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Research Article

Galectin-3 mediates the pulmonary arterial hypertension–induced right ventricular remodeling through interacting with NADPH oxidase 4

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Abstract

Pulmonary arterial hypertension (PAH) is a progressive disorder that affects both pulmonary vasculature and the heart. The response of the right ventricle (RV) to the increased afterload is an important determinant of the PAH final outcome. Galectin-3 (Gal-3), a novel biomarker in left cardiac remodeling, takes part in multiple pathophysiological processes including the inflammation, fibrosis, immunity, and oxidative stress. The levels of Gal-3 are elevated in PAH patients, although the exact mechanisms underlie the PAH-induced right ventricular structural changes remain unclear. Our results showed that the serum Gal-3 and NADPH oxidase 4 (Nox4) levels were significantly elevated and correlated in 26 human PAH patients when compared with 14 age- and sex-matched healthy controls. In the monocrotaline-induced PAH rat models of right ventricular hypertrophy and fibrosis, the Gal-3 and Nox4 expressions were both significantly upregulated compared with the controls. Moreover, the Gal-3 positive areas were co-localized with the collagen III-specific staining and the Gal-3 and Nox4 were partly co-localized in the intercellular area. The exogenous Gal-3 recombinant protein stimulated the proliferation, differentiation, collagen deposition, and Nox4 expression of cardiac fibroblasts. These simulations were blocked by the Gal-3 knockdown. The profibrotic effects of transforming growth factor-β1 (TGF-β1) on the cardiac fibroblasts were partially mediated by the Gal-3. Subsequently, our results showed that Gal-3 mediated the TGF-β1–induced cardiac fibrotic process through interacting with the Nox4 and Nox4-derived oxidative stress. Therefore, Gal-3 plays an important role in the PAH-induced right ventricular remodeling through interacting with the Nox4 and Nox4-derived oxidative stress. Gal-3 may become a RV-specific diagnostic and therapeutic target for clinics. J Am Soc Hypertens 2017;■(■):1–15.

Keywords: Galectin-3; NADPH oxidase 4; oxidative stress; right ventricular remodeling.

Introduction

Pulmonary arterial hypertension (PAH) is a deadly disease with a progressively increased pulmonary vascular resistance caused by both vasoconstriction and vascular remodeling. The right ventricular adaptation to chronic pressure overload associating with PAH is a complex and multifaceted response.[1,2] The survival rate of PAH patients is crucially dependent on the ability of the right ventricle (RV) to cope with the increased afterload.[3,4] However, the cellular and molecular mechanisms that underlie right ventricular remodeling during the course of PAH were less well-characterized.

Galectin-3 (Gal-3) is a soluble β-galactoside–binding animal lectin, which appears to be mainly expressed and

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secreted by macrophages, fibroblasts, mast cells, and neutrophils.[5,6] Gal-3 could also cause superoxide to be released from monocytes[7] and mast cells.[8] Gal-3 takes part in multiple pathophysiological processes including the inflammation, fibrosis, immunity,[9–11] and oxidative stress.[12] Gal-3 is recognized as a promising new biomarker which has been proved to be effective for diagnosing and evaluating the prognosis of the left heart failure.[13–15] The level of Gal-3 was elevated in both idiopathic PAH and connective tissue diseases related to PAH patients[16]; however, the role of Gal-3 in the PAH-induced right ventricular morphologic changes remains less known.

The inflammation, fibrosis, and oxidative stress all contribute to the development of the right ventricular remodeling and even heart failure in PAH.[2,17,18] Additional evidence of oxidative stress affecting the cardiac remodeling process has been showed in human heart failure[19] and various animal models of cardiac hypertrophy (induced by angiotensin II,[20] aldosterone,[21] myocardial infarction,[22] and aortic coarctation[23]). Among the potential sources of ROS, NADPH oxidase 4 (Nox4) is thought to be the major enzyme to produce superoxide (O2•−) and hydrogen peroxide (H_2O_2) in the heart.[24] It has been found that the expressions and activities of Nox4 were upregulated in various models of cardiac hypertrophy.[25] A recent study showed that Nox4 was prominently expressed in the adventitia of pulmonary arteries that contributed to the altered fibroblast behavior, the hypertensive vascular remodeling, and the development of PAH.[26] Therefore, we raise the hypothesis that Gal-3 mediates PAH-induced right ventricular remodeling by interacting with the Nox4 and Nox4-dependent superoxide productions.

The present study was designed to investigate the role and the underlying mechanisms of Gal-3 during the fibrotic process of the PAH-induced right ventricular remodeling in the monocrotaline (MCT) induced-PAH rats and isolated neonate rat primary cardiac fibroblasts.

Materials and Methods

Animals and Hemodynamic Evaluation

All animal procedures were approved by the Institutional Animal Care and Use Committee. Adult male Sprague–Dawley (SD; 250–300g, $n = 4$) rats were induced by a single intraperitoneal injection of MCT (60/mg/kg), which produced a progressive and severe pulmonary arterial hypertension after 3 weeks of MCT exposure.[27] Adult age-matched male SD (250–300 g, $n = 4$) rats were used as the controls. Rats were anesthetized and measured for right ventricular systolic pressure (RVSP) with pressure transducers. The hearts and lungs were flushed with normal saline and removed immediately after euthanasia. The heart was removed and the weight of heart was recorded, the free wall of the RV, left ventricle (LV), and septum (S) were carefully dissected and weighed respectively to calculate the RV/LV + S ratio (Fulton index) as an index of right ventricular hypertrophy. A half portion of the RV muscle from each rat was dissected and frozen in liquid nitrogen for preparation of homogenates and further Western blotting analysis. The remaining half portion of the right ventricular muscle was fixed in 4% paraformaldehyde solution for the following histology staining.

Histology Staining

The right lungs and a half portion of right ventricular muscle were removed and fixed in situ in the distended state by infusion of 4% paraformaldehyde solution for 24 hours and then were placed in 0.2% sodium azide solution before being embedded in paraffin. The right lung and right ventricular muscle were dehydrated and embedded in paraffin and sliced into 5-μm thick sections. Lung sections were stained with hematoxylin and eosin, and morphometric analyses were performed in pulmonary arteries with an external diameter of 50–100 mm. The medial wall thickness was calculated by the following formula: medial thickness (%) = medial wall thickness/external diameter × 100. For quantitative analyses, 20 vessels from each rat were counted, and the average was calculated.[28] The right ventricular sections were stained with hematoxylin and eosin and Masson's trichrome staining as per manufacturer's instructions. The quantification of medial thickness of pulmonary artery, cardiomyocytes cross-sectional areas, and fibrotic areas were measured using ImageJ. The Gal-3 and Nox4 immunohistochemistry were performed on the right ventricular muscle with the following primary antibodies used: rabbit anti-galectin-3 antibody (1:150, Proteintech, US, Cat. No. 14,979-1-AP) and rabbit anti-Nox4 antibody (1:200, Novus, US, Cat. No. NB110-58849). The right ventricular sections were treated with the Gal-3/Nox4 co-staining and the Gal-3/Collagen III co-staining as per manufacturer's instructions. The staining were performed with the following primary antibodies used: mouse anti-galectin-3 antibody (1:100; Abcam, UK, Cat. No.AB2785), rabbit anti-Nox4 antibody (1:100; Novus, USA, Cat. No. NB110-58849), and rabbit anti-collagen III (1:000; Abcam, UK, Cat. No. AB7778). The secondary antibodies were used as follow: CY3-conjugated goat anti-rabbit IgG (1:300; Servicebio, China, Cat. No. GB21303) and Alexa Fluor 488-labeled goat anti-mouse IgG (1:400; Servicebio, China, Cat. No. GB25301).

Cell Culture and Treatments

Rat primary cardiac fibroblasts were isolated from the neonate male SD rats (1–3 days), as described previously.[29] Cells were cultured in high-glucose Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 20% fetal bovine serum, along with 50 U/mL penicillin and