Abstract

**Rationale:** Pulmonary Arterial Hypertension (PAH) is a progressive, debilitating, and eventually lethal disease that is resistant to current therapeutics. A defining characteristic of PAH is the excessive cellular proliferation and remodeling of pulmonary arteries (PA) that results in increased vascular resistance and stiffness, and eventually failure of the right ventricle and death. Ablent vascular remodeling in PAH originates from diverse, poorly characterized pathways in the intima, media, and adventitia and involves musculuarization of intima and adventitia, and adipogenesis and inflammation. A microscopic analysis of isolated pulmonary arteries (PA) revealed Galectin-3 (Gal-3) upregulation in isolated PA which was confirmed by qRT-PCR and WB. Gal-3 is a β-galactoside binding lectin that is implicated in multiple signaling pathways regulating cell proliferation, apoptosis, inflammation and fibrosis. However, the therapeutic utility of targeting cell-mediated Gal-3 in PAH and the mechanisms by which it influences pulmonary vascular remodeling are not yet known. **Methods:** We found that Gal-3 is robustly upregulated in PA from multiple models of PAH including monocrotaline (MCT), TGF-β, and Sugen/Hypoxia rats, and Sugen/Hypoxia rats and correlates with disease severity. In animals and humans with PAH robust induction of Gal-3 was observed in the media where it overlaps with smooth muscle markers. To establish the functional relevance of Gal-3 in PAH, we have obtained selective inhibitors of Gal-3 and show that they attenuate and reverse PA remodeling and functional indices of PAH in MCT-treated rats in vivo. In cultured human pulmonary arterial smooth muscle cells (HPASMC), increased Gal-3 expression promotes proliferation and resistance to apoptosis whereas silencing Gal-3 reduces proliferation and collagen expression (fibrosis). **Conclusions:** Based on these findings, we hypothesize that Gal-3 is a central regulator of proliferation and fibrosis in PA and contributes to pathologic vascular remodeling in PAH.

Introduction

Pulmonary Arterial Hypertension (PAH) is a disease that results in a progressive narrowing of the pulmonary arteries leading to increased vascular resistance and the elevation of pulmonary blood pressure that ultimately leads to the right ventricle failure. It is currently thought that the primary cause of the permanent elevated pulmonary vascular resistance that occurs in PAH is due to physical obstruction from the progressive accumulation of a number of vascular events that are responsible for the treatment of PAH, but none of the current therapeutics offers long-term success for survival. Galectin-3 (Gal-3), the lectin family of proteins which recognize specific carbohydrate motifs on glycosylated proteins. A pathologic role for Gal-3 is observed in cancer and inflammatory and fibroproliferative disorders such as pulmonary, cardiac and hepatic fibrosis. Although Gal-3 blood levels are elevated in human PAH, it is not yet known if Gal-3 contributes to the pathogenesis of PAH, and which cell types express Gal-3 in the vasculature. Vascular smooth muscle proliferation, especially in the smaller pulmonary arteries, is a prominent feature of PAH, and many growth factors that play a central role in the hyper-proliferation of other diseases such as fibrosis and cancer are also increased in PAH. Further, Gal-3 is a potent regulator of cellular proliferation, migration, and resistance to apoptosis. In light of these previous findings, we hypothesized that Gal-3 is a potential stimulator of pulmonary arterial smooth muscle proliferation in experimental models of PAH.

**Results**

**Figure 1.** Gal-3 expression is increased in hypertensive pulmonary arteries (PA). (A) Western blot analysis of Gal-3 expression in PA from MCT-treated rats. (B) qRT-PCR analysis of Gal-3 mRNA normalized to GAPDH in control and MCT-treated rats. (C) Western blot analysis of Gal-3 expression in PA from MCT-treated (4 wks) rats.

**Figure 2.** WB of Gal-3 expression in PA from SUGEN/HYP treated rats. Figure 3. Severity of PAH (Fulton Index) correlates with Gal-3 expression in MCT-treated (4 wks), and MCT+Pn (pneumaticoctomy; Pn) rats, p<0.05 n = 5 for each group.

**Figure 3.** Silencing Gal-3 decreases collagen 1 in Human (B) PASMNC (B) Gal-3 inhibitors decrease collagen expression and proliferation in 4 wk-MCT treated lung explants. Figure 4. Cultured PASMNC from MCT-treated rats exhibit increased proliferation and Gal-3 expression. *Significantly different from Control, p<0.05 (n=5 for each group)

**Figure 5.** Gal-3 inhibition with GM-CT-01 and GR-MD-02 reduces PAH-induced right ventricle hypertrophy and pulmonary artery remodeling in MCT-treated rats. (A) RVSP, (B) Fulton Index. (C) total blood flow (Velocity Time Integral; VTI); (D) ultrasound scan (representative) in 4 wk-treatment with MCT and RV vitamin over time to MCT using Pulsed-Wave Doppler (Vevo 2100). (E) RVSP; (F) Fulton Index. For reversal, rats were given vehicle or GM inhibitor at week 4 and sacrificed at week 6. *Significantly different versus control, p < 0.05 (n = 5-6 per group).

**Methods**

**Experimental protocol:**
- Control group (Sprague-Dawley rats)
- Single dose of MCT (60mg/kg IP) in Sprague-Dawley rats
- MCT and Gal-3 inhibitors (GM-CT-01 or GR-MD-02; 60mg/kg i.v.) given 2x weekly for 4 weeks at the start of single MCT injection in Sprague-Dawley rats (inhibition protocol) or post-3wks MCT treatment (reversal protocol)
- Left pneumonectomy (3 wks) followed by MCT treatment (4 weeks)
- Sugen 5416 (20mg/kg SQ) Hypoxia (10% O2; 3 wks) treated rats followed by normoxia (21% O2) for 10 weeks
- Intramyocardial blood vessels, fibrosis, and inflammation using Hematoxylin and Eosin (H & E) or Trichrome staining
- In situ pulmonary arterial vessel immunofluorescence using alpha-actin and Gal-3 antibodies
- In vivo cardiopulmonary hemodynamic measurements using the Vevo 2100 imaging ultrasound system and PowerLab data acquisition system
- Measurement of RV/LV + S ratio (Fulton Index) to assess right ventricular hypertrophy as a marker for PAH
- Western Blot (WB) and qRT-PCR analysis

**Conclusions**

The results of this study demonstrate that experimental rat models of PAH have significantly increased RVSP, right ventricular hypertrophy, and pulmonary arterial smooth muscle proliferation as well as increased right ventricular wall thickness. Lower pulmonary blood flow. Further, in rats and humans with PAH robust induction of Gal-3 expression was observed in the medial layer. The alterations in cardiopulmonary function and vascular proliferation as well as fibrosis were significantly reduced by Gal-3 inhibitors treatment with specific Gal-3 inhibitors. These findings indicate that Gal-3 signaling contributes to the compromised pulmonary vascular function and prominent pulmonary arterial remodeling that occurs in PAH, which suggests that Galectin-3 is a hypotensive and viable target for treatment of PAH and other related pulmonary vascular diseases.

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**Figures 6, 7, 8**

**Figure 6.** Gal-3 expression is abundant in the medial layer of rat and human pulmonary arteries in experimental and human PAH. Confocal images of lung sections from control, 4-wk treated MCT rats, and human PAH (patients with IPAH undergoing lung transplant). Sections were stained with Gal-3 and alpha-actin antibodies.

**Figure 7.** (A) Silencing Gal-3 decreases collagen 1 in Human (B) PASMNC (B) Gal-3 inhibitors decrease collagen expression and proliferation in 4 wk-MCT treated lung explants. Figure 8. Cultured PASMNC from MCT-treated rats exhibit increased proliferation and Gal-3 expression. *Significantly different from Control, p<0.05 (n=5 for each group)