Glucose-6-phosphate dehydrogenase plays a critical role in hypoxia-induced CD133+ progenitor cells self-renewal and stimulates their accumulation in the lungs of pulmonary hypertensive rats

Sukrutha Chettimada,1 Sachindra Raj Joshi,1 Abdallah Alzoubi,2 Sarah A. Gebb,3 Ivan F. McMurtry,2,4 Rakhee Gupte,1 and Sachin A. Gupte1,4

1Department of Biochemistry and Molecular Biology, University of South Alabama, Mobile, Alabama; 2Department of Pharmacology, College of Medicine, University of South Alabama, Mobile, Alabama; 3Department of Cell Biology and Neuroscience, University of South Alabama, Mobile, Alabama; and 4Center for Lung Biology, University of South Alabama, Mobile, Alabama

Submitted 28 October 2013; accepted in final form 22 July 2014

Chettimada S, Joshi SR, Alzoubi A, Gebb SA, McMurtry IF, Gupte R, Gupte SA. Glucose-6-phosphate dehydrogenase (G6PD) activity was increased threefold in hypoxic CD133+ cells. The increased G6PD activity was required for CD133+ cell proliferation, and their growth was arrested by G6PD inhibition or knockdown. G6PD activity upregulated expression of HIF1α, cyclin A, and phospho-histone H3, thereby promoting CD133+ cell differentiation and self-renewal and altering cell cycle regulation. When CD133+ cells were cocultured across a porous membrane from pulmonary artery smooth muscle cells (PASMCs), G6PD-dependent H2O2 production and release by PASMCs recruited CD133+ cells to the membrane, where they attached and expressed smooth muscle markers (α-actin and SM22α). Inhibition of G6PD reduced smooth muscle marker expression in CD133+ cells under normoxia but not hypoxia. In vivo, CD133+ cells colocalized with G6PD+ cells in the perivascular region of lungs from rats with hypoxia-induced pulmonary hypertension. Finally, inhibition of G6PD by dehydroepiandrosterone in pulmonary arterial hypertensive rats nearly abolished CD133+ cell accumulation around pulmonary arteries and the formation of occlusive lesions. These observations suggest G6PD plays a key role in increasing hypoxia-induced CD133+ cell survival in hypertensive lungs that differentiate to smooth muscle cells and contribute to pulmonary arterial remodeling during development of pulmonary hypertension.

Molecular oxygen is essential for most life on Earth, functioning as the final electron acceptor during the oxidation of glucose to produce energy in the form of ATP. Ironically, hypoxia promotes the survival and expansion capacity of embryonic stem cells, cancer stem cells (16, 40), and neuronal crest stem cells (30, 44), as well as the differentiation potential of bone marrow-derived CD133+ progenitor cells (33, 40).

CD133+ progenitor cells are capable of differentiating into hematopoietic, endothelial, smooth muscle, and neuronal cell types. They play a role in tissue genesis during gestation (35) and in the angiogenesis that promotes wound healing (4). CD133+ and bone marrow-derived stem cells also contribute to tumorigenesis (39, 47, 54) and pathogenic remodeling that reduces conductance in coronary (49) and pulmonary arteries (13, 28, 48, 51), thereby contributing to chronic hypoxia-induced pulmonary hypertension (23, 41). In response to hypoxia, circulating bone marrow-derived CD34+/CD133+/Kit+ and c-kit+ cells are recruited to the adventitial, medial or intimal compartments, where they assume mesenchymal or smooth muscle cell-like characteristics (29, 38, 43). In vitro, these cells acquire the morphology and phenotype of the cells with which they are cocultured (11). For example, CD133+ cells cocultured with isolated human pulmonary arterial segments migrate through the intima and differentiate into smooth muscle cells. More interestingly, transplantation of bone marrow-derived CD133+ progenitor cells from patients with pulmonary arterial hypertension (PAH) into mice resulted in angiproliferative pulmonary vascular remodeling, right ventricular failure, and death (3). However, the molecular mechanisms that underlie the expansion and differentiation of hypoxic CD133+ progenitor cells remain unclear.

Self-renewal of progenitor cells is mediated by Wnt-Notch (4, 22, 36) and TGF-β-SMAD signaling pathways (25, 50), and NADPH oxidase (NOX)-derived reactive oxygen species (ROS) are also involved in the differentiation of stem/progenitor cells to endothelial and smooth muscle cells (26). For example, mobilization of endothelial progenitor cells is dependent on NOX2-derived ROS (9, 33, 37). In addition, TGF-β upregulates glucose-6-phosphate dehydrogenase (G6PD) expression in fetal brown adipocytes and increases levels of NOX4-derived ROS (45). Because production of superoxide anion from NOX is dependent on G6PD-derived NADPH (18, 20), we hypothesized that increases in G6PD-dependent NADPH redox and NOX-derived ROS act in concert to mediate hypoxia-induced CD133+ cell expansion and differentiation.
Our findings indicate that G6PD activity promotes 1) hypoxia-induced CD133+ cell hyperproliferation via a TGF-β- and ROS-independent pathway, and 2) the expression of smooth muscle contractile proteins in CD133+ cells cocultured with pulmonary artery smooth muscle cells (PASMCs). Furthermore, oral administration of dehydroepiandrosterone (DHEA), an endogenous 17α-ketosteroid that blocks G6PD activity (19), to PAH rats for 5 wk inhibited accumulation of CD133+ cells around their pulmonary arteries and reduced the formation of occlusive neointimal and plexiform lesions.

MATERIALS AND METHODS

Chemicals. The TGF-β receptor blocker SB525334 [6-[2-((1,1-dimethylthyl)-5-(6-methyl-2-pyridinyl)-1H-imidazol-4-yl)quinoxaline] was purchased from Selleckchem (Houston, TX). All other chemicals were from Sigma Aldrich (St. Louis, MO), unless indicated otherwise.

CD133+ cell culture. Human CD133+ progenitor cells were purchased from PromoCell and expanded in hematopoietic progenitor cell expansion medium (PromoCell) supplemented with Cytokine mix E (PromoCell) containing human thrombopoietin, stem cell factor,flt-3 ligand, and IL-3. Cells were expanded for 1 wk in 75-cm2 flasks (Corning, Corning, NY) at 37°C in a humidified CO2 incubator before use.

Cell coculture. CD133+ cells were labeled with a nontransferable dye, Alexa Fluor 594-conjugated AcLDL (10 μg/ml Ac-LDL AS94, Life Technologies, Grand Island, NY) at 37°C and seeded (2,800 cells/Transwell insert) onto membranes (0.4-μm pores) in Transwell inserts in a 12-well plate (Corning). Forty-eight hours prior to seeding the CD133+ cells, PASMCs were seeded on the reverse side of the Transwell membrane at a density of 50,000 cells/Transwell insert. After seeding the CD133+ cells, the coculture setup was incubated under normoxia or hypoxia for 8 days. CD133+ cells cultured alone served as a negative control for coculture. PASMCs cultured on Transwell inserts without CD133+ cells served as a positive control for smooth muscle phenotype markers.

Hypoxia treatment. CD133+ progenitor cells cultured in 25-cm2 flasks (Corning), (n = 6 flasks/condition) were incubated for 72 h in a hypoxic chamber (InviV02 300, Ruskin Technology) under 2% to 10% O2) for 3 wk. They were returned to normoxia (21% O2) for an additional 5 wk (total 8 wk after SU5416 injection). DHEA was administered to rats in food (1% of daily diet; Teklad Custom Research Diet) for a period of 5 wk (weeks 3 to 8). The dosage of DHEA was based from previous studies (32). The number of CD133+ cells, around 10 vessels (50–200 μm diameter), per whole left lobe cross section (5 sections/rat) including the hilum from three groups of rats (6 rats/group) were counted by investigators who were unaware of the source of the sections. The number of CD133+ cells was normalized for the total number of perivascular cells. Staining for CD133 was done with a Vectastain kit. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of South Alabama. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Statistical analysis. Values are presented as means ± SE. ANOVA and post hoc Student’s t-tests were used for analysis. Values of P < 0.05 were considered significant. In all cases, the number of experimental determinations (n) was equal to the number of rats from which pulmonary arteries were harvested for this study.
RESULTS

CD133<sup>+</sup> cell numbers increase under hypoxia. A remarkable property of CD133<sup>+</sup> cells is that they self-renew and differentiate. To determine the effect of hypoxia, we compared CD133<sup>+</sup> cell numbers/self-renewal between cells cultured under hypoxia (Hx, 3% O<sub>2</sub>, 5% CO<sub>2</sub>) or normoxia (Nx, 21% O<sub>2</sub>, 5% CO<sub>2</sub>). Both expression and cell numbers of CD133 were increased in cultures exposed to hypoxia (Fig. 1, A and B), as did cell size (Fig. 1C). To determine whether the observed increases in cell number and size reflected a reduction in the incidence of cell death, we used LDH-cell cytotoxicity assays to assess necrotic cell death and found no difference between normoxic and hypoxic cells (data not shown). CD133<sup>+</sup> cells apparently thrive under hypoxic conditions.

Increased G6PD activity contributes to increased cell growth in hypoxia. DHEA and 6-aminonicotinamide (6AN), both known to inhibit G6PD (19), suppressed hypoxia-induced increases in G6PD activity in CD133<sup>+</sup> cells (Fig. 2A). This inhibition of G6PD also suppressed the hypoxia-induced increases in cell numbers (Fig. 2B) and cell size (Fig. 2C) and tended to increase cytotoxicity (Fig. 2D). This suggests G6PD activity makes a significant contribution to cell growth. We then further confirmed the positive effect of G6PD activity on CD133<sup>+</sup> cell growth by knocking down G6PD expression under hypoxic conditions using an adenoviral vector (10<sup>13</sup> pfu) harboring a GFP-tagged shRNA targeting G6PD (Fig. 2, E and F). Both G6PD activity and cell numbers were reduced in CD133<sup>+</sup> cells transfected with G6PD-shRNA but were unaffected in cells transfected with a vector harboring GFP alone (Fig. 2, G and H).

TGF-β and ROS signaling do not contribute to increased CD133<sup>+</sup> cell numbers in hypoxia. TGF-β signaling is important for self-renewal of stem cells (25), which is mediated via activation of downstream SMAD2/3 signaling (50). To determine whether the same pathway is involved in the self-renewal of CD133<sup>+</sup> cells under hypoxia, we treated the cells with or without the TGF-β receptor blocker 6-((2-(1,1-dimethylethyl)-5-((6-methyl-2-pyridinyl)-1H-imidazol-4-yl)quinoxaline (SB525334; 1, 5 and 10 μM, Selleckchem, Houston, TX) (6). SB525334 (1 μM) reduced phosphorylation of SMAD2 but not SMAD3 in CD133<sup>+</sup> cells (Fig. 3, A and B). Under normoxia, SB525334 (1 μM) effectively inhibited CD133<sup>+</sup> cell growth, but it had no effect on the increase in cell numbers caused by hypoxia (Fig. 3C). In addition, lucigenin chemiluminescence assays showed that ROS (superoxide) levels declined under hypoxia and that inhibition of TGF-β signaling had no effect on superoxide levels under normoxia or hypoxia, compared with untreated controls (Fig. 3D). On the other hand, G6PD activity increased under hypoxia, and SB525344 treatment further increased that activity (Fig. 3E).

G6PD inhibition reduces expression of HIF1α and cell cycle proteins. Because Notch-HIF1α signaling mediates stem cell self-renewal and is required to maintain stem cells in a dedifferentiated state under hypoxia (22), we assessed the levels of HIF1α expression in CD133<sup>+</sup> cells cultured under normoxia or hypoxia. We found that HIF1α expression increased under hypoxia was reduced by G6PD inhibition with 6AN (Fig. 4). Moreover, cyclin A and phospho-histone H3 that promote cell proliferation were also elevated in hypoxia and inhibition of G6PD suppressed this increase (Fig. 4).

Differentiation of CD133<sup>+</sup> cells into smooth muscle-like cells is influenced by G6PD-dependent signaling. To determine whether G6PD-dependent signaling influences differentiation of CD133<sup>+</sup> to PASM-like cells, we cocultured CD133<sup>+</sup> cells and PASMCs in a Transwell chamber on opposite sides of a porous membrane (0.4-μm pores) that allowed transmembrane movement of small molecules but not cells (Fig. 5A). We then examined the expression of smooth muscle cell phenotype markers in the CD133<sup>+</sup> cells. Immunofluorescent staining showed that CD133<sup>+</sup> cells expressed the smooth muscle markers SM22α and α-actin when cocultured with PASMCs but remained undifferentiated in suspension when cultured alone for the same period (Fig. 5, B and C). In addition, significantly more (7.5-fold) CD133<sup>+</sup> cells were attached to the membrane after 8 days of coculture with PASMCs than were attached after culture alone for the same period (Fig. 5E). Our next goal was to determine whether PASMCs released G6PD-dependent factors that affected the recruitment and differentiation of CD133 cells. Inhibiting G6PD in PASMCs cultured in the lower chamber (see schematic; Fig. 5A) using 6AN or DHEA reduced the number of CD133<sup>+</sup> cells attached to the membrane and prevented their expression of SM22α (Fig. 5C). Consequently, the SM22α<sup>-</sup>-to-total cell ratio was reduced by inhibition of G6PD (Fig. 5, C and F).

Fig. 1. Immunoblot showing an increase in CD133 expression under hypoxia (Hx) (A), which correlated with increases in cell number (B) and cell size (C). Nx, normoxia.
Fig. 2. A: hypoxia increased glucose-6-phosphate dehydrogenase (G6PD) activity in CD133⁺ cells, but the effect was suppressed by dehydroepiandrosterone (DHEA) and 6-aminonicotinamide (6AN). Inhibition of G6PD with DHEA or 6AN also suppressed hypoxia-induced increases in cell number (B) and cell size (C) but slightly increased the incidence of necrotic cell death (D). Fluorescence micrographs showing CD133⁺ cells transfected with an adenoviral vector encoding green fluorescent protein (GFP) with G6PD-shRNA or GFP alone (E and F). G: G6PD activity was reduced in cells transfected with G6PD shRNA. H: transfection with G6PD shRNA also reduced CD133⁺ cell numbers.
but the CD133\(^+\)-to-total cell ratio was unaffected (data not shown). Interestingly, the SM22\(^+\)-to-total cell ratio was not reduced by inhibition of G6PD in cocultures exposed to hypoxia (Fig. 5, D and F).

\(H_2O_2\) acts as a chemotactic factor that recruits CD133\(^+\) cells to PASMCs. Because G6PD-derived NADPH fuels production of NOX-dependent ROS (12, 42), we tested whether \(H_2O_2\) was involved in the observed recruitment of CD133\(^+\) cells to the Transwell membrane in the presence of PASMCs. When we treated the PASMCs in the lower chamber with pegylated catalase (100 g/ml), a \(H_2O_2\) scavenger (Fig. 6A, bright-field; Fig. 6B, DAPI-stained nuclei of CD133\(^+\) cells), fewer CD133\(^+\) cells attached to the membrane (Fig. 6C). By contrast, attachment of CD133\(^+\) cells cultured alone was stimulated by \(H_2O_2\) (50 \(\mu\)mol/l; Fig. 6D) added to the lower chamber. This suggests that the PASMC-related recruitment of CD133\(^+\) cells was mediated, at least partly, by \(H_2O_2\).

G6PD and CD133 colocalize in the pulmonary arterial adventitia in hypoxic rats. When we used immunohistochemistry to examine the distributions of G6PD and CD133 in consecutive sections of paraffin-embedded lungs from rats exposed to normoxia or hypoxia for 3 wk, we found that cells positive for CD133 and G6PD colocalize in the adventitia (or perivascular space) of pulmonary arteries from chronically hypoxic lungs (Fig. 7A, arrows). By contrast, the normoxic control lungs were negative for CD133. The pulmonary wall thickness, determined as medial wall thickness-to-diameter ratio, increased by 1.92-fold in lungs of hypoxic compared with normoxic control rats. Concurrently, right ventricle-to-left ventricle + septum ratio increased (normoxia: 0.169 ± 0.008 and hypoxia: 0.240 ± 0.009; \(P < 0.05\)) in hypoxic rats was reduced by DHEA treatment (0.148 ± 0.005; \(P < 0.05\) vs. hypoxia).
Co-culture experiments

CD133+ progenitor cells

Expansion – 1 week

Label with fluorescent non-transferable dye AcLDL-A594

2 days

8 days Normoxia or Hypoxia

3 hrs @ 37 °C

Microscopy

PASMC

CD133+ cells

Porous membrane

PASMC: DHEA/6AN/Peg-Catalase

CD133+ Cells

PASMC+ CD133+ Cells

6AN+PASMC+ CD133+ Cells

SM22α

Ac-LDL A594

α-Actin

Merge

Cell Number/Field

Nx-CD133

Nx-6AN

Hx-Con

Hx-6AN

*P<0.05 vs Nx- & Hx-CD133+

#P<0.05 vs Nx- & Hx-CO

SM22+ / Total cells

Nx-con

Hx-con

Nx-6AN

Hx-6AN

@P<0.05 vs Nx- & Hx-con

*P<0.05 vs Nx- & Hx-6AN
DHEA reduces CD133+ cell numbers and plexiform lesions in the lungs of PAH rats. CD133+ cells have been implicated in the pathogenesis of PAH. Because these cells accumulate around the abluminal side of the pulmonary arteries in hypertensive rat and human lungs, we speculated that they participate in the pathogenesis of the occlusive neointimal and plexiform lesions associated with the PAH. Immunohistochemical analysis of sections of paraffin-embedded lungs from rats exposed to SU5416/H11001 hypoxia for 3 wk plus reexposure to normoxia for 5 wk (SU/Hx/Nx 8 wk) revealed that the numbers of CD133+ cells, mainly around occlusive lesions, were observed in lungs from hypertensive rats (Fig. 7B). Because...
increased G6PD activity contributes to increased CD133\(^+\) proliferation (Fig. 2), we speculated that inhibiting G6PD could decrease CD133\(^+\) cell number and reduce occlusive lesion formation. Treating the PAH rats for 5 wk (from week 3 through week 8) with DHEA (1% of total food intake), which blocks G6PD activity in CD133\(^+\) cells (Fig. 3A) and other cell types, including vascular smooth muscle cells (10), decreased the accumulation of CD133\(^+\) cells (Fig. 7, B and C). Coincidentally, DHEA decreased wall thickness (in \(\mu\)l/min normoxia: 0.066 ± 0.014; SU/Hx/Nx: 0.128 ± 0.021; SU/Hx/
CD133⁺ CELLS, G6PD, HYPOXIA, SELF-RENEWAL, DIFFERENTIATION

Nₓ + DHEA: 0.068 ± 0.012; P < 0.05 normoxia vs. SU/Hx/Nₓ and SU/Hx/Nₓ vs. SU/Hx/Nₓ + DHEA) and increased cardiac output (normoxia: 34,460 ± 3,725; SU/Hx/Nₓ: 21,133 ± 2,502; SU/Hx/Nₓ: 29,101 ± 1,805; P < 0.05 normoxia vs. SU/Hx/Nₓ and SU/Hx/Nₓ vs. SU/Hx/Nₓ + DHEA).

Ultrasound- and sonographically-guided CD133⁺ cells appear to be increased under hypoxia, and so contribute to the pathogenesis of hypoxia-associated diseases like cancer and PAH. However, the signaling pathways that lead to CD133⁺ cell growth under hypoxia remain obscure. In the present study, we 1) identified G6PD as a pivotal mediator involved in the proliferation and differentiation of CD133⁺ cells, 2) described a G6PD-associated pathway via which proliferation and differentiation of CD133⁺ cells appear to be increased under hypoxia, and 3) demonstrated that inhibition of G6PD reduced both CD133⁺ cell numbers and occlusive lesions in the lungs of PAH rats.

Among the products generated from glucose catabolism is glucose-6-phosphate, which generates electron donors that reduce molecular oxygen to water and produce energy in the mitochondrial respiratory chain. And when the cell has surplus energy, glucose-6-phosphate is shunted into the pentose phosphate pathway to produce NADPH, which is required to protect the cell from oxidative damage and to synthesize fatty acids and cholesterol, as well as the ribose required for de novo synthesis of RNA and DNA. The pentose phosphate pathway is also oxygen sensitive. In coronary arterial smooth muscle, the pentose phosphate pathway activity is decreased by reductions in Po₂ (21), but in the pulmonary arterial smooth muscle (10, 18) and in solid tumors (15) the activity is increased by hypoxia. Similarly, we found that G6PD activity is enhanced in hypoxic CD133⁺ cells, compared with normoxic cells, and inhibition or knockdown of G6PD reduces the hyperproliferation of CD133⁺ cells seen under hypoxia. Consistent with that finding, increased activity in the pentose phosphate pathway appears to promote growth in other cell types (14, 52). Moreover, changes in cellular energy metabolism and hypoxia promote proliferation and differentiation of neural crest and central nervous system (CNS) stem cell population (30, 44). Collectively, these findings suggest that adaptive metabolic changes mediate the increases in stem cell proliferation induced by hypoxia and that the pentose phosphate pathway plays a key role. We observed that hypoxia had no effect on the incidence of apoptosis in CD133⁺ cells (data not shown); nonetheless, both G6PD inhibition and knockdown reduced cell numbers without significantly increasing the incidence of necrotic cell death. This finding is consistent with the notion that G6PD plays a role in regulating the growth and death of cells (7) and demonstrates for the first time that activation of G6PD is crucial for mediating sustained CD133⁺ cell proliferation under hypoxia.

TGF-β upregulates G6PD expression and increases levels of NOX4-derived ROS (45), which in turn stimulate G6PD activity in a variety of cell types, including erythrocytes, hepatocytes, and smooth muscle myocytes. We found that TGF-β receptor blockade significantly reduced CD133⁺ cell numbers under normoxia but not hypoxia. This suggests the TGF-β pathway does not mediate proliferation of CD133⁺ cells under hypoxia and that G6PD does not play a role in TGF-β-mediated self-renewal of CD133⁺ cells under normoxia. In addition, our observation that TGF-β receptor blockade did not affect ROS production in CD133⁺ cells under either normoxia
or hypoxia suggests that TGF-β-associated signaling does not mediate ROS generation in CD133+ cells. Indeed, ROS production was reduced in hypoxic CD133+ cells. This indicates that neither hypoxia-evoked G6PD overactivation nor hyperproliferation of CD133+ cells was mediated by TGF-β/SMAD or ROS signaling. This is not unprecedented, as oxidative stress is known to reduce stem cell growth (5). Moreover, we found that inhibition of G6PD stalled cell growth and reduced hypoxia-induced expression of HIF1α, phospho-histone H3 and cyclin A. Under hypoxia, Notch interacts with HIF1α, recruiting it to Notch-responsive promoters, and prevents differentiation of C2C12 and neural crest stem cells (22). G6PD-derived NADPH stabilizes HIF1α in human mesangial cells (34) and activates NADPH-dependent thioredoxin reductase that promotes HIF-responsive element (HRE) activity in rat PASMCs (46). Taken together with those findings, our results suggest that hypoxia-induced activation of G6PD and elevation of G6PD-derived NADPH promotes CD133+ cell growth, perhaps through upregulation of cell cycle proteins and HIF1α associated with Notch pathways. Besides HIF-Notch pathway the role of other HIF-dependent transcription factors (ETs, Oct3/4, and SOX) that activate CD133 promoter activity (24, 27, 31), in hypoxia-induced growth/self-renewal of CD133+ cells cannot be ruled out.

Consistent with the fact that CD133+ cells differentiate into the vascular endothelial and smooth muscle cell lineages (8, 53), we found that significantly more (7.5-fold) CD133+ cells expressed SM22α and α-actin when cocultured in Transwells with PASMCs. Like neural crest and CNS stem cells, which respectively differentiate into the sympathoadrenal and dopaminergic lineages under hypoxia (22, 30, 44), the numbers of CD133+ cells attaching to the membrane and expressing smooth muscle cell markers significantly increased in hypoxia compared with normoxia. Inhibition of G6PD in PASMCs by 6AN or DHEA reduced the numbers of CD133+ cells recruited to Transwell membranes as well as the numbers expressing SM22α under normoxia but not hypoxia. This likely reflects our finding that G6PD-derived NADPH supports NOX-mediated production of PASMCs ROS/H2O2 (18), which acts as a chemoattractant to recruit CD133+ cells. Collectively, then, these findings suggest that CD133+ cell expansion and differentiation are dependent on a G6PD-activated redox pathway under normoxia (Fig. 8). However, although their expansion under hypoxia is also critically dependent on a G6PD-activated redox pathway, interestingly their differentiation is mediated through a G6PD-independent pathway.

In hypoxia-induced pulmonary hypertension, circulating progenitor cells infiltrate the arteries and migrate to the adventitia or media. These progenitor cells signal to resident smooth muscle cells to dedifferentiate and proliferate, or they themselves differentiate into smooth muscle cells, leading to pulmonary arterial hypertrophy and hyperplasia (3, 13, 23, 28, 41, 48, 51). Therefore, to determine whether CD133+ contributes to pulmonary vascular remodeling, we used both moderate hypoxia-induced pulmonary hypertension and severe Sugen5416/hypoxia/normoxia-induced PAH models in which, respectively, medial thickening and occlusive neointimal lesions develop. We observed that CD133+ cell proliferation increased in a G6PD-dependent manner under hypoxia (Fig. 7), leading to the accumulation of CD133+/G6PD+ cells within the lungs of moderate and severe pulmonary hypertension rats.

Treatment with DHEA, a well-tolerated steroid hormone widely used to inhibit G6PD in vivo, reduced the numbers of CD133+ cells around the pulmonary arteries of PAH rats. DHEA treatment also reduced occlusive lesions and RV remodeling (2), which typically are seen in SU/Hx/Nx-treated rat lungs (1).

In summary, our findings indicate that G6PD, the rate-limiting enzyme in the pentose phosphate pathway, plays a fundamental role in hypoxia-induced expansion/growth and differentiation of CD133+ cells. Supporting that finding are the observations that 1) cultured cells hyperproliferate and grow in size under hypoxia; 2) hyperproliferation is mediated by over-activation of G6PD via a TGF-β- and ROS-independent pathway; 3) inhibition or knockdown of G6PD downregulates HIF1α and cell cycle promoting proteins and reduces cell number; and 4) treating PAH rats with DHEA, a G6PD blocker, inhibits pulmonary accumulation of CD133+ cells and reduces remodeling of the pulmonary arteries. We therefore propose that the development of second-generation drugs that potently and selectively block G6PD and the pentose phosphate pathway is a promising approach to the treatment of PAH.

ACKNOWLEDGMENTS

Present address for S. Chettimada: Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA. Present address for S. R. Joshi, R. Gupta, and S. A. Gupte: Department of Pharmacology and Pulmonary Hypertension Center, New York Medical College, Valhalla, NY.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

S.C., S.R.J., and A.A.Z. performed experiments; S.C. and S.A. Gebb analyzed data; S.C., S.A. Gebb, I.F.M., R.S.G., and S.A. Gupte interpreted results of experiments; S.C., S.R.J., and A.A.Z. prepared figures; S.C. and S.A. Gupte drafted manuscript; S.R.J., S.A. Gebb, I.F.M., R.S.G., and S.A. Gupte edited and revised manuscript; I.F.M., R.S.G., and S.A. Gupte conception and design of research; I.F.M., R.S.G., and S.A. Gupte approved final version of manuscript.

REFERENCES


