Golgi dysfunction is a common feature in idiopathic human pulmonary hypertension and vascular lesions in SHIV-nef-infected macaques

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Sehgal PB, Mukhopadhyay S, Patel K, Xu F, Almodóvar S, Tuder RM, Flores SC. Golgi dysfunction is a common feature in idiopathic human pulmonary hypertension and vascular lesions in SHIV-nef-infected macaques. Am J Physiol Lung Cell Mol Physiol 297: L729–L737, 2009. First published July 31, 2009; doi:10.1152/ajplung.00087.2009.—Golgi dysfunction has been previously investigated as a mechanism involved in monocrotaline-induced pulmonary hypertension (PAH). In the present study, we addressed whether Golgi dysfunction might occur in pulmonary vascular cells in idiopathic PAH (IPAH) and whether there might be a causal relationship between trafficking dysfunction and vasculopathies of PAH. Quantitative immunostaining for the Golgi tethers giantin and p115 on human lung tissue from patients with IPAH (n = 6) compared with controls demonstrated a marked cytoplasmic dispersal of giantin- and p115-bearing vesicular elements in vascular cells in the proliferative, obliterative, and plexiform lesions in IPAH and an increase in the amounts of these Golgi tethers/matrix proteins per cell. The causality question was approached by genetic means using human immunodeficiency virus (HIV)-Nef, a protein that disrupts endocytic and trans-Golgi trafficking. Macaques infected with a chimeric simian immunodeficiency virus (SIV) containing the H-I nef gene (SHIV-nef), but not the nonchimeric SIV virus containing the endogenous SIV-nef gene, displayed pulmonary arterial vasculopathies similar to those in human IPAH. Giantin and p115 levels and their subcellular distribution in pulmonary vascular cells in lungs of SHIV-nef infected macaques (n = 4) were compared with SIV-infected (n = 3) and an uninfected macaque control. Only macaques infected with chimeric SHIV-nef showed pulmonary vascular lesions containing cells with dramatic cytoplasmic dispersal and an increase in giantin and p115. Specifically, the HIV-Nef-positive cells showed increased giantin, p115, and the activated transcription factor PY-STAT3. These data represent the first test of the Golgi dysfunction hypothesis in IPAH and place trafficking and Golgi disruption in the chain of causality of pulmonary vasculopathies in the macaque model.

pulmonary arterial hypertension; endothelial cells; smooth muscle cells; intra-Golgi tethers; human immunodeficiency virus; giantin; p115; signal transducer and activator of transcription 3

IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION (IPAH) is an unrelentingly progressive disease characterized by proliferative, enlarged, and vacuolated endothelial and smooth muscle cells resistant to apoptosis (23, 30). The cellular and subcellular mechanisms underlying the pathogenesis of the “idiopathic” disease are poorly understood. While the role of particular vascular growth factors, respective cell-surface receptors, caveolar proteins, and signaling pathways in the pathobiology of IPAH has been investigated at length over the years (5, 10, 23, 30–32), different investigators have emphasized one or other mechanistic aspect in its pathogenesis. As one example, mutations in BMPRII are associated with familial and sporadic PAH (11, 25). Other studies (5, 10, 11, 17, 20, 23, 25–30) have reported changes in individual cell surface receptors (e.g., Tie-2), lipid raft proteins such as caveolin-1 and eNOS, vascular growth factors (e.g., Ang-1, VEGF, and PDGF), or membrane-associated endocytic signaling pathways such as those utilized by activated Smads or activated STAT3. Alternatively, dysfunction of intracellular trafficking may disrupt several different pathways all at once (11a, 15–18, 27–29).

In the present studies, we asked whether there might be a relationship between Golgi dysfunction and vasculopathies of PAH in humans (23, 30) and in a macaque model of pulmonary arterial vasculopathies (14). We assayed for the cytoplasmic dispersal of the Golgi tethers giantin and p115 and the per cell content of giantin and p115 as readouts of the underlying functional Golgi disruption (11a, 27–29). To investigate whether there might be a causal relationship between trafficking disruption and PAH vasculopathies, we used human immunodeficiency virus (HIV)-nef as an in vivo disrupter of intracellular and trans-Golgi trafficking using a virus-vector approach in a nonhuman primate model (2, 3, 9, 13, 14, 24).

HIV-nef is a viral protein expressed early in infection that interferes with intracellular trafficking along the endocytic internalization and the Golgi-to-plasma membrane trafficking pathways (2, 3, 9, 24). A chimeric virus expressing HIV-nef in the context of a simian immunodeficiency virus (SIV) backbone (13) was previously associated with PAH-like pulmonary vasculopathies in macaques (14). We infected a new cohort of macaques with a chimeric SIV containing the HIV-nef gene (SHIV-nef) and asked whether vascular cells in pulmonary lesions would exhibit Golgi dysfunction in Nef-positive cells.

The results from the human and macaque studies are presented side by side to show the similarities between the two. Taken together, these data provide the first evidence of Golgi dysfunction in human IPAH and in the SHIV-nef-macaque model of pulmonary vascular remodeling.

MATERIALS AND METHODS

Immunofluorescence studies of lungs from patients with PAH. Serial sections (5 μm) of formalin-fixed, paraffin-embedded lung tissue from patients with idiopathic PAH and from controls without PAH (n = 6 each) were obtained from the Johns Hopkins University School of Medicine (Baltimore, MD) in compliance with the guidelines of the Institutional Review Board (Supplemental Table 1; supplemental data for this article are available online at the Am J Physiol Lung Cell Mol Physiol website). Processing of lung sections for indirect immunofluorescence included the recovery of antigens by boiling for 8–10 min in PBS and indirect immunofluorescence stain-
ing carried out as described earlier (17, 29), with respective rabbit pAbs or murine mAbs as the primary antibodies and donkey Alexa-Fluor 488 or AlexaFluor 594-tagged (Molecular Probes, Eugene, OR) pAbs as secondary antibodies. Nuclei were demarcated using DAPI. To minimize the confounding effects of autofluorescence, which is significant in green in formalin-fixed lung tissue, most of the experimental variables were evaluated using red secondary antibodies along with appropriate controls. Fluorescence images were captured using a Zeiss epifluorescence microscopy system (Axioskop 2 Plus) with a red-green-blue camera (Photometrics CoolSNAPPix, Roper Scientific, Tucson, AZ) and RS Image V1.7.3 software and saved in a 24-bit format. Images within each group of sections from control and PAH patients were collected at identical exposure settings using either the “normal” or “raw/uncorrected” settings in the RS Image software. The number of images collected in this study (typically as individual 3-color epifluorescence images) and the pulmonary vascular profiles evaluated for giantin and p115 are summarized in Supplemental Table 3. Images were deconvolved using the Iterative 3D deconvolve plugin of the NIH Image J software to eliminate out-of-focus glare. Representative sections were also stained with hematoxylin-eosin (H&E) and imaged in visible light together with imaging using autofluorescence in green to show elastin.

Higher magnification representations of areas from within selected images (e.g., insets and side sets) were derived using the zoom plug-in software in Adobe Photoshop 7.0.

**Immunofluorescence studies of lungs from macaques infected with SHIV-nef or SIV.** Lung tissues were obtained from a new cohort of monkeys infected with SHIV-nef (n = 4; as distinct from those in Ref. 14) housed at the California National Primate Research Center that were followed longitudinally and necropsied at various time points postinfection in compliance with the Institutional Animal Care and Use Committee (Supplemental Table 2). Tissue samples were formalin fixed and paraffin embedded. Monkeys infected with native SIV (n = 3) or uninfected (n = 1) were used as controls (Supplemental Table 2). Serial sections (5 μm) were processed for indirect immunofluorescence and pulmonary arterial segments, and vascular lesions within each section were systematically imaged. The number of images collected and the pulmonary vascular profiles evaluated for giantin and p115 are summarized in Supplemental Table 3. Respective sections were also stained with H&E and imaged in brightfield alongside a green filter to show autofluorescence of elastin fibers.

**Image analyses.** Quantitative image analyses were performed on a per cell basis using NIH Image J (available as a free download from http://rsb.info.nih.gov/ij/) as described earlier (11, 17). For quantitation, images were uniformly thresholded to reduce “background” pixel intensity to 0 and the Golgi elements were then clearly discerned next to the DAPI-stained nuclei. Due to the thresholding of pixel intensities, it was not necessary to outline individual cell boundaries. Integrated intensities of thresholded pixels surrounding each DAPI-stained nucleus were derived (11a, 17, 18). In this process, the intercellular tissue “background” was adjusted to 0 pixel intensity, which reduces variance in intensities between experimental and control sets.

**Antibody reagents.** Rabbit pAbs to giantin and p115 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A murine mAb to giantin was a gift from Dr. Adam Linstedt (Carnegie-Mellon University, Pittsburgh, PA), and a second rabbit pAb to p115 was a gift from the late Dr. Dennis Shields (Albert Einstein College of Medicine, New York, NY). Murine mAb to smooth muscle α-actin (SMA) was from Sigma-Aldrich (St. Louis, MO) and to HIV-NeF was from Advanced Biotechnologies (Columbia, MD). Rabbit pAb to human von Willebrand factor (vWF) was from Dako Cytomation (Carpinteria, CA), and the human vWF antigen for competition assays was from Haematologic Technologies (Essex Junction, VT).

**Statistical analyses.** Statistical analyses were performed using the two-tailed Student’s t-test and single-factor ANOVA methods with Microsoft Excel software. Multiple group comparisons were evaluated using the Tukey-Kramer post hoc test (NCSS 2007 software).
Fig. 2. Increased accumulation and cytoplasmic dispersal of the Golgi matrix protein/tether giantin in vascular cells in human PAH. A: medium magnification images of giantin immunofluorescence in vascular cells in proliferative, obliteratorive, and plexiform lesions in patients with PAH compared with control pulmonary arterial segments. Scale bar = 75 μm. Proliferative arterial segment from PAH-B (second from left) is the same arterial segment with neointimal proliferation shown in Supplemental Fig. S1, left, and Fig. 1, left. B, top: higher magnification images of sections of proliferative arterial lesion from PAH-B shown in A, second from left, imaged following H&E staining or after immunostaining for giantin and p115 or processed without primary antibody. Elastin is illustrated by autofluorescence in green. Scale bar = 25 μm. B, bottom row: boxed insets in B, top row, at higher magnification. Scale bar = 4 μm. C: triple-label immunofluorescence imaging analyses of sections of proliferative arterial lesion in PAH-B shown in A, second from left, probed for giantin using a pAb and SMA using a mAb showing increased/dispersed giantin in the smooth muscle α-actin (SMA)-negative luminal lining endothelial cells and in the SMA-positive arterial wall cells. Scale bar = 25 μm. D: triple-label immunofluorescence imaging analyses of sections of the proliferative arterial lesion in PAH-B shown in A, second from left, probed for giantin using a mAb and von Willebrand factor (vWF) using a pAb showing increased/dispersed giantin in vWF-positive arterial lining cells. Scale bar = 25 μm.

Fig. 3. Increased accumulation of the Golgi matrix proteins/tethers giantin and p115 in cellular elements in pulmonary arterial vasculopathies in human PAH and in the chimeric SHIV-infected macaque model. A: representative images of respective vasculopathies probed for giantin or p115 compared with representative controls. Scale bar = 85 μm. B: representative higher magnification images of giantin and p115 immunostaining from analyses as in A. Scale bar = 10 μm. SIV-F, p115 segment at top right is same as in Fig. 1, SIV-F, right.
RESULTS

Increased accumulation and cytoplasmic dispersal of Golgi matrix proteins/tethers giantin and p115 in pulmonary arterial endothelial cells (PAECs) and hypertrophic medial smooth muscle cells (PASMCs) in pulmonary vasculopathies in humans and macaques. Supplemental Tables 1 and 2 summarize the origins of the human and macaque lung sections evaluated in this study. Briefly, serial sections from respective formalin-fixed paraffin blocks corresponding to lung areas with known histological evidence of vasculopathy (proliferative, obliterative, and plexiform arterial lesions) from six patients (“PAH-A” to “PAH-F”) were evaluated for cellular content and subcellular localization of the Golgi matrix proteins/tethers giantin and p115 and colocalization analyses were performed with known endothelial (vWF) or smooth muscle SMA cell markers. Similar lung sections from four individuals without PAH were used as controls (“Control-A” to “Control-F”). For the macaque portion of the study, serial sections from respective formalin-fixed paraffin blocks corresponding to lung areas with known histological evidence of vasculopathy (proliferative, obliterative, and plexiform arterial lesions) derived from four macaques infected with a chimeric SHIV-nef virus (“SHIV-A” to “SHIV-D”) were compared with sections from three macaques infected with the native SIV (“SIV-A” to “SIV-C”) and to one uninfected rhesus monkey (“Control”). Figure 1 and Supplemental Fig. S1 illustrate representative H&E images of human and macaque pulmonary vascular lesions included in the present study. The pulmonary vascular lesions in both the human and macaque tissues included cellular elements that were vWF positive (14, 30; Fig. 2; also see Fig. 4; some data not shown).

Figure 2A illustrates representative low-magnification immunofluorescence images revealing a dramatic increase in giantin in cells present in the proliferative, obstructive, and plexiform lesions in human PAH. Figure 2B illustrates a higher magnification H&E view of the proliferative arterial segment shown in Fig. 2A, PAH-B as well as immunostaining of the same arterial segment for giantin and p115, and a control in which the primary antibodies were omitted. It is apparent from Fig. 2B, top row, that luminal endothelial cells as well as cellular elements in the proliferative intima and media showed an increase in the Golgi tethers giantin and p115. Figure 2B, bottom row, illustrates the respective insets depicted in Fig. 2B, top row, at higher magnification. These higher magnification images show clearly the marked vesicular dispersal of the two Golgi tethers throughout the cytoplasm. The triple-label im-

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A Human: Giantin pAb/DAPI

B Human: Giantin mAb/DAPI

Fig. 4. Golgi tether dysfunction in PAH includes SMA-negative and SMA-positive as well as vWF-positive vascular cells. A: triple-label immunofluorescence imaging analyses of sections of a proliferative arterial segment probed for giantin using a pAb and SMA using a mAb showing increase in and cytoplasmic dispersal of giantin in the SMA-negative luminal lining endothelial cells and in the SMA-positive arterial wall cells. Scale bars = 25 μm for left two columns and 4 μm for higher magnification side sets (i, ii) at right. B: triple-label immunofluorescence imaging analyses of sections of vascular lesions in PAH probed for giantin using an mAb and for vWF using a pAb showing increase in and cytoplasmic dispersal of giantin in vWF-positive cells. Scale bars = 25 μm for left two columns and 4 μm for higher magnification side sets (i, ii) at right.
Fig. 5. Golgi tether giantin dysfunction in the SHIV-macaque model includes SMA-negative and SMA-positive vascular cells, but p115 dysfunction is more prominent in SMA-negative arterial lining cells. A: triple-label immunofluorescence imaging analyses of a proliferative lesion in lung of an SHIV-infected macaque probed for giantin and SMA showing increase in and cytoplasmic dispersal of giantin in both SMA-negative luminal lining endothelial cells and in SMA-positive cells. Ctrl-E corresponds to an uninfected animal. Scale bars = 25 μm for left two columns and 4 μm for higher magnification side sets (i, ii) at right. B: triple-label immunofluorescence imaging analyses of a proliferative lesion in lung of an SHIV-infected macaque probed for p115 and SMA showing increase in and cytoplasmic dispersal of p115 prominently in SMA-negative luminal lining cells. Ctrl-E corresponds to an uninfected animal. Scale bars = 25 μm for left two columns and 4 μm for higher magnification side sets (i, ii) on right.

Fig. 6. Simultaneous dysfunctions of both Golgi tethers giantin and p115 in the same obliteratorive pulmonary arterial lesion in an SHIV-infected macaque. Several sections of the same obliteratorive lesion in lung of macaque SHIV-A were imaged using H&E and elastin by autofluorescence (far left) or following triple-label immunostaining for giantin or p115 together with SMA and DAPI (right). Scale bars = 50 μm.
muonfluorescence images in Fig. 2C and corresponding higher magnification images (not shown) revealed increased and dispersed giantin in both SMA-negative and SMA-positive cells. The triple-label images in Fig. 2D and corresponding higher magnification images (not shown) show that the vWF-positive lining endothelial cells have increased and dispersed giantin.

Figure 3 shows a side-by-side comparison of giantin and p115 in the various cell components in pulmonary vascular lesions observed in human PAH and in macaques infected with the chimeric SHIV virus. Giantin and p115 were increased as well as cytoplasmically dispersed in cellular elements in vascular lesions in IPAH (Fig. 3, A, left, and B, left; for quantitation also see Fig. 7). In the SHIV-infected macaques, giantin and p115 were increased in cells in the obliterator and proliferative lesions (Fig. 3, A and B; also see Fig. 6). All four SHIV-infected macaques showed increased p115 in the pulmonary arterial endothelium compared with the three SIV-infected macaques and the uninfected rhesus controls (Fig. 3A, far right; for quantitation also see Fig. 7).

The higher magnification images in Fig. 3B, derived largely from images such as in Fig. 3A, revealed that while the normal Golgi was a compact juxtanuclear punctum in controls, the Golgi tethers giantin and p115 in cellular elements in vascular lesions all displayed enlarged and dispersed Golgi. Moreover, an analysis using an anti-giantin mAb and anti-vWF pAb shown in Fig. 4B revealed that vWF-positive cells lying within an obliterator lesion also displayed enlarged and dispersed Golgi in PAH.

Cells with enlarged and dispersed Golgi were vascular vWF- and SMA-positive cells. Figure 4A shows triple-label imaging of sections of a pulmonary artery segment assayed for giantin using a pAb and for SMA using a mAb together with DAPI to show nuclei. It is evident that SMA-negative luminal endothelial lining cells as well as SMA-positive cells in the vascular lesions all displayed enlarged and dispersed Golgi. In enumerating the number of cells in vascular lesions with the normal punctate juxtanuclear Golgi complex, 75–90% of the cells showing both a giantin and a DAPI nuclear staining in the categories indicated in Fig. 7 contained dispersed Golgi comparable with the high-magnification insets/aside sets in Figs. 2–5.

In enumerating the number of cells in vascular lesions with the normal punctate juxtanuclear Golgi compared with a dispersed Golgi complex, 75–90% of the cells showing both a giantin and a DAPI nuclear staining in the categories indicated in Fig. 7 contained dispersed Golgi comparable with the high-magnification insets/aside sets in Figs. 2–5.

An enumeration of the quantitative immunomorphometry data derived from each individual is presented in Supplemental Figs. S2 and S3. There was patient-to-patient and macaque-to-macaque variation in the integrated pixel intensity data and variation among arterial segments imaged in the same section. In human PAH, there were individuals with marked increases in giantin compared with all the controls (e.g., PAH-B, -C, and -D in respective categories) and others in which the increase was more limited. As another example of inter-patient variation, the PAEC p115 data in Fig. 7A in the PAH column are derived from PAH-B, -C, and -E with PAH-A, -F, and -D, showing little increase in p115 in the luminal endothelium (data not shown). Moreover, within PAH-C increased p115 in PAECs was observed in one large arterial segment in the
section evaluated but not in other segments in the same section (data not shown). Despite such variations, there was elevated giantin and p115 in the cellular elements in the observed vasculopathies (Fig. 7). In the case of the macaque model, animal SHIV-A, which had the most extensive vasculopathies, also had the most marked increases in giantin and p115 in vascular cell elements. Strikingly, all four macaques infected with the chimeric SHIV virus (SHIV-A to -D) had increased levels of p115 in PAECs compared with all four control animals, including all three macaques infected with the non-chimeric SIV (SIV-F to -H; Supplemental Fig. S3, B).

Golgi tether dysfunction in Nef-positive vascular cells. Since HIV-Nef interferes with intracellular trafficking (2, 3, 9, 24), we investigated whether changes in the Golgi were evident with the chimeric SHIV virus (SHIV-A to -D) had increased levels of p115 in PAECs compared with all four control animals, including all three macaques infected with the non-chimeric SIV (SIV-F to -H; Supplemental Fig. S3, B).

Fig. 8. HIV-Nef-positive vascular cell elements have increased giantin. A: lung sections from an SHIV-infected macaque with increased giantin in the arterial lining endothelium and obliterator vascular lesion were probed for HIV-Nef. Scale bar = 25 μm. B: higher magnification images of insets depicted in A. Scale bar = 4 μm.

Fig. 9. Increased accumulation of PY-STAT3 in obliterator lesion together with HIV-Nef-positive cells in an SHIV-infected macaque. Lung sections from an SHIV-infected macaque with obliterator vascular lesions were probed for HIV-Nef and PY-STAT3 and compared with an arterial segment from the uninfected control. Lesion illustrated is the same shown by H&E in Fig. 1, right, SHIV-A, Oblit. Scale bar = 25 μm.
specifically in the Nef-positive cells in vascular lesions. Macaque cells positive for HIV-Nef showed accumulation and dispersal of giantin (Figs. 8). Similarly, HIV-Nef-positive endothelial cells showed increased accumulation of dispersed p115 (data not shown). We have previously pointed out that vascular cells in the proliferative vessel walls and plexiform lesions in IPAH display increased accumulation of the activated Tyr-phosphorylated form of the promitogenic transcription factor PY-STAT3. Figure 9 shows that proliferative/obliterative lesions in the SHIV-infected macaque that were Nef-positive were also PY-STAT3 positive.

**DISCUSSION**

We investigated whether Golgi dysfunction would be found in cells in pulmonary vasculopathies seen in IPAH and in a new cohort of SHIV-nef-infected macaques with pulmonary vascular lesions. Immunofluorescence microscopy of the Golgi matrix proteins/tethers giantin and p115 in lung sections from patients with IPAH showed a marked increase in and cytoplasmic dispersal of giantin- and p115-bearing vesicular elements in vascular cells in the proliferative, obliterative, and plexiform lesions, suggesting a functional trafficking dysfunction syndrome in the pathogenesis of IPAH. Moreover, macaques infected with chimeric SHIV-nef showed pulmonary vascular lesions with cells with dramatically increased and punctate cytoplasmic dispersal of p115 and giantin. At the single cell level, it was the HIV-Nef-positive vascular cells that displayed increased giantin, p115, and PY-STAT3. The macaque results implicate trafficking and Golgi dysfunction in the chain of causality culminating in pulmonary arterial vasculopathies. Impressively, the SHIV-nef-infected macaque model showed almost identical Golgi histopathologies as in human PAH. The increase in p115 specifically in PAECs in all four macaques infected with chimeric SHIV but not in the three infected with nonchimeric SIV was particularly noteworthy. The presence of HIV-Nef in vascular cells is probably due to cell-to-cell transmission and targeting to endocytic vesicles rather than by productive infection. Why HIV-Nef but not SIV-Nef affects Golgi function is not clear. Intriguingly, despite the known sequence and functional similarities between the two proteins, it is known that HIV-Nef has a much higher affinity for proteins with SH3 domains like the protein-tyrosine kinase hemopoietic cell kinase (Hck; Ref. 4). Furthermore, Hiyoshi et al. (9) recently reported the accumulation of active Hck at the Golgi. Together, these reports suggest that Nef perturbations of intracellular trafficking involving the Golgi may be more prominent with HIV- and not SIV-Nef (9).

The present data add a novel subcellular trafficking disruption aspect to discussions of the molecular pathogenesis of PAH. For instance, in discussions of the mechanistic bases of mutations in BMPRII to cause PAH, it now becomes necessary to consider the effect of such a mutation on, perhaps, dominant-negative trans-Golgi trafficking in the context of the basket of tethers, SNAREs and SNAPs, and sorting nexins that exist in a particular individual. Sorting nexin 6 (SNX6) has been shown to specifically interact with the transforming growth factor-β family of receptors (especially TβRI/Alk5), and overexpression of SNX6 inhibits productive transcriptional signaling by transforming growth factor-β1 and activin (22). Thus investigations of likely “second hit” candidates should include consideration of molecules that are involved in and that regulate intracellular membrane trafficking. That PAH-related BMPRII mutants are trapped in the endoplasmic reticulum/ Golgi apparatus and endosomal compartments and can have a dominant-negative effect on the trafficking of wild-type BMPRII has already been reported (19, 25).

It has been suggested that IPAH is an hypo-NO disease state at the level of the pulmonary arterial wall (1, 6–8, 12, 21, 33). While there is some controversy on this issue (23, 30), we note that scavenging NO from cultures of PAECs leads to enlarged cells, an increase in per-cell amounts and cytoplasmic dispersal of the Golgi markers giantin and p115, as well as a global trafficking dysfunction syndrome encompassing both anterograde and retrograde trafficking (11a). Notably, scavenging NO markedly enhances proliferation of endothelial cells (11a). The possibility that, as with our cell culture studies (11a), a hypo-NO state underlies both the subcellular Golgi dysfunction and the exuberant cell proliferation in IPAH is intriguing.

To summarize, the present data support the hypothesis that Golgi dysfunction and defective intracellular trafficking (11a) are likely players in the pathogenesis of pulmonary vascular remodeling and pulmonary hypertension. These data represent a paradigm shift toward consideration of disrupted subcellular membrane trafficking mechanisms, Golgi enlargement/dispersal, global alterations in vasorelevant receptors and signaling, and increased mitosis as a coordinated basket of changes in this disease (11a).

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