Leptin knockout attenuates hypoxia-induced pulmonary arterial hypertension by inhibiting proliferation of pulmonary arterial smooth muscle cells

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Pulmonary arterial hypertension (PAH) is a fatal disease characterized by excessive vascular smooth muscle cells proliferation in small pulmonary arteries, leading to elevation of pulmonary vascular resistance with consequent right ventricular (RV) failure and death. Recently, emerging evidence has shown that leptin signaling is involved in different cardiac pathologies; however, the role of leptin remains limited in the setting of PAH. Thus, in this study, we tested the hypothesis of direct involvement of leptin in the development of PAH. Our data show that leptin activity in plasma and protein level in the lung were higher in hypoxia- and monocrotaline-induced PAH models compared with control animals. Wild-type (WT) and C57BL/6J-Lep^{ob} (*ob/ob*) male mice were exposed to normobaric hypoxia (10% O_2) or normoxia (21% O_2). After 2 and 4 weeks of chronic hypoxia exposure, WT mice developed PAH as reflected by the increased values of RV systolic pressure, RV hypertrophy index, the medial wall thickness of pulmonary arterioles, and muscularization of pulmonary arterioles. And, all these alterations were attenuated in *ob/ob* mice treated with hypoxia. Leptin could stimulate the proliferation of pulmonary arterial smooth muscle cells (PASMCs) by activating extracellular signal–regulated kinase (ERK), signal transducer and activator of transcription 3 (STAT3), and Akt pathways. These data suggest that the leptin signaling pathway is crucial for the development of PAH. Leptin activates ERK, STAT, and Akt pathways and subsequently PASMCs proliferation, providing new mechanistic information about hypoxia-induced PAH. (Translational Research 2015;166:772–782)

Abbreviations: PAH = pulmonary arterial hypertension; PASMC = pulmonary arterial smooth muscle cells; MCT = monocrotaline; RVSP = right ventricular systolic pressure; RVHI = right ventricular hypertrophy index; SMCM = smooth muscle cell medium; FBS = fetal bovine serum; CFSE = 5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; α-SMA = α-smooth muscle actin; vWF = von Willebrand factor

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a devastating, life-threatening disorder characterized by elevated pulmonary vascular resistance and pulmonary arterial pressure with increased muscularization of small arteries, thickening or fibrosis of the intima, and the presence of plexiform lesions.[1,2] The specific mechanisms involved in the development of PAH remain unknown. Abnormal proliferation and phenotypic switching of pulmonary arterial smooth

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AT A GLANCE COMMENTARY

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Background

Leptin, a 16-kDa nonglycosylated protein encoded by the obese (*ob*) gene, has been implicated in the regulation of different cardiac pathologies; however, the role of leptin remains limited in the setting of pulmonary arterial hypertension (PAH).

Translational Significance

In this article, we demonstrated that leptin knockout attenuates hypoxia-induced PAH through inhibition the proliferation of pulmonary arterial smooth muscle cells. Thus, pharmacologic inhibition of extracellular signal–regulated kinase, signal transducer and activator of transcription 3, and Akt pathways involved in leptin signaling pathways may be beneficial for the prevention and therapeutic treatment of PAH.

muscle cells (PASMCs) from a contractile, differentiated phenotype to a synthetic, undifferentiated phenotype are major events in this disease.[3-5] Therapies for PAH include prostacyclins, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors, which fail to fully reverse this disease. The mortality remains unacceptably high, and survival for incident patients continues to be as low as 54.9% 3 years after diagnosis.[6] Novel approaches targeting the proliferation of PASMCs are urgently needed for the treatment of PAH.

Leptin is a 16-kDa nonglycosylated protein encoded by the obese (*ob*) gene and mainly synthesized and secreted by adipocytes.[7] In addition, recent study suggested that bronchial epithelial cells, type II pneumocytes, and alveolar macrophages are significant sources of pulmonary leptin.[8] Leptin exerts its biological actions through the activation of membrane receptors which exist in at least 6 isoforms, *Ob*-Ra through *Ob*-Rf.[9] As an anorexic peptide, leptin is primarily known for its role as a hypothalamic modulator of food intake, body weight, and fat stores. Recently, emerging evidence has shown that leptin signaling is involved in the promotion of atherosclerosis, including endothelial dysfunction, platelet aggregation, vascular smooth muscle cells proliferation, and inflammatory vascular response.[10–15] As well, clinical studies have suggested that pulmonary endothelial cells from patients with idiopathic PAH can produce leptin, which may participate in the development of PAH.[16,17] These reports advocate the critical role of leptin in the progression or setting of PAH, but the underlying mechanism has not been fully understood. Based on this background, we hypothesized that leptin is a critical determinant during the development of pulmonary hypertension by regulating PASMCs proliferation. To test this hypothesis, we (1) determined any changes in leptin level in PAH animal models, including hypoxia- and monocrotaline (MCT)-induced PAH; (2) knocked out the leptin gene to evaluate the effect of leptin in the development of hypoxia-induced PAH; (3) investigated whether the effect of leptin on PAH affects the proliferation of PASMCs; and (4) elucidated the possible signaling pathways involved in the effect of leptin on PASMCs.

MATERIALS AND METHODS

Materials. MCT was purchased from Sigma-Aldrich (St. Louis, Mo). Recombinant rat leptin was purchased from PeproTech. Smooth muscle cell medium (SMCM), penicillin/streptomycin, fetal bovine serum (FBS), and smooth muscle cell growth supplement were purchased from ScienCell Research Laboratories (Carlsbad, Calif). Radioimmunoprecipitation assay buffer was purchased from Applygen Technologies (Beijing, China). Protease and phosphatase inhibitors were purchased from Roche Applied Science (Basel, Switzerland). IRDye800-conjugated goat antirabbit immunoglobulin G secondary antibody was purchased from Odyssey LI-COR (Lincoln, Neb). U0126 was from Cell Signaling Technology (Beverly, Mass). Ruxolitinib (INCB018424) was purchased from Selleck Chemicals (Houston, Tex). LY294002, 5(6)-Carboxyfluorescein N-hydroxysuccinimidyl ester (CFSE) stock solution, and von Willebrand factor (vWF) were purchased from Abcam (Cambridge, United Kingdom). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from AMRESCO (Solon, Ohio). Antibody for Ob was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif). Dimethyl sulfoxide, anti–α-smooth muscle actin (anti–α-SMA) antibody, and anti–β-actin antibody were purchased from Sigma-Aldrich (St. Louis, MO). Antibodies for phosphorylated extracellular signal–regulated kinase (pERK-Thr202/Tyr204), anti-ERK, phosphorylated signal transducer and activator of transcription 3 (pSTAT3-Tyr705), anti-STAT3, phosphorylated AKT1 (pAKT-Ser473), and anti-AKT were purchased from Cell Signaling Technology. Mounting medium with 4′,6-diamidino-2-phenylindole, tetramethylrhodamine isothiocyanate–conjugated goat antirabbit, and fluorescein isothiocyanate–conjugated goat antimouse secondary antibodies were purchased

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