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Galectin-3-centered paracrine network mediates cardiac inflammation and fibrosis upon β-adrenergic insult

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Rapid over-activation of β-adrenergic receptors (β-AR) following acute stress initiates cardiac inflammation and injury by activating interleukin-18 (IL-18), however, the process of inflammation cascades has not been fully illustrated. The present study aimed to determine the mechanisms of cardiac inflammatory amplification following acute sympathetic activation. With bioinformatics analysis, galectin-3 was identified as a potential key downstream effector of β-AR and IL-18 activation. The serum level of galectin-3 was positively correlated with norepinephrine or IL-18 in patients with chest pain. In the heart of mice treated with β-AR agonist isoproterenol (ISO, 5 mg kg^{-1}), galectin-3 expression was upregulated markedly later than IL-18 activation, and *Nlrp3*$^{-/-}$ and *Il18*$^{-/-}$ mice did not show ISO-induced galectin-3 upregulation. It was further revealed that cardiomyocyte-derived IL-18 induced galectin-3 expression in macrophages following ISO treatment. Moreover, galectin-3 deficiency suppressed ISO-induced cardiac inflammation and fibrosis without blocking ISO-induced IL-18 increase. Treatment with a galectin-3 inhibitor, but not a β-blocker, one day after ISO treatment effectively attenuated cardiac inflammation and injury. In conclusion, galectin-3 is upregulated to exaggerate cardiac inflammation and injury following acute β-AR activation, a galectin-3 inhibitor effectively blocks cardiac injury one day after β-AR insult.

galectin-3, interleukin-18, β-adrenergic receptor, macrophage, inflammation, fibrosis

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INTRODUCTION

Sympathetic stress is associated with increased risk of cardiovascular disease-associated mortality and morbidity (Manolis et al., 2014). Acute sympathetic hyperactivity activates β-adrenergic receptors (β-ARs), triggering cardiac injury and adverse events such as myocardial infarction, arrhythmias, and stress-induced cardiomyopathy (Kivimäki and Steptoe, 2018; Lyon et al., 2021). Stress-induced cardi-

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omyopathy (SIC) is a typical example of acute stress-related myocardial injury (Lyon et al., 2021). Although SIC is characterized by reversible myocardial injury, it has a poor prognosis with a mortality rate similar to that of myocardial infarction (Akashi et al., 2015; Ghadri et al., 2018; Redfors et al. 2015). Use of β-blockers in the acute phase of SIC did not reduce mortality (Isogai et al., 2016), which indicates that β-blockers could not effectively reverse the pathological effect of acute stress. Therefore, the mechanisms of acute stress-induced cardiac injury require further investigation to identify novel therapeutic targets.

Our previous study showed that acute β-adrenergic receptor (β-AR) activation triggers cardiac inflammation and dysfunction (Xiao et al., 2018). This process involves activation of NLR family pyrin domain containing 3 (NLRP3) inflammasomes and downstream interleukin-18 (IL-18) in cardiomyocytes within 1 h following β-AR activation. Activation of IL-18 initiates cardiac inflammation through upregulating chemokines to promote macrophage infiltration, resulting in cardiac fibrosis and diastolic dysfunction. Whereas, inhibition of IL-18 1 h after onset of β-AR activation alleviates cardiac injury, but not after one day. This finding suggests IL-18 is critical for the initiation of acute stress-induced cardiac inflammation, rather than the subsequent inflammation cascades. The mechanism that exaggerates cardiac inflammation upon acute stress remains unknown.

The aim of the present study is to identify key factors associated with NLRP3/IL-18 activation and cardiac inflammation to identify critical molecules contributing to the amplification of cardiac inflammation. Potential downstream effectors of NLRP3/IL-18 were screened using the Gene Expression Omnibus database. The identified gene and the underlying mechanisms by which it contributes to acute β-AR activation-induced cardiac inflammatory injury are investigated. Our findings clarify the mechanism of cardiac inflammation amplification upon acute stress and provide a novel target for treatment of acute stress-induced cardiac injury.

RESULTS

Bioinformatic analysis identified galectin-3 as a potential downstream target of NLRP3 inflammasome/IL-18 activation

To identify novel downstream targets of NLRP3 inflammasome/IL-18 activity, we performed analysis of RNA sequencing (RNASeq) datasets, including isoproterenol (ISO, β-AR agonist) treated mice (PRJNA746703), *Nlrp3*^{A350V} mice (GSE140742), and *Il18*$^{-/-}$ mice (GSE5129). The *Nlrp3*^{A350V} mutant knock-in mice with an alanine 350 to valine (A350V) substitution are constitutively-activated NLRP3, which was used as a mouse model of NLRP3-inflammasome activation (Schuster-Gaul et al., 2020). We found 285 upregulated genes in ISO-treated mice, 638 upregulated genes in *Nlrp3*^{A350V} mice, and 870 downregulated genes in *Il18*$^{-/-}$ mice in comparison to the appropriate non-treated or wild-type controls. Among the upregulated genes in ISO-treated mice and downregulated genes in *Il18*$^{-/-}$ mice, *Lgals3* ranked first. In addition, *Lgals3* ranked fifth among genes upregulated in ISO-treated and *Nlrp3*^{A350V} mice (Figure 1A), which suggests that galectin-3 is a downstream effector of the NLRP3 inflammasome/IL-18 signaling pathway.

Isoproterenol induces sustained galectin-3 expression in mouse hearts following IL-18 activation

Mice were subcutaneously injected with a single 5 mg kg^{-1} dose of ISO. The galectin-3 mRNA levels in cardiac tissue increased at the 12th hour following ISO treatment, and remained elevated through day 1 (Figure S1A in Supporting Information). The galectin-3 protein level was upregulated on day 1 and day 3, whereas the IL-18 protein level was increased at 1 h after ISO treatment (Figure 1B). Immunohistochemical staining showed that galectin-3-positive staining in the heart was increased on day 1 (Figure S1B in Supporting Information). Isoproterenol induced galectin-3 expression on day 1 in a dose-dependent manner as shown by RT-PCR, ELISA, and immunohistochemical staining results (Figure S2A–C in Supporting Information). Treatment with 5 mg kg^{-1} ISO was the minimum dose that significantly upregulated galectin-3 expression, and this dose was chosen for further experiments.

Galectin-3 levels are increased in response to sympathetic overactivation in patients

Clinical and experimental studies have shown that circulating levels of galectin-3 reach their peak at 24 h post-MI (Nguyen et al., 2018a; Sharma et al., 2017). To investigate whether circulating galectin-3 levels were increased in human patients in response to sympathetic overactivation, we measured norepinephrine (NE) and galectin-3 levels in the plasma of patients diagnosed with unstable angina (UA) and non-ST-segment elevation myocardial infarction (NSTEMI). Plasma samples were collected on admission within one day after the onset of chest pain. The baseline characteristics of patients are summarized in Table S1 in Supporting Information. A positive correlation was observed between levels of norepinephrine and galectin-3 (Figure 1C). Furthermore, a positive correlation was observed between levels of galectin-3 and IL-18 activity (Figure 1D). In addition, higher galectin-3 content was associated with lower ejection fraction (EF), which reflects reduced cardiac systolic function (Figure 1E). The patients were divided into

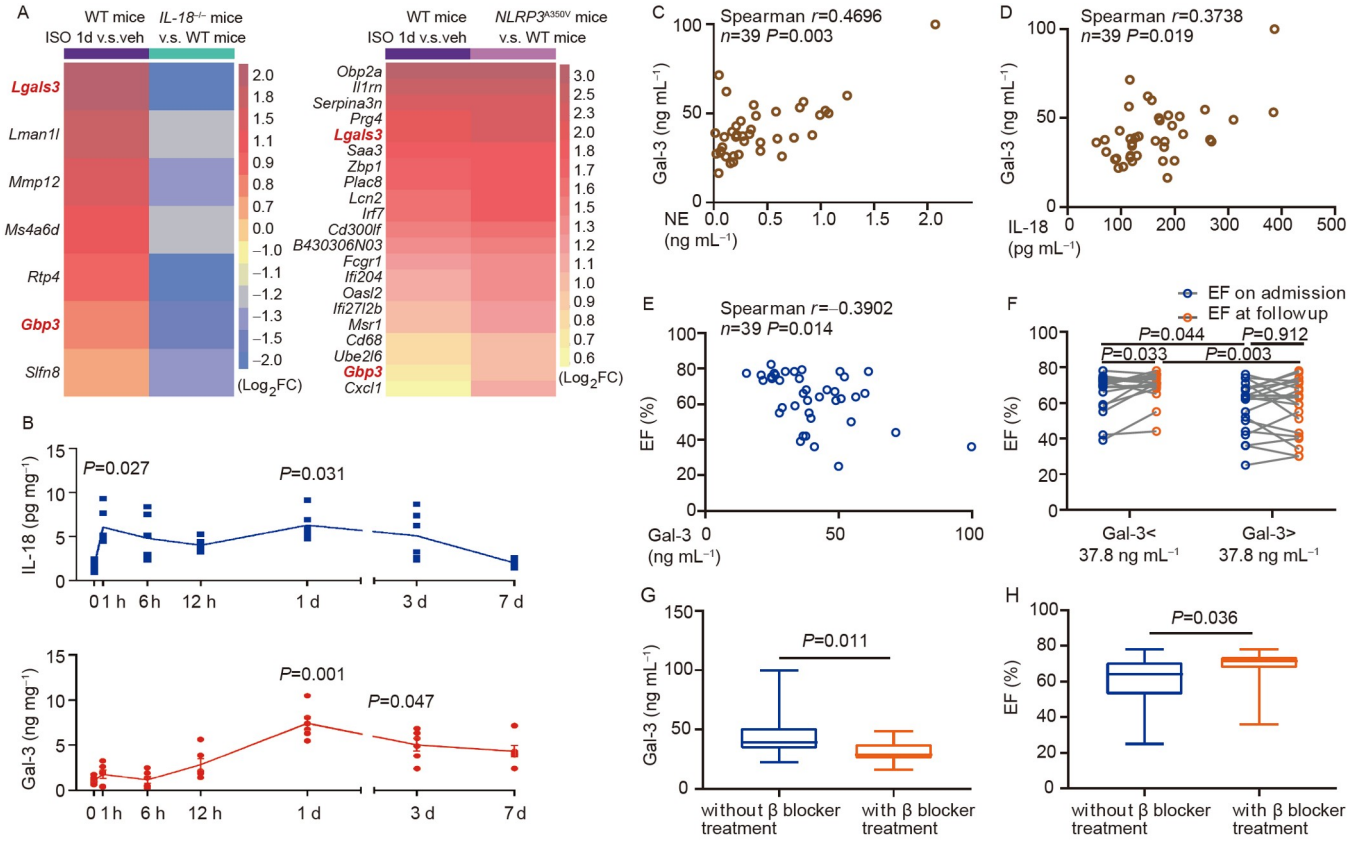

Figure 1 Galectin-3 is a downstream target of β-adrenergic receptor activation-induced NLRP3 inflammasome/IL-18 activity. A, The upregulated genes in ISO-treated mice (P<0.05 and Fold change>2), the down-regulated genes in *Il18*$^{-/-}$ mice (Fold of change<1/2), and the upregulated genes in *NLRP3*^{A350V} mice (P<0.05 and Fold change>2) were screened out. The common genes were ranked by their fold change. B, Interleukin-18 (IL-18) and galectin-3 protein levels in heart tissue of wild-type mice following isoproterenol (ISO) treatment at 1, 6, and 12 h, and on days 1, 3, and 7 were detected using ELISA (n=6). C–H, Plasma samples were collected from patients diagnosed with non-ST-elevation myocardial infarction (n=21) and unstable angina (n=18) on admission within 24 h after onset of chest pain. Galectin-3, norepinephrine, and IL-18 levels were measured using ELISA. C, The correlation between plasma levels of galectin-3 and norepinephrine (NE) (n=39). D, The correlation between plasma levels of galectin-3 and IL-18 (n=39). E, The correlation between plasma levels of galectin-3 and ejection fraction (EF) on admission (n=39). F, The patients were divided into two groups based on galectin-3 levels, and ejection fraction on admission or at follow-up are shown. G, Galectin-3 levels in patients with or without β-blocker treatment. H, The ejection fractions (EF) of patients with or without β-blocker treatment. WT mice, wild-type mice; veh, vehicle; FC, fold of change; Gal-3, Galectin-3; data are presented as the mean ±SEM. Kruskal-Wallis ANOVA combined with post hoc Dunn's multiple comparison test (B), Spearman correlation (C, D, and E), or a Mann-Whitney U test with exact method (F, G, and H) was used.

two groups based on galectin-3 content, and EF was determined after 0.5–2 years (median follow-up time was 12 months). The results showed that patients with acute coronary syndrome (ACS) with higher galectin-3 content on admission had poorer EF recoveries (Figure 1F). We also investigated galectin-3 levels and EF among patients who were receiving regular β-blocker treatment and those who were not based on the medical history. The baseline characteristics of these patients are summarized in Table S2 in Supporting Information. Patients who were receiving β-blocker had lower galectin-3 levels and better cardiac function than patients without β-blocker treatment (Figure 1G and H). These results suggest that galectin-3 was upregulated in patients with sympathetic overactivation and high IL-18 activity, and high galectin-3 levels reflected cardiac dysfunction on admission and poor long-term prognosis. Furthermore, β-blocker treatment is associated with lower galectin-3 levels and better cardiac function.

The expression of galectin-3 following ISO treatment is attenuated in *Nlrp3*$^{-/-}$ and *Il18*$^{-/-}$ mice

Nlrp3$^{-/-}$ and *Il18*$^{-/-}$ mice were used to investigate whether ISO-induced expression of galectin-3 in the heart tissue was regulated by NLRP3-dependent inflammasome/IL-18 activation. ISO-induced expression of galectin-3 was inhibited in the hearts of *Nlrp3*$^{-/-}$ mice and *Il18*$^{-/-}$ mice as shown using Western blotting and ELISA (Figure 2A–F). Immunohistochemistry results also showed reduced expression of galectin-3 in the hearts of *Nlrp3*$^{-/-}$ and *Il18*$^{-/-}$ mice (Figure 2G–H). Moreover, ISO-induced cardiac fibrosis and diastolic dysfunction were alleviated in *Nlrp3*$^{-/-}$ and *Il18*$^{-/-}$ mice compared with those in wild-type mice (Figure S3 and S4 in Supporting Information).

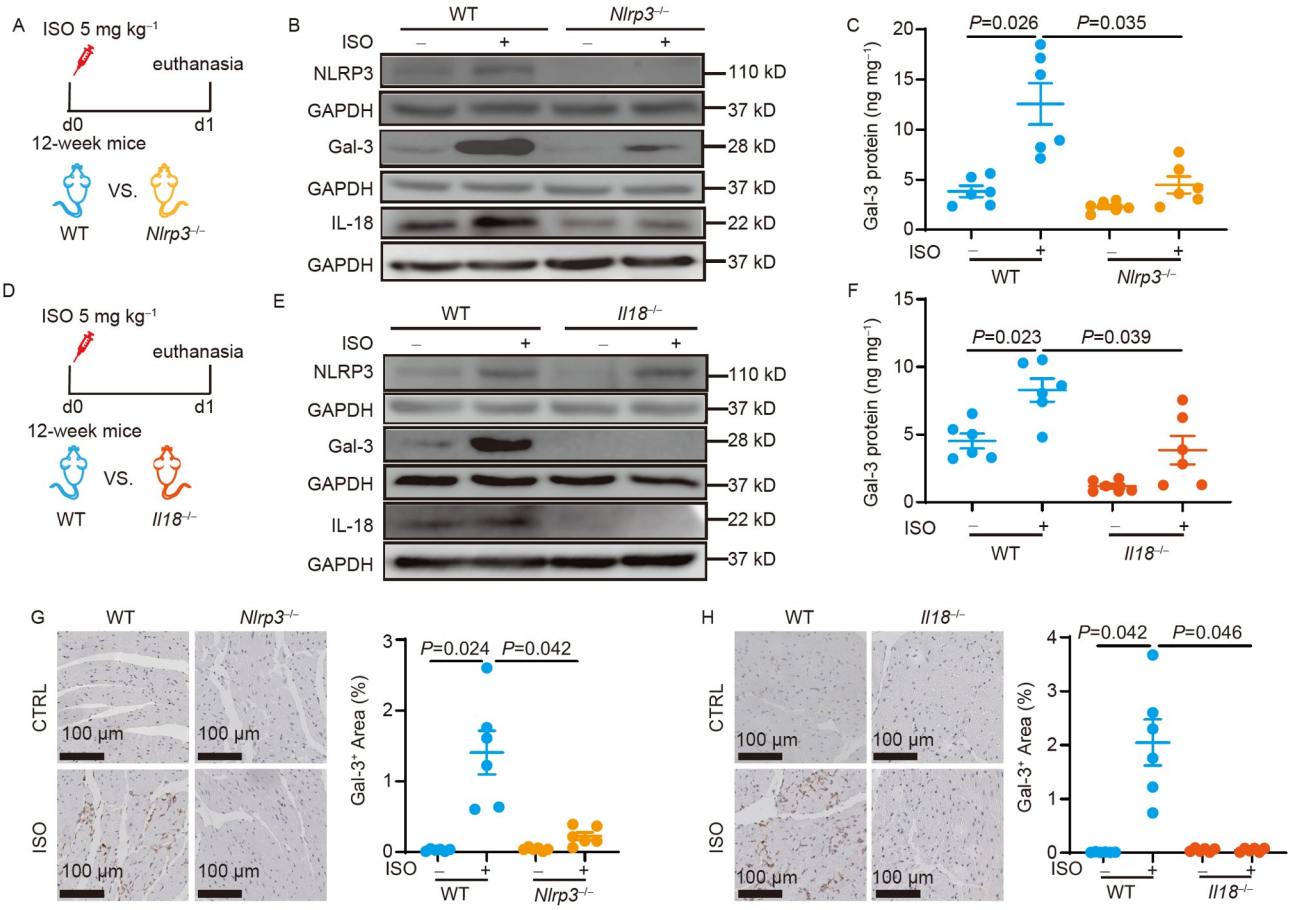

Figure 2 Isoproterenol-induced expression of galectin-3 was blocked in *Nlrp3*$^{-/-}$ and *Il18*$^{-/-}$ mice. A, Wild type mice or *NLRP3*$^{-/-}$ mice were treated with a single 5 mg kg^{-1} dose of ISO, and heart galectin-3 content was measured on day 1. Western blot of NLRP3, galectin-3 and IL-18 (B), and ELISA of galectin-3 (C) in hearts on day 1 after ISO stimulation of *Nlrp3*$^{-/-}$ mice and their wild-type littermates (n=6). D, Wild type mice or *Il18*$^{-/-}$ mice were treated with a single 5 mg kg^{-1} dose of ISO, and heart galectin-3 content in hearts was measured on day 1. Western blot of NLRP3, IL-18 and galectin-3 (E), and ELISA of galectin-3 (F) in heart lysates on day 1 after ISO treatment of *Il18*$^{-/-}$ mice and their wild-type littermates (n=6). The expression of galectin-3 following ISO stimulation was measured on day 1 by immunohistochemistry staining of heart cross-sections of *Nlrp3*$^{-/-}$ mice (G) and in *Il18*$^{-/-}$ mice (H), and their wild-type littermates (n=6). Scale bar in G and H: 100 μm. WT, wild-type; Gal-3, galectin-3; CTRL, control. Data are presented as the mean±SEM. Welch's ANOVA with the Games-Howell post hoc test was used.

Cardiac inflammatory injuries induced by ISO are suppressed in *Lgals3*$^{-/-}$ mice

The function of galectin-3 was further investigated in *Lgals3*$^{-/-}$ mice (Figure 3A). Galectin-3 deletion did not block the increase of IL-18 content in the myocardium following ISO treatment (Figure 3A and B). However, upregulation of the inflammatory cytokines monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-5 (MCP-5), and IL-6 on day 1 after ISO stimulation both on mRNA and protein level was inhibited in *Lgals3*$^{-/-}$ mice compared with that in wild-type mice (Figure S5 in Supporting Information and Figure 3B). Isoproterenol-induced macrophage infiltration and cardiac fibrosis were reduced in *Lgals3*$^{-/-}$ mice (Figure 3C and D). The *E*/*E*′ ratio, which reflects diastolic function of the left ventricle, showed that ISO-induced cardiac diastolic function was inhibited in *Lgals3*$^{-/-}$ mice (Figure 3E and Table S3 in Supporting Information). These are consistent with the results from galectin-3 inhibitor (modified citrus pectin) pretreatment (Figure S6 and Table S4 in Supporting Information). To elucidate the functional effects of galectin-3, we treated macrophages, cardiomyocytes, and cardiac fibroblasts with mouse recombinant galectin-3 (1 ng mL^{-1}) (Figure S7A–C in Supporting Information). The mRNA levels of MCP-1 and IL-6 were increased in macrophages, cardiomyocytes, and cardiac fibroblasts 3 hours after the treatment (Figure S7D–F in Supporting Information). In addition, MCP-1 and IL-6 protein levels in culture media supernatants were increased 6 h after recombinant galectin-3 protein treatment (Figure S7G–I in Supporting Information).

Galectin-3 expression in macrophages is upregulated by cardiomyocyte-produced of IL-18 after ISO administration

To further investigate the cellular source of galectin-3, im-

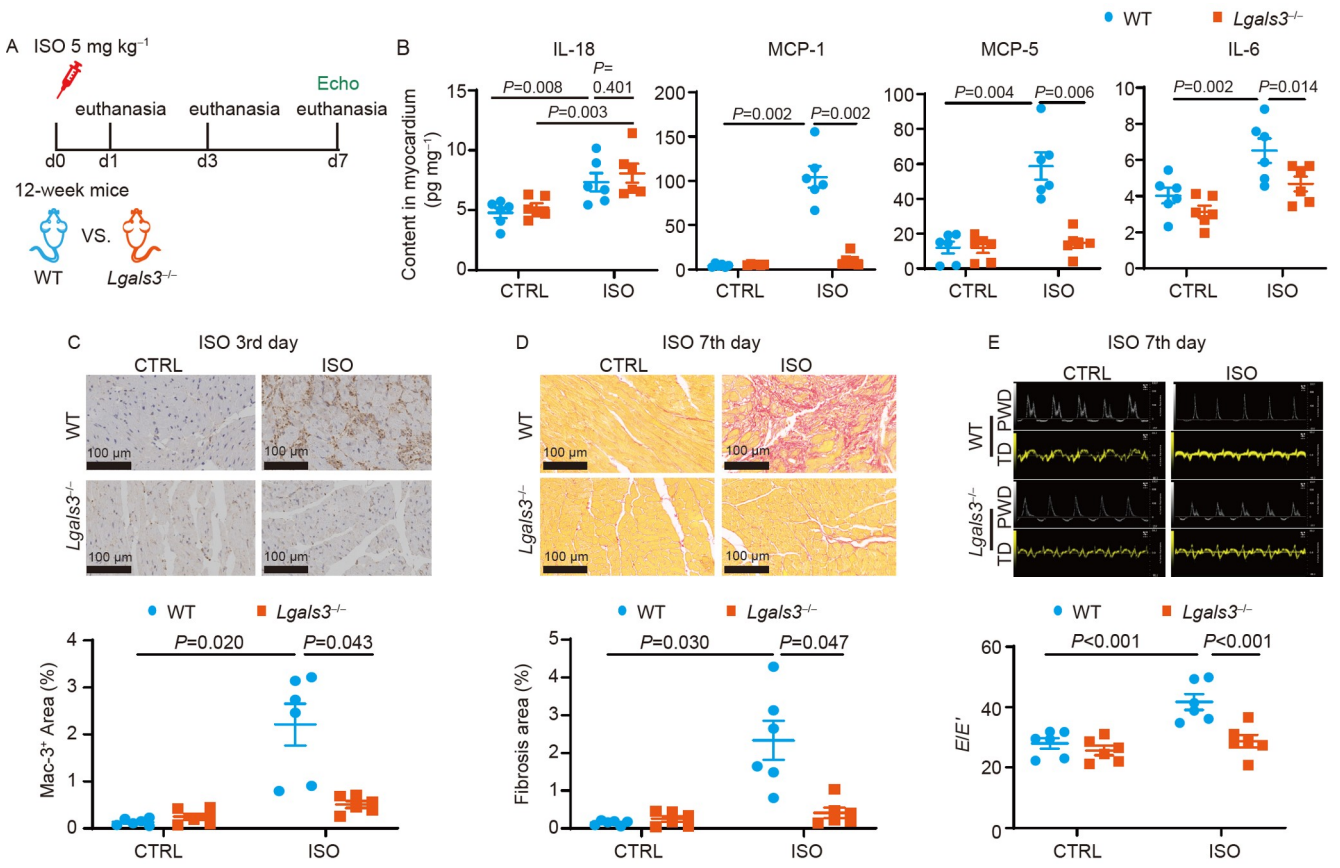

Figure 3 Isoproterenol-induced cardiac inflammatory injuries were suppressed in galectin-3 knockout mice. A, Wild type mice or *Lgals3*$^{-/-}$ mice were treated with a single 5 mg kg^{-1} dose of ISO, and heart galectin-3 content was measured on day 1. B, Myocardial IL-18, MCP-1, MCP-5, and IL-6 levels in *Lgals3*$^{-/-}$ and wild-type mice on day 1 after ISO treatment were measured using ELISA (n=6). C, Cardiac macrophage infiltration on day 3 following ISO treatment was detected using immunohistochemistry (n=6). D, Cardiac fibrosis on day 7 following ISO treatment was detected using picrosirius red staining (n=6). E, Cardiac function on day 7 after ISO treatment was measured using echocardiographic measurements, with E/E' index indicating cardiac diastolic function (n=6). Scale bars in (C) and (D): 100 μm. WT, wild-type; CTRL, control; Gal-3, galectin-3. Data are presented as the mean±SEM. One-way ANOVA with LSD post hoc test (B, first and fourth diagram and E) and Welch's ANOVA with Games-Howell post hoc test (B, second and third diagrams, C, and D) were used.

munofluorescence staining was performed to label galectin-3 in frozen heart cross-sections. The increased expression of galectin-3 following ISO treatment was mainly localized in macrophages as shown by colocalization with the macrophage marker CD68 (Figure 4A). Whereas, ISO (1 μmol L^{-1}) did not increase the expression or secretion of galectin-3 in cultured macrophages, isolated neonatal mouse cardiomyocytes or cardiac fibroblasts (Figure 4B and Figure S8 in Supporting Information). However, when cocultured with cardiomyocytes, macrophages showed elevated galectin-3 protein expression and secretion 6 h after ISO treatment (Figure 4C and Figure S9 in Supporting Information). Our previous study revealed that ISO can activate IL-18 production in cardiomyocytes (Xiao et al., 2018). Here we also found that IL-18 was not colocalized with cardiac fibroblasts or endothelial cells in heart tissue after ISO treatment (Figure S10 in Supporting Information). Therefore, we next investigate if cardiomyocyte-originated IL-18 promotes galectin-3 expression in macrophages. Inhibition of IL-18 in the coculture system with neutralizing antibodies (0.5 μg mL^{-1}) suppressed galectin-3 upregulation in macrophages and galectin-3 secretion into the co-culture media (Figure 5A). Interleukin-18-deficient cardiomyocytes did not show ISO-induced expression of galectin-3 in cocultured macrophages (Figure 5B). Furthermore, recombinant mouse IL-18 directly induced macrophage expression and secretion of galectin-3 (Figure 5C and D, Figure S11 in Supporting Information), but did not induce increased galectin-3 protein expression in cardiomyocytes (Figure 5D). Besides, Inhibiting NF-κb P65 phosphorylation or silencing P65 suppressed IL-18-induced galectin-3 expression (Figure 5E and F).

Galectin-3 inhibitor, but not β-AR antagonist, treatment on day 1 after ISO administration alleviated ISO-induced cardiac inflammation injuries

We investigated whether a galectin-3 inhibitor could

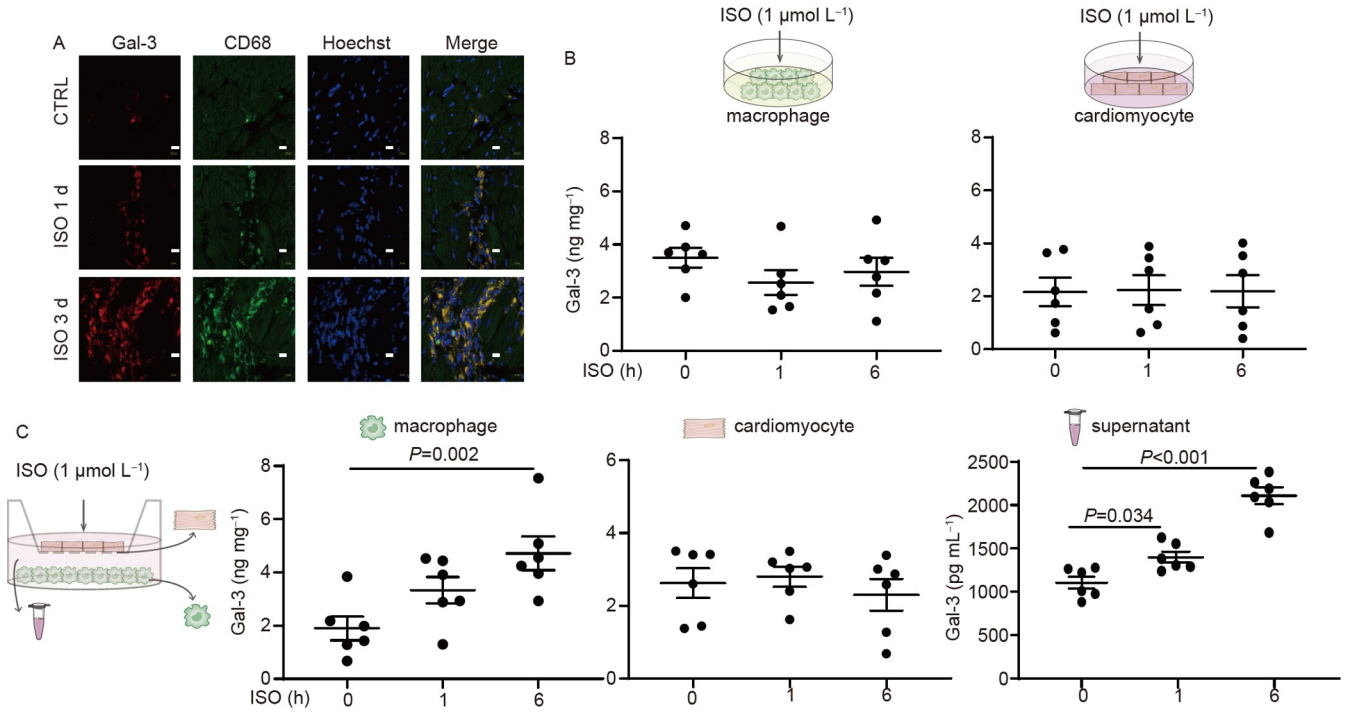

Figure 4 Isoproterenol increased galectin-3 expression only in macrophages cocultured with cardiomyocytes. A, The localization of galectin-3 (Gal-3, green) was evaluated in frozen heart tissue sections 1 and 3 days after ISO stimulation using immunofluorescence. The macrophages were labeled with an antibody against macrophage-specific CD68 (red). The nuclei were stained with Hoechst 33342 (blue). Scale bar, 10 μm. B, Galectin-3 content in isolated neonatal cardiomyocytes (n=6) and bone marrow-derived macrophages (BMDMs) (n=6) at 1 and 6 h after ISO administration was determined using ELISA. C, In a Transwell assay, isolated neonatal mouse cardiomyocytes were plated in the upper chamber and macrophages were plated in the lower chamber, and the two chambers shared the same culture medium. Galectin-3 content in cell lysates of macrophages and cardiomyocytes, and in cell culture supernatants was measured at 1 and 6 h using ELISA (n=6). Gal-3, galectin-3. Data are presented as the mean±SEM. One-way ANOVA with the LSD post hoc test was used.

alleviate cardiac inflammation and fibrosis after ISO injection, and compared the effects of galectin-3 inhibition with the effects of a non-selective β-AR antagonist, propranolol. We treated mice with a galectin-3 inhibitor (400 mg kg^{-1}) via intragastric administration, and propranolol (5 mg kg^{-1}) via intraperitoneal injection on day 1 after stimulation with ISO (Figure 6A). The galectin-3 inhibitor treatment inhibited ISO-induced expression of MCP-1 and IL-6 (Figure 6B). Although pretreatment with propranolol suppressed ISO-induced IL-18 and galectin-3 expression and cardiac inflammation (Figure S12 in Supporting Information), the treatment of propranolol one day after ISO administration did not inhibit increased expression of IL-18, galectin-3, MCP-1, and IL-6 (Figure 6C and Figure S13 in Supporting Information). The galectin-3 inhibitor treatment decreased macrophage infiltration (Figure 6D) and alleviated cardiac fibrosis (Figure 6E) and diastolic dysfunction (Figure 6F and Table S5 in Supporting Information). In contrast, the propranolol posttreatment did not decrease ISO-induced macrophage infiltration (Figure 6G), cardiac fibrosis (Figure 6H), or cardiac dysfunction (Figure 6I and Table S6 in Supporting Information).

DISCUSSION

The present study showed that galectin-3 was upregulated in macrophages by cardiomyocyte-derived IL-18 following acute sympathetic stress. As showed in Figure 7, upregulation of galectin-3 induced increased expression of chemokines and inflammatory cytokines in multiple cardiac cells, resulting in cardiac inflammation amplification and subsequent cardiac fibrosis. Treatment with a galectin-3 inhibitor, but not a β-blocker, one day after β-AR overactivation inhibited cardiac inflammation and fibrosis. These findings suggest that galectin-3 may play a critical role in acute stress-induced cardiac injury and have potential as a therapeutic target.

We showed that galectin-3 was upregulated by IL-18 upon acute sympathetic stress, which involves intercellular communication between cardiomyocytes and macrophages. Secretion of cytokines and interactions between cardiac cells have been observed previously in studies of cardiac inflammation and injury (Christia and Frangogiannis, 2013; Shen et al., 2020; Wu et al., 2019). Cardiomyocytes are the most susceptible cells in the heart to acute sympathetic stress, and proinflammatory signals are initiated in cardiomyocytes by activation of NLRP3 inflammasomes and IL-18

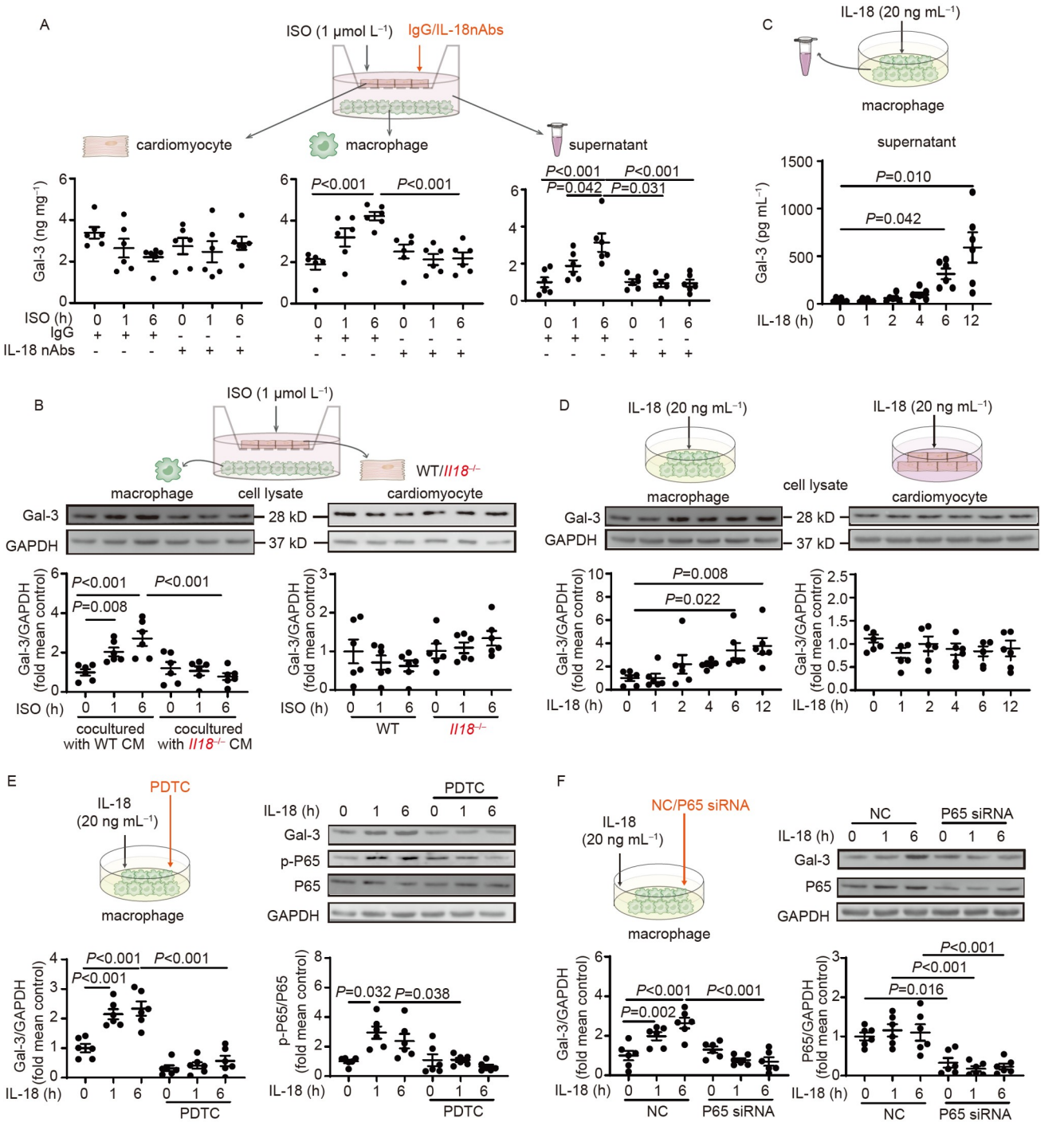

Figure 5 Cardiomyocyte-derived IL-18 is required for ISO-induced increases in galectin-3 expression in macrophages. A, An IL-18 neutralizing antibody (IL-18 nAbs, 0.5 μg mL^{-1}) was used to block the function of IL-18. Galectin-3 content in cell lysates or supernatants of cardiomyocytes and macrophages following ISO (1 μmol L^{-1}) treatment for 1 or 6 h, as determined using ELISA (n=6). B, *Il18*$^{-/-}$ cardiomyocytes were used to block the function of IL-18, and galectin-3 expression in cardiomyocytes and macrophages following ISO (1 μmol L^{-1}) treatment for 1 or 6 h was measured using western blotting (n=6). C, Galectin-3 levels in BMDM culture media following treatment with recombinant mouse IL-18 (20 ng mL^{-1}) (n=6). D, Western blot analysis of galectin-3 expression in BMDMs and neonatal cardiomyocytes following treatment with recombinant mouse IL-18 (20 ng mL^{-1}) at different time points (n=6). E, Western blot analysis of galectin-3, p-P65, and P65 in BMDMs pretreated with NF-κb P65 phosphorylation inhibitor (Pyrrolidinedithiocarbamate ammonium, PDTC; 10 μmol L^{-1}) for 30 min following treatment with recombinant mouse IL-18 (20 ng mL^{-1}) at different time points (n=6). F, NF-κb P65 in macrophages was knockdown by P65 siRNA. Galectin-3 and P65 expression in BMDMs was analyzed by western blotting following treatment with recombinant mouse IL-18 (20 ng·mL^{-1}) at different time points (n=6). Gal-3, galectin-3; WT, wild-type; NC, negative control. Data are presented as the mean±SEM. One-way ANOVA with an LSD post hoc test (A right diagram, B, E left diagram and F left diagram), Welch's ANOVA with the Games-Howell post hoc test (D right diagram, E right diagram and F right diagram), and Kruskal-Wallis ANOVA combined with post hoc Dunn's multiple comparison test (A left diagram, A middle diagram, C and D left diagram) were used.

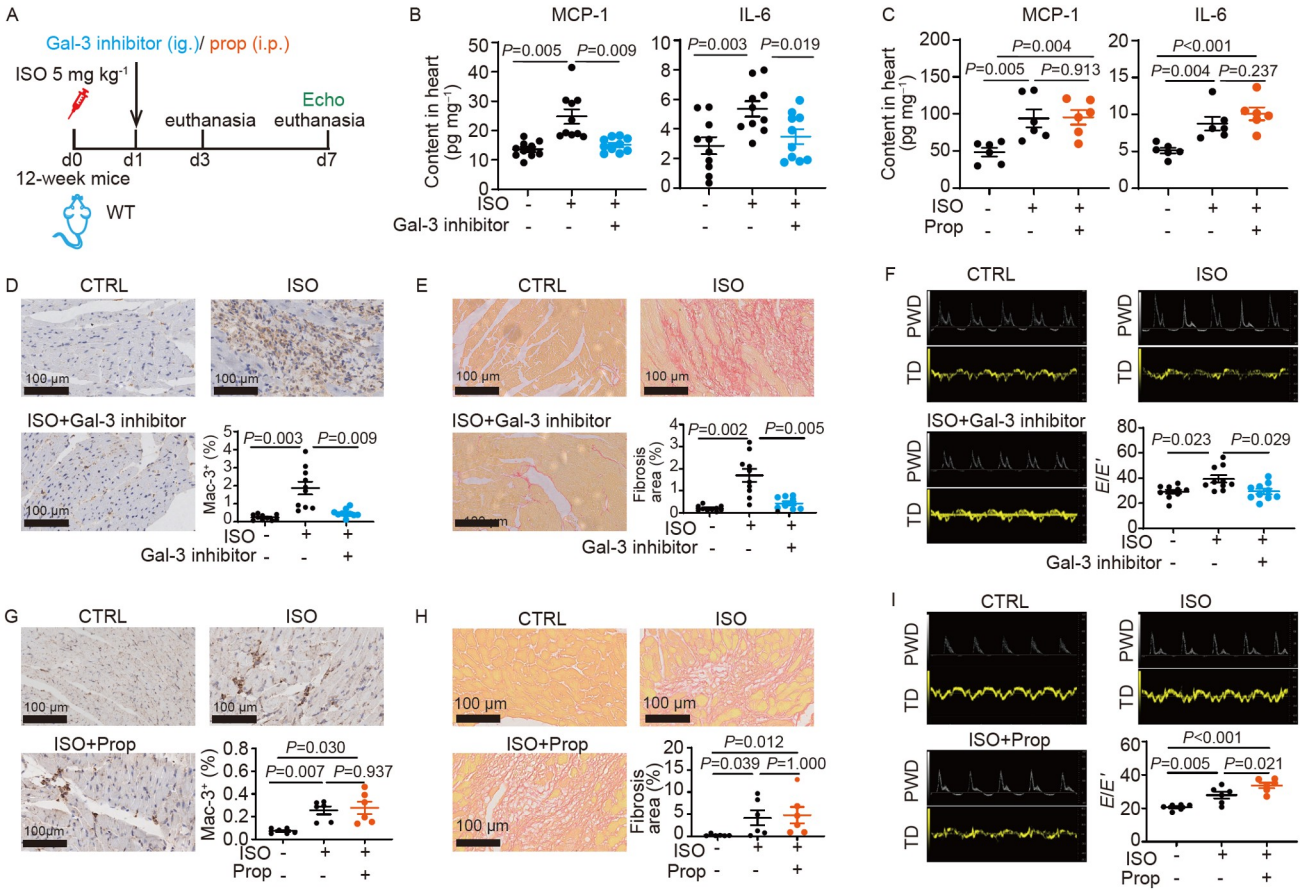

Figure 6 A galectin-3 inhibitor, but not a β-AR antagonist, alleviated ISO-induced cardiac inflammatory injury. A, A galectin-3 inhibitor or the β-AR antagonist propranolol (Prop) was given one day after ISO injection, and mice were sacrificed on day 3 or day 7. MCP-1 and IL-6 levels were detected in mouse hearts after galectin-3 inhibitor treatment (B, n=10) or Prop treatment (C, n=6) using ELISA. Cardiac macrophage infiltration on day 3 (D), and cardiac fibrosis (E) and cardiac function (F) on day 7 after ISO treatment were determined in mice treated with or without Gal-3 inhibitor (n=10). Cardiac macrophage infiltration (G), cardiac fibrosis (H), and cardiac function (I) were determined in mice treated with or without Prop (n=6). Scale bar in D, E, G, and H: 100 μm. Gal-3, galectin-3; i.g., intragastric; i.p., intraperitoneal; CTRL, control. Data are presented as the mean±SEM. One-way ANOVA with LSD (B right diagrams, C, and I), Welch's ANOVA with Games-Howell post hoc test (B left diagrams, D, E, and, G), and Kruskal-Wallis ANOVA combined with post hoc Dunn's multiple comparison test (F and H) were used.

as shown in our previous study (Xiao et al., 2018). Cardiomyocyte-derived IL-18 induces increased secretion of MCP-1 and MCP-5 from fibroblasts, promoting macrophage infiltration (Xiao et al., 2018). We showed in the present study that activated IL-18 resulted in prolonged increased expression of galectin-3 in macrophages in the heart. Upregulation of galectin-3 in macrophages was further verified in cultured macrophages following treatment with IL-18 or in macrophages co-cultured with cardiomyocytes. Moreover, ISO-induced upregulation of galectin-3 was inhibited in NLRP3- or IL-18-deficient mice *in vivo*. These findings suggest that macrophages are the targets of cardiomyocyte-derived IL-18, and the resulting sustained upregulation of galectin-3 promoted cardiac inflammation.

Galectin-3 induced the expression of inflammatory cytokines in cardiomyocytes, cardiac fibroblasts, and macrophages (Figure S7). This multi-target effect of galectin-3 is expected to enhance its role in promoting cardiac inflammation. Galectin-3 induced secretion of MCP-1 and IL-6 from various cell types, contributing to cardiac inflammation, fibrosis, and dysfunction. This is consistent with previous studies on the effect of galectin-3 on human monocytes (Sano et al., 2000), glial cells (Burguillos et al., 2015; Jeon et al., 2010), and synovial fibroblasts (Arad et al., 2015). By binding to IFN-γ receptor 1, TLR4 or CD98 (Cheng et al., 2022) and activating inflammatory signaling pathway, galectin-3 could induce inflammatory factor secretion in different kinds of cells. In addition, galectin-3 also exerts a profibrotic effect. Cardiac fibroblasts differentiation into myofibroblasts is a crucial cause of cardiac fibrosis, which eventually leads to heart failure (Li et al., 2021). Galectin-3 can facilitate the transition of fibroblasts into myofibroblasts, and induces production of matrix proteins such as collagen and contribute to collagen maturation and cross-linking (de Boer et al., 2014; Henderson et al., 2006; Sharma et al., 2004; Yu et al., 2013). Thus, galectin-3 plays an important role in acute stress-induced cardiac injury via its multifunctional effects on various cell types.

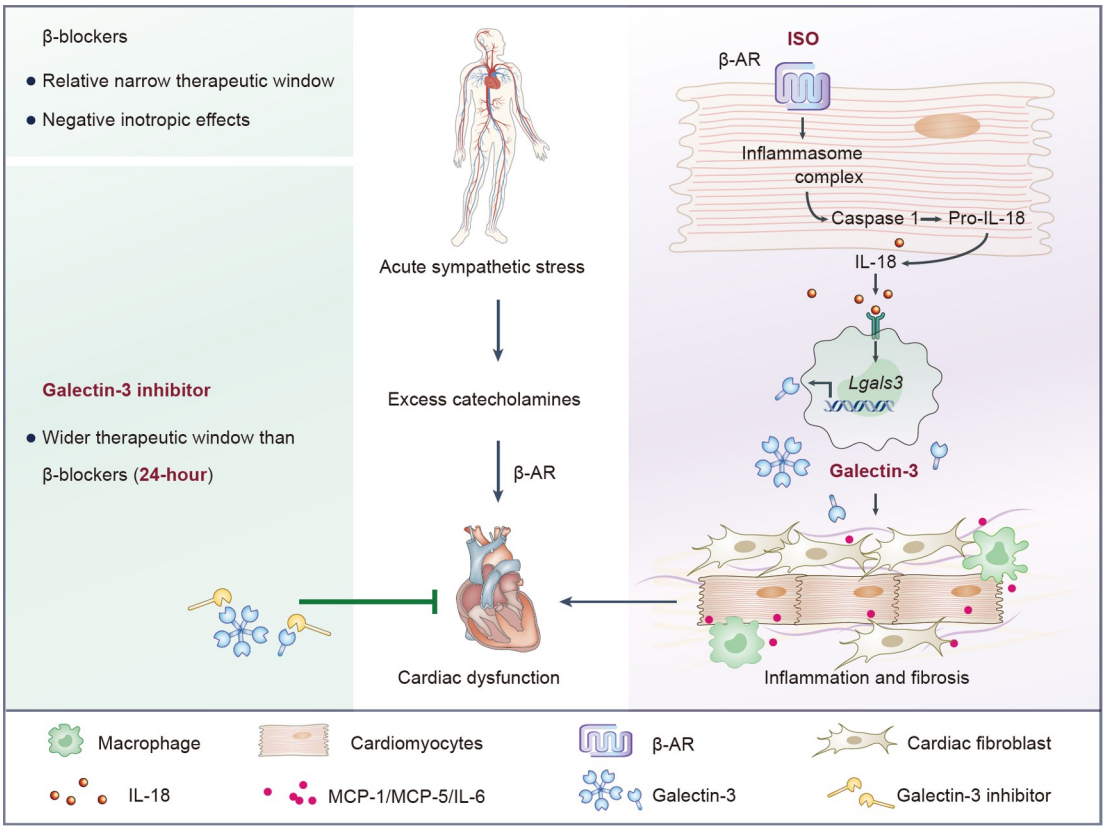

Figure 7 Illustration showing galectin-3-centered paracrine network mediates cardiac inflammation and fibrosis upon β-adrenergic insult. Galectin-3 upregulated both in patients and mice upon acute sympathetic overactivation. IL-18/galectin-3 axis mediates the cardiac inflammatory injuries induced by acute β-adrenergic receptor activation. Galectin-3 inhibitor, but not a β-blocker, treatment one day after β-AR insult can successfully block cardiac inflammatory injuries upon acute β-adrenergic receptor overactivation.

We characterized the temporal expression profiles of IL-18 and galectin-3 in the heart following a single ISO injection. The upregulation of galectin-3 expression peaked on day 1 following treatment with a β-adrenergic receptor agonist. Galectin-3 was upregulated subsequent to increase in IL-18 expression, which occurred within the first hour. This time course was consistent with the notion that galectin-3 is a critical molecule in the later amplification of cardiac inflammation. Indeed, galectin-3 deficiency blocked ISO-induced cardiac inflammation, but showed little effect on IL-18. Following acute β-AR activation, the peak of IL-18 expression was transient and decreased quickly after the first hour, whereas increased expression of galectin-3 was sustained from day 1 to day 3. The early activation of IL-18 may result in sustained proinflammatory signaling in the heart through promoting sustained expression of galactin-3. A positive correlation between IL-18 and galectin-3 was observed in the plasma of patients with UA and NSTEMI, which are often accompanied by acute sympathetic stress. Higher galectin-3 levels are associated with poor cardiac function, supporting our hypothesis that the galectin-3 contributes to a prolonged effect on cardiac dysfunction.

The galectin-3 is a potential target for therapeutic intervention of acute stress-induced cardiac injury. Therefore, we investigated the effect of blockade of galectin-3, and β-AR in mouse models. Prolonged expression of galectin-3 provides a target for therapeutic intervention at a later timepoint. Treatment with a galectin-3 inhibitor on day 1 following ISO treatment alleviated subsequent cardiac inflammation, fibrosis, and dysfunction. Pretreatment with propranolol to block β-AR inhibited acute ISO-induced cardiac injury (Figure S12 in Supporting Information). However, treatment with propranolol on day 1 after ISO stimulation failed to inhibit cardiac inflammation, fibrosis, or dysfunction. These results suggest that acute stress-induced cardiac injury is more dependent on galectin-3 increase than on sustained activation of β-AR. Beta blockers are routinely prescribed to patients with heart failure or myocardial infarction (Joseph et al., 2019). Here the patients undergoing β blocker treatment had lower galectin-3 content and improved cardiac function (Figure 1G and H), which is consistent with the results of β blockers pretreatment in the ISO-induced mouse model (Figure S12 in Supporting Information). Our research revealed that the administration of β-blocker 1 day after ISO treatment could not alleviate ISO-induced cardiac inflammation and fibrosis. Similarly, post-stress β-blocker treatment (on day 1 or 2) has a limited beneficial effect on in-hospital mortality in patients with SIC (Isogai et al., 2016).

Use of β-blockers at hospital discharge did not show a beneficial effect on 1-year survival in patients with SIC in a large-scale multinational study (Templin et al., 2015). Our findings provide a new option for the post-stress treatment of acute stress-induced cardiac injury.

Acute stress is a risk factor for many cardiovascular diseases, such as myocardial infarction. Both the NLRP3 inflammasome (Duewell et al., 2010; Xiao et al., 2013) and galectin-3 (MacKinnon et al., 2013; Nachtigal et al., 2008) are involved in the pathogenesis of atherosclerosis, a major cause of myocardial infarction. The NLRP3 inflammasome is also activated in the hearts of mice following acute myocardial infarction (Mezzaroma et al., 2011). Plasma galectin-3 was reported to predict clinical outcomes in patients with ST-segment elevation (STEMI) and NSTEMI, including all-cause mortality and major adverse cardiac and cerebrovascular events (Obeid et al., 2020). Interestingly, high-dose ISO can be used to construct a mouse model of myocardial ischemia. Following treatment with high-dose ISO, ischemia-induced left ventricular systolic dysfunction and fibrosis were alleviated by galectin-3 inhibition (Vergaro et al., 2016). The present study showed a positive association between galectin-3 and NE or IL-18 in patients with UA and NSTEMI. These findings suggest the potential role of IL-18 and galectin-3 in myocardial infarction.

Sympatho-adrenergic activation in chronic conditions causes deleterious cardiac remodeling, including hypertrophy, fibrosis, cardiac dysfunction, and eventual heart failure (Li et al., 2014). The NLRP3 inflammasome (Wu et al., 2021) and galectin-3 (Chen et al., 2020; Rabkin and Tang, 2021) play important roles in development of heart failure. Galectin-3 was previously evaluated in a study of sustained ISO treatment with an osmotic minipump in which expression of galectin-3 was increased in hearts via activation of the Hippo pathway (Zhao et al., 2019). Sustained ISO treatment-induced expression of inflammatory cytokines and cardiac fibrosis-related genes, and cardiac systolic dysfunction were inhibited in galectin-3 knock-out mice (Zhao et al., 2019). Both β_1-AR and β_2-AR mediated upregulation of galectin-3 in the hearts of mice administered ISO using an osmotic minipump (Zhao et al., 2019). Neither galectin-3 inhibitors nor galectin-3 deletion alleviated cardiac fibrosis or dysfunction in β_2-AR transgenic mice (Nguyen et al., 2018b), which indicated that chronic β_2-AR activation-induced cardiac fibrosis and dysfunction was independent of galectin-3. During acute sympathetic stress, ISO-induced NLRP3 inflammasome activation and IL-18 release were mainly mediated by β_1-AR but not β_2-AR (Xiao et al., 2018), which suggests that the increased galectin-3 response to acute stress is mediated by acute β_1-AR activation. The β_1-AR-mediated IL-18 activation may also contribute to chronic sympathetic stress-induced galectin-3 expression and cardiac inflammation, but the exact effect requires further investigation.

In conclusion, following acute sympathetic activation, galectin-3 was upregulated in macrophages by cardiomyocyte-derived IL-18, and promoted cardiac inflammation cascades. Galectin-3 mediated cardiac inflammatory amplification, and is a potential therapeutic target to intervene following acute sympatho-β-AR activation. Treatment with a galectin-3 inhibitor allows wider therapeutic window than β-blockers and is a potential therapeutic candidate for cardiac diseases involving β-adrenergic toxicity.

MATERIALS AND METHODS

Human blood sample collection

Human blood sample collection was approved by the Human Ethics Committee, Peking University Third Hospital (2014177, LM2021284). The study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. Written consent was obtained from the participants or their guardians.

Plasma samples were prepared from the initial admission blood draws of those patients who attended the Department of Cardiology, Peking University Third Hospital within one day after the onset of chest pain. Patients diagnosed with NSTEMI and UA were included according to the ESC Guidelines for management of ACS (Roffi et al., 2016). Patients with one or more of the following criteria were excluded: prior surgery or trauma within one month prior to admission; autoimmune disease; severe infection; and malignancy. Eighteen patients with UA, 21 patients with NSTEMI were included in this study. Norepinephrine, IL-18, and galectin-3 plasma levels were determined using ELISA kits (Norepinephrine, IBL International, Hamburg, Germany; IL-18, MBL, Nagoya, Japan; Galectin-3, Abcam, Cambridge, UK).

Animal and ethics statement

All animal care and experimental procedures complied with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health and were approved by the Committee of Peking University on Ethics of Animal Experiments (LA 2018-112). Male C57BL/6N mice at 12 weeks of age, weighing 28–30 g, were purchased from the department of laboratory animal science, Peking University Health Science Center (Beijing, China). Male NLRP3 knockout mice (*Nlrp3*$^{-/-}$, C57BL/6J background), IL-18 knockout mice (*Il18*$^{-/-}$, C57BL/6 background), and Galectin-3 knockout mice (*Lgals3*$^{-/-}$, C57BL/6 background) at 12 weeks of age, weighing 28–30 g, were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). A maximum of five mice were housed in a single cage. All mice were housed in a specific pathogen-free environment under a 12:12 h light-dark cycle and fed rodent diet ad libitum.

Mouse models of acute β-AR activation

The acute β-AR activation models used in this study were described previously (Xiao et al., 2018). Male 12-week-old C57BL/6N mice were randomly divided into seven groups and were subcutaneously injected with a single dose of isoproterenol (ISO, a selective agonist of β-AR; 5 mg kg^{-1} body weight) to mimic acute sympathetic activation at different time points (1, 6, and 12 h, and 1, 3, and 7 days) (n=6). Male 12-week-old male *Nlrp3*$^{-/-}$, *Il18*$^{-/-}$ and *Lgals3*$^{-/-}$ mice and their littermates were divided into different groups and injected with a single dose of ISO (5 mg kg^{-1} body weight) subcutaneously at different time points (1, 3, and 7 days) (n=6).

Modified citrus pectin administration after ISO treatment

A galectin-3 inhibitor, modified citrus pectin (Allergy Research Group, Alameda, USA; 400 mg kg^{-1} body weight) dissolved in saline, was given at a single dose of 400 mg kg^{-1} body weight by intragastric administration one day after ISO treatment. The hearts were collected on day 3 or 7 following ISO treatment.

Propranolol administration after ISO treatment

One day after ISO treatment, 5 mg kg^{-1} of propranolol (Sigma-Aldrich, St. Louis, USA) was given via intraperitoneal injection. The mice were sacrificed with an overdose of sodium pentobarbital, and the hearts were collected on day 3 or day 7 following ISO treatment.

Data and statistical analysis

Data are represented as the mean±SEM, and data analyses were performed by individuals blinded to experimental conditions. Sample size estimation was performed using PS Power and Sample Size Calculations program version 3.0. Statistical analyses were performed using SPSS 23.0 software (IBM SPSS Statistics for Windows, Armonk, USA). For parametric data, Student's t-test (two groups) or one-way ANOVA (three or more groups) was used to analyze the differences among groups if the data were normally distributed. In addition, LSD post hoc test was conducted only when F-test resulted in P<0.05 and there was no significant variance in homogeneity. For data from more than 2 groups with unequal variances, Welch's ANOVA with Games-Howell post hoc test was performed. For nonparametric data, Mann-Whitney U test with exact method (two groups) or Kruskal-Wallis ANOVA combined with post hoc Dunn's multiple comparison test (three or more groups) was performed.

Data and materials availability statement

All data associated with this study are present in the paper or the Supplementary Materials. Any other relevant data are available from the corresponding author upon reasonable request.

Compliance and ethics *Dr. Marschall Runge is a member of the Board of Directors at Eli Lilly and Company. The other authors declare no conflict of interest.*

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SUPPORTING INFORMATION

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