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Original Article

Histone demethylase JARID1B regulates proliferation and migration of pulmonary arterial smooth muscle cells in mice with chronic hypoxia-induced pulmonary hypertension via nuclear factor-kappa B (NFkB)

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ABSTRACT

Chronic hypoxia-induced pulmonary hypertension (PH) is a disorder that is characterized by increased pulmonary arterial pressure resulting from lung diseases or shortage of oxygen in the body. Excess proliferation of pulmonary vascular cells such as pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs) plays a critical role in the pathogenesis of PH. Recent evidence indicates that, in addition to genetic predisposition and environmental factors, epigenetic mechanisms play a pivotal role in etiology of PH. In this study, we investigated the possible role played by jumonji AT-rich interactive domain 1B (JARID1B), a histone demethylase, in regulating the proliferation of vascular smooth muscle cells in chronic hypoxia-induced PH condition. Quantitative polymerase chain reaction analysis of samples from rats with PH showed an elevated expression of JARID1B in their PASMCs, positively correlating with increased nuclear factor-kappa B (NFkB) expression. Further functional studies in vitro indicated that overexpression of JARID1B increased the proliferation and migration of PASMCs, which were inhibited by depletion of NFkB. Genomewide transcriptional analysis revealed that the JARID1B regulated NFkB signaling pathway by directly binding to its promoter. We have also shown that JARID1B indirectly regulates the expression of vascular endothelial growth factor via NFkB signaling and hence may also play a crucial role in controlling PAECs, leading to changes in vascular architecture in PH. Our findings could lead to further studies on the role of JARID1B in PH etiology and therefore could lead to a potential therapeutic target for chronic hypoxia induced pulmonary hypertension.

1. Introduction

Chronic hypoxia-induced pulmonary hypertension (PH) is a disease of the pulmonary vasculature which ultimately leads to right ventricular failure and death [1–3]. Risk factors that cause include chronic obstructive pulmonary disease (COPD), sleep apnea, living at high altitudes, lung developmental abnormalities, etc. [4,5]. PH, in general, is defined by a resting mean pulmonary artery pressure (PAP) >25 mmHg and pulmonary vascular resistance (PVR) >3 [1,6]. The clinical manifestations of PH result from physical narrowing of the pulmonary arteries, driven by various complications such as excess vasoconstriction, thrombosis, intimal fibrosis, inflammation, and obstructive remodeling of the pulmonary artery wall [7]. Similar to other arteries, the pulmonary artery wall comprises of an external layer of connective tissue, extracellular matrix (ECM), nerve cells, and fibroblasts, and a secondary layer consisting of pulmonary artery vascular smooth muscle cells (PASMCs) which encloses a single layer of endothelial cells (ECs), while numerous elastic lamina are interspersed throughout the media of the vessel wall [8]. The ECs which are in contact with circulating blood communicate with SMCs to maintain homeostasis within the pulmonary circulation. The SMCs play a crucial role in maintaining the structural integrity and regulating the vascular tone and vessel diameter in response to stimuli [8–10]. Excess cellular proliferation and apoptosis resistance of PASMCs lead to hypertrophy, ECM, and collagen deposition, resulting in an increase in the external diameter of the pulmonary arteries leading to vascular remodeling [11]. Consequently, PVR increases dramatically, which requires right ventricular compensation leading to progressive elevation of PAP, right ventricular stress, and ultimately right-sided heart failure and death [11,12].

Over the past three decades, many mediators and signaling pathways, along with their downstream effectors, involved in PH pathobiology have been identified, leading to the development of targeted therapies and improved patient outcomes in many ways [13]. Yet, the

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pathogenic pathways that drive regulation of pulmonary vascular activity, endothelial function, PASMC proliferation, vascular inflammation, or thrombosis are still not completely understood at a molecular level.

Epigenetic processes play crucial role in the regulation of normal development, differentiation, and proliferation of distinct cell lineages by modifying chromatin structure and cell-type-specific gene expression [14]. Mounting evidence indicates that various epigenetic processes are largely involved in the regulation of PH initiation, progression, and establishment by affecting differentiation, proliferation, and migration of PASMCs. Recent findings such as variations in DNA methylation levels at superoxide dismutase 2 [15] and granulysin gene loci [16], anomalous expression of histone deacetylases [17] and bromodomain-containing protein 4 [18], reduction in nuclear histone H1 levels [19], and dysregulated network of microRNAs [20] have been shown to be involved in PH pathogenesis.

Histone methylation is an important epigenetic mechanism that regulates chromatin dynamics and transcriptional activity of genes [21]. Methylation of histones on specific lysine/arginine residues by enzymes such as histone methyl transferases (HMTs) controls gene expression by making promoter region of the gene accessible or inaccessible for transcription factors to bind. Aberrant histone methylation has been reported to play a role in multiple human malignancies and other diseases [21]. It was shown that increased levels of enhancer of zeste homolog 2, an HMT, in mice led to apoptotic resistance, as well as increased proliferation and migration of PASMCs in PH [22].

Like many other epigenetic mechanisms, histone lysine methylation is also reversible. A family of histone demethylases known as KDM5/JARID1 can effectively remove the di- and trimethyl marks from the lysine on histone H3 [23]. Jumonji AT-rich interactive domain 1B (JARID1B), also known as KDM5B or PLU1, is a member of KDM5 family and is known to play a significant role in repression of gene transcription at various promoters of many genes related to cell cycle, proliferation, differentiation, stem cell renewal, and other developmental processes [24]. JARID1B is present at low levels in adult tissues and is predominantly expressed in the testis and brain. Recent studies showed that elevated expression of JARID1B promotes tumor initiation, invasion, and metastasis in multiple cancers such as breast, lung, prostate, bladder, and other tumors [24,25]. JARID1B has been shown to be involved in the regulation of genes associated with G2/M checkpoint, late M phase, spindle assembly, chromosomal condensation, and transition through the late stages of mitosis [26].

Despite the advances in epigenetics technology and established role of JARID1B in cell proliferation, there are no studies so far on the role of JARID1B in the regulation of PASMC proliferation or PH pathogenesis when exposed to hypoxia. In this study, we analyzed the role of elevated JARID1B expression on the proliferation and migration features of PASMCs, which are necessary for vascular regeneration after injury induced by chronic hypoxia.

2. Materials and methods

2.1. Animal model of hypoxia-induced PH

All experimental protocols described in mice were reviewed and approved by the Animal Care and Use Committee at the Harbin Medical University. Mice were cared for in accordance with the Harbin Medical University Guide for the Care and Use of Laboratory Animals. The mice model of chronic hypoxia induced PH was established as previously described by Yang et al. [27]. Briefly, newborn C57BL/6 mice pups (16 mice/group) were exposed to normobaric hypoxia (11% oxygen) shortly after birth for 2 weeks. Control pups were raised in normoxia condition. Eight mice were sacrificed after the completion of exposure to normoxia or hypoxia at postnatal day 14. The remaining 8 mice were moved to regular room air (21%) for another 4 weeks.

2.2. Measurement of systolic right ventricular pressures (RVPs)

Six-week-old C57BL/6 mice exposed to either normoxia or normobaric hypoxia conditions were anesthetized with isoflurane. RVP pressure was measured as described previously [27]. The heart was exposed, and a Scisense pressure transducer (Transonic Systems, Ithaca, NY, USA) was introduced apically into the right ventricle. The transducer was advanced to just outside the pulmonary outflow tract for the assessment of RVP.

2.3. Measurement of ventricular weights

C57BL/6 mice were weighed and then sacrificed. The right ventricle (RV) was dissected from the left ventricle and interventricular septum (LV + S), and the ratios of their weights [RV/(LV + S)] (Fulton's index) and [RV/body weight] were calculated as indices of right heart hypertrophy. Values are represented as mean ± S.E.M.

2.4. Isolation and culture of PASMC cells

Mice PASMCs were isolated following an optimization of the protocol described by Jia et al. [28]. Mice were sacrificed by cervical dislocation methods, and the pulmonary artery was biopsied. The fibrous tunica externa and the tunica intima were removed, and the smooth muscle tissues of the vascular middle layer of the artery were then extracted. The tissue was cut into 1-mm^3 blocks and seeded into the culture flasks for 2 h at 37°C and 5% CO_2. Following 2 h of adherent conditioning, M199 medium containing 10% fetal bovine serum was added, and the blocks are cultured for another week at 37°C and 5% CO_2. When the cells grown out of the tissue blocks covered 70% of the area of the flask, they were passaged. The third-generation cells were the confirmed for the expression α-smooth muscle actin using immunohistochemistry and identified as pure PASMCs and subjected to further experiments. For hypoxia culture, PASMCs were cultured in 1% oxygen for 24 h.

2.5. Gene expression

For gene overexpression, the PASMC cell lines were cultured overnight in six-well plates (1.0×10^6 cells/well) followed by transfection with 10 μg/ml plasmid pcDNA-JARID1B (Genepharma, Shanghai, China) or empty plasmid using 5 μl of Lipofectamine 2000 reagent (Invitrogen, USA). The cells were incubated for 24 h, and positive clones were isolated by G418 (Sigma, USA) selection using 600 μg/ml G418. JARID1B shRNA (cat. 150,247) and NFkB plasmid (cat. 160,235) were purchased from Genepharma (Shanghai, China).

2.6. Protein extraction, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and Western blot

Total cellular proteins were extracted using radioimmunoprecipitation analysis lysis buffer. Equal amounts of proteins were separated by SDS-PAGE and then transferred onto nitrocellulose membranes. The membranes were then blocked with 5% nonfat milk for 1 h followed by incubation with primary antibodies at 4°C overnight. Antibodies for JARID1B, NFκB, and vascular endothelial growth factor (VEGF)-A were purchased from Abcam, USA. The antibody against β-actin (Santa Cruz Biotechnology, CA, USA) was used as a loading control. Subsequently, the blots were washed and probed with horseradish-peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, CA, USA) for 2 h, and complexes were detected by enhanced chemiluminescence (PerkinElmer Life Sciences).