Differential Regulation of Pulmonary Vascular Cell Growth by Hypoxia-Inducible Transcription Factor-1 α and Hypoxia-Inducible Transcription Factor-2 α

Aftab Ahmad¹, Shama Ahmad¹, Kenneth C. Malcolm³, Stacy M. Miller¹, Tara Hendry-Hofer¹, Jerome B. Schaack², and Carl W. White¹

¹Department of Pediatrics, and ²Department of Microbiology, School of Medicine, University of Colorado at Denver, Aurora, Colorado; and

³Department of Medicine, National Jewish Health, Denver, Colorado

Hypoxia-inducible transcription factors HIF-1 α and HIF-2 α can contribute to pulmonary hypertension and vascular remodeling, but their mechanisms remain unknown. This study investigated the role of HIF-1 α and HIF-2 α in pulmonary artery endothelial and smooth muscle cells. The exposure of human pulmonary artery endothelial cells (HPAECs) to hypoxia (10% O₂ or 5% O₂) increased proliferation over 48 hours, compared with cells during normoxia (21% O₂). The adenovirus-mediated overexpression of HIF-2 α that is transcriptionally active during normoxia (mutHIF-2 α) increased HPAEC proliferation, whereas the overexpression of HIF-1 α , which is transcriptionally active during normoxia (mutHIF-1 α), exerted no effect. The knockdown of HIF-2 α decreased proliferation during both hypoxia and normoxia. Both HIFs increased migration toward fibrinogen, used as a chemoattractant. In an angiogenesis tube formation assay, mutHIF-2 α -transduced cells demonstrated increased tube formation, compared with the mutHIF-1 α -transduced cells. In addition, the tubes formed in HIF-2 α -transduced cells were more enduring than those in the other groups. In human pulmonary artery smooth muscle cells (HPASMCs), chronic exposure to hypoxia increased proliferation, compared with cells during normoxia. For HPASMCs transduced with adenoviral HIFs, HIF-1 α increased proliferation, whereas HIF-2 α exerted no such effect. Thus, HIF-1 α and HIF-2 α exert differential effects in isolated cells of the human pulmonary vasculature. This study demonstrates that HIF-2 α plays a predominant role in the endothelial growth pertinent to the remodeling process. In contrast, HIF-1 α appears to play a major role in pulmonary smooth muscle growth. The selective targeting of each HIF in specific target cells may more effectively counteract hypoxic pulmonary hypertension and vascular remodeling.

Keywords: HIF-2 α ; hypoxia; proliferation; endothelial; pulmonary

Adaptation to hypoxia is associated with biochemical and physiological changes that can cause either decreased or increased cellular proliferation (1, 2). Hypoxia also occurs during various lung diseases, and can cause pulmonary hypertension (PH) (3). Hypoxia-induced PH causes vascular remodeling, and growing evidence suggests that the hypoxia-inducible transcription factors (HIFs) HIF-1 α and HIF-2 α contribute to this process (4–6). Vascular remodeling involves several linked processes, including

CLINICAL RELEVANCE

Hypoxia-inducible transcription factors (HIFs) are critical contributors to vascular remodeling associated with hypoxia-induced pulmonary hypertension. The unique roles of HIF-1 α and HIF-2 α in endothelial and smooth muscle cells of the vasculature, as defined in this study, provide novel insights into pathways that could be targeted for therapy.

the proliferation and migration of endothelial as well as vascular smooth muscle cells.

HIF-1 α and HIF-2 α bind to the same hypoxia-response element and activate the transcription of a more or less common set of genes, notably vascular endothelial growth factor (VEGF). HIF-1 α may contribute to the proliferative phenotype of endothelial cells derived from patients with PH (7). Other studies using isolated adventitial fibroblasts and human pulmonary artery smooth muscle cells (HPASMCs), however, suggest that hypoxic proliferation is mediated through HIF-2 α (8, 9). Although these studies underscore the importance of hypoxia and HIFs in vascular remodeling, an understanding of the direct effects by HIF-1 α and HIF-2 α on individual human vascular cell types is lacking.

In this study, we investigate the roles of hypoxia and the hypoxia-inducible transcription factors HIF-1 α and HIF-2 α in human pulmonary artery endothelial cells (HPAECs) and HPASMCs. Through selective genetic manipulation, we show that each HIF isoform plays distinct roles in each cell type.

MATERIALS AND METHODS

Materials

Primary HPAECs and primary HPASMCs were obtained from Lonza (Walkersville, MD). The fluorescent dye DiI₁₂ (3) and FluorBlok inserts were obtained from BD Biosciences (Bedford, MA). Human fibrinogen was obtained from the Sigma Chemical Company (St. Louis, MO).

Cell Culture

HPAECs were cultured in endothelial cell basal medium (EBM-2) supplemented with VEGF, human fibroblast growth factor (FGF), human epidermal growth factor (EGF), hydrocortisone, ascorbic acid, insulin-like growth factor-1, GA-1000 (gentamycin/amphotericin-B), and 5% FBS according to the supplier's protocol (Lonza). HPASMCs were cultured in Media-231 containing 5 ng/ml heparin, 5 μ g/ml insulin, 0.5 ng/ml human EGF, 2 ng/ml human FGF- β , 0.2 μ g/ml BSA, 5% FBS, penicillin, and streptomycin.

Immunoblot Assay

Nuclear cell extracts were prepared from primary HPAECs, as described earlier (10). Additional details are available in the online supplement.

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Correspondence and requests for reprints should be addressed to Aftab Ahmad, Ph.D., Department of Pediatrics, University of Colorado at Denver, Aurora, CO 80045. E-mail: aftab.ahmad@UCDenver.edu

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RNA Interference and Hypoxic Exposures

The knockdown of HIF-1 α and HIF-2 α in HPAECs was performed using predesigned SmartPool small interfering RNA (siRNA) purchased from Dharmacon (Lafayette, CO), as detailed in the online supplement.

Cellular Proliferation

The proliferation of HPAECs and HPASMCs was measured using [3 H]-thymidine incorporation, as we described previously (11). Hypoxic proliferation in HPASMCs was performed essentially as described by Cooper and Beasley (12). Additional details are provided in the online supplement.

Migration Assay

Endothelial cell migration assays were performed using a modified Boyden chamber and BD FluoroBlok inserts (BD Biosciences). HPAECs were transduced with adenoviral vectors encoding HIF-1 α or HIF-2 α (11) and labeled with DiI $_{C12}$ dye before plating 300,000 cells per insert. Migration was monitored using an FLx 800 fluorescence plate reader (BioTek Instruments, Winooski, VT). Additional details are provided in the online supplement.

Angiogenesis Tube Formation Assay

Endothelial tube formation assays were performed in 24-well plates using BD Matrigel matrices, as described elsewhere (11). Briefly, cells were transduced with the adenoviral constructs at a multiplicity of infection of 10 plaque-forming units per cell in EBM-2 medium supplemented with growth factors. Eighteen hours after transduction, cells were detached from the plates using HyQase solution (HyClone, Logan, UT). Before plating the cells, 300 μ l of Matrigel matrix were added to each well of a 24-well plate. The cells were washed, and 100,000 cells in a total volume of 400 μ l were plated on the insert in reduced EBM-2 medium containing hydrocortisone, ascorbic acid, and GA-1000. Twenty-four hours after plating, bright-field images were collected at $\times 4$ magnification, using a DS-Fi1 camera (Nikon Instruments Inc., Melville, NY) connected to a Nikon TS100 inverted microscope (Nikon Instruments). For quantification, images were analyzed with the Image Pro Premier software (Media Cybernetics, Rockville, MD), using a macro developed to calculate branch points, tube length, and tube area.

Real-Time PCR

For assessing mRNA concentrations, total RNA was isolated from cells, cDNA was synthesized, and real-time (RT)-PCR was performed with gene-specific primers and TaqMan probes, as we described previously (11).

Statistical Analysis

All statistical analyses were performed with JMP software (SAS Institute, Cary, NC). Data are represented as the means $\pm$ SEMs of $n \geq 3$ experiments, and were compared according to ANOVA, followed by the Tukey-Kramer test for multiple comparisons. $P < 0.05$ was considered significant.

RESULTS

Hypoxic signaling promotes the proliferation of vascular cells and subsequent remodeling (13, 14). To understand the role of HIFs in this process, we assessed proliferation in HPAECs and HPASMCs. To determine endothelial proliferation, we first exposed HPAECs to normoxia (21% O $_2$) or hypoxia (10% O $_2$ or 5% O $_2$), and measured [3 H]-thymidine incorporation over 48 hours. Hypoxia (10% O $_2$ and 5% O $_2$) increased the proliferation of HPAECs during 48 hours (Figure 1A). In separate experiments, cell counts were performed at 48 hours after exposure to the different oxygen concentrations. As expected, an increase was evident in the number of cells in both of the hypoxia-exposed groups, compared with the control group at 21% O $_2$ (Figure 1B). Because HIFs are implicated in hypoxia-induced

vascular proliferation, we assessed their presence in nuclear protein, and found that both HIFs were increased in 5% O $_2$, whereas only HIF-2 α was increased in 10% O $_2$ (Figure 1C and Figure E1 in the online supplement). Quantification of the band intensities from several blots showed similar results. HIF-1 α and HIF-2 α degrade rapidly under normoxic conditions (21% O $_2$), but mutations in critical proline residues help stabilize the proteins in 21% O $_2$. Adenovirus-encoded, constitutively active HIF-1 α (Ad.mutHIF-1 α) and HIF-2 α (Ad.mutHIF-2 α) constructs that are stable during normoxia (11) were expressed, and their proliferation was assessed in comparison with cells transduced with Ad.LacZ. Although enhanced HIF-1 α expression did not affect thymidine incorporation, thymidine incorporation was elevated twofold in response to HIF-2 α overexpression (Figure 1E). Similar results were obtained when cell counts were performed in which cells transduced with Ad.mutHIF-1 α exhibited minimal proliferation, whereas cells transduced with Ad.mutHIF-2 α exhibited considerably greater proliferation (Figure 1F). To validate the functional HIF constructs, VEGF-A was measured after the transduction of these cells. As expected, both HIF-1 α and HIF-2 α increased steady-state VEGF mRNA (Figure 1G) as well as VEGF protein concentrations (Figure 1H). Interestingly, HIF-1 α was a more potent inducer of VEGF-165, the most common VEGF-A isoform, than was HIF-2 α .

We next determined the effects of HIF-1 α and HIF-2 α knockdown in these cells during hypoxia. We used 10% O $_2$, because this concentration is more physiologic in terms of its relevance to disease. As expected, hypoxia increased the proliferation of HPAECs (Figure 2A). Efficiencies of the knockdown of HIF-1 α and HIF-2 α were assessed at steady-state mRNA concentrations by RT-PCR (Figure 2B) and by the assessment of protein concentrations in nuclear lysates, using Western blots (Figure 2C). The knockdown of HIF-2 α mRNA by approximately 90% decreased cell proliferation under normoxic as well as hypoxic conditions. The knockdown of HIF-1 α also decreased proliferation under these conditions.

To elucidate the physiological role of HIFs in endothelial cells, migration assays were performed using the adenoviral-mediated overexpression of HIF-1 α , HIF-2 α , or LacZ as control. Fibrinogen, a chemoattractant for endothelial cells, was used as a stimulant (15). Both HIF-1 α and HIF-2 α overexpression increased endothelial cell migration in response to fibrinogen versus control nontransduced cells or adenoviral control LacZ-expressing cells (Figure 3A). Areas under the curve showed that the overexpression of both HIFs increased migration (Figure 3B). To confirm the identity of migrated cells, we measured downstream targets of HIF-1 α and HIF-2 α using RT-PCR. As expected, hexokinase-2 (HK2) was increased in HIF-1 α -overexpressing cells whereas the adenosine A $_{2A}$ receptor was increased in HIF-2 α -overexpressing cells, confirming the presence of functional HIF-1 α and HIF-2 α in the migrated cells (Figure 3C).

To explore the physiological role of HIFs in endothelial cells further, angiogenesis tube formation assays were performed using a Matrigel matrix and the adenoviral-mediated overexpression of HIF-1 α , HIF-2 α , or empty vector control. Twenty-four hours after plating cells on the Matrigel matrix, significantly increased branching was evident in the Ad.mutHIF-2 α -transduced cells, compared with all other groups (Figures 4A and 4B). The number of vessels (Figure 4C) and the areas covered by these vessels (Figure 4D) were also significantly increased. Representative images are shown in Figure 4A. Similar results were obtained at the 48-hour time point (data not shown). In addition, after 1 week, a few tubes still remained in only the HIF-2 α group (data not shown).

In the vasculature, hypoxia also increases the proliferation of PSMCs. Therefore, we assessed the proliferation of HPASMCs under different oxygen tensions. Initial studies showed no

Figure 1. Effects of hypoxia and hypoxia-inducible transcription factors (HIFs) on proliferation of human pulmonary artery endothelial cells (HPAECs). (A) HPAECs were exposed to 21% O₂, 10% O₂, or 5% O₂ for the indicated times. ^3H -thymidine was added 24 hours before harvest, and the incorporated radioactivity was measured. (B) HPAECs were similarly exposed to 21% O₂, 10% O₂, or 5% O₂ for 48 hours, and cells were counted after trypsinization. (C) Immunoblots from HPAECs were exposed similarly for 48 hours. Blots were probed with anti-HIF-2 α , anti-HIF-1 α , and β -actin antibodies. (D) For densitometry, blots were imaged using a Bio-Rad Gel Doc system (Bio-Rad, Hercules, CA), and band-quantitated using Image Lab software (Media Cybernetics). (E) HPAECs were transduced with control Ad.LacZ or adenovirus vectors encoding constitutively active HIF-1 α (Ad.mutHIF-1 α) or constitutively active HIF-2 α (Ad.mutHIF-2 α) at a multiplicity of infection of 10 plaque-forming units/cell. ^3H -thymidine was added 6 hours after transduction, and proliferation was assessed 24 hours after transduction. (F) Cells were also counted after adenoviral transductions. (G) In parallel experiments, RNA was isolated, and real-time-PCR was performed for human vascular endothelial growth factor (VEGF)-A. (H) In adenoviral-transduced cells, total VEGF-165 was measured in media and cell lysates. * $P < 0.05$, compared with the 21% O₂ control cells (A and B) or control or Ad.LacZ-treated cells (D, E, and H). # $P < 0.05$, compared with the Ad.LacZ-treated cells (F).

proliferative response to hypoxia (72 h; not shown). However, chronic hypoxia caused increased proliferation, consistent with other reports (12). HPASMCs that were pre-exposed to chronic hypoxia (96 h) showed increased proliferation, both at 10% O₂ and 3% O₂ (Figure 5A).

To assess the role of HIFs in the hypoxic proliferation of HPASMCs, we initially assessed concentrations of HIF-1 α and HIF-2 α in the nuclear lysates of hypoxia-exposed cells. Compared with HPAECs, HPASMCs showed a higher basal concentration of HIF-1 α , even in 21% O₂. In response to lower oxygen tensions, HIF-1 α increased further in these cells (Figures 5B and 5C). As expected, HIF-2 α also increased with decreasing oxygen tensions.

Next, we evaluated the effects of individual HIF isoforms on the proliferation of HPASMCs. We used the same adenoviral-based overexpression of constitutively active HIF-1 α (Ad.mutHIF-1 α) and HIF-2 α (Ad.mutHIF-2 α) (11). HIF-1 α increased the proliferation of HPASMCs, whereas HIF-2 α exerted no effect (Figure 5D). These HIFs are transcriptionally active in HPASMCs, as demonstrated by increased VEGF steady-state mRNA (Figure 5E).

DISCUSSION

Vascular lesions of patients with PH demonstrate increased HIF-1 α expression (7). Compared with wild-type mice, both HIF-

Figure 2. Effects of HIF knockdown on the proliferation of HPAECs. HPAECs were transfected with control siRNA (siCtrl) or siRNA against HIF-1 α (siHIF-1 α) or HIF-2 α (siHIF-2 α). Twenty-four hours after transfection, cells were exposed to 21% O₂ or 10% O₂. (A) ³[H]-thymidine was added after 24 hours of exposure, and proliferation was assessed at 48 hours. (B) In parallel experiments, RNA was isolated and RT-PCR was performed for human HIF-1 α and HIF-2 α . (C) In parallel experiments, an immunoblot of nuclear lysates of cells transfected with siRNAs was probed with anti-HIF-2 α , anti-HIF-1 α , and β -actin antibodies. Ctrl, control. * P < 0.05 versus normoxia-exposed control or siCtrl cells. # P < 0.05 versus respective control cells during normoxia or hypoxia.

1 α ^{+/-} and HIF-2 α ^{+/-} mice demonstrated reduced vascular remodeling in hypoxia-induced PH (4, 5). However, the role of each protein in individual cell types remained unclear. The proliferation of pulmonary vascular cells is characteristic of the remodeling process, and HIFs can play important roles. Our results indicate that HIF-2 α promotes endothelial proliferation, whereas HIF-1 α promotes smooth muscle cell proliferation. These findings are intriguing because both HIF-1 α and HIF-2 α can induce angiogenic genes such as VEGF, which can act as endothelial cell mitogens. Although our experiments show that HIF-2 α increases VEGFA mRNA concentrations more potently, they

also show that HIF-1 α is a major inducer of VEGF-165 protein. This difference may have arisen because the primer-probe pair detects all isoforms of VEGFA at the mRNA level, whereas the protein assay detects only the VEGF-165 isoform. Although isoform differences may play a role, it appears that VEGF may not influence HIF or hypoxic proliferation. This is consistent with other reports that have shown no change in hypoxia-mediated proliferation when VEGF signals were blocked using neutralizing antibodies (16). Several studies have indeed shown that HIF-1 α and HIF-2 α can differentially influence growth and proliferation in specific cases (17–19). The

Figure 3. Effects of HIFs on the migration of HPAECs. HPAECs were transduced with control Ad.LacZ, Ad.mutHIF-1 α , or Ad.mutHIF-2 α . Cells were stained with DilC₁₂ (3) (BD Biosciences, Bedford, MA), and 300,000 cells were plated on FluoroBlok inserts (BD Biosciences). Plates were incubated at 37°C in a cell-culture incubator, and the migration toward fibrinogen (50 μ g/ml) was recorded using a fluorescence plate reader. (A) Changes in fluorescence with time and (B) areas under the curve were calculated. (C) RT-PCR of the migrated cells. * P < 0.05 versus control or Ad.LacZ-transduced cells.

Figure 4. Effects of HIFs on the angiogenic activity of HPAECs. HPAECs were either untreated or transduced with an empty adenovirus vector (Ad.Empty Vector), or the constitutively active HIF-1α (Ad.mutHIF-1α) or the constitutively active HIF-2α (Ad.mutHIF-2α) adenoviral constructs at a multiplicity of 10 plaque-forming units/cell. Eighteen hours after transduction, HPAECs were split and plated at 100,000 cells/well in a 24-well plate coated with Matrigel (BD BioSciences). After 24 hours, images were taken at $\times 4$ magnification. (A) Representative images. An average of 25 images per condition was analyzed, using Image Pro Premier software (Media Cybernetics). The average number of branch points per field (B), the average number of tubes per field (C), and the average area (in μm^2) covered by tubes per field (D) were calculated and plotted. * $P < 0.05$, compared with nontransduced control cells. # $P < 0.5$, compared with the empty vector control cells. $^S P < 0.05$, compared with Ad.mutHIF-1α.

effects of HIF-2α could be mediated through increased c-Myc function (20) or through the adenosine A_{2A} receptor, a proliferative target of HIF-2α in pulmonary endothelial cells (11). Additional mechanisms by which HIF-1α and HIF-2α

could potentially influence proliferation and growth were summarized in a recent review (21).

The knockdown of HIF-2α decreased the proliferation of HPAECs under both hypoxic and normoxic conditions. This

Figure 5. Effects of hypoxia and HIFs on the proliferation of human pulmonary artery smooth muscle cells (HPASMCs). (A) HPASMCs were pre-exposed (PE) for 4 days to 3% O_2 and replated. Twenty-four hours after plating, cells were exposed to 21% O_2 , 10% O_2 , or 3% O_2 for the indicated times. ^3H -thymidine was added 24 hours before harvest, and the incorporated radioactivity was measured. (B) Immunoblot with nuclear lysate from HPASMCs exposed similarly. Blots were probed with anti-HIF-1α, stripped, and reprobed with anti-HIF-2α, followed by stripping and probing with β -actin antibodies. (C) For densitometry, blots were imaged using a Bio-Rad Gel Doc system, and band-quantitated using Image Lab software (Media Cybernetics). (D) HPASMCs were transduced with control Ad.LacZ, Ad.mutHIF-1α, or Ad.mutHIF-2α constructs. ^3H -thymidine was added 6 hours after transduction, and proliferation was assessed 24 hours after transduction. (E) In parallel experiments, RNA was isolated, and RT-PCR was performed for human VEGF-A. * $P < 0.05$ versus normoxia-exposed cells (A) or control and Ad.LacZ-treated cells (D and E).

is consistent with findings that HIF-2 α , compared with HIF-1 α , is more highly expressed at the protein level under normoxic conditions in most cell types, and is abundant in the endothelium (22, 23). Besides rendering a proliferative phenotype, HIF-2 α also helps maintain endothelial homeostasis and vessel integrity (24, 25). Decreased proliferation with HIF-1 α knockdown could be related to its secondary effect in knocking down HIF-2 α expression as well. A lesser role for HIF-1 α in endothelial proliferation could be attributed to its ability to induce cell-cycle arrest (26–28) or to the inhibition of transcriptional activity by mini-chromosome maintenance proteins (29). Our results on the effects of HIF-1 α on HPAEC proliferation are at variance with a report showing increased proliferation with HIF-1 α overexpression (30). Part of this variance could be attributed to the C-terminus-deleted HIF-1 α construct used (30), compared with the full-length construct used in our experiments. Similar effects of HIF-2 α have been shown in fibroblasts derived from pulmonary arteries (8). Interestingly, the hypoxic proliferation of fibroblasts was performed in 1% O₂, an oxygen tension where we observed decreased endothelial cell proliferation (data not shown). Therefore, the mechanisms by which hypoxia influences fibroblast proliferation are likely to differ from the endothelial proliferation (at 5% O₂ and 10% O₂) reported in this study.

Cellular migration is an integral part of vascular remodeling. Our findings of increased migration by HIF-1 α -overexpressing and HIF-2 α -overexpressing HPAECs are consistent with the proangiogenic profile of both HIFs, and are also in line with other reports showing similar effects (8). Interestingly, our results showed that HIF-2 α exerted a profound effect on angiogenesis tube formation, and that HIF-1 α exerted a minimal effect compared with the control samples. In addition, tubes formed from HIF-2 α -transduced cells were more enduring than those in the other groups. This highlights yet another vital difference between the two HIF isoforms in pulmonary endothelial cells, and underscores the importance of HIF-2 α in these cells. Similar findings were reported in endothelial-specific deletions of HIF-2 α , where animals exhibited defective angiogenesis (25).

Unlike the proliferation of endothelial cells during moderate hypoxia, only chronic hypoxia caused the proliferation of HPASMCs. PSMCs may involve lower O₂ tensions than endothelial cells, given that the pulmonary arterial walls are many layers thick, especially in PH, and a decreasing O₂ gradient is evident across the vessel wall (31). These findings are understandable in light of previously reported *in vivo* observations that the proliferation of endothelial cells in hypoxia precedes that of PSMCs (13). In sharp contrast to the increased proliferation of endothelial cells caused by HIF-2 α , HPASMC proliferation was mediated by HIF-1 α . Several lines of evidence support the current findings, and indicate the importance of HIF-1 α in influencing PSMCs. First, PSMCs have a higher basal level of HIF-1 α under normoxic conditions than other pulmonary cells and smooth muscle cells from the systemic circulation (32). Second, HIF-1 α can promote growth factor-induced proliferation in PSMCs (33). Third, a HIF-1 α ^{+/-} murine model of PH showed decreased medial thickening of muscularized vessels of heterozygotes, compared with wild-type animals (5). Moreover, HIF-1 α ^{+/-} mice exhibited impaired electrophysiological responses in smooth muscle cells. Therefore, the effects of HIF-1 α on vascular remodeling are likely mediated mainly through its action on PSMCs. The mechanisms by which HIF-1 α influences the growth of PSMCs are unclear. Emerging evidence suggests a role for myelocytomatosis viral oncogene (Myc) in this process, where increased concentrations of Myc can reverse HIF-1 α -mediated growth inhibition (34). This is consistent with another report indicating that Myc suppression in smooth muscle cells decreased

proliferation (35). Thus, factors influencing Myc could also affect HIF-1 α -mediated proliferation.

The role of individual HIF isoforms in human vascular remodeling is not well understood. Several studies have shown that gain-of-function mutations in HIF-2 α lead to erythrocytosis (36–38). Individuals with increased erythrocytosis, a characteristic of Chuvash polycythemia, are at increased risk of developing pulmonary hypertension (39, 40). Three recent studies reported a high allelic variability in the HIF-2 α gene locus in Tibetans living at high altitude, and that variability correlated with a blunted erythropoietic response and lower hematocrit concentrations (41–43). Contrary to these findings, no evidence for the enrichment of HIF-2 α variants was observed in Andean highlanders who exhibited higher hematocrit concentrations, a dampened hypoxic ventilatory response, and increased pulmonary artery pressures (44). Furthermore, humans with gain-of-function mutations in HIF-2 α were associated with pulmonary hypertension, increased heart rate, and increased cardiac output (45). These findings underscore the potential importance of hypoxic factors such as HIF-2 α in pulmonary hypertension. Although these studies strongly suggest a role for HIF-2 α in pulmonary hypertension and hypoxic/high-altitude adaptation, the mechanisms by which they influence the process are not yet fully understood (46, 47). The present study highlights some of the potential ways by which HIF-2 α could influence vascular remodeling.

Our study highlights the distinct roles of HIF isoforms in response to hypoxic signals. Given the nature of induction of HIFs by hypoxia and their co-occurrence in cells, conflicting results in the literature may be explained by the overlapping and often opposing nature of the two HIF signals, as illustrated in this study. In addition, our experiments demonstrate only the autocrine effects of HIF-1 α and HIF-2 α on pulmonary vascular cells. Because cell-cell interactions contribute to the remodeling process, the paracrine effects of HIFs can also influence this process and warrant further investigation (48–50). Our present findings, together with previous reports stating that both HIFs play important roles in hypoxia-induced PH (4, 5), suggest that targeting both HIFs simultaneously could exert a greater impact in this disorder. These results also underscore the potential importance of targeted therapies in cases where one HIF isoform is selectively up-regulated, as may occur with mutations and/or the nonhypoxic stabilization of HIFs.

Author disclosures are available with the text of this article at www.atsjournals.org.

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