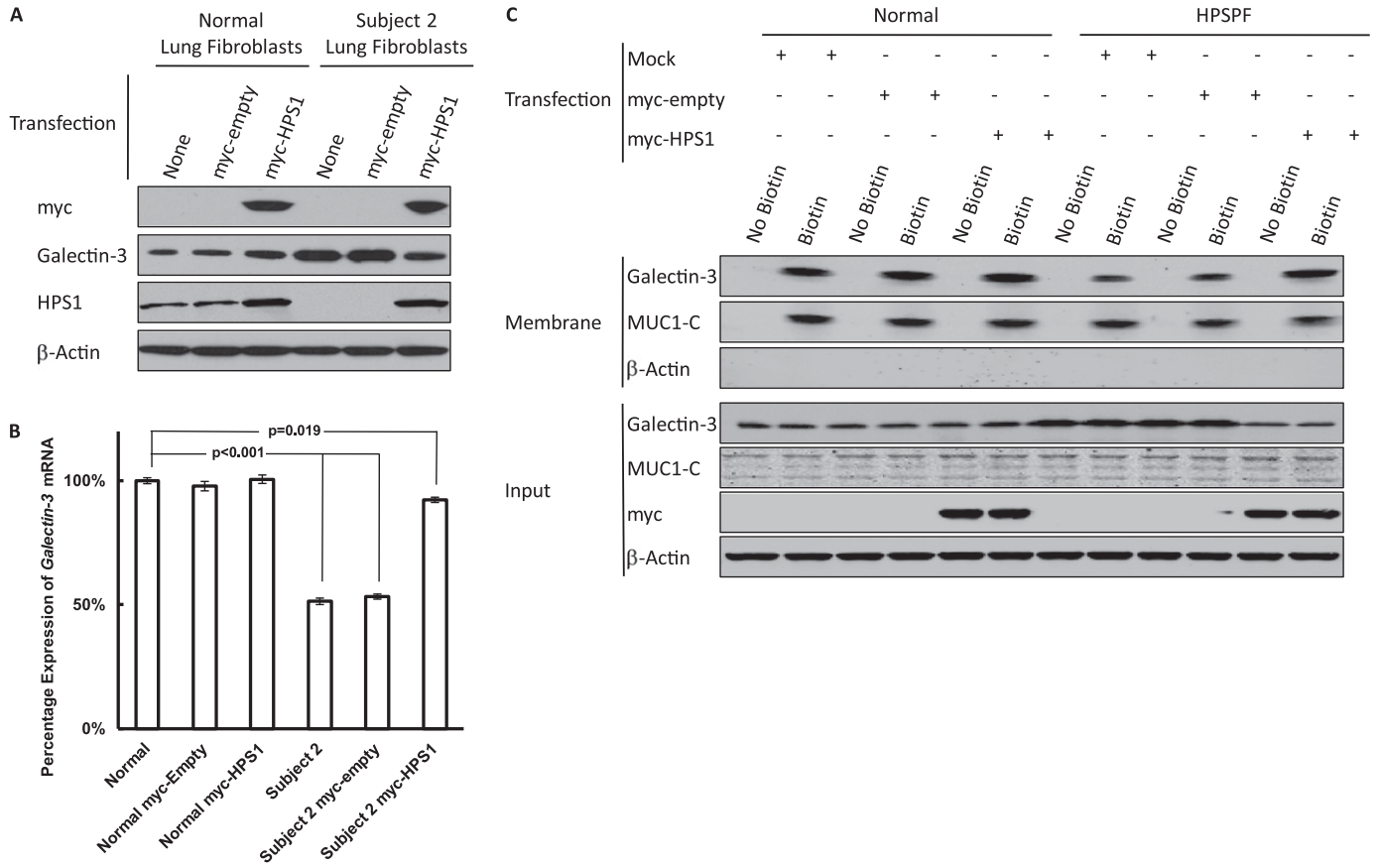

Figure 6. Normalization of galectin-3 expression in HPS-1 lung fibroblasts reconstituted with HPS1. (*A*) Immunoblots of transfected lung fibroblasts. Normal and HPS-1 lung fibroblasts were either mock transfected or transfected with either a myc-empty or myc-HPS1 vector and allowed to recover for 2 days before whole-cell lysates were extracted. Immunoblotting was performed using primary anti-myc, anti-HPS1, or anti–galectin-3 antibodies; primary anti–β-actin antibody was used as a loading control. Galectin-3 expression is normalized in HPS-1 fibroblasts that are reconstituted with myc-HPS1, and not with the myc-empty vector. (*B*) *Galectin-3* mRNA levels of transfected lung fibroblasts. qRT-PCR results show significantly increased expression (to near-normal levels) of *Galectin-3* mRNA in HPS-1 lung fibroblasts reconstituted with myc-HPS1, and not with the myc-empty vector. Values shown are mean percentage expression of *Galectin-3* mRNA normalized to ACTB and relative to mock transfected normal cells (n = 3 in three independent assays; *error bars*, ± 1 SEM; *P* values by two-tailed *t* test). (*C*) Rescue of galectin-3 trafficking to the plasma membrane. Plasma membrane protein biotinylation assay of HPS-1 lung fibroblasts transfected with myc-HPS1 shows increased galectin-3 protein in the plasma membrane to levels similar to that of control. MUC1-C levels did not change. Purity of membrane fractions is shown by absence of β-actin.

late endosome biogenesis (2). We also showed that HPS-1 cells defective in BLOC-3 have abnormal plasma membrane galectin-3 expression, reflecting mistrafficking of galectin-3. Reconstitution of HPS1 in HPS1-defective cells normalizes intracellular galectin-3 levels. Taken together, these findings indicate that intracellular galectin-3 is regulated by BLOC-3 or AP3.

We also found that high concentrations of galectin-3 in BAL fluid correlate with severity of HPSPF. Based on our findings, alveolar macrophages and type 2 cells are potential sources of galectin-3, because these cells are capable of secreting proteins into the alveolar space. The absence of colocalization of galectin-3 and pro–surfactant protein C suggests that galectin-3 may not localize to type 2 cell lamellar bodies, which are lysosome-related organelles. Galectin-3 is reported to accumulate intracellularly in the endosomal recycling compartment of polarized and nonpolarized cells (40). Further studies are indicated to localize intracellular galectin-3 in HPS lung cells.

Multiple mutations have been reported in *HPS1* and *HPS4* (1, 2, 4). We found that whole-cell lysates from HPS-1 and HPS-4 dermal fibroblasts with either a missense or null allele express higher levels of galectin-3 compared with normal dermal fibroblasts. These data indicate that high levels of galectin-3 are found in cells from subjects with different genetic mutations in *HPS1* and *HPS4*, and are consistent with the reported association of progressive HPSPF with multiple mutations in either of these two genes (1, 2, 4).

Overall, these results provide additional insights into the regulation of galectin-3 and its potential role in the pathogenesis of HPSPF. Specifically, BLOC-3 and the AP3 complex appear to be important in the normal intracellular trafficking of galectin-3. Studies investigating galectin-3 as a therapeutic target in HPSPF will further elucidate the role of galectin-3 in the pathogenesis of this progressive disorder. ■

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