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RESEARCH ARTICLE

Galectin-3 Expression Promotes Pulmonary Hypertension Through Multiple Mechanisms

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ABSTRACT
Pulmonary Hypertension is a progressive vascular disease resulting from the tapering of pulmonary arteries causing high pulmonary arterial blood pressure and ultimately right ventricular failure. A defining characteristic of Pulmonary Hypertension is the excessive remodeling of pulmonary arteries that includes increased proliferation, vascular fibrosis and inflammation. There is no outward cure for Pulmonary Hypertension nor are there interventions that effectively impede or reverse pulmonary arterial remodeling, and pulmonary vascular research over the past several decades has sought to identify novel molecular mechanisms to target for therapeutic benefit. Galectin-3 is a carbohydrate binding lectin that is unique for its chimeric structure, comprised of an N-terminal oligomerization domain and a C-terminal carbohydrate-recognition domain. Galectin-3 is a regulator of modifications in cell behavior that contribute to aberrant pulmonary arterial remodeling including cell proliferation, inflammation, and fibrosis, but its role in Pulmonary Hypertension is poorly understood. In this review, we define Galectin-3 and summarize specific topics regarding the role of Galectin-3 expression in the development of Pulmonary Hypertension by providing evidence which supports the ability of Galectin-3 to influence reactive oxygen species production, NADPH enzyme expression, vascular inflammation and vascular fibrosis, all phenomena which contribute to pulmonary arterial remodeling and the development of Pulmonary Hypertension.

INTRODUCTION

Pulmonary Hypertension

Pulmonary Hypertension (PH) is a pathophysiological condition of the lung vasculature that is functionally characterized by a sustained elevation of pulmonary arterial pressure[1], defined as a mean pulmonary artery pressure at rest $\geq$ 20mmHg[2]. Continued progression of this disease increases pulmonary vascular resistance initially causing compensatory right ventricular (RV) hypertrophy[1,3], but eventually leading to RV failure. Medial wall cellular proliferation of pulmonary arteries (PA) is a hallmark feature of PH[4], which eventually elicits vessel luminal occlusion[5]. In PH, muscularization of small distal PA occurs[6], further characterized by excessive arterial proliferation, fibrosis, and inflammation, causing medial remodeling, and loss of vascular compliance [5,7-9]. Increased resistance to perfusion via loss of PA compliance contributes to the failure of the right ventricle (RV)[10,11], and the response of the RV by the increased afterload due to PH increases cardiac hypertrophy, end-diastolic volume, alters contractile function, subsequently leading to muscle dilation, cardiac fibrosis and eventual ventricular decompensation[12]. Ultimately, increased RV volume (diastolic and systolic) combined with increased intraluminal cardiac pressure leads to an unsustainable increase in wall stress that culminates in right heart failure and ultimately death[13-15].

In PH, within the vascular wall, endothelial cells become dysfunctional and vascular smooth muscle cells undergo a phenotypic switch from a contractile phenotype to a 'synthetic' quiescent phenotype that is characterized by a decrease in contractile smooth muscle genes and proteases as well as increased cellular proliferation[16,17]. In addition, there is an increase in pro- inflammatory and pro-fibrotic molecules, which promotes vascular fibrosis, inflammation and the deposition of extracellular matrix[18-22].

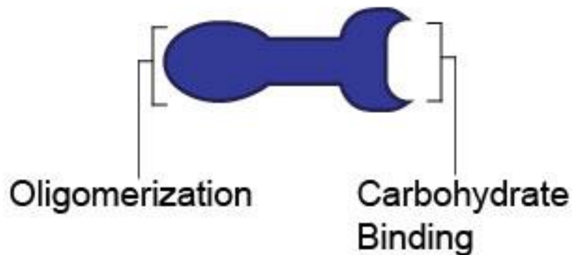
In this review, we attempt to discuss what is currently known about the role of Galectin-3 (Gal-3) expression as a major contributor to PH. Specifically, this review will focus on the role of Gal-3 on Reactive Oxygen Species (ROS) signaling, cellular inflammation, and vascular fibrosis in the development of PH.

Galectin-3: A Unique Lectin

Galectin-3 (Gal-3; *LGALS3*, Mac-2) is a member of the lectin family of proteins, which recognize and bind to specific carbohydrate motifs on glycosylated proteins and lipids [23]. Gal-3, first identified in the 3T3 mouse fibroblast cell line[24], is robustly expressed in the lung[25], and changes in Gal-3 mRNA expression in fibroblasts has been observed in response to growth factors[26]. Gal-3 is present in both the cell cytoplasm and cell nucleus, with higher protein expression in the nucleus of proliferating cells[27], which appears to be age-dependent with robust expression induced by growth factors in juvenile cells, diminishing in matured cells and those with replicative senescence[28]. Approximately 30 years ago, the macrophage surface antigen, Mac-2 was determined to be identical to Gal-3 and observed to be expressed in high concentrations by specific subpopulations of pro-inflammatory macrophages and secreted into the extracellular space[29,30]. As the name for the moiety denotes, Mac-2 expression was extensively used to identify macrophages[31]. It is now known that Gal-3 expression is also expressed in fibroblasts (where it was originally discovered), smooth muscle cells[32], endothelial cells[33], activated T cells[34] epithelial cells [35,36] and different varieties of tumor cells[37].

Gal-3 belongs to a family of 16 (related) members that all share an evolutionarily conserved carbohydrate recognition domain (CRD) that can bind β-galactosides and lactose but differ in their ability to bind more complex saccharides. Gal-3 'family' members can be broadly classified into three types: the prototypes which contain one CRD and are monomers or homodimers (includes galectins- 1, 2, 5, 7, 10, 11, 13, 14, 15, and 16), the chimeras (Gal-3 is the only member) which contain one CRD and a self-association domain, and the tandem-repeat galectins (galectin- 4, 6, 8, 9, and 12), which have two CRDs connected by a linker peptide. As the only chimeric galectin, Gal-3 is comprised of a C-terminal CRD that is present in all members of the galectin family but has a unique N-terminal domain that include glycine and proline-rich domains that enable Gal-3 to oligomerize with other Gal-3 molecules (**Figure 1A**) or to engage in protein-protein interactions with other proteins. Gal-3 is initially expressed as a monomer but can self-assemble into dimers and higher order structures in response to diverse stimuli. Cysteine 173 (previously referred to as cysteine 186) is a critical residue that enables disulfide bonds to link between homodimers[38]. Carbohydrate binding to the C-terminal CRD of Gal-3 triggers a structural change in the N-terminus to enable oligomerization into pentamers (**Figure 1B**)[39,40], and specific monoclonal antibodies targeting the N-terminus of Gal-3 facilitate the multimerization of Gal-3. Further, the C-terminal CRD can initiate self-assembly within the CRD[40,41], which can modify the

N-terminal domain and thus impact oligomerization and substrate binding[42]. Tissue transglutaminase can also directly promote Gal-3 oligomerization, which may increase and stabilize interactions with substrates[43,44].



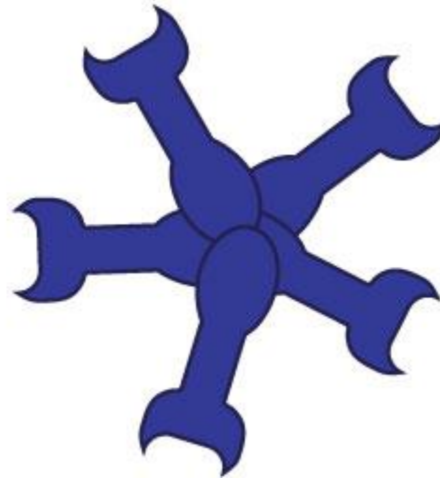


Figure 1. Schematic illustration of Gal-3 monomer (A) and oligomer (B). Gal-3 is understood to be initially expressed as a monomer that assembles into a larger multimer in response to carbohydrate binding and other post-translational modifications. Reprinted with copyright permission from *Antioxidants and Redox Signaling*, Volume 31, Issue 14, Mary Ann Liebert, Inc., New Rochelle, NY (Publisher).

As previously stated, Gal-3 is found in the cell cytosol, nucleus, and extracellular space, but how Gal-3 traffics to these different intracellular locations remains poorly understood although hypotheses involve post-translational modification, protein binding or vesicular trafficking. Cytosolic Gal-3 can regulate intracellular signaling and apoptosis/cell survival[45]; in the nucleus, Gal-3 affects RNA processing, and in the extracellular space, Gal-3 binds to numerous ligands including receptors and integrins to control cell to cell and cell to matrix signaling. Gal-3 does not contain a signal peptide and its secretion to the extracellular space is inhibited by methylamine and increased by heat shock and calcium mobilizing agents, which suggests that exocytosis is a major export pathway[46]. Despite this information, several questions remain as to whether this pathway accounts for the export of both free and encapsulated Gal-3, as secreted Gal-3 is reported to be predominantly free from being packaged into extracellular vesicles[47]. Through utilizing a CRISPR-Cas9 genomic screen, another proposed mechanism for Gal-3 secretion involves binding to N-linked glycosylated proteins with signal peptides that are enroute to the plasma membrane, although N-linked glycosylation is not required for secretion but essential for extracellular membrane binding[47]. An alternative mechanism for secretion is the reported ability of Gal-3 to penetrate lipid bilayers allowing the moiety to enter/exit cells, as well as traffic to the nucleus or other intracellular organelles[48].

Gal-3 is also involved in several post-translation modifications. As a case in point, it is cleaved by matrix metalloproteinases 2 and 9 between Ala62 and Tyr63 to yield intact CRD and N-terminal peptides, which results in increased carbohydrate binding and reduced oligomerization[49]. Gal-3 is also a substrate for other proteases including MMP-7, MMP-13, MT1-MMP, and PSA, and is primarily phosphorylated on Ser6, Ser12[35] and Tyr107[50,51], which can impact the subcellular localization of Gal-3 by promoting translocation from the nucleus to the cytoplasm[52], thereby shaping its ability to regulate apoptosis in the cytoplasm[53]. Finally, Ser6 phosphorylation can impact the ability of Gal-3 to recognize carbohydrate motifs, and the phosphorylation of Tyr107 may impair protease-dependent cleavage[54].

Gal-3 impacts a variety of biological processes including RNA splicing proliferation, altered signaling, migration, apoptosis, fibrosis and inflammation[45,55-59]. Towards this end, a pathogenic role for Gal-3 has been proposed in numerous diseases such as cancer[60,61], inflammatory[62,63] and fibroproliferative disorders in various organs such as pulmonary, cardiac and hepatic fibrosis[57,64-68].

Pulmonary Hypertension is Associated with Increased Galectin-3 Expression

Increasing evidence supports a role for Gal-3 in the development of PH. In humans with PH, circulating Gal-3 is elevated and correlates with RV ejection fraction, and end diastolic and systolic volumes[12], which is supported by showing that Gal-3 levels correlate with the severity of PH, is a biomarker of disease progression[69], and a strong predictor of mortality in PH[70]. Circulating levels of Gal-3 correlate with RV dysfunction[71], with a reported role for Gal-3 as an indicator of left-sided cardiac failure[72,73]. Gal-3 expression is also upregulated in different established experimental rat models of PH. Luo *et al*[74] reported that Gal-3 is increased in lung tissue from the hypoxia-induced rat model of PH, and Barman and colleagues observed increased Gal-3 expression within the medial smooth muscle layer in human PH as well as in both the MCT-treated rat model and the Sugen5416/Hypoxia rat model of PH[75], which induce both pulmonary vascular inflammation and fibrosis [6, 76-78]. Elevated Gal-3 expression has also been reported in the hypoxia-induced mouse model of PH[79]. Similarly, in the hypoxia-induced rat model of PH, both mPAP and RVSP, as well as the Fulton Index (RV/LV+S, an index of RV hypertrophy) were increased by hypoxia, but inhibited by N-Lac, a non-selective galectin inhibitor[74]. In addition, Luo and colleagues found that Gal-3 inhibition by N-Lac attenuated medial hypertrophy as well as collagen deposition in the PA, suggesting that Gal-3 expression is involved in both PA proliferation and fibrosis, possibly via a TGF-β1 signaling pathway[74]. To provide a complementary genetic approach that is more selective, Gal-3 was knocked out in the Sprague-Dawley (SD) rat using CRISPR Cas9 technology, and noninvasive indices of PAH were assessed *in vivo* using high resolution digital ultrasound in both wild-type (WT) and Gal-3 KO rats treated with or without MCT. It was observed that MCT-treated WT rats exhibited a time-dependent increase in PH that was absent in Gal-3 KO rats[75]. In addition, while RSVP was significantly increased in WT rats exposed to Sugen5416/Hypoxia, there was no difference in RVSP between control WT rats and Sugen5416/Hypoxia exposed Gal-3 KO rats[75]. Collectively, these results advance the hypothesis that Gal-3 expression is increased in PH from rodent models (and human PH), which contributes to the vascular remodeling of PA leading to the development of PH.

Galectin-3 Promotes Reactive Oxygen Species in Pulmonary Hypertension

Abundant evidence supports increased levels of reactive oxygen species (ROS) in both human and experimental models of PH[23-29]. ROS that are produced in the pulmonary vasculature include superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\cdot$), and hydroperoxyl radical (HO_2)[30]. Numerous mechanisms have been hypothesized to account for increases in ROS including altered NADPH oxidase (Nox) enzyme expression and activation, and steady state levels of ROS reflect the balance between ROS generation and oxidant scavenging, with evidence supporting variations in both pathways in PH[31]. Of the ROS produced, O_2^- and H_2O_2 activate multiple signaling pathways promoting cell proliferation and apoptosis,—elevated vascular tone, fibrosis, and inflammation, which are all hallmark pathophysiological indications of PH[30]. The human genome encodes five NOX isoforms of which NOX1, NOX2, NOX4 and NOX5 are expressed in the pulmonary vasculature. NOX4 is unique in that it is a constitutively active enzyme that produces levels of H_2O_2 which is primarily controlled by changes in gene expression[32,33]. Increased expression of NOX4 occurs in human PH[34], and strong evidence supports an important role for NOX4 in the pathogenesis of PH in both rat and human[34,35] but this premise is less well-defined in mice[36-38]. In addition, NOX4 has been reported to be a major NADPH oxidase homolog expressed in human pulmonary arterial smooth muscle cells (PASMCs)[39], and its expression at the mRNA and protein level is significantly increased in lungs from patients with idiopathic pulmonary arterial hypertension (IPAH) compared to healthy lungs[34], suggesting a relationship between NOX4 and the development of PH.

The association between Gal-3 and oxidative stress has been demonstrated in vitro with the treatment of monocytes with phorbol myristate acetate, an NADPH oxidase-dependent inducer of reactive oxygen species, which produced an increase in Gal-3 mRNA and protein expression[80]. In addition, Gal-3 stimulates the superoxide levels from neutrophils through activation of NADPH II[81]. Gal-3 also induces ROS through the release of O_2^- in cultured mast cells, an effect that was blocked by the antioxidant enzyme superoxide dismutase[82]. Further, plasma Gal-3 was increased in patients with vascular disease, which correlated with F2-isoprostanes, a serologic marker of oxidative stress [83].

Experimentally, in the MCT-model of PH, we found increased expression of NOX1, NOX2

and NOX4 mRNA in isolated PA (**Figure 2A-C**), and pre-treatment with a specific inhibitor of Gal-3 that ameliorates PH[79] lead to significant reductions in NOX1, NOX2 and NOX4 expression (**Figure 2A-C**). Increased intracellular and extracellular Gal-3 can contribute to superoxide production, and transduction of mouse peritoneal macrophages with a Gal-3 adenovirus resulted in increased phorbol myrisate acetate (PMA)-stimulated superoxide production (**Figure 2D**). Alternatively, extracellular recombinant Gal-3 increased superoxide production in mouse peritoneal macrophages, which was accompanied by increased expression of NOX2, the major oxidoreductase in immune cells (**Figure 2E**). To assess whether Gal-3 contributes to vascular ROS production in PH, we measured the expression levels of 8-hydroxy deoxyguanosine, a molecular footprint of DNA damage due to ROS, in lungs from control rats, rats treated with MCT and MCT in the presence of a Gal-3 inhibitor. MCT increased ROS levels as estimated by 8-hydroxy deoxyguanosine, and pre-treatment with the Gal-3 inhibitor reduced ROS levels to control values (**Figure 2F**). Collectively, these results suggest that *in vivo* Gal-3 contributes to the elevation of ROS via upregulation of multiple NOX isoforms that promote aberrant vascular remodeling towards the development of PH.

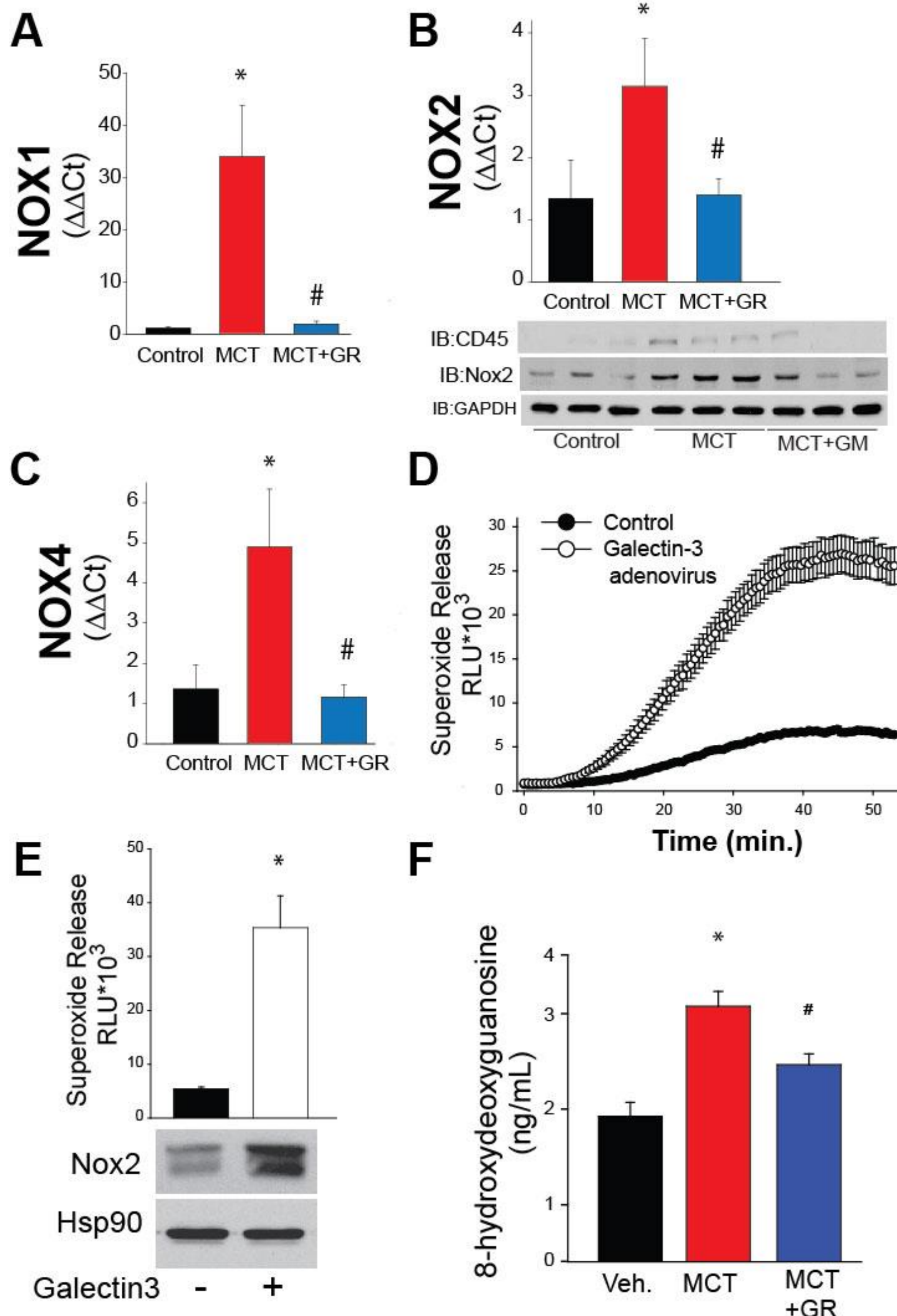


Figure 2. Galectin-3 increases expression of NOX enzymes and ROS production in pulmonary arteries from a rat model of PH. The expression of NOX enzymes was determined in pulmonary arteries (PA) isolated from rats treated with MCT for four weeks. Relative expression of (A) NOX1 mRNA, (B) NOX2 mRNA and protein and (C) NOX4 mRNA was determined in PA isolated from control, MCT and MCT-treated with the Gal-3 inhibitor, GR by real time PCR. In D, mouse peritoneal macrophages were transduced with control (GFP) or Gal-3 adenovirus and the ability to generate reactive oxygen species was determined using enhanced L-012 chemiluminescence. In E, mouse peritoneal macrophages were incubated with recombinant Gal-3 (10µg/ml) and 24h later basal superoxide production was determined using L-012 versus NOX2 expression. In F, the levels of 8-Hydroxydeoxyguanosine, a molecular footprint of ROS production *in vivo* was measured by ELISA in lung tissue isolated from control, MCT-treated rats and MCT-treated rats plus the Gal-3 inhibitor GR. Reprinted with copyright permission from *Antioxidants and Redox Signaling*, Volume 31, Issue 14, Mary Ann Liebert, Inc., New Rochelle, NY (Publisher).

The Role of Galectin-3 in Cellular Inflammation

Chronic vascular inflammation is frequently accompanied with vascular fibrosis, loss of compliant tissue composition, and subsequent organ failure[84]. Vascularized tissue and individual cells respond to injury, infection, and irritation by initiating an inflammatory response. While acute (early) inflammation usually resolves itself to enable the transition to the process of healing, chronic inflammation is the failure of acute inflammation to resolve, resulting in a deleterious environment usually through persistence of an inflammatory stimulus[85]. Gal-3 is an important cellular regulator of the immune system, and is highly expressed in myeloid cells including monocytes, macrophages, dendritic cells, and neutrophils, which contributes to both acute and chronic pulmonary vascular inflammation.

Gal-3 directly binds to CD11b on macrophages[86], and CD66 on neutrophils to regulate inflammatory cell extravasation[87], which elicits immune cell differentiation as well as the binding of these cells to numerous pathogens including LPS (the endotoxin from gram-negative bacteria)[88], *H. pylori*[89], pathogenic fungi and *Trypanosoma cruzi*[90]. Gal-3 can also function as a pattern-recognition receptor (PRR) and a danger-associated molecular pattern (DAMP)[91] that can promote the assembly of inflammasomes to produce IL-1β and IL-18, which amplifies inflammatory responses by potentiating NFκB among other pathways. In the pulmonary vasculature, Gal-3 is generally considered to be a pro-inflammatory molecule, and has been reported to activate T and B lymphocytes[92], mast cells,[93] monocytes and macrophages[94] and neutrophils[95]. Gal-3 is expressed on the surface of human monocytes and increased expression levels elicit cellular differentiation to macrophages. In addition, Gal-3 is important in promoting macrophage divergence towards the M2 phenotype, and macrophages lacking Gal-3 show an impaired ability to express M2 genes in response to IL-4[96]. Gal-3 can also function as a chemoattractant, and high levels promote the inward migration of monocytes and macrophages[94] leading to vascular inflammation in PH.

PH is accompanied by increased vascular inflammation [7,97,98] and recruitment of inflammatory cells[99]. As Gal-3 is closely involved in the function of immune cells, to assess the role of Gal-3 in regulating vascular inflammation we measured the expression level of inflammatory markers in isolated PA from control, MCT and MCT plus Gal-3 inhibitor treated rats. We found that MCT-induced PH was associated with increased expression of IL-6 (pro-inflammatory cytokine), CD45 (pan leukocyte marker), CD68 (monocytic cell marker) and CD4 (T-cell marker) in isolated PA, and inhibition of Gal-3 pharmacologically significantly attenuated MCT-induced vascular inflammation (**Figure 3 A-D**). In addition, silencing Gal-3 caused reduced expression of IL-6 (**Figure 3E**). To determine a mechanism by which Gal-3 impacts vascular inflammation, we treated HPASMC with LPS with and without recombinant Gal-3, and found that LPS induced phosphorylation of p65, a transcription factor that orchestrates many traits of inflammatory signaling. Further, in cells pretreated with recombinant Gal-3, p65 phosphorylation was increased in control cells, suggesting priming and the subsequent response to LPS was enhanced (**Figure 3F**).

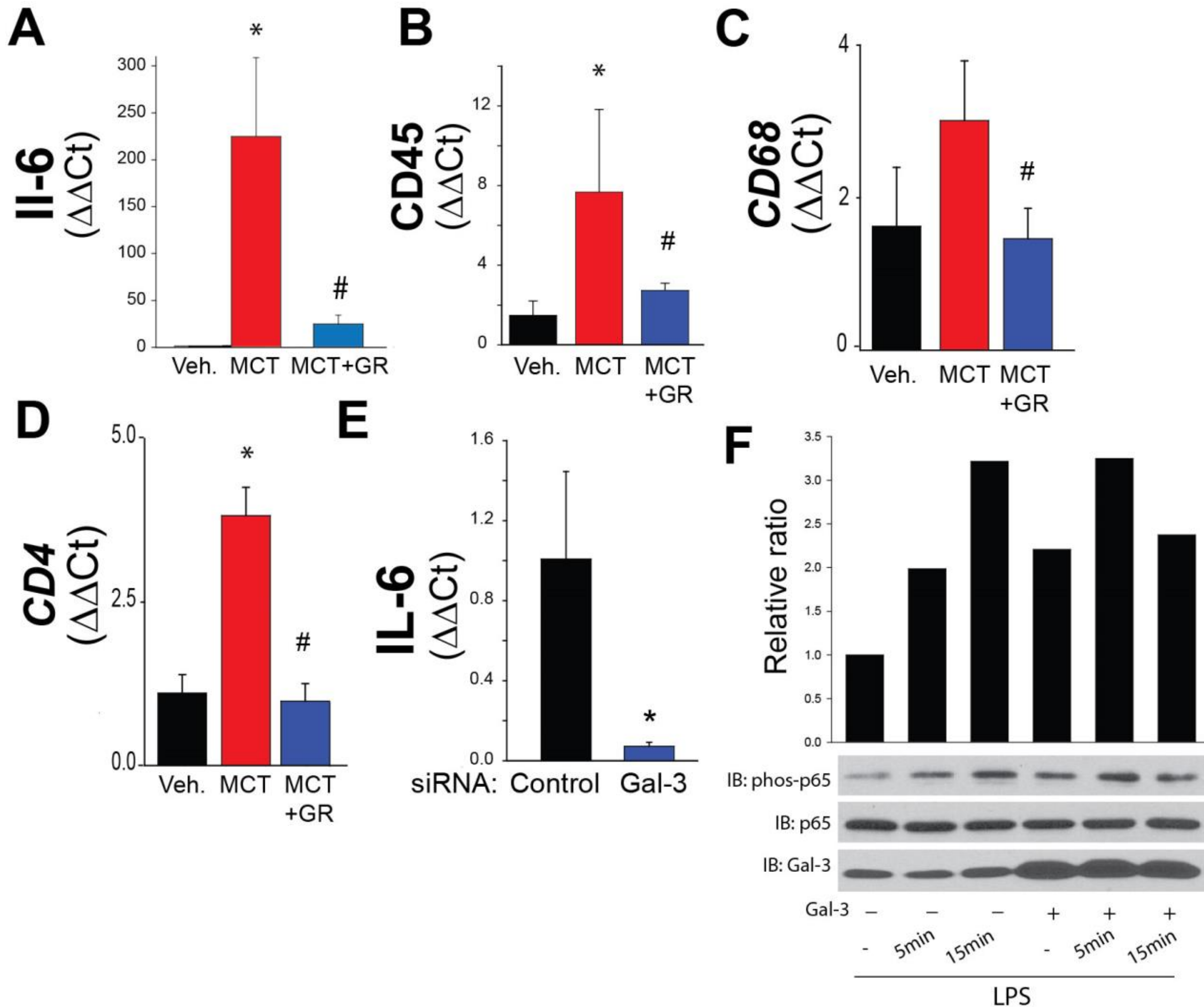


Figure 3. Galectin-3 promotes inflammation in hypertensive pulmonary arteries. The expression of proinflammatory genes was determined in pulmonary arteries (PA) isolated from rats treated with MCT for four weeks. Relative expression of **(A)** Il-6 mRNA, **(B)** CD45 mRNA **(C)** CD68 mRNA and (**D**) CD4 mRNA was determined in PA isolated from control, MCT and MCT-treated with the Gal-3 inhibitor, GR by real time PCR. In **(E)** silencing Gal-3 in human pulmonary artery smooth muscle cells (HPASMC) reduced IL-6 mRNA expression. In **(F)**, Gal-3 regulates NF-κB activity. HPASMC were pretreated with recombinant Gal-3 (10μg/ml) and then exposed to vehicle or LPS and time-dependent changes in the levels of phosphorylated p65, total p65 and Gal-3 determined by Western blot. n=3-4 per group; Reprinted with copyright permission from *Antioxidants and Redox Signaling*, Volume 31, Issue 14, Mary Ann Liebert, Inc., New Rochelle, NY (Publisher).

Galectin-3 and Vascular Fibrosis

Fibrosis refers to the deposition of excessive amounts of connective tissue as part of a reparative process, often secondary to inflammation, that results in the scarring of a tissue or organ impairing the ability to function efficiently. Gal-3 has long been identified as mediator of tissue and organ fibrosis[100], and by activating fibroblasts, induces secretion of collagen leading to fibrosis[57,58] . In PH, fibrosis occurs in both the lung vasculature and the right ventricle[101], and pulmonary vascular fibrosis results from a diverse range of stimuli including oxidative stress, inflammatory cell signaling, release of inflammatory cytokines, compromised endothelial function, and the production of endothelium-derived vasoactive substances including the renin-angiotensin aldosterone system[69]. Collagen I expression is increased by Gal-3 in rat vascular smooth muscle, and in hypertensive aldosterone-treated rats, Gal-3 expression increases vascular hypertrophy, inflammation, and fibrosis, which is reversed in the presence of pharmacological Gal-3 inhibition and absent in Gal-3 KO mice[62]. Wang and colleagues[102] observed increased pulmonary

vascular fibrosis in the MCT-treated rat model of PH, and Gal-3 also mediated TGF-□1-induced vascular fibrosis via the STAT3 and MMP9 signaling pathways[102]. Other proposed mechanisms for Gal-3 mediating vascular fibrosis include activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) and protein kinase C (PKC) pathways[103,104], as well as directly increasing the production of extracellular matrix (ECM) proteins[105]. In the setting of PH, the right ventricle (RV),undergoes changes in cardiac morphology including the development of fibrosis[106], and increased circulating levels of Gal-3 in cardiac (right heart) fibrosis, may provide benefit as a clinical biomarker providing diagnostic information for the potential onset and pathophysiological manifestations of PH and eventual heart failure[107 108]. In mice, knockout or pharmacological inhibition of Gal-3 reduces cardiac fibrosis and improves function[109], while in rats, infusion of recombinant Gal-3 for four weeks promoted cardiac fibroblast proliferation, collagen production, and cyclin D1 expression leading to ventricular dysfunction[110]. Mechanistically, hyaluronic acid has been reported to be a major component of cardiac fibrosis, and Gal-3 upregulates CD44, which increases levels of hyaluronic acid[111,112].

As stated earlier, fibrosis contributes to the stiffening and compromised function of organs and blood vessels, and PH is accompanied by increased pulmonary artery stiffness[99,113], increased deposition of matrix[8] and increased numbers of vascular fibroblasts[114]. Gal-3 is a potent regulator of fibrosis and has been identified as a contributing factor to idiopathic pulmonary fibrosis[68], liver fibrosis[66], renal fibrosis[58], cardiac fibrosis[108] and vascular fibrosis[59]. To investigate a possible pathogenic role of Gal-3 in regulating vascular fibrosis in a model of PH, we measured indices of fibrosis in PA from control, MCT-treated, and MCT-treated with a Gal-3 inhibitor. We found that MCT-induced PH resulted in increased expression of CD90 (a marker of fibroblasts) and Grem1 (a marker of fibrosis). Subsequently, pre-treatment with the Gal-3 inhibitor significantly reduced these markers of vascular fibrosis (**Figure 4A-B**), and in isolated lung fibroblasts, recombinant Gal-3 and TGFβ increased collagen expression. However, there was no significant interaction between Gal-3 and the actions of TGFβ (**Figure 4C**) as recombinant Gal-3 failed to increase the expression of fibroblast NOX4 and ACTA2 (a marker of myofibroblasts), which were robustly increased by TGFβ. These data suggest that Gal-3 contributes to the vascular fibrosis seen in hypertensive pulmonary arteries, but that its actions on fibroblasts are distinct from those of TGFβ.

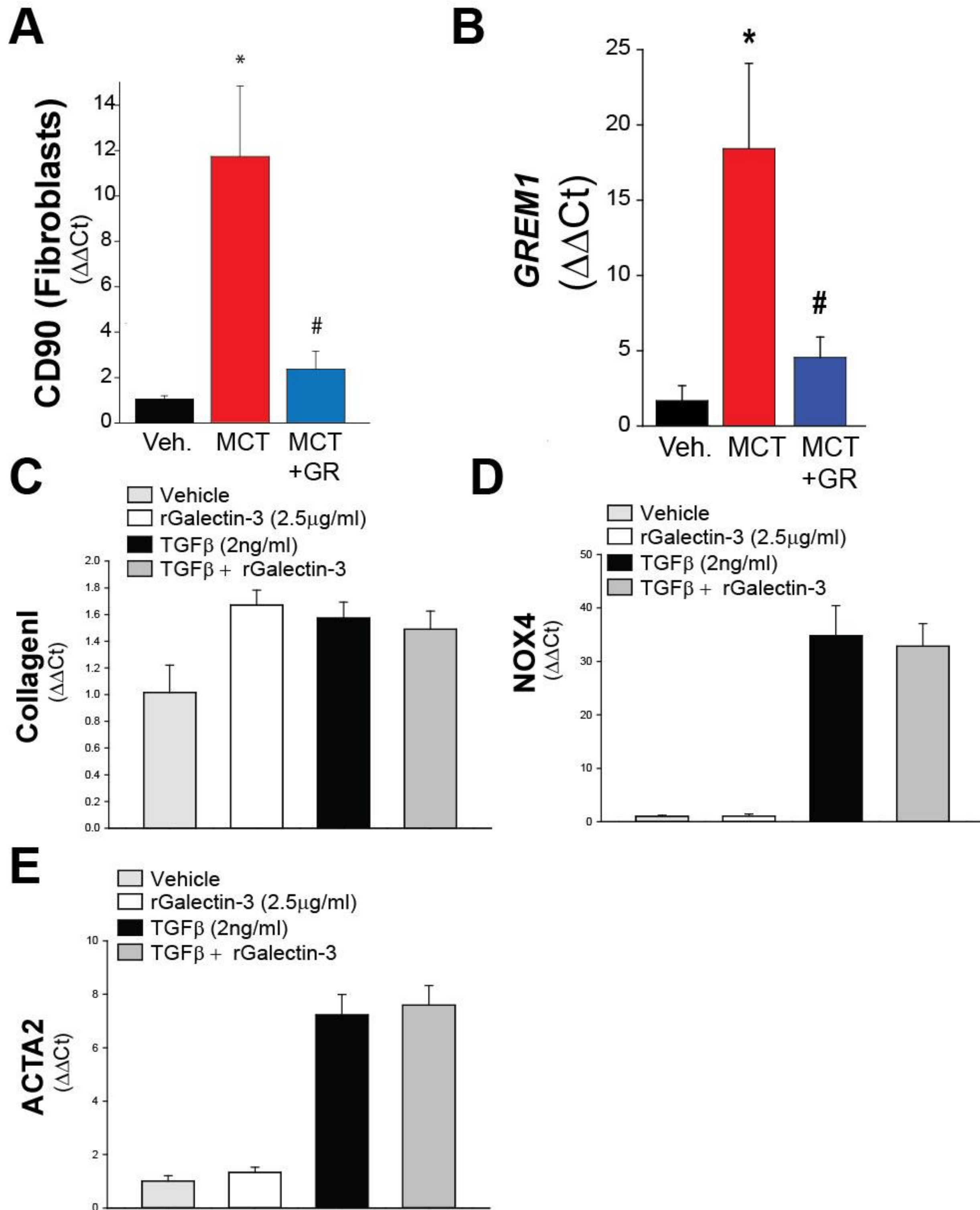


Figure 4. Galectin-3 promotes vascular fibrosis in hypertensive pulmonary arteries. The expression of pro-fibrotic markers was determined in pulmonary arteries (PA) isolated from rats treated with MCT for four weeks. Relative expression of (A) CD90 (Thy1, fibroblast marker) and (B) GREM1 mRNA was determined in PA isolated from control, MCT and MCT-treated with fibroblasts was determined. In (C), recombinant Gal-3 (10µg/ml) increased collagen expression in fibroblasts but did not modify the ability of TGF-β1. In (D) recombinant Gal-3 did not increase NOX4 expression or alter the ability of TGF-β1 to robustly increase NOX4 expression. In (E), recombinant Gal-3 did not increase the expression or smooth muscle actin or alter the ability of TGF-β1 to robustly increase expression. n=3-4 per group; Reprinted with copyright permission from *Antioxidants and Redox Signaling*, Volume 31, Issue 14, Mary Ann Liebert, Inc., New Rochelle, NY (Publisher).

Conclusions

Numerous studies thus far support the premise that Gal-3 expression is increased in both rodent and human PH. Although circulating levels of Gal-3 likely originate from increased expression in the right ventricle, increases in expression in isolated PA suggest local mediated-effects of Gal-3 to promote PA remodeling through changes in cell proliferation, increased ROS, inflammation, and fibrosis (**Figure 5**). Gal-3 is expressed in many cell types and influences a variety of mechanisms to alter cell function, which contributes to the changes in cellularity as well as in pulmonary vascular and RV function seen in PH. Given that PH is a complex disease originating from diverse mechanisms in multiple cells types, the experimental evidence strongly suggests that targeting Gal-3 may be a useful therapeutic approach. Taking advantage of recent studies insinuating that Gal-3 serves as a circulating biomarker in humans that tracks PH severity and progression, the ability of Gal-3 inhibitors to affect multiple cellular pathways may be advantageous in the approach to treating complex vascular proliferative diseases like PH. In addition, specific Gal-3 inhibitors may also have benefit as part of a combination strategy that has significantly greater potential to delay and ameliorate the progression of PH and other pulmonary vascular diseases[115].

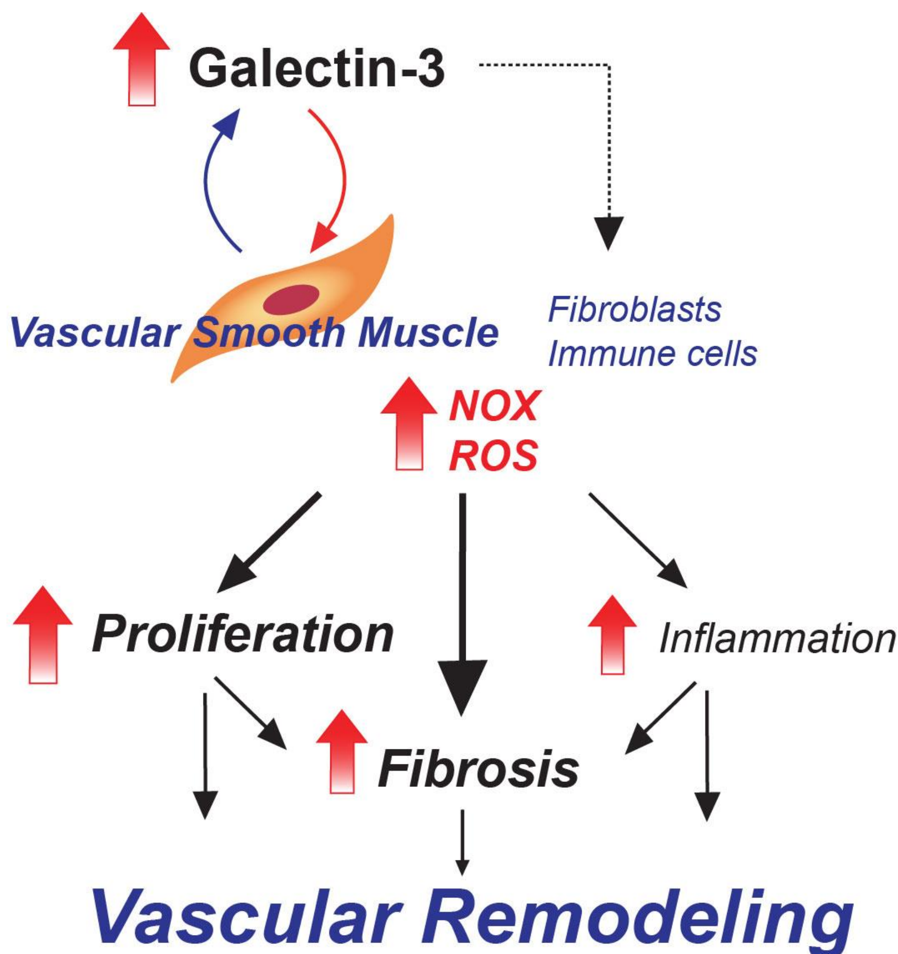


Figure 5. Summary of the proposed mechanisms by which Gal-3 promotes NOX and ROS- mediated vascular remodeling in PA to induce PH. Gal-3 increases cell proliferation, inflammation, and fibrosis (matrix deposition) via paracrine and autocrine functions via different cell types. Reprinted with copyright permission from *Antioxidants and Redox Signaling*, Volume 31, Issue 14, Mary Ann Liebert, Inc., New Rochelle, NY (Publisher).

Conflicts of Interest Statement
The authors have no conflicts of interest to declare.

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