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β-arrestin 2 attenuates cardiac dysfunction in polymicrobial sepsis through gp130 and p38

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ABSTRACT

Sepsis is an exaggerated systemic inflammatory response to persistent bacteria infection with high morbidity and mortality rate clinically. β-arrestin 2 modulates cell survival and cell death in different systems. However, the effect of β-arrestin 2 on sepsis-induced cardiac dysfunction is not yet known. Here, we show that β-arrestin 2 overexpression significantly enhances animal survival following cecal ligation and puncture (CLP)-induced sepsis. Importantly, overexpression of β-arrestin 2 in mice prevents CLP-induced cardiac dysfunction. Also, β-arrestin 2 overexpression dramatically attenuates CLP-induced myocardial gp130 and p38 mitogen-activated protein kinase (MAPK) phosphorylation levels following CLP. Therefore, β-arrestin 2 prevents CLP-induced cardiac dysfunction through gp130 and p38. These results suggest that modulation of β-arrestin 2 might provide a novel therapeutic approach to prevent cardiac dysfunction in patients with sepsis.

1. Introduction

Sepsis, a significant clinical problem, is one of the leading causes of death in intensive care units throughout the world [1]. Sepsis is the No.1 cause of morbidity and mortality in intensive care units (ICUs), and about 60% of patients admitted to the ICU have cardiac dysfunction [2–4]. When accompanied by heart dysfunction, survival for sepsis is only 30% [1,2]. An average of 7.5 million incidences of severe sepsis are recorded in the United States yearly, and the number is rising at a steady rate. The prognosis of sepsis is different from person to person. However, the mortality rate is nearly 40% in an advanced aged patient under severe sepsis in spite of aggressive treatment [1,2]. Cardiac dysfunction plays a critical role in the high morbidity and mortality of this condition [2–4]. Therefore, it is urgent to elucidate the mechanisms by which sepsis modulates cardiac dysfunction and generate more efficient ways to improve the prognosis.

β-arrestin 2, a universally expressed member of arrestin family in many tissues with especially high expression in nervous and cardiovascular tissues [5–7], is an essential negative regulator of the G-protein-coupled receptor (GPCR) signaling [5,7–9]. β-arrestin 2 not only facilitates G-protein associated 7 TMR desensitization/internalization but also mediates intracellular signal transduction independently [5,9]. In addition to these established functions, β-arrestin 2 increasingly represents an active line of investigation where β-arrestin 2 binds with various target molecules and thus modulates a broad range of biological processes [10–12]. Recent evidence has shown that β-arrestin 2 is functionally involved in the regulation of immune responses by modulating various signaling pathways [11,12]. β-arrestin 2 stimulation protects against acute cardiac injury [13,14]. However, the effect of β-arrestin 2 on cardiac function during sepsis is not yet known.

The affinity between β-arrestin 2 and mitogen-activated protein kinases (MAPKs) exhibited in numerous cases of GPCR signaling [15–18]. Others and we recently reported that β-arrestin 2 scaffolds MAPK components such as the MAP kinases extracellular-signal regulated kinase (ERK) and c-Jun-N-terminal kinase (JNK), leading to phosphorylation, activation and accumulation of MAPKs in defined cellular compartments [15,18]. To examine the mechanisms by which β-arrestin 2 modulates cardiac functions, we focused on investigation of β-arrestin 2 to regulate glycoprotein 130 (gp130) and p38 MAPK signaling during sepsis.

In the present study, we demonstrated that overexpression of β-arrestin 2 enhances survival and attenuates cardiac dysfunction in septic mice. Additionally, β-arrestin 2 overexpression prevents elevated levels of myocardial gp130 and p38 MAPK phosphorylation in polymicrobial sepsis.

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2. Materials and methods

2.1. Experimental animals

Wild-type (WT) C57BL/6J mice were ordered from Jackson Laboratory (Bar Harbor, ME). β-arrestin 2 knockout (KO) mice on a C57BL/6 background were kindly provided by Dr. Robert Lefkowitz (Duke University) and bred at East Tennessee State University (ETSU) [18]. β-arrestin 2 over-expression (TG) mice were generated as previously described [19]. Briefly, full-length human β-arrestin 2 cDNA from brain cDNA/λphage library was cloned into pcDNA3 (BamHI-EcoRI) with HA tag (HindIII-BamHI) under the control of a human cytomegalovirus (CMV) promoter. Then the DNA constructions were injected into fertilized mice eggs with the C57BL/6 J background. The integration of variable copies of a transgene into the genomes of founder mice and their offspring was verified. Real-time PCR analysis was used to check the mRNA expression of the transgene. The genomic DNA primers used to identify transgenic mice were β-arrestin 2, sense 5′-CAGCCAGGACCAGAGGACA-3′, antisense 5′-TGATAAGCCGCACAGAGTT-3′. There is no difference between physical appearance, activity, productivity and life span in WT, β-arrestin 2 KO, and β-arrestin 2 TG mice. Male and female mice aged 11–12-week were used in survival and Western Blot analysis. Only male mice were utilized in cardiac function analysis. All mice were maintained in the Division of Laboratory Animal Resources at ETSU, a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). The ETSU Committee on Animal Care approved all animal studies.

2.2. Cecal ligation and puncture (CLP) polymicrobial sepsis

CLP was performed to induce sepsis in mice as described in our previous studies [20]. Briefly, mice were initially anesthetized by 5.0% isoflurane inhalation in 100% O_2 in a closed chamber and then maintained by 3% isoflurane inhalation during surgery. A small incision was made in the anterior abdomen, and the cecum was ligated 1 cm proximal to the terminal of cecum with a size 2-0 sutures. The cecal puncture was done with a 20-gauge needle and the content was extruded from two holes. The abdomen was then closed layer by layer. Mice without ligation and puncture were served as control. Immediately following CLP or sham surgery, 40 ml/kg pre-warmed saline was administrated by intraperitoneal injection.

2.3. Cardiac functional analysis

Cardiac function was detected by use of the SPR-839 instrument (Millar Instruments, Houston, TX, USA) as described previously by us [21]. Briefly, a microtip pressure–volume catheter (SPR-839; Millar Instruments, Houston, TX, USA) was inserted through a 25-gauge apical stab into the LV to measure the steady-state cardiac function. At the completion of the study, 10 μL of hypertonic saline (15%) was injected into the right atrium to calibrate Vp, the parallel volume. The signals were continuously recorded at a sampling rate of 1000 s^{-1} using an ARIA pressure–volume conductance system (Millar Instruments) coupled to a Powerlab/4SP A/D converter (AD Instruments, Mountain View, CA, USA). All pressure–volume loop data were analyzed with a cardiac pressure–volume analysis program (PVAN3.4; Millar Instruments). At the end of the functional analysis, the hearts were removed and perfused for 2 min as Langendorff preparations to remove the remaining blood before Western blot analysis.

2.4. Western blot analysis

Western blot analysis was performed according to established protocols [18,22]. Briefly, proteins extracted from heart tissue lysis were loaded to 10–15% SDS-PAGE, and then transferred to a nitrocellulose membrane (Bio-Rad). The blocking solution was composed of 3% BSA dissolved in 1 × TBS; blocking the membrane for 1 h at room temperature. The membrane was incubated for 2 h at room temperature in primary antibody and 1 h in secondary antibody, both in 1.5% BSA dissolved in 1 × TBS. The signals were detected with the ECL system (Amersham Biosciences), and the signals were quantified by scanning densitometry and computer-assisted image analysis. Pan p38, phospho-p38, pan ERK, phospho-ERK, pan JNK, phospho-JNK, pan STAT3, and phospho-STAT3 antibodies were from Cell Signaling Technology (Beverly, MA). Pan gp130, phospho-gp130, and GAPDH antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

2.5. Statistical analysis

Comparisons of data from multiple groups were carried out using one-way analysis of variance and Newman-Keuls multiple comparison tests. Means were compared by Student's *t*-test between two groups. All data were expressed as mean ± SEM. The Kaplan-Meier method was used to generate the survival curves, and the significance of differences was ascertained using the Log-rank (Mantel-Cox) test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Overexpression of β-arrestin 2 in mice enhances animal survival following CLP

First, we investigated the effect of the multifunctional protein β-arrestin 2 [5,18] on animal survival after sepsis. WT, β-arrestin 2 TG, and β-arrestin 2 KO mice were subjected to CLP, and mortality were monitored for 120 h. As shown in Fig. 1, death occurred with the highest frequency 18–24 h after sepsis. The survival rate 24 h after CLP was 40% for WT mice, 80% for β-arrestin 2 TG mice, and 13.3% for β-arrestin 2 KO mice. At the end of the observation period, the survival rates were 20% in WT, 53.3% in TG, and 6.7% in β-arrestin 2 KO group. There were no deaths in sham control mice (data not shown). These results suggest that β-arrestin 2 contributes to animal survival following CLP.

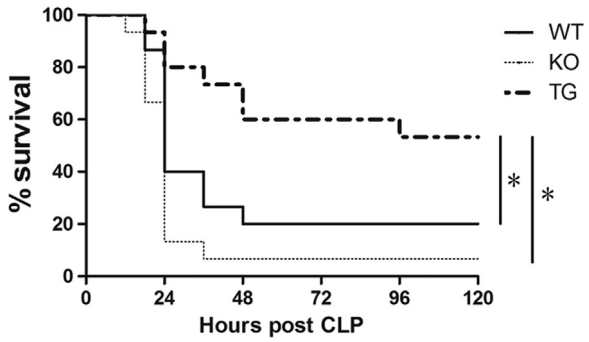

Fig. 1. β-arrestin 2 TG mice are less susceptible to CLP-induced polymicrobial sepsis. WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (N = 15 per group) were subjected to CLP and monitored up to 120 h. *$P < 0.05$.

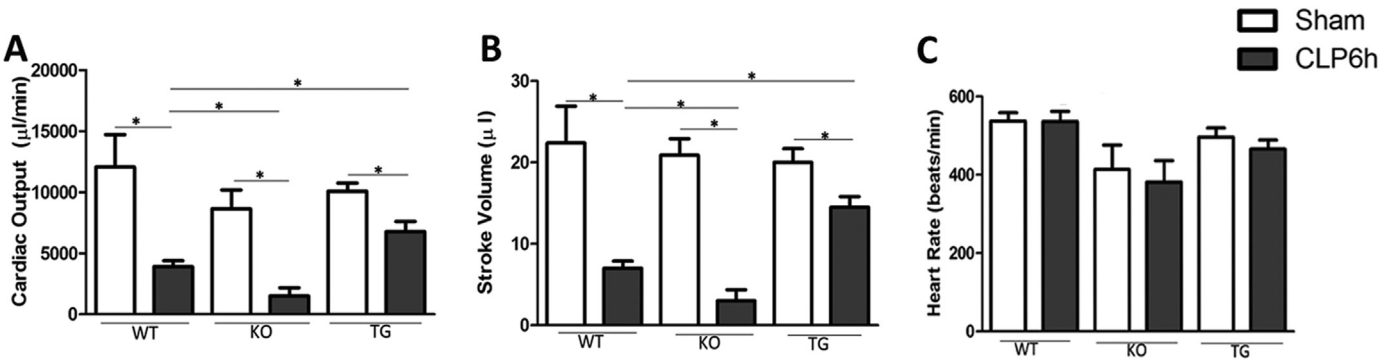

Fig. 2. Overexpression of β-arrestin 2 in mice attenuates CLP-reduced cardiac output and stroke volume. We subjected WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (N=6 per group) to CLP or sham operations. At 6 h CLP, hemodynamic parameters were measured by cardiac functional analysis. (A) CO, cardiac output. (B) SV, stroke volume. (C) HR, heart rate. CO, SV, and HR from KO and TG group after CLP and sham treatment were compared to WT CLP group. $^{*}P < 0.01$.

3.2. *β-arrestin 2 overexpression attenuates cardiac dysfunction induced by CLP*

3.2.1. *Overexpression of β-arrestin 2 diminishes CLP-reduced cardiac output and stroke volume*

Very recently, it has been shown that sepsis induces cardiac dysfunction [23]. However, it is not known whether β-arrestin 2 plays a role in sepsis-induced cardiac dysfunction. To evaluate the effect of β-arrestin 2 on cardiac function following sepsis, we collected hemodynamics parameters by pressure-volume loop measurement 6 h after sepsis in WT, β-arrestin 2 KO, and β-arrestin 2 TG mice. As shown in Fig. 2(A), 67% of cardiac output was preserved in β-arrestin 2 TG mice while 32% was preserved in WT and 17% was preserved in β-arrestin 2 KO mice. We found the similar results in stroke volume (Fig. 2(B)). However, the CLP had no significant effect on mice heart rate (HR), except a slight decrease in β-arrestin 2 KO group (Fig. 2(C)). Therefore, HR was most likely not the main contributor to the decreased cardiac output in CLP groups. The similar results were observed by echocardiography analysis (data not shown). Taken together, overexpression of β-arrestin 2 attenuates sepsis-reduced cardiac output and stroke volume.

3.2.2. *β-arrestin 2 overexpression attenuates CLP-reduced end diastolic volume (EDV)*

End-diastolic volume (EDV) represents the extent of ventricular filling in sepsis-induced cardiac dysfunction. EDV decreased by 39.6% and 49.5% in WT and β-arrestin 2 KO mice after CLP (Fig. 3 (A)), respectively. Importantly, EDV decreased by only 16.6% in β-arrestin 2 TG mice. Therefore, β-arrestin 2 overexpression significantly blocks sepsis-reduced EDV. Neither sepsis nor β-arrestin 2 had an effect on LV end-systolic volume (ESV) (Fig. 3(B)). The similar results were obtained by echocardiography analysis (data not shown).

3.2.3. *Overexpression of β-arrestin 2 enhances left ventricular contractility following CLP*

We then measured left ventricle pressure-related parameters after sepsis in WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (Table 1). End systolic pressure (ESP) was severely reduced in β-arrestin 2 KO mice after sepsis (36 mmHg) as compared to sham mice (71 mmHg). In contrast, ESP was slightly increased in septic WT mice (105 mmHg) and maintained in β-arrestin 2 TG mice (90 mmHg). However, the end diastolic pressure (EDP) was not changed in CLP treated groups. In addition, β-arrestin 2 TG mice showed less decrease in dP/dt_{max} and dP/dt_{min} after sepsis (decrease by 15% and 5%, respectively) compared to WT mice (decrease by 37% and 29%, respectively) and β-arrestin 2 KO mice (decrease by 70% and 72% respectively).

3.3. *Increased β-arrestin 2 expression in septic heart*

To investigate the effect of β-arrestin 2 on septic heart, we first examined the expression level of β-arrestin 2 in heart tissue after sepsis. Although elevated cardiac β-arrestin 2 expression was observed in both WT and β-arrestin 2 TG mice at 6 h after sepsis, β-arrestin 2 expression was still higher in TG mice (Fig. 4). The interference of β-arrestin 2 expression from non-residential cells (blood cells, macrophages) in the heart was minimized by sufficient saline rinse before and after tissue harvest.

3.4. *Effect of β-arrestin 2 on the levels of phospho-gp130 and phospho-p38 MAPK following CLP*

Glycoprotein 130 (gp130), a common part of membrane-bound receptor for IL-6 family, and an essential signal transducer, has been considered to be involved in sepsis [24]. Hence, we studied gp130 activation in the myocardium of β-arrestin 2 KO and

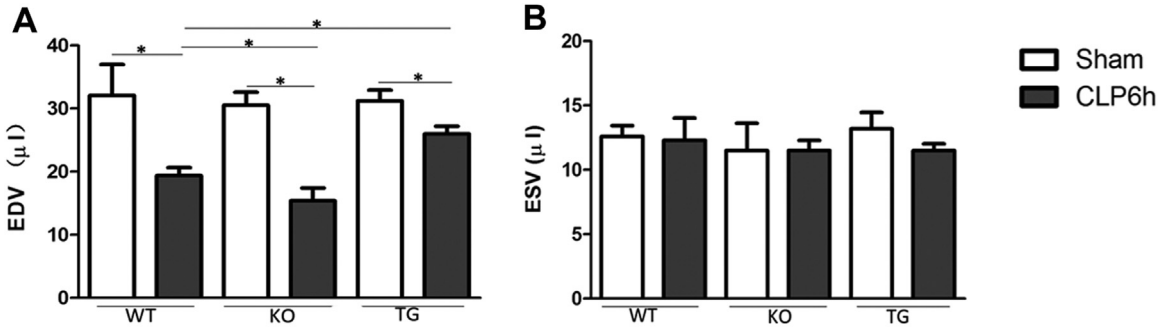

Fig. 3. β-arrestin 2 overexpression in mice diminishes CLP-reduced end diastolic volume (EDV). WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (N=6 per group) were subjected to CLP or sham operations. Hemodynamic parameters were determined by cardiac functional analysis 6 h after CLP as in Fig. 2. (A) EDV, LV end-diastolic volume. (B) ESV, LV end-systolic volume. EDV and ESV from KO and TG group after CLP and sham treatment were compared to WT CLP group. $^{*}P < 0.05$.

Table 1
Cardiac systolic and diastolic functions 6 h after cecal ligation and puncture.

Parameter	WT		KO		TG	
(P–V loop)	Sham	CLP	Sham	CLP	Sham	CLP
EF, (%)	66 ± 4.4	37 ± 6.5*	66 ± 3.8	25 ± 5.8‡,†	63 ± 3.6	56 ± 2.9§,†
ESP, mmHg	91 ± 7.2	105 ± 8.9*	71 ± 10.6*	36 ± 8.2‡,†	97 ± 11.1	90 ± 4.3†
EDP, mmHg	7 ± 3.0	4 ± 2.2	6 ± 1.7	7 ± 3.5	6 ± 2.3	6 ± 2.8
LVDevP, mmHg	92 ± 8.3	104 ± 7.8	75 ± 7.5*	34 ± 9.7‡,†	97 ± 9.3	89 ± 7.6†
dP/dt_{max}, mmHg/s	10209 ± 2342	6393 ± 469*	5647 ± 1297*	1702 ± 377‡,†	9782 ± 1333	8320 ± 1311†
dP/dt_{min}, mmHg/s	9143 ± 1200	6524 ± 1107*	4713 ± 934*	1310 ± 367‡,†	7863 ± 794*	7468 ± 779
Ea (mmHg/μL)	4.2 ± 0.68	14.7 ± 2.73*	3.5 ± 0.73	10.3 ± 2.47‡,†	4.8 ± 0.93	6.2 ± 0.72†
Tau-Weiss (msec)	7.0 ± 1.28	9.2 ± 1.34	9.6 ± 1.12	20.4 ± 5.01‡,†	7.6 ± 0.99	8.0 ± 1.18

Values present with means (± S.D.). N=6 for each group.

* P < 0.05, versus WT-Sham;

† P < 0.05 versus WT-CLP;

‡ P < 0.05 versus KO-Sham;

§ P < 0.05 versus TG-Sham. EF, ejection fraction; ESP, LV end-systolic pressure; EDP, LV end-diastolic pressure; LVDevP, LV developed pressure = $P_{max} - P_{min}$.

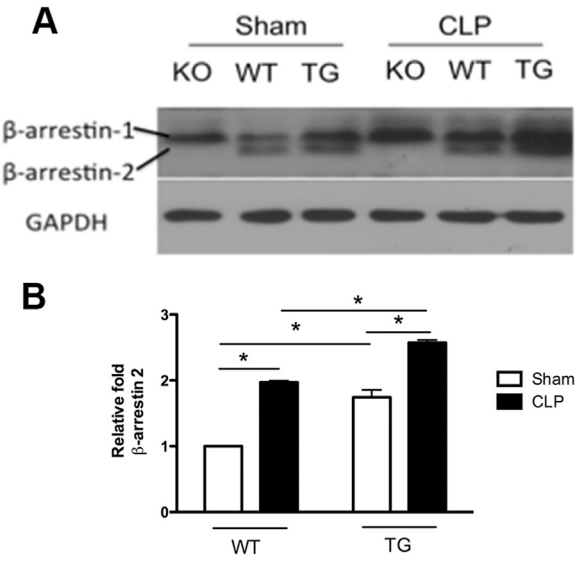

Fig. 4. Figure 2.6 β-arrestin 2 expression in septic heart. The protein level of β-arrestin 2 in saline rinsed heart tissue from mice 6 h hour after treated with Sham or CLP were examined by Western blot with loading control GAPDH. Data are representative of at least three independent experiments. Values present means ± SEM.). *$P < 0.05$.

β-arrestin 2 TG and WT mice following CLP. Gp130 Ser^{782} phosphorylation at Ser^{727} was significantly enhanced in septic WT and β-arrestin 2 KO mice compared with their control mice (Fig. 5(A)) at 6 h after CLP. Interestingly, the activation of gp130 was strikingly decreased in β-arrestin 2 TG septic mice as compared with WT and β-arrestin 2 KO mice.

Results showed p38 and gp130 can still be phosphorylated in inflammation-induced myocardial depression in the absence of β-arrestin 2, which is consisted with the impaired cardiovascular function in both WT and β-arrestin 2 knockout mice. Results of β-arrestin 2 knockout suggested β-arrestin 2 was not an essential mediator in the development of uncontrolled inflammation. Further than that, WT level of β-arrestin 2 expression was unable to prevent the CLP-induced stimulation of signaling transduction pathways mediated by p38 and gp130. Only β-arrestin 2 overexpression before sepsis showed positive results in anti-inflammation.

Phosphorylation of gp130 on Ser^{782} accelerated the internalization of membrane-bound gp130 [28]. Our results showed correlated beta-arretin2 overexpression and phosphorylation of gp130 in TG mice following sham treatment, which indicated a ligand-independent regulation of IL-6 receptors by β-arrestin 2.

Our results suggested lowered threshold for the activation of p38 due to overexpression of β-arrestin 2. Therefore, the mild stimulation of sham treatment was able to moderately enhance p38 phosphorylation in TG mice compared to WT and knockout mice. The molecular mechanism between β-arrestin 2 and p38 is unknown.

We recently reported that β-arrestin 2 inhibits Toll-like receptor 4 by targeting p38 in lipopolysaccharide-stimulated cell culture studies [18]. The effect of β-arrestin 2 on p38 activation (phospho-p38) in sepsis remains to be elucidated. In the present study, we tested whether β-arrestin 2 in the myocardium of CLP mice can modulate p38 activation. Fig. 5(B) shows that CLP-induced sepsis significantly enhanced the level of phospho-p38 in WT and β-arrestin 2 KO mice, compared with sham control. Notably, overexpression of β-arrestin 2 prevented CLP-enhanced myocardial phospho-p38 levels.

3.5. The effects of β-arrestin 2 on signal transducer and activator of transcription 3 (STAT3) phosphorylation after sepsis

To understand the signaling pathway downstream to gp130, we then examined levels of phosphorylated STAT3 (Tyr^{705} and Ser^{727}), a possible effector of gp130 mediated signaling pathway in septic myocardium [24].

Results showed that STAT3 phosphorylation at Tyr^{705} was dampened in KO mice (Fig. 6), indicating β-arrestin 2 was required in STAT3 Tyr^{705} phosphorylation. STAT3 Ser^{727} phosphorylation was enhanced in all three genotypes after CLP including KO group, suggesting the involvement of β-arrestin 2 independent inflammatory signaling pathways.

In consistent with increased gp130 phosphorylation in TG mice with sham treatment, increased STAT3 phosphorylation on Ser^{727} was also observed.

However, STAT3 phosphorylation on Tyr^{705} was not elevated in TG sham group.

The unbalanced STAT3 phosphorylation on two sites suggested different signaling transduction pathways were involved.

3.6. The effects of β-arrestin 2 on ERK and JNK phosphorylation after sepsis

As shown in result 3.4, β-arrestin 2 regulated p38 MAPK activation. To explore possible downstream effectors of β-arrestin 2 after CLP-induced myocardial dysfunction, we also examined the phosphorylation level of ERK and JNK. Increased activation of both ERK and JNK was observed after CLP-induced sepsis. However, the results of phosphorylation were not correlated with the expression

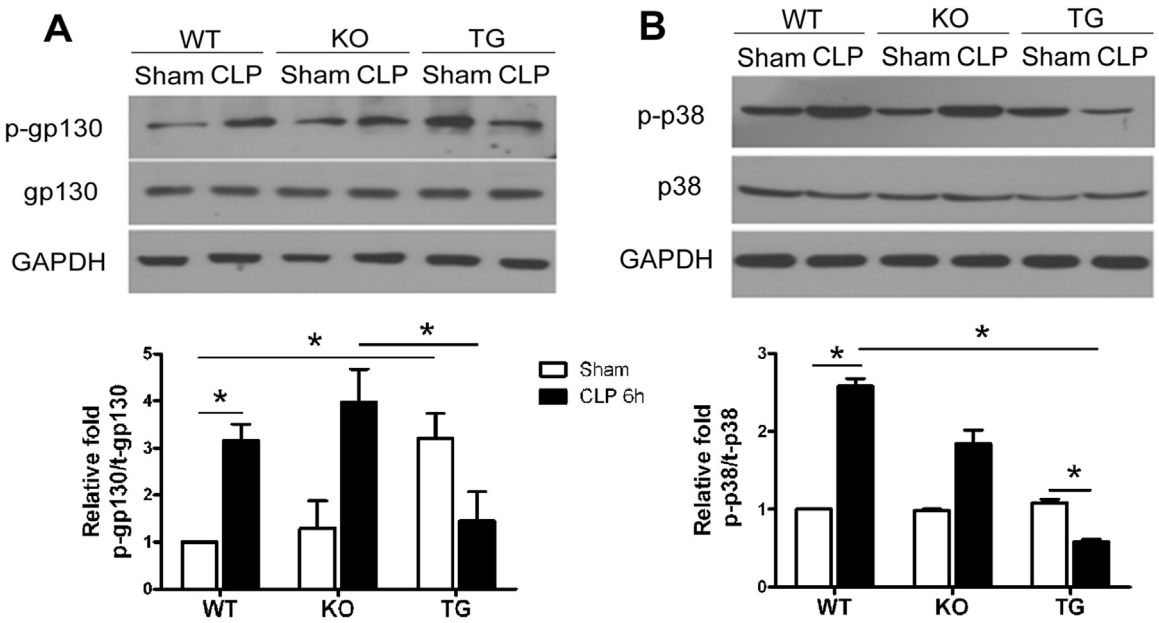

Fig. 5. Overexpression of β-arrestin 2 in mice blocks CLP-induced the levels of gp130 and p38 phosphorylation. WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (N=6 per group) were subjected to CLP or sham operations as in Fig. 2. After cardiac functional analysis, mice hearts were harvested and cellular proteins were prepared. The levels of phosphorylation of gp130 (A) and p 38 (B) were determined by Western blot with specific antibodies. Representative results are shown above the graph. $^{*}P < 0.05$.

level of β-arrestin 2 in both sham and CLP treated conditions (Fig. 7). Therefore, at the time point of 6 h after CLP, ERK and JNK were unlikely the downstream effectors of β-arrestin 2.

4. Discussion

Sepsis is a major clinical problem with more than a 40% mortality rate [1,3]. Sepsis is the most important cause of morbidity and mortality in intensive care units (ICUs), and about 60% of patients admitted to the ICU have cardiac dysfunction [2–4,23]. Cardiac dysfunction plays a fundamental role in the high morbidity and mortality of this condition [2–4,23]. Thus, it is urgent to elucidate the mechanisms by which sepsis modulates cardiac dysfunction and generate more efficient ways to improve the prognosis. In this study, we have demonstrated that β-arrestin 2 plays a critical role in the regulation of sepsis-triggered cardiac dysfunction through gp130 and p38 MAPK. Following sepsis, overexpression of β-arrestin 2 in mice increases animal survival. Importantly, β-arrestin 2 overexpression in mice abolishes sepsis-induced cardiac dysfunction. The role of β-arrestin 2 in regulating gp130 and p38 MAPK activation is significant, as β-arrestin

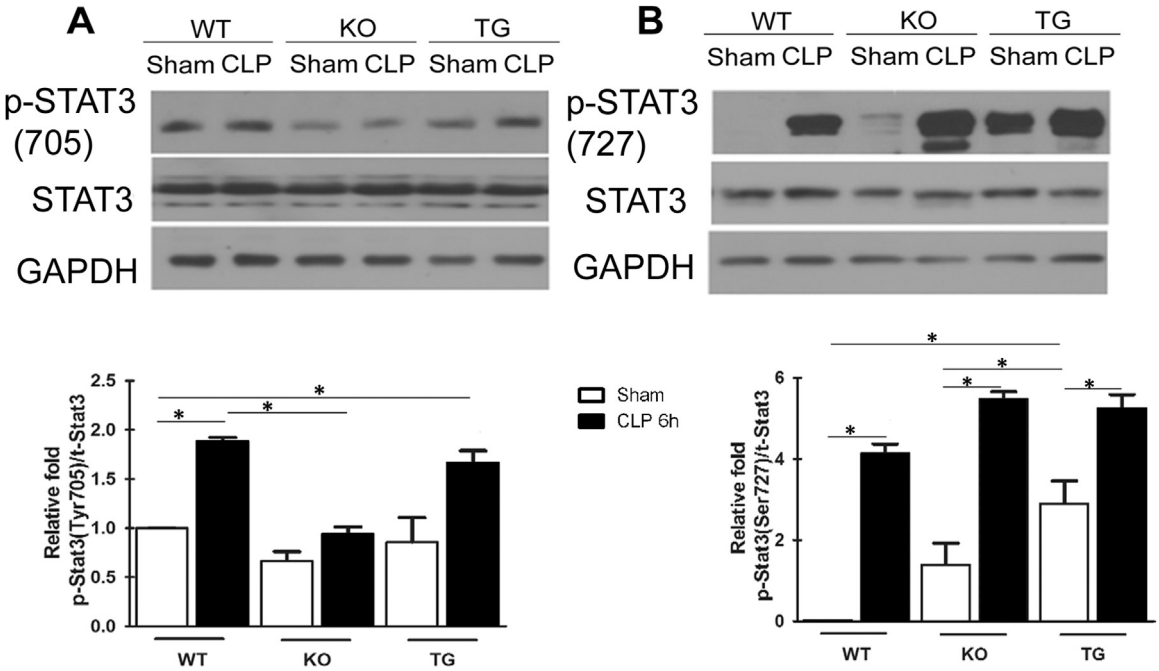

Fig. 6. The effect of β-arrestin 2 on STAT3 phosphorylation in CLP-induced myocardial dysfunction. WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (N=6 per group) were subjected to CLP or sham operations as in Fig. 2. After cardiac functional analysis, mice hearts were harvested and cellular proteins were prepared. The levels of phosphorylation on site Tyrosine705 (A) and Serine727 (B) were determined by Western blot with specific antibodies. Representative results are shown above the graph. $^{*}P < 0.05$.

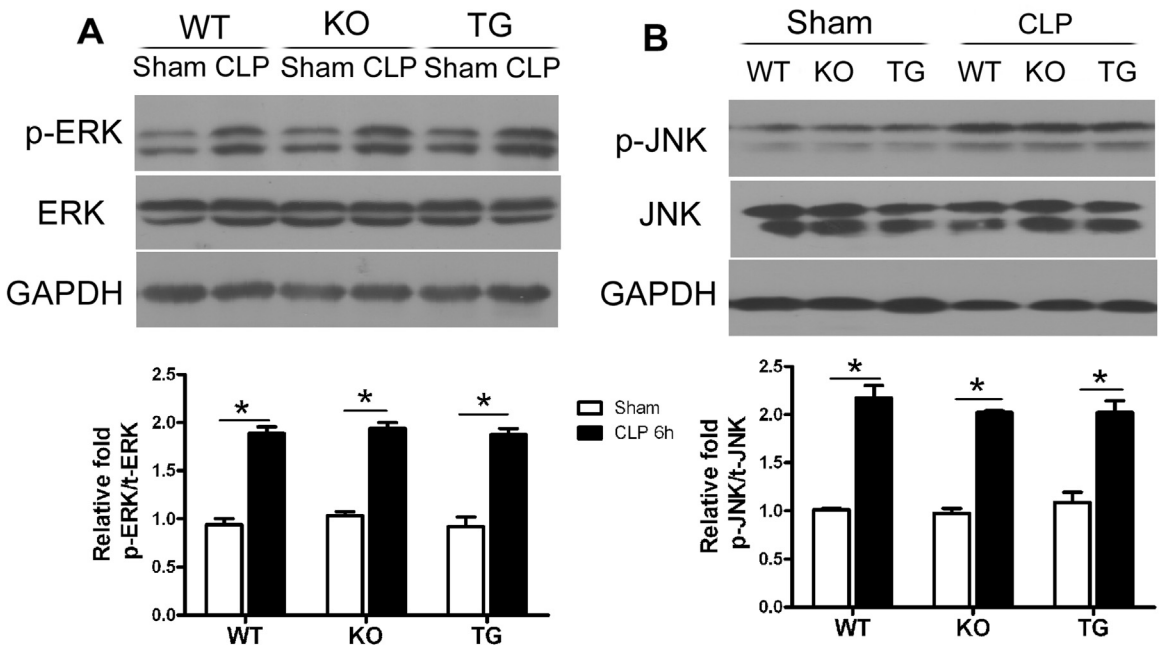

Fig. 7. The role of β-arrestin 2 in CLP-induced ERK and JNK phosphorylation. WT, β-arrestin 2 KO, and β-arrestin 2 TG mice (N=6 per group) were subjected to CLP or sham operations as in Fig. 2. After cardiac functional analysis, mice hearts were harvested and cellular proteins were prepared. The levels of phosphorylation of ERK (A) and JNK (B) were determined by Western blot with specific antibodies. Representative results are shown above the graph. $^{*}P < 0.05$.

2 overexpression results in lower gp130 and p38 phosphorylation after sepsis stimulation. Our results implicate that overexpression of β-arrestin 2 may form the basis of a new strategy for the clinical treatment of sepsis.

Increasing evidence suggests that β-arrestin 2 has the ability to modulate inflammatory responses through a few mechanisms. For instance, β-arrestin 2 modulates immune functions during the development of allergic asthma [25]. Another prior study indicates that β-arrestin 2 participates in the regulation of inflammatory responses in sepsis [26]. In previous studies, sepsis was associated with decreased cardiac output, decreased end diastolic volume or diastolic diameter, and decreased ejection fraction (EF). Decreased heart contractility was found 18 h as well after CLP using Millar instruments for cardiac functional analysis [23]. In our study, WT mice showed significant cardiac dysfunction 6 h after CLP, consistent with results from these studies [23,27]. For example, EDV were dramatically decreased in WT and β-arrestin 2 KO mice and moderately reduced in β-arrestin 2 TG mice after CLP, with apparently non-affected ESV (Fig. 3). However, 6 h after CLP is a relatively early time point, heart dysfunction may become more deleterious at a later time point (e.g., 12 h). Eventually, both EDV and ESV would be declined to undetectable levels when approached to mortality. With the highest mortality rate after CLP on day one, the mice in β-arrestin 2 KO group would be expected to show declined ESV at an earlier time point compared to the mice in WT and β-arrestin 2 TG groups. Impaired vascular contractility and decreased sympathetic tone in sepsis has been demonstrated in several studies [3,27]. In this study, we also confirmed the involvement of vascular factors by echo-cardiovascular measurement before CLP and 6 h after CLP (N=9) (data not shown). We found decreased cardiac output after sepsis, most likely due to combined cardiomyocyte dysfunction and decreased cardiac preload. The decreased mortality and preserved cardiac function in β-arrestin 2 overexpression mice suggests that agents increasing β-arrestin 2 expressions may protect the cardiac and vascular system from sepsis-induced injury. In the present study, we found that overexpression of β-arrestin 2 increases animal survival in sepsis. Notably, a new and novel role for β-arrestin 2 was revealed in the prevention of sepsis-induced cardiac dysfunction. Thus, attenuation of cardiac dysfunction might be an important mechanism by which β-arrestin 2 enhances animal survival during sepsis. While investigating the role of β-arrestin 1 in cardiac dysfunction induced by sepsis is beyond the scope of the current study and it will be elucidated in the future.

In this study, we examined phosphorylation of gp130, a key signal transducer. We found that the levels of gp130 phosphorylation in the myocardium were significantly decreased in β-arrestin 2 TG mice following CLP, while the opposite results were observed in WT and β-arrestin 2 KO mice. Gp130 phosphorylation at Ser^{782} is involved in the internalization of membrane-bound gp130 [28]. Recent studies have shown that β-arrestin 2 function as adaptors to connect the receptors to the cellular trafficking machinery, such as scaffolding GPCRs activation [18,22], as well as the signal transduction of non-GPCRs such as Toll-like receptors [8,18,30,31]. The scaffolding protein β-arrestin 2 has been conventionally associated with receptor internalization. β-arrestin 2 can scaffold different sets of molecules that determine different and even opposite effects on the same signaling cascade dependent on the receptor activated [29,30].

Our studies show that in the septic animal model, the overexpression of β-arrestin 2 reduces phospho-gp130, which is associated with animal survival. Our results suggest a possible connection between β-arrestin 2 and gp130 internalization. Our studies did not determine the specific membrane receptors that are involved in the modulation of β-arrestin 2 phosphorylation gp130 in sepsis. While determining the exact membrane receptors is beyond the scope of the current study and it will be investigated in the future.

P38 and ERK, members of MAPKs family, are important cellular protein kinases. They can be activated by a series of extracellular signals and then induce cell responses, including cell proliferation, differentiation, survival and apoptosis [22]. Activation of p38 and ERK modulates different cell responses depending on stimulus [22,31]. However, the effect of β-arrestin 2 on p38 and ERK activation in sepsis remain to be established. In the current study, we observed that CLP significantly induced p38 phosphorylation in

the myocardium in WT and β-arrestin 2 KO mice. Interestingly, the level of phospho-p38 was significantly diminished in β-arrestin 2 TG mice following CLP. However, we observed that β-arrestin 2 was not involved in ERK phosphorylation in the myocardium following CLP. In addition, the phosphorylation level of another MAPK, JNK, was also examined, which showed no correlation with β-arrestin 2 expression levels. Taken together, our results suggest that β-arrestin 2 may specifically activate myocardial p38 during sepsis.

Previous studies have suggested p38 as a crucial modulator for gp130 Ser^{782} phosphorylation and internalization in the crosstalk between IL-1β and IL-6 signaling pathways during inflammation [28,32]. In acute inflammation of sepsis, overestimation of the IL-6 signaling pathway, which is mediated by gp130, could be negatively regulated by the activation of p38. On the other side, p38 activation could be controlled by β-arrestin 2 in various conditions. Without inflammation, stress-induced p38 activation could be facilitated by the overexpression of β-arrestin 2, which might serve as an explanation for moderately increased p38, gp130, and STAT3 phosphorylation in the sham group of transgenic mice. During sepsis, p38 activation could be achieved by β-arrestin 2 dependent as well as β-arrestin 2 independent pathways, followed by accelerated gp130 phosphorylation/internalization and STAT3 activation. However, we suspect an opposite function of β-arrestin 2 on p38 activation, when the accumulation of β-arrestin 2 exceeds the threshold, which could serve as a signal or a direct effector for the suppression of p38 activation. At 6 h after CLP, the suppression of p38 action was first achieved in β-arrestin 2 transgenic mice. Although the network among p38, β-arrestin 2, and gp130 could be complicated and variable in the development of sepsis, evidence revealed from this work could be useful for future studies.

In summary, the data presented herein demonstrated for the first report, to the best of our knowledge, a key role for β-arrestin 2 in sepsis-induced cardiac dysfunction. The protective effects could be mediated at least partially by down-regulation of gp130 and p38 activation in β-arrestin 2 TG mice. These findings implicate the beneficial effect of β-arrestin 2 overexpression in sepsis and open a novel promising target for the management of sepsis.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.05.021.

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