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Protein kinase C promotes cardiac fibrosis and heart failure by modulating galectin-3 expression

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ABSTRACT

Protein kinase C (PKC) and galectin-3 are two important mediators that play a key pathogenic role in cardiac hypertrophy and heart failure (HF). However, the molecular mechanisms and signaling pathways are not fully understood. In this study, we explored the relationship between and roles of PKC-α and galectin-3 in the development of HF. We found that activation of PKC by phorbol dibutyrate (PDB) increased galectin-3 expression by ~180%, as well as collagen I and fibronection accumulation in cultured HL-1 cardiomyocytes. Over-expression of galectin-3 in HL-1 cells increased collagen I protein production. Inhibition of galectin-3 by β-lactose blocked PDB-induced galectin-3 and collagen production, indicating that galectin-3 mediates PKC-induced cardiac fibrosis. In rats subjected to pulmonary artery banding (PAB) to induce right ventricular HF, galectin-3 was increased by ~140% in the right ventricle and also by ~240% in left ventricle compared to control. The elevated galectin-3 is consistent with an increase of total and activated (phosphorylated) PKC-α, α-SMA and collagen I. Finally, we extended our findings to examine the role of angiotensin II (Ang II), which activates the PKC pathway and contributes to cardiac fibrosis and the development of HF. We found that Ang II activated the PKC-α pathway and increased galectin-3 expression and collagen production. This study provides a new insight into the molecular mechanisms of HF mediated by PKC-α and galectin-3. PKC-α promotes cardiac fibrosis and HF by stimulation of galectin-3 expression.

1. Introduction

Heart failure (HF), which is primarily characterized by a loss of cardiac function, afflicts approximately 5 million people and causes 300,000 deaths per year in the United States alone [1]. The prognosis of patients with HF is poor, with a 50% 4-year mortality [2]. HF that affects the left side of the heart is more common, but the incidence of HF affecting the right side of the heart is increasing due to the increased incidence of pulmonary hypertension and increased survival of children with congenital heart disease into adulthood [3–5]. HF is a complex disorder in which a number of physiological systems participate, acting on both the cardiomyocytes and interstitial cells. Although significant therapeutic progress has been made over the past decades, the molecular mechanisms and signaling events that lead to HF remain unsolved.

Protein kinase C (PKC) is a group of serine/threonine kinases. Approximately 15 different isozymes comprise the PKC family. Increasing evidence demonstrates the association of PKC activation with cardiac hypertrophy, HF, ischemic injury, or agonist stimulation [6–10]. Among the PKC family, PKC-α is the most abundant isoform and is expressed in mouse, human, and rabbit hearts, whereas PKC-β and PKC-γ are both detectable but expressed at substantially lower levels [10,11]. Activation of PKC-α has been shown to be necessary and sufficient to induce cardiomyocyte hypertrophy in cultured neonatal cardiomyocytes [12]. The critical impact of PKC-α in heart disease has been demonstrated in PKC-α knock-out mice where genetic deletion of PKC-α protects against HF induced by pressure overload [13] as well as against dilated cardiomyopathy [6]. In contrast, PKC-α transgenic mice showed reduced ventricular performance at 4 months of age and increased cardiac hypertrophy at 6 months [6].

Acting as a crucial HF mediator, PKC-α has gained tremendous attention as a potential novel therapeutic target for the treatment of HF [1,10,14]. Pharmacological inhibition of PKC-α in rodents or

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utilization of a dominant negative PKC-α mutant, was beneficial and antagonized the development of HF [1,11]. Clinical trials utilizing ruboxistaurin to inhibit PKC activity have shown promise in treating heart disease [1,15–17]. Furthermore, Connelly et al. [17] also demonstrated that ruboxistaurin attenuated diastolic dysfunction, myocyte hypertrophy, collagen deposition, and preserved cardiac contractility in a rat diabetic HF model. Blockade of PKC-α/β with ruboxistaurin enhanced cardiac contractility and attenuated HF induced by myocardial infarction in pigs [18].

Galectin-3 is a small lectin-like protein that is emerging as a key player with a substantial role in the process of HF development. Galectins are a family of soluble β-galactoside-binding lectins that are involved in the regulation of inflammation, immunity, and cancer. Notably, in a comprehensive microarray study, Sharma et al. utilized the homozygous transgenic Ren-2 rat model that develops HF and identified cardiac galectin-3 as the most strongly overexpressed gene [19]. Galectin-3 is the only chimeric protein in the galectin family based on its special structure. It is found in a wide range of species and tissues and can interact with cell surface receptors and glycoproteins to initiate transmembrane signaling pathways for different cellular functions. As a mediator of cardiac fibrosis, galectin-3 has been documented as significantly up-regulated in several experimental studies [19–21]. In a rat model of HF, galectin-3 expression is increased at an early stage of hypertrophy, prior to the development of HF [19]. Additionally, Liu et al. showed that infusion of the galectin-3 inhibitor As-SDKP into the pericardial sac, not only inhibited fibrosis and inflammation, but also improved cardiac function [20]. Disruption of the galectin-3 gene also prevents fibrosis formation in heart [21]. In a large study in patients with HF, circulating levels of galectin-3 were found to be a marker of cardiac fibrosis and were associated with an increased risk for incident HF [22]. Furthermore, clinical data shows that plasma galectin-3 is increased in acute and chronic HF [22,23], suggesting that galectin-3 may be of particular value as a biomarker for HF severity.

Although both PKC-α and galectin-3 have been recognized as important players in the development and progress of HF, the molecular mechanism of how PKC-α and galectin-3 participate in these processes remains largely unknown. In addition, it is not clear whether PKC-α and galectin-3, which are very disparate types of molecules, work synergistically or have separate modes of action. Thus, in this study, we present new findings showing that these two important HF factors act in concert in the development of HF. We found that activation of the PKC pathway increases galectin-3 expression and also that inhibition of galectin-3 blocks PKC-stimulated collagen production. This study demonstrates that PKC-α promotes cardiac fibrosis and HF by altering galectin-3 expression.

2. Materials and methods

2.1. Plasmid constructs

A 789-bp coding region gene of rat galectin-3 was PCR amplified from kidney cDNAs and subcloned into a p3xFLAG-CMV-10 vector (Sigma). Oligonucleotide primers were designed based on the rat galectin-3 gene (accessory number NM_031832). The forward primer including Hind III site: 5′-CAAGCTTATGGCAGACGGCTTCTCACTTAATG-3′; the reverse primer including XbaI site: 5′-CTCTAGACTTAGATCATG GCGTGGGAAGCGCT-3′. The constructed p3xFlag-galectin-3 was verified by nucleotide sequence analysis. pcDNA3-PKC-α has been described previously [24].

2.2. Cardiomyocyte culture

Mouse HL-1 cardiomyocytes were kindly provided by Dr. William C. Claycomb from Louisiana State University Health Sciences Center. HL-1 cell medium has been optimized by Dr. Claycomb's group. Claycomb medium (Sigma, 51800C) was supplemented with 10% fetal bovine serum (Sigma, F2442), 4 mM L-glutamine, 0.1 mM norepinephrine (Sigma, A0937) and 1% penicillin/streptomycin. Cells were grown in culture flasks, dishes or plates pre-coated with 1 mg/mL fibronectin (Sigma, F1141) dissolved in a 0.02% gelatin solution at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The medium was changed every 24–48 h.

2.3. Cell transfection and treatment

For plasmid transfection, HL-1 cells were grown into 6-well plates at 90% confluence and transfected with p3xFLAG-galectin-3 or pcDNA3-PKC-α with Lipofectamine 2000 (Invitrogen). After 48 h, cells were collected for western blot analysis. For pharmacologic cell treatment, HL-1 cells were grown to confluence and serum starved for 12 h. The cells were then treated with 2 μM PKC activator phorbol dibutyrate (PDB, Sigma, P1269), 2 μM PKC inhibitor chelerythrine (Sigma, C2932), 1 μM angiotensin II (Sigma, A9525), or galectin-3 inhibitor β-lactose (Sigma, L3750) for the indicated time.

2.4. HF animal model creation

All animal protocols were approved by the Emory University Institutional Animal Care and Use Committee. A rat model of right ventricular HF was created by banding of the pulmonary artery (PAB) as described with slight modifications [25]. Male Sprague Dawley rats with an initial weight of 150–200 g were utilized. Anesthesia was induced and maintained with isoflurane; the rats were intubated and placed on an adjustable heating pad. The chest was open via a left thoracotomy through the fourth intercostal space, and the ribs and pectoral muscles were gently retracted to expose the heart. The pericardium was opened and the pulmonary trunk was carefully separated from the aorta. The main pulmonary artery was partially ligated over an 18 gauge angiocatheter to ensure consistent stenosis. The sizer was promptly removed to allow for antegrade flow through the banded area. The thoracotomy was closed in multiple layers under positive pressure ventilation to evacuate pleural air. Similarly, sham operated animals underwent the same procedure with exposure of the pulmonary artery but without banding. Echocardiography under isoflurane anesthesia was performed 12–17 weeks post-surgery for both PAB and sham animals, prior to sacrifice. Hearts were collected for Western blot analysis.

2.5. Echocardiography

To evaluate the heart function, echocardiography was performed on PAB and sham rats 12–17 weeks after surgery. Transthoracic echocardiography was performed by the Emory Children's Animal Physiology Core utilizing a Vevo 2100 digital high-frequency ultrasound system (FujiFilm Visualsonics Inc, Toronto, Canada) equipped with a probe suited for rat imaging. The system includes capabilities for standard echocardiographic exams including pulsed-wave (PW) and color Doppler. Pulmonary artery pressure gradient and myocardial performance index (MPI) were measured using PW Doppler. Left ventricular ejection fraction and tricuspid annular plane systolic excursion (TAPSE) were determined in M mode. Right ventricular wall thickness and diastolic dimension were measured in B mode.

2.6. Western blot analysis

Total proteins were prepared from either HL-1 cell extracts or cardiac homogenates from HF and control rats. Protein concentration was determined by the Bradford method using BioRad protein assay. Equal amounts of proteins (50–100 μg/lane) were loaded and separated by SDS-PAGE gels and transferred to Nitrocellulose Membranes (Bio-Rad). After blocking with 5% non-fat milk in PBST, membranes were incubated with primary antibodies overnight at 4 °C, followed by HRP-conjugated secondary antibody. The protein abundance was detected using enhanced

chemiluminescence ECL system (Amersham Biosciences). Band density was analyzed with ImageJ software (National Institutes of Health). The results were expressed as percentage of control group.

The following antibodies were used in this study: galectin-3 hybridoma (TIB-166; ATCC), PKC-α (sc-8393; Santa Cruz), PKC-α (phospho Thr497) (GTX61959; GeneTex), PKC-α (phospho Thr638/641) (9375; Cell signaling), α-SMA (A2547; Sigma), Col Iα1 (sc-8784; Santa Cruz), fibronectin (F3648; Sigma), FLAG (F1804; Sigma), HRP anti-rabbit IgG (NA934; Fisher), and HRP-goat anti-mouse IgG (115-036-062; Jackson Immuno Research).

2.7. Statistical analysis

The protein levels quantified by densitometry were expressed as mean ± SD. The statistically significant differences were assessed by ANOVA with post-hoc Tukey HSD test for three or more groups and Student's *t*-test for two groups.

3. Results

3.1. PKC stimulation promotes galectin-3 expression in cardiomyocytes

Both PKC and galectin-3 are implicated in the development of cardiac hypertrophy and HF [6,19]. To explore the possible linkage of PKC and galectin-3, we explored the galectin-3 expression in mouse cardiomyocyte HL-1 cells when the cells were treated with the PKC activator phorbol dibutyrate (PDB). As seen in Fig. 1A, HL-1 cardiomyocytes expressed galectin-3 (Gal-3) and following PDB treatment (24 h) galectin-3 was significantly increased by 179 ± 16% ($P < 0.01$). PKC inhibitor chelerythrine (Chel) treatment slightly reduced basal galectin-3 protein levels by 30 ± 13%. To investigate the time course of galectin-3 upregulation, HL-1 cells were treated with 2 μM PDB for increasing durations of 0, 4, 8, and 24 h. PDB treatment time-dependently increased galectin-3 expression and this effect started as early as 4 h (Fig. 1B).

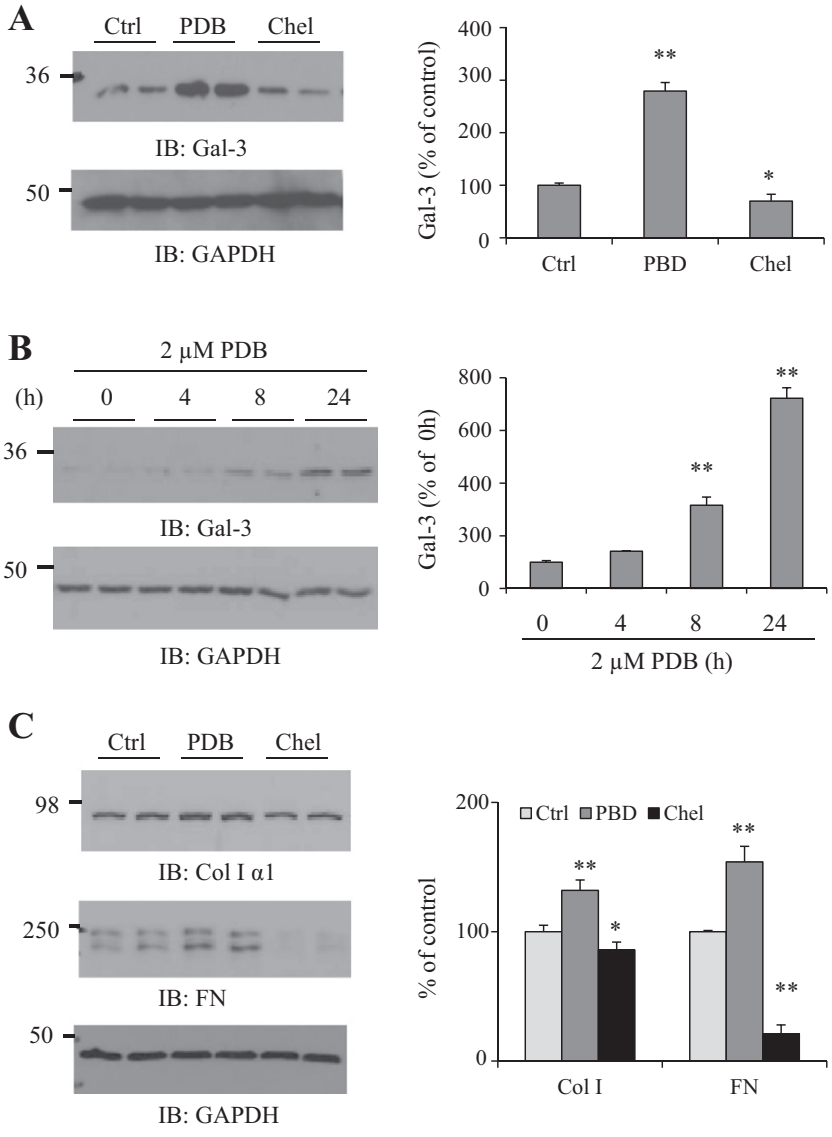

Fig. 1. Effect of PDB on galectin-3 expression and matrix production in HL-1 cells. A. Rat cardiomyocyte HL-1 cells were treated with PKC activator phorbol dibutyrate (PDB, 2 μM) or PKC inhibitor chelerythrine (Chel, 10 μM) for 24 h. Cells were solubilized in RIPA buffer and processed for Western blot analysis with galectin-3 antibody. B. HL-1 cells were treated with 2 μM PDB for the indicated times and galectin-3 protein expression was examined by Western blot with galectin-3 antibody. C. HL-1 cells were treated with PDB or Chel for 24 h. Collagen I (Col Iα1) and fibronectin (FN) expression was measured by western blot with antibodies to Col Iα1 and FN. For loading control, the same samples were re-probed for GAPDH. Western blot signals were quantified by NIH ImageJ (compared to control, * $P < 0.05$, ** $P < 0.01$, $n = 4$).

3.2. Activation of PKC stimulates collagen I and fibronectin expression in HL-1 cells

Chronic HF has increased matrix accumulation. To confirm the role of PKC in HL-1 cardiomyocyte matrix synthesis, we examined collagen I and fibronectin production after PDB treatment. As demonstrated in Fig. 1C, PDB treatments led to increased collagen I and fibronectin by 32 ± 8% ($p < 0.01$) and 54 ± 12% ($p < 0.01$), respectively. In contrast, PKC inhibitor chelerythrine treatment decreased collagen I by 15 ± 6% and fibronectin by 79 ± 7%.

3.3. PKC-α increases galectin-3 expression in HL-1 cells

To ensure that PDB treatment indeed activated PKC and stimulated galectin-3 expression, HL-1 cells were pre-incubated with PKC inhibitor chelerythrine for 30 min prior to treatment with PDB. Chelerythrine reduced PDB-stimulated galectin-3 protein expression (Fig. 2A). PKC-α is the major isoform expressed in heart [10–12]. To examine the direct role of PKC-α in the regulation of galectin-3 expression, PKCα cDNA was transfected into HL-1 cells. Overexpression of PKC-α increased galectin-3 expression by 65 ± 3% ($P < 0.01$) and collagen I abundance by 107 ± 15% ($P < 0.01$) (Fig. 2B).

3.4. Galectin-3 promotes collagen I protein production

Galectin-3 is thought to augment fibrosis, a pivotal process in both maladaptive cardiac remodeling and HF [19]. To directly show that galectin-3 affects cardiac fibrosis, HL-1 cells were transfected with p3xFLAG-galectin-3. Galectin-3 expression was verified by Western blot using FLAG antibody. Overexpression of galectin-3 enhanced collagen I production (Fig. 3A).

To determine whether PKC-stimulated collagen accumulation is mediated through galectin-3, HL-1 cells were pre-treated with galectin-3 inhibitor β-lactose followed by PDB treatment. As shown in Fig. 3B, β-lactose blocked PDB-induced collagen I production (68 ± 6% vs 136 ± 9, $p < 0.01$).

3.5. Echocardiographic assessment of cardiac function in HF induced by PAB

To further explore our in vitro findings in experimental heart disease, we generated right ventricular HF in rats by PAB which induces pressure overload of the right ventricle (RV). This model does not just induce RV hypertrophy but also induces overt HF [25]. Cardiac function was assessed with echocardiography prior to sacrifice and a summary of these findings is shown in Fig. 4. PAB resulted in a pulmonary artery pressure gradient of 63.5 ± 15.1 mm Hg, demonstrating RV pressure overload. This resulted in a ~100% increase in RV wall thickness and a ~60% increase in RV diastolic dimension. In addition, TAPSE, a measure of RV dysfunction, was significantly decreased compared to sham (by ~33%). Although there were no changes in left ventricular (LV) ejection fraction, there was indication of LV dysfunction as measured by LV myocardial performance index (MPI). MPI has been shown to correlate with invasive measures of cardiac function in a spontaneously hypertensive rat model [26]. We found that MPI was increased in HF rats nearly ~50% compared to sham. These data show that both RV and LV function was diminished in this model of RV failure.

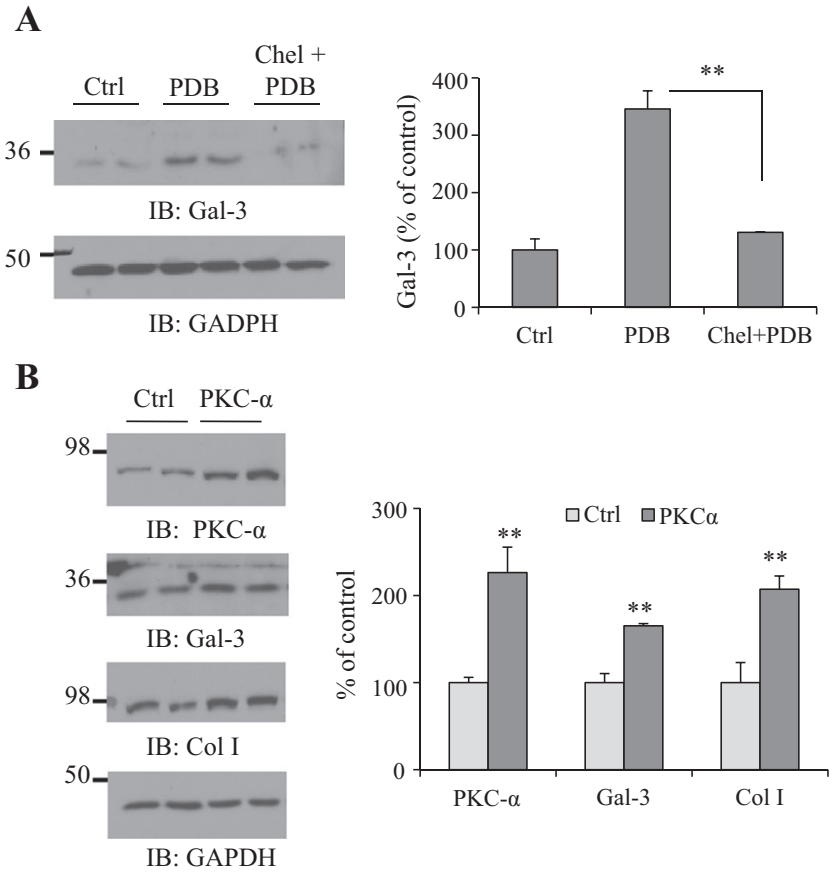

Fig. 2. Effect of PKC-α on galectin-3 expression in HL-1 cells. A. HL-1 cells were pre-incubated with 10 μM chelerythrine (Chel) for 30 min then treated with 2 μM phorbol dibutyrate (PDB). Cells were solubilized in RIPA buffer and processed for Western blot analysis with galectin-3 antibody. B. HL-1 cells grown in a 6-well plate were transfected with 1 μg/well of pcDNA3-PKC-α or vector alone. Two days later, cells were lysed and PKC-α, galectin-3, and collagen I expression were examined by western blot. Western blot signals were quantified by NIH ImageJ (compared to control, ** $P < 0.01$, $n = 4$).

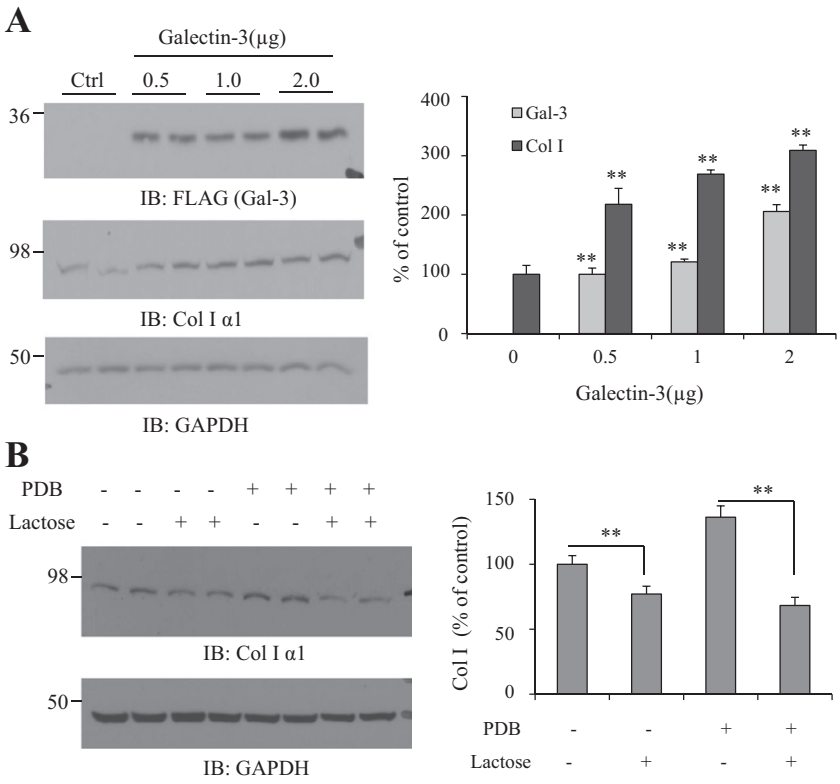

Fig. 3. Galectin-3 increases collagen I expression in HL-1 cells. A. HL-1 cells grown in 6-well plate were transfected with p3xFlag-galectin-3 at different concentration. Two days later, cells were lysed and processed for analysis of collagen expression by Western blot with collagen antibody. Galectin-3 expression was evaluated by FLAG antibody. B. HL-1 cells were pre-treated with or without 50 mM β-lactose for 2 h, followed by PDB for 6 h. Collagen I and galectin-3 expression were examined by Western blot with appropriate antibodies. Western blot signals were quantified by NIH ImageJ (compared to control, ** $P < 0.01$, $n = 4$).

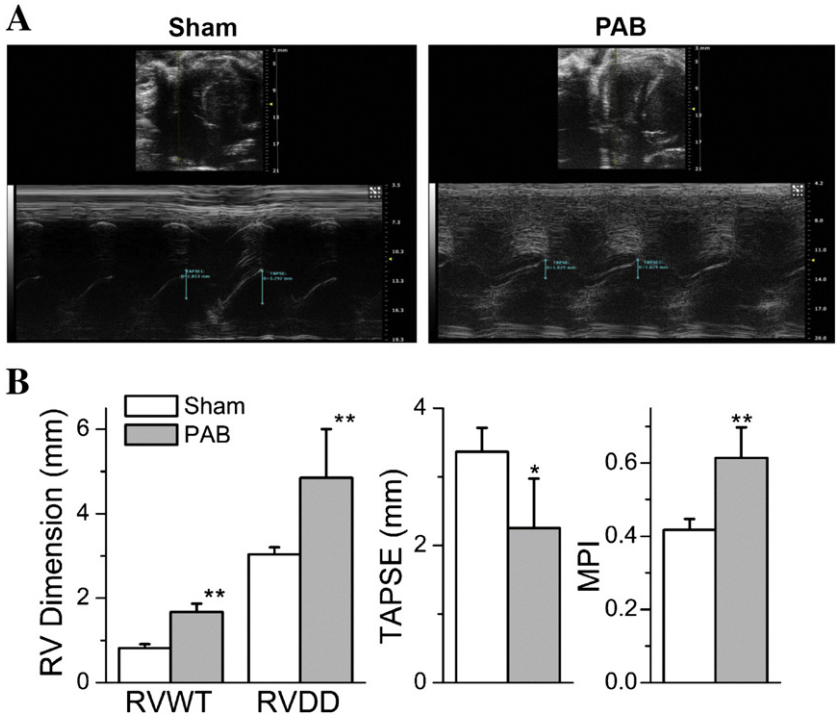

Fig. 4. Abnormal cardiac function in HF rats. A. M-mode echocardiographic images taken from Sham and PAB rats using a Vevo 2100 high frequency ultrasound system. Tricuspid annular plane systolic excursion (TAPSE) measurements are shown in blue, with decreased TAPSE in PAB indicating decreased right ventricular (RV) function. B. Summary of echocardiographic measures of cardiac function. White bars are data from sham operated rats (Sham) and gray bars are data from rats with HF induced by pulmonary artery banding (PAB). Dimensions of RV wall thickness (RVWT) and RV diastolic dimension (RVDD) were measured in B mode. TAPSE was measured in M mode. Myocardial performance index (MPI) was measured with pulsed-wave (PW) Doppler. Data were analyzed using the Vevo 2100 software (compared to control, * $P < 0.05$, ** $P < 0.01$, $n = 5–7$).

3.6. Increased galectin-3 expression in HF

LV and RV were dissected from control and PAB HF rats and processed for western blot analysis of galectin-3 protein expression. Galectin-3 was significantly increased in both RV and LV (Fig. 5A). Galectin-3 often forms dimers. Interestingly, galectin-3 expressed in heart tissue showed significant amounts of the dimerized form and this was dramatically increased by ~130% in HF RV relative to the control ventricles. Similarly, galectin-3 expression was increased by ~240% in LV from HF animals. Consistent with increased galectin-3 expression, the cardiomyocyte activation marker α-smooth muscle actin (α-SMA), and actin as well as fibrotic protein collagen I were increased. The marked increase in galectin-3, collagen type I and fibronectin expression reflects the development of cardiac fibrosis in the HF rats.

3.7. Enhanced PKC-α expression in HF

PKC-α is the predominant PKC isoform expressed in heart and a key player in the development of HF [6]. We examined PKC-α expression in PAB HF. As illustrated in Fig. 6, total PKC-α protein levels were dramatically increased in both LV (by 130 ± 8.7%) and RV (by 117 ± 10.3%) in HF compared to controls. We then specifically investigated activated PKC-α with antibodies to specific phosphorylated threonine sites of PKC-α. Phospho-PKC-α at Thr497 and phospho-PKC-α at Thr638/641 were increased in the HF group in both LV and RV.

3.8. Angiotensin II promotes galectin-3 and collagen expression in HL-1 cells

Angiotensin II (Ang II) is a well-known hormone that activates the PKC pathway and increases in Ang II levels are critical to myocardial remodeling and progression of HF [27,28]. Thus, we tested the hypothesis that galectin-3 might mediate Ang II-induced cardiac fibrosis. Treatment with Ang II at 10^{-6} M induced a 61 ± 21% increase of total PKC-α ($p < 0.01$). Interestingly, Ang II predominantly activated phospho-PKC-α at Thr638/641 by 79 ± 12.6% ($p < 0.01$) but had little effect on phospho-PKC-α (Thr497) activation (Fig. 7A). As expected, Ang II-treatment stimulated HL-1 cell galectin-3 expression and this increase is consistent with increased collagen I accumulation. This effect was prevented by pretreatment with PKC inhibitor chelerythrine (Fig. 7B), suggesting that Ang II may promote heart fibrosis, at least in part, through activating the PKC–galectin-3 pathway.

4. Discussion

PKC is a family of protein kinase enzymes that are involved in many cellular physiological and pathological processes. Cardiomyocytes express multiple PKC isoforms, of which PKC-α is the most abundant in heart. In fetal and neonatal hearts, the level of PKC expression is high and decreases with age [29]. However, PKC expression and activity are increased by cardiac injury suggesting a critical role for PKC in the development of cardiac disease [1]. The increased activity of PKC isozymes has been observed in multiple types of experimental heart disease including agonist-induced cardiomyocyte hypertrophy, ischemic heart disease, myocardial infarction, and HF [1,6–9,30]. Belin et al. first reported upregulation of PKC-α expression and activity in a rat model of end-stage HF [11]. Deletion of PKC-α can prevent cardiomyopathy and increase cardiac function in experimental HF [6]. In the current study, we discovered that PKC-α was upregulated in rat hearts with PAB induced HF. By utilizing antibodies specific for phosphorylated PKC-α, we detected increased phosphorylation of PKC-α phosphorylated at Thr497, Thr638 and Thr641 in the HF rats, reflecting an increase in both PKC-α expression and PKC-α activation in PAB HF.

The role for PKC-α in cardiac hypertrophy and HF is substantial. However, the signaling pathways and the underlying mechanisms remain

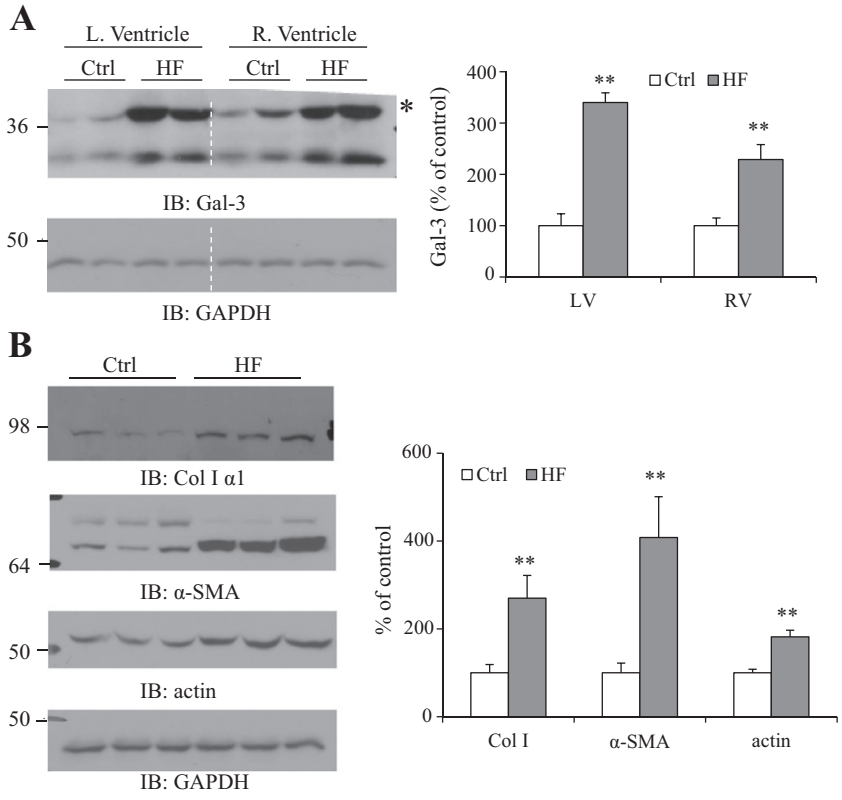

Fig. 5. Expression of galectin-3, α-SMA and collagen in HF rat heart. A. Cardiac tissue was collected from left and right ventricles from control and HF rats. The tissue was solubilized in ice-cold RIPA buffer. Galectin-3 expression was assessed by Western blot (asterisk indicates the dimer galectin-3). B. Right ventricular tissues were collected from control and HF rats and analyzed for α-SMA and Col Iα1 expression. Western blot signals were quantified by NIH ImageJ (compared to control, ** $P < 0.01$, $n = 4$).

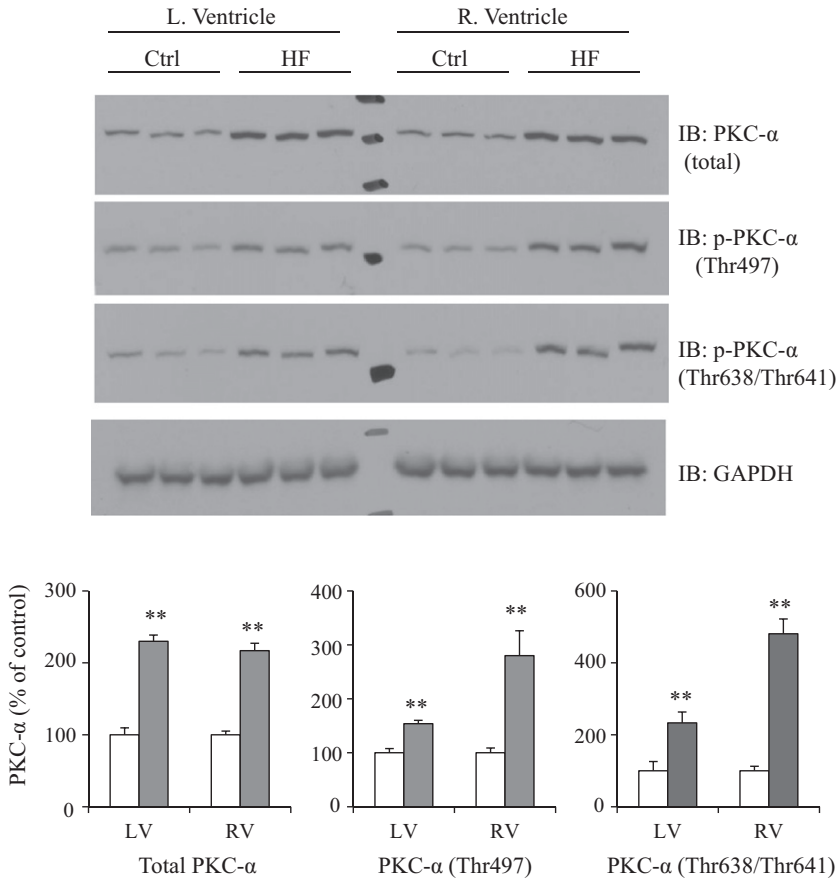

Fig. 6. Changes of PKC-α expression in HF rat heart. Ventricular tissue was collected from control and HF rats. Total and activated PKC-α was examined by Western blot with appropriate antibodies. Western blot signals were quantified by NIH ImageJ (compared to control, ** $P < 0.01$, $n = 3$).

unclear. A number of specific molecules, such as sarcoplasmic reticulum Ca2 + ATPase (SERCA2), G-protein-coupled receptor kinase 2 (GRK2), and myofilament protein cardiac troponin I (cTnI), have all been identified as targets of PKC-α. It is notable that all of these targets are associated with modulation of cardiac contractility [1,14]. In this study, for the first time, we report that activation of PKC promotes galectin-3 expression in HL-1 cardiomyocytes and in HF hearts. This finding suggests that galectin-3 may act as an important effector in PKC-α-stimulated cardiomyocyte hypertrophy and the development of HF. Indeed, inhibition of galectin-3 using β-lactose blocked PDB-induced collagen type I synthesis. Therefore, elevated PKC-α may affect cardiac remodeling and fibrosis by stimulating expression of galectin-3.

Galectin-3 is an active contributor to cardiac remodeling, including myocardial fibrogenesis, and the development of HF [21,22,31]. The important role for galectin-3 in HF was first recognized when Sharma et al. identified galectin-3 as the strongest HF predictor among 48 genes in HF [19]. In normal rat, murine and human hearts, the expression of galectin-3 is low, but expression is rapidly and significantly up-regulated in response to injury [32]. Patients with HF had markedly elevated serum galectin-3 values as compared to normal controls [31]. Therefore, galectin-3 has been examined as a potential diagnostic and prognostic marker for HF in humans [2,22,31,33], but the myocardial localization of galectin-3 had not previously been elucidated. Immunohistochemistry and confocal microscopy analyses of hypertrophied rat myocardium revealed that galectin-3 binding sites were localized predominantly to the myocardial matrix, in fibroblasts and macrophages [19,21,32,34]. In the present study, we demonstrate that cultured cardiomyocyte HL-1 cells express galectin-3 and its expression is dramatically increased in the presence of PKC activation (Fig. 1A). We postulate that cardiomyocytes may also serve as the major source of galectin-3 in heart, at least in diseased conditions.

Myocardial galectin-3 is upregulated in a number of rodent models of heart disease, such as HF-prone hypertensive rats [19], interferon γ-induced murine chronic active myocarditis and cardiomyopathy [35], rat streptozotocin-induced diabetic cardiomyopathy [36], and rat angiotensin II-induced hypertension [37]. In the current study, we found that galectin-3 protein expression is also significantly increased in PAB-induced HF in both LV and RV (Fig. 5A).

Recent studies indicating that galectin-3 promotes HF development suggest involvement of multiple mechanisms including stimulatory effect of galectin-3 on macrophage migration, cardiac fibroblast proliferation, collagen deposition, and the development of fibrosis [19,33,36,37]. Of these, galectin-3 shows a tremendous effect on fibrosis. Indeed, increased circulating galectin-3 has been correlated with an increase in fibrogenesis eventually leading to organ failure including chronic kidney disease [38], chronic liver disease [39], and pulmonary fibrosis [40]. We have shown direct evidence that overexpression of galectin-3 by transfection of exogenous galectin-3 enhanced HL-1 cell collagen I production (Fig. 3A). This is in agreement with our in vivo data showing that increased galectin-3 expression was accompanied by increased collagen I expression in PAB HF (Fig. 5B).

Angiotensin II (Ang II), the principal mediator of the renin-angiotensin system, exerts both short-term and long-term effects in the pathophysiology of cardiovascular disease. With HF, Ang II levels are often increased. In wild-type mice, Ang II causes LV hypertrophy, decreased fractional shortening, and increased LV end-diastolic pressure and fibrosis [27,28]. Furthermore, Ang II has been shown to activate the PKC signaling pathway. Activation of AT1 receptor by Ang II

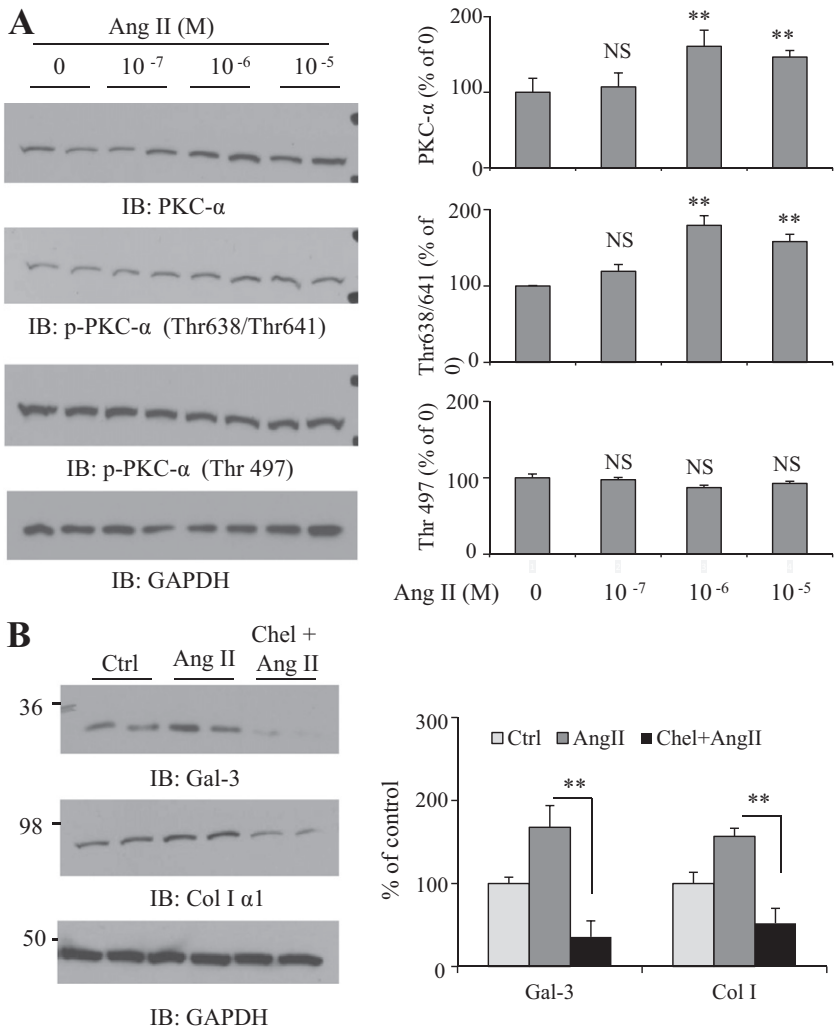

Fig. 7. Effect of angiotension II (AngII) on galectin-3 and collagen expression. A. HL-1 cells were treated with different doses of Ang II for 48 h. Total and activated PKC-α was examined by Western blot with appropriate antibodies. B. HL-1 cells were treated with 10 μM AngII or pre-treated with chelerythrine for 30 min, then treated with AngII for 48 h. Galectin-3 and collagen I α1 proteins were examined by Western blot. Protein expression was quantified by NIH ImageJ (compared to control, NS = no significance, ** $P < 0.01$, $n = 4$).

stimulates phospholipase C (PLC) and subsequently activates PKC [27,28]. Our results are in agreement in that we found Ang II treatment increased galectin-3 expression and collagen I synthesis in HL-1 cells and this effect was prevented by PKC inhibition, suggesting that PKC–galectin-3 pathway might mediate Ang II-induced heart hypertrophy and the development of HF.

5. Conclusions

Numerous animal and human studies have demonstrated that PKC-α activation or an increase in PKC-α expression is associated with HF and that inhibition of PKC-α is cardioprotective. In this study, we report the new finding that PKC-α regulates galectin-3, another crucial mediator of cardiac remodeling, cardiac fibrosis and HF, independent of contractility regulation. The distinction of the current study is the reconciliation of these two important but different kinds of HF mediators and the discovery that they work synergistically in the process of HF development. We thus propose that with the onset of heart disease, augmented PKC-α increases galectin-3 expression which subsequently promotes cardiac fibrosis and HF. We also found that the action of Ang II, a well-known stimulator of cardiac hypertrophy and remodeling, may be mediated, in part, by the activation of the PKC–galectin-3 pathway.

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Disclosures

None.

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