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Heart failure-inducible Suppression of Protein Phosphatase 1β Partially Prevents Cardiac Remodeling in Experimental Pressure Overload-induced Heart Failure

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Abstract Aberrant increases in protein phosphatase 1(PP1) activity have been shown to be associated with inefficient sarcoplasmic reticulum Ca^{2+} cycling, leading to cardiac dysfunction in the failing heart. In the present study, we investigated whether BNP promoter-inducible suppression of PP1β would ameliorate progression of pressure overload-induced heart failure in mice, a clinically relevant animal model. An Adeno-associated virus 9 (AAV9) vector encoding PP1βshRNA and a negative control (NC) shRNA driven by a brain natriuretic peptide (BNP) promoter with an emerald green fluorescent protein expression (EmGFP) cassette were used to test the hypothesis. AAV9 vectors (AAV9-BNP-EmGFP-PP1βshRNA and AAV9-BNP-EmGFP-NCshRNA) were introduced into the in vivo heart via the tail vein injection ($4x10^{11}$ GC/mice) in 8-week-old C57BL6J mice, followed by transverse aortic constriction (TAC) 2 weeks after the AAV9 vector injection. Post TAC cardiac function was sequentially assessed every 2 week by echocardiography, followed by hemodynamic assessment at 1 month. AAV9-BNP-EmGFP-PP1βshRNA treatment suppressed myocardial PP1β expression by 15% compared with the NCshRNA group ($p<0.001$). The fractional shortening (%FS) of the left ventricle in the PP1βshRNA-treated group was significantly larger than the NCshRNA-treated group (21% ± 1.0% vs. 15% ± 0.01, $p<0.01$). The ratios of heart weight (HW) / body weight (BW) and lung weight (LW) / BW in the PP1βshRNA group were significantly smaller than those of the NCshRNA group (HW/BW: 9.20 ± 0.49 vs. 10.6 ± 0.45 mg/g; $p<0.05$, LW/BW: 9.27 ± 0.99 vs. 13.3 ± 1.29 mg/g; $p<0.05$, respectively). Moreover, PP1βshRNA treatment induced a significant decrease in both LV end-diastolic pressure (28.8 ± 1.20 to 17.2 ± 3.93 mmHg, $p<0.05$) and BNP mRNA expression (40% decrease compared to the control vector treated group). Survival analysis of animals receiving PP1βshRNA treatment for 7 months showed a tendency to extend life, but this did not attain the statistical significance

compared with the control vector treated group.In conclusion, heart failure-inducible molecular targeting of PP1β may be a therapeutic option for improving cardiac function and preventing cardiac remodeling in the failing heart, at least in a constrained period of a week to a month.

Key words: protein phosphatase 1β(PP1β), adeno-associated virus 9 (AAV9) vector, brain natriuretic peptide (BNP) promoter, AAV9-BNP-EmGFP-PP1βshRNA, transverse aortic constriction (TAC)

Introduction

Heart failure (HF) is now a global pandemic, affecting approximately 26 million people, 1 million annual hospitalizations worldwide.[1] Despite significant advances in both medical treatment and devices over the last 2 decades, large numbers of patients undergo repeated readmission due to exacerbation of HF, with negative impact on health costs, daily activities and eventual outcome. Currently, one critically important clinical issue is the lack of an effective treatment strategy for HF with preserved ejection fraction (EF), a feature that is more common in the elderly.[1] Although the fundamental mechanism of HF with preserved EF has yet to be elucidated, disease pathophysiology may include impaired diastolic function and ventricular filling, which are influenced by a variety of both cardiac and non-cardiac comorbidities, including patients who were previously treated for HF with reduced EF by EBM-based therapy.[2] Of these, impaired sarcoplasmic reticulum (SR) mediated calcium cycling is an essential characteristic of these failing hearts. The key components of calcium cycling are sarcoplasmic calcium ATPase 2a (SERCA2a) and phospholamban (PLN), with intricate regulation provided by protein kinases and phosphatases (PPs).[3] These key kinases includes A-kinase (PKA) and calcium/calmodulin-dependent kinase II (CAMKII), whereas the PPs consist mainly of PP1, PP2A, and PP2B.[3] It has been reported that overactivation of PP1 causes a decreased phosphorylation of PLN, resulting in inefficient Ca^{2+} cycling, at least in adult failing hearts.[4,5] The PP1 catalytic subunit consists of three distinct genes: ppp1ca (PP1α), ppp1cb (PP1β/δ) and ppp1cc (PP1γ).[6] We previously demonstrated that PP1 is the key isoform that dominantly suppresses SR mediated calcium cycling in cardiomyocytes.[7] Indeed, there are lines of evidence showing that PP1 inhibition is a potential molecular target for the treatment of heart failure by upregulating intracellular Ca^{2+} cycling.[8,9] We and others have shown that gene transfer of endogenous PP1 inhibitors, such as constitutively active inhibitor-1 (INH-1c) or inhibitor-2 (INH-2) significantly improved cardiac function and extended survival time in animal models of heart failure. Furthermore,[8,10] we previously were able to mitigate heart failure progression by brain natriuretic peptide (BNP) promoter-driven therapeutic gene suppression of PP1 using an adeno-associated virus 9 (AAV9) vector-mediated short hairpin RNA (shRNA) in genetic mice cardiomyopathy.[11] In the present study, we investigated whether such heart failure-inducible suppression of PP1 would provide a similar beneficial effect on pressure overload-induced heart failure, clinically relevant animal model.

Materials and Methods

Animals

All of the animal protocols were approved by the Yamaguchi University School of Medicine Animal Experiment Committee (institutional permission #24-014). The animals were treated according to the Guide for the care and use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). The 10 week-old C57/BL6J mice were obtained from Kyudo Co. Ltd.,(Saga, Japan).

Preparation of AAV9 vectors for shRNA

An AAV9 vector encoding PP1βshRNA driven by the human BNP promoter was employed to control therapeutic gene regulation, as previously described.[11] The negative control

sequence shRNA AAV9 was used as a control vector. Heart failure inducible suppression of PP1β was confirmed by expression of emerald green fluorescent protein (EmGFP) was connected to the 3' end of the BNP-promotor sequence. AAV9 vectors encoding the above expression cassette (AAV9-BNP-EmGFP-NCshRNA, AAV9-BNP-EmGFP-PP1βshRNA) were produced in the vector core facility at University of Pennsylvania.

Generation of the Pressure Overloaded Heart Failure Mice Model

Twelve week-old mice were anesthetized with 2~3 % isoflurane inhalation, with ketamine (100mg/kg) and xylazine (5mg/kg) added as appropriate, intubated, ventilated, and received a small thoracotomy at the second intercostal space. TAC was performed as described previously.[12] Briefly, the aortic arch was exposed through a small window of the intercostal space and TAC performed by tying the transverse aorta using a blunt 27G blunted needle and 4-0 nylon suture at the point between the brachiocephalic artery and left subclavian artery, followed by immediate release of the blunt needle. A successful TAC operation was confirmed by observing the difference in pulse between the right and the left carotid arteries. The chest was then closed and extubated and the animals subsequently fed ad libitum. Proper recovery from anesthetics was confirmed, and normal saline solution was injected under the skin (0.5ml/body) as appropriate.

In vivo gene transfer of AAV9 and Transverse Aorta Constriction

Ninety six C57/BL6J mice at 10 week-old of age underwent in vivo AAV9 gene transfer as described in Figure 1. Briefly, as shown in Figure 1A, 48 mice were randomly assigned into two groups; one group (n=24) received an injection of control AAV9 vector (AAV9-BNP-EmGFP-NCshRNA, Figure 2A), while the other (n=24) received an injection of AAV9 encoding PP1βshRNA (AAV9-BNP-EmGFP-PP1βshRNA Figure 2B). In each animal, a 0.6 mL volume of the 4.0x1011GC AAV9 viral vector was injected through the tail vein. Two weeks later, both groups of mice underwent the TAC procedure (n=24 in both the AAV9-BNP-EmGFP-NCshRNA AAV9-BNP-EmGFP-PP1βshRNA groups), followed by serial echocardiography, along with hemodynamic (N=6~8) and biochemical assessment (n=14~15) at 4 weeks after TAC. Nine mice each were used for the saline injection and sham operation for additional control analysis. Cardiac function was monitored every 2 weeks by cardiac ultrasonography equipped with a 15 MHz linear probe (HDI-5000 SonoCT, Philips, Netherlands) using 1.5% isoflurane and spontaneous ventilation. For hemodynamic analysis, mice were anesthetized with 2% isoflurane, mechanically ventilated, and the right carotid artery cannulated with a 1F Millar micro-tip catheter placed in the left ventricle (Millar instruments, Houston, TX). Maximum and minimum LV dP/dt and the time constant relaxation, tau (using the exponential function), were calculated from the LV pressure, as previously described.[10]

As shown in Figure 1B, another 48 C57/BL6J mice were randomly assigned into two groups and received an AAV9-vector injection through the tail vein (n=24 AAV9-BNP-EmGFP-NCshRNA and n=24 AAV9-BNP-EmGFP-PP1βshRNA) and TAC at 2 weeks, then were fed ad libitum under a 12:12 LD cycle to follow up survival of the animals for 7 months.

Analysis of Cardiac Gene Transfer Efficiency

AAV transfected hearts were minced and homogenized with a buffer containing (in mM) 25 Tris-HCl (pH 7.4), 50 NaCl, 300 sucrose, 1 EDTA, 1 EGTA, 50 NaF, 1 Na3VO4, 1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 0.02% 2-mercaptoethanol and 1% protease inhibitor cocktail (PIC) (Sigma, St. Louis, MO).[10] Protein concentrations were calculated by the Bradford assay, followed by immunoblotting and image analysis with an LAS-4000 (GE Healthcare Japan, Tokyo), then by immunoblotting of the protein expression levels of EmGFP, which represents the BNP promoter activity of the AAV shuttle vector plasmid.

The AAV9vector-transfected hearts were frozen in plastic containers using isopentane precooled with liquid N2 and stored at -80℃ for further sectioning. Hearts were sectioned at a thickness of 6 μm, fixed with 2% paraformaldehyde in phosphate buffered saline and

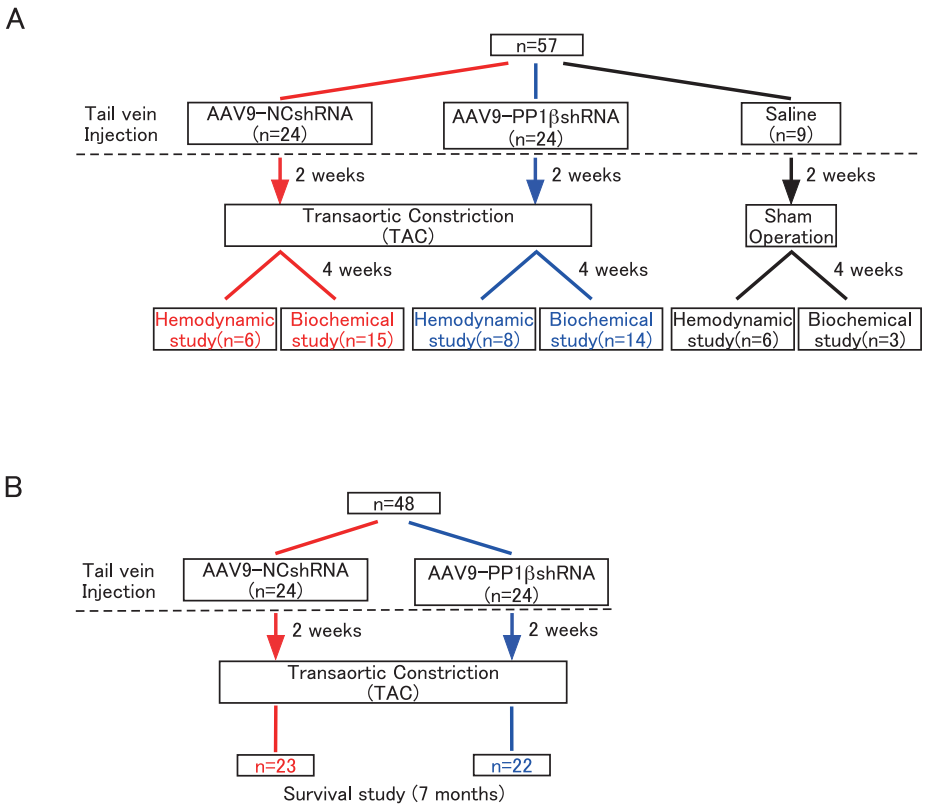

Fig. 1 Experimental designs.
(A) The experiment for assessing the hemodynamic and biochemical effects of in-vivo AAV9-BNP promoter-mediated heart failure condition-specific PP1β shRNA gene transfer compared to the negative control shRNA and sham-operated mice in trans-aortic constriction (TAC)-induced pressure overload heart failure mouse model.
(B) The experiment assessing the effect on long term survival after in-vivo AAV-BNP-mediated heart-failure-specific shRNA gene transfer followed by the TAC operation.

immunostained with an anti-GFP antibody (ab290, Abcam, Cambridge, UK), followed by chemical reaction performed with a VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA) and 3,3'-diaminobenzidine (DAB).

Analysis of SR protein phosphorylation

Hearts were homogenized with the buffer described above for the assessment of the SR protein phosphorylation level. The phosphorylation levels of PLN at Ser16 and Thr17, the ryanodine receptor (RyR) at Ser2808 and the cardiac troponin I (TnI) at Ser22 and 23 were normalized to the total protein levels.

Antibodies

The following antibodies were obtained from commercially available sources: the antibodies for PP1β (ab16369, ab53315), and PLN (ab2865, clone 2D12: Abcam, Cambridge, UK); phosphorylated-PLN at Ser16, PLN, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon International, Billerica, MA), phosphorylated-PLN at Thr17, and phoshphorylated-RyR2 at Ser2808 (Badrilla, Leeds, UK); RyR2 (clone C3-33: Sigma-Aldrich, St. Louis, MO), SERCA2a (clone N-19: Santa Cruz Biotechnology, Santa Cruz, CA), cardiac troponin I (cTn-I: clone 19C7), phospho-TnI at Ser22,23 (clone 5E6: Genetex, San Antonio, TX), GFP(ab290: Abcam) and protein phosphatase regulatory subunit 12B (MYPT)(04-386, MerckMillipore, Darmstadt, Germany). The antibodies for MLC2v and phosphorylated MLC2v were provided by Dr. Hideko Kasahara of the University of Florida.

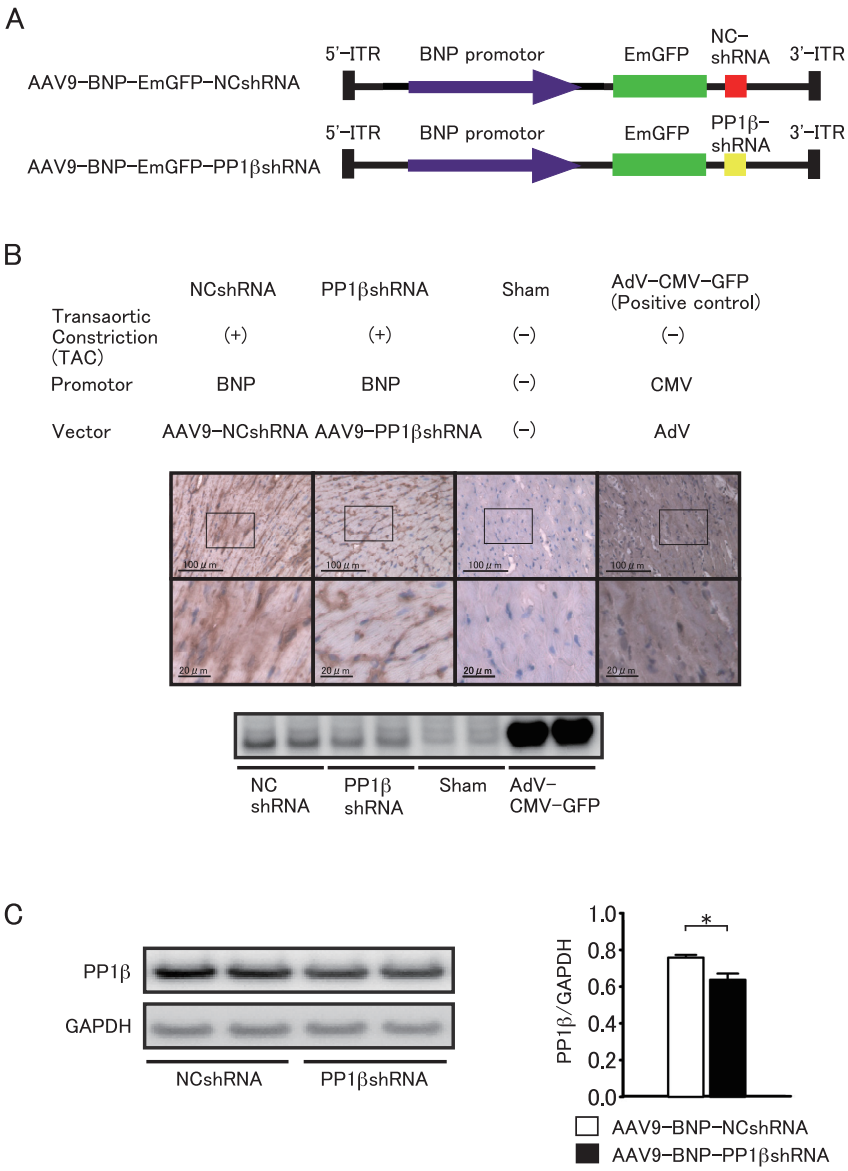

Fig. 2
(A) Design of the AAV9 vectors encoding BNP promoter mediated heart-failure-specific shRNA with marker gene expression indicated by emerald green fluorescent protein (EmGFP).
(B) Immunohistochemical staining and immunoblotting of EmGFP in AAV9-BNP promoter-mediated shRNA vectors in tranfected- and cytomegalovirus promoter-mediated adenoviral vector (positive control) transfected hearts.
(C) Immunoblot analysis of myocardial PP1β expression in AAV9-PP1βshRNA treated- and AAV9-NCshRNA treated hearts. Quantitative analysis of PP1β expression normalized by the protein expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). "*" indicates $p<0.01$ compared to NCshRNA treated mice.

Statistical analysis

Comparisons between the two groups were made with Student's t-test. Comparisons between repeated measurements were performed with ANOVA, followed by the post hoc test (the Student-Newman-Keuls method was used to compare the two groups when appropriate). A value of $p<0.05$ was considered statistically significant. Data are expressed as the mean ± SEM.

The results were analyzed by LogRank test (SigmaPlot Ver.12.0 Systat Software Inc. San Jose, CA).

Results

The design of the gene transfer procedure using AAV9 is summarized in Figure 1. All mice survived after tail vein injection of the AAV9 vector. In protocol A, which assessed the cardiac function and biochemical characteristics of the gene transferred hearts, 3 mice in the AAV9-NCshRNA treated group and 3 mice in the AAV-PP1βshRNA treated group died within 5 days of TAC. No mice died in the saline injection sham-operated group. In protocol B assessing survival after the TAC operation, No animals died for at least 1 week, indicating complete recovery from the TAC procedure and anesthesia.

AAV9-mediated inducible PP1βshRNA partially reduced PP1β expression

In AAV9 vector-transfected hearts, immunohistochemical analysis revealed EmGFP-positive perinuclear staining in cardiomyocytes throughout the transverse heart section, as shown in Figure 2B, suggesting that AAV-mediated gene transfer induced gene suppression in the TAC operated mice hearts in a BNP promoter dependent manner. In contrast, the sham operated hearts did not show any positive staining. Pressure overload-induced expression of EmGFP was also confirmed by immunoblotting with the anti-GFP antibody as shown in the lower panel of Figure 2B, because this AAV vector harbors an EmGFP gene expression cassette at the conjugation site of the microRNA fragment. Interestingly, the expression levels of EmGFP appeared to be weaker in the AAV9-PP1βshRNA transfected hearts compared to the AAV9-NCshRNA transfected hearts. In Figure 2C are shown the protein expression levels of PP1β that were quantified by immunoblotting in the AAV9 vector-transfected hearts. PP1β expression was reduced by approximately 15% in the AAV-PP1βshRNA-treated hearts compared with the AAV-NCshRNA treated hearts, showing that BNP promoter mediated PP1βshRNA partially suppressed PP1β gene expression by AAV-microRNA vector-mediated RNA interference.

AAV9-BNP-promoter-mediated PP1β shRNA significantly improved LV systolic and diastolic function in TAC-operated mice

As shown in the representative Figure 3A, the AAV9-PP1βshRNA treated heart (the rightmost panel) exhibited smaller left ventricular diastolic (LVDd) and systolic dimensions (LVDs) compare to the AAV9-NCshRNA treated hearts (the center panel), although both dimensions were larger than that of the sham operated heart (the leftmost panel). The averaged data revealed a significantly smaller LVDd/LVDs in the AAV9-PP1βshRNA treated group compared to the AAV9-NCshRNA treated group, resulting in a significantly greater % of fractional shortening of the LV in the AAV9-PP1βshRNA treated hearts at 2 and 4 weeks after the TAC operation compared to the AAV9-NCshRNA treated group (Fig. 3B).

As shown in Figure 4A, the PP1βshRNA-treated mouse exhibited an improved LV pressure tracing pattern compared to that of the NCshRNA-treated mouse; namely, the elevated LV end-diastolic pressure was mitigated, and the first derivative curve of the LV pressure showed greater positive and negative dP/dt amplitudes, whereas that of the NCshRNA-treated mouse a smaller and dull LV pressure curve, with elevated end-diastolic pressure. Figure 4B shows that the AAV9-PP1βshRNA treated mice had a significantly greater LV peak pressure, larger negative dP/dt of the LV amplitude and smaller LV end-diastolic pressure compared to the NCshRNA-treated mice, although these results are significantly inferior to those obtained in the sham operated animals. There was also a tendency of improved maximum

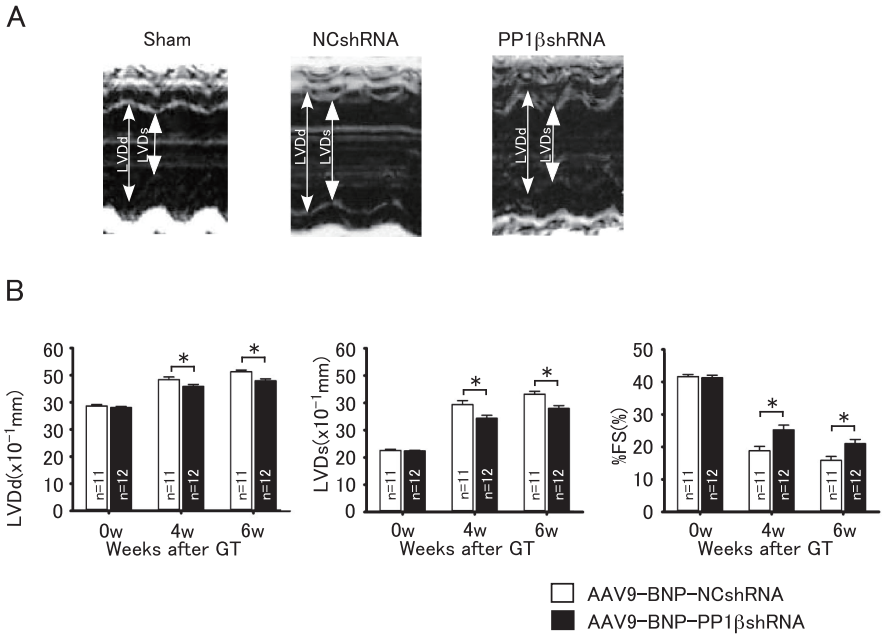

Fig. 3
(A) Representative echocardiographic assessment of cardiac contractility in the sham-operated, AAV9-BNP-NCshRNA treated- and AAV9-BNP-PP1βshRNA treated mouse hearts 1 month after TAC.
(B) Comparison of end-diastolic (LVDd) and end-systolic diameter (LVDs) diameter of the left ventricle, and the fraction shortening (%FS) of the LV at 2 weeks before and 2 and 4 weeks after TAC. "*" indicates $p<0.01$ compared to (those of) AAV9-BNP-NCshRNA treated mice.

positive dP/dt in the AAV9BNP-PPβshRNA treated hearts, but it did not attain statistical significance. There were no significant differences in the heart rate among the three groups of AAV-PP1βshRNA-treated-, AAV-NCshRNA-treated- and sham operated-mice. These data indicate that AAV9-BNP mediated PP1βshRNA mainly ameliorated LV diastolic function and helped prevent adverse left ventricular remodeling in the TAC-operated mice for at least 4 weeks after TAC induced pressure overload.

AAV9-PP1βshRNA-mediated changes in biochemical parameters

The AAV-PP1βshRNA- and AAV9-NCshRNA treated mice exhibited significant increases in heart and lung weight as normalized by body weight compared the sham operated mice, as shown in Figure 5. The AAV9-PP1βshRNA treated mice exhibited a smaller increase in both heart and lung compared to the AAV-NCshRNA treated mice (Fig. 5A). In addition, the AAV-PP1βshRNA treated TAC mice exhibited a smaller increase in BNP mRNA expression compared the AAV-NCshRNA treated TAC mice (Fig. 5B). These data indicate that AAV-PP1βshRNA treated mice underwent less cardiac hypertrophy and adverse chamber remodeling in response to the TAC operation.

In the heart homogenates, the phosphorylation levels of PLN at Ser16 in the AAV9-PP1βshRNA treated TAC hearts was significantly increased compared with the NCshRNA-treated ones (Fig. 6). In contrast, there was no change in the phosphorylation level of the RyR at Ser2808, and no change in the expression level of PLN or RyR. There was a tendency of greater expression of EmGFP in the AAV9-NCshRNA treated TAC mouse hearts compared to the AAV9-PP1βshRNA treated hearts. There was also no change in the phosphorylation levels of cardiac troponin I (TnI) at Ser22/23 and no change in the expression levels of TnI,

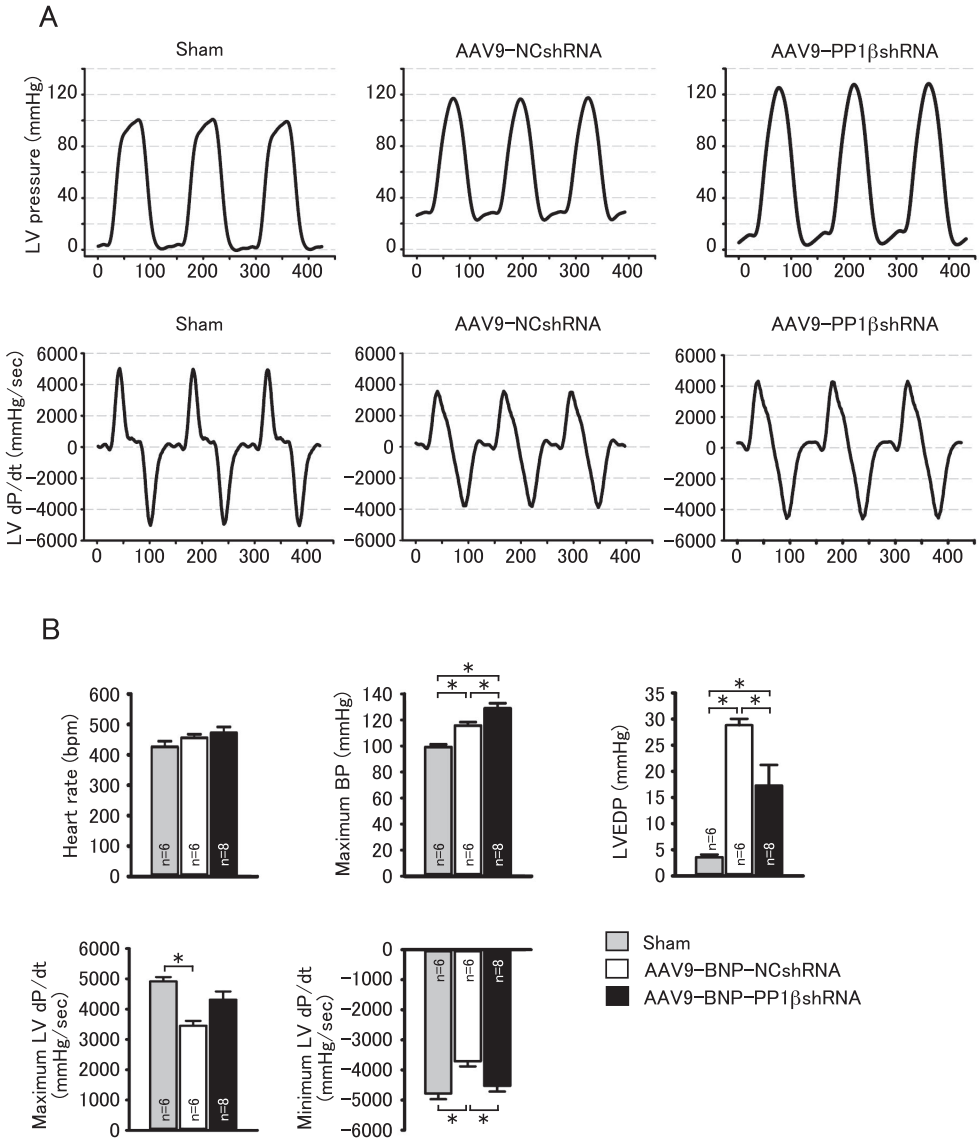

Fig. 4
(A) Representative LV pressure (LVP) and its dP/dt in the sham-operated, AAV9-BNP-NCshRNA treated- and AAV9-BNP-PP1βshRNA treated mouse hearts 1 month after TAC.
(B) Comparison of the heart rate, LV peak-systolic pressure (max LVP), LV end-diastolic pressure (LVEDP) and maximum and minimum LV dP/dt.
"*" indicates $p<0.01$ compared to the AAV9-BNP-NCshRNA treated mice or the sham-operated mice.

SERCA2a and GAPDH. As PP1β has been shown to affect myosin light chain phosphorylation by antagonizing myosin light chain kinase, we looked at the phosphorylation levels of MLC2v together with the MYPT protein expression level. There were no changes in the phosphorylation level of MLC2v or the expression level of MYPT induced by PP1βshRNA compared with the negative control vector.

In the survival analysis, the AAV9-PP1βshRNA treated mice initially showed a better tendency to survive after the TAC operation, but this did not attain statistical significance at 7 months post procedure (Fig. 7, $p=0.09$).

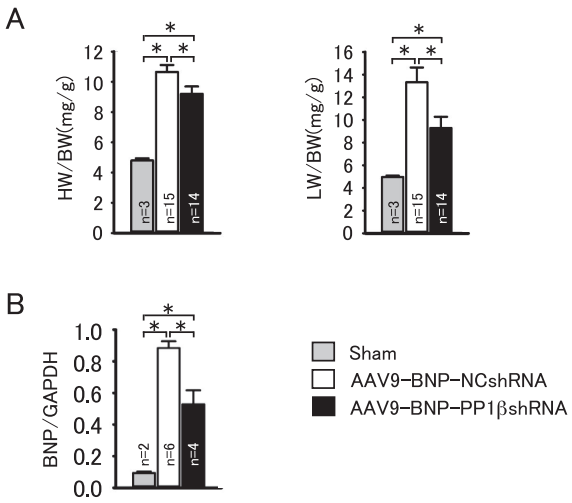

Fig. 5
(A) Comparison of heart weight (HW) and lung weight (LW) normalized by body weight (BW) at 1 month after TAC among the sham-operated, AAV9-BNP-NCshRNA treated- and AAV9-BNP-PP1βshRNA treated-hearts.
(B) BNP expression analysis was performed by using real-time RT-PCR at 1 month after TAC among sham-operated, AAV9-BNP-NCshRNA treated- and AAV9-BNP-PP1βshRNA treated-hearts.
"*" indicates $p<0.01$ compared to the AAV9-BNP-NCshRNA treated mice or the sham operated mice.

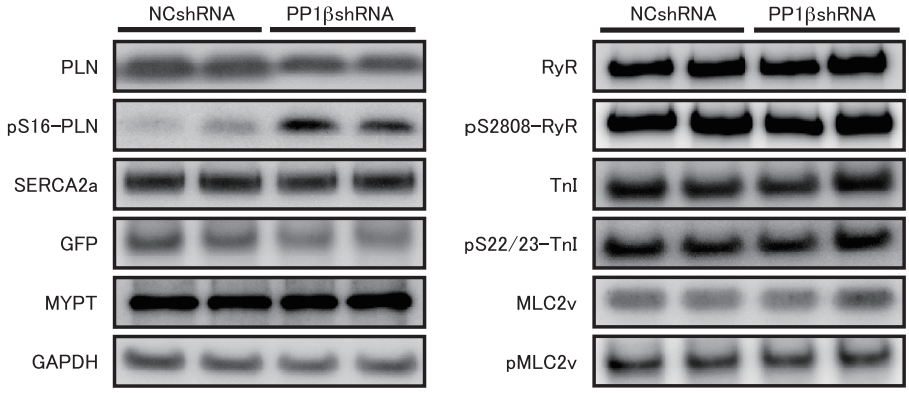

Fig. 6 Immunoblots of the key SR phosphoproteins and myofilament protein,s and analysis of the phosphorylation levels using phosphospecific antibodies in LV homogenates at 1 month after TAC.

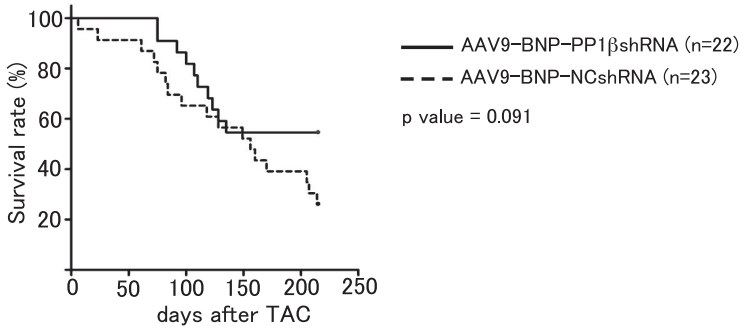

Fig. 7 Survival analysis using log rank test between AAAV9-BNP-PP1bshRNA (n=22) and AAV9-BNP-NCshRNA(n=23) treated mice. The p value between these groups did not attain statistical significance (p= 0.09).

Discussion

Protein phosphatase 1 has been reported to be the major serine/threonine phosphatases in the heart.[13] It has been suggested that an aberrant increase in PP1 activity is associated with progressive cardiac dysfunction and impaired phosphorylation of the key sarcoplasmic reticulum (SR) protein PLN, an endogenous inhibitor of SERCA2a in cardiomyocytes.[4] Modifying PP1 activity by targeting endogenous inhibitors in the failing heart has therefore become an attractive target for restoring a proper phosphorylation balance and improving SR-mediated calcium cycling.[8-10,14] In this regard, there are three PP1 catalytic subunit isoforms (PP1α, PP1β, and PP1γ) encoded by distinct genes that are approximately 90% homologous to one another.[15] We previously reported that PP1β is the most critically important isoform in cardiomyocytes,[7] regulating SR calcium cycling and cardiomyocyte contractility by modifying the phosphorylation of PLN. It has also been reported that PP1β is a critical isoform in the regulation of the dephosphorylation of myofilaments, myosin light chain 2v (MLC2v), in response to myosin light chain kinase phosphorylation,[16-18] thereby regulating cardiomyocyte contractility. Since the phosphorylation level of MLC2v has been shown to be decreased in human and experimentally induced heart failure,[16,19] imbalances of kinases and phosphatases seem to play a critical role in myofilament dynamics in the ailing heart. We previously reported that BNP promoter inducible suppression of PP1β significantly improved LV diastolic function and mitigated heart failure progression in muscle LIM protein (MLP) deficient mice cardiomyopathy. In the present study, we further extended the hypothesis that BNP promoter regulated PP1β inhibition may ameliorate heart failure progression in trans-aortic constriction (TAC) by carrying out an investigation in a clinically relevant pressure overload heart failure mouse animal model. We demonstrated in TAC-induced heart failure mice that AAV9 vector-mediated BNP promoter-inducible PP1βshRNA treatment improved LV systolic and diastolic function, and helped prevent progressive left ventricular remodeling at 1 month. Based on the biochemical characterization, the mechanism underlying the improved contraction and relaxation was attributable to increased phosphorylation of PLN at Ser16 and subsequent augmentation of SR-mediated calcium cycling. These data support the notion that PP1β is the key isoform that acts as the pivotal phosphatase during cardiomyocyte contraction and relaxation in the pressure overloaded hypertrophied heart, and its transition toward heart failure. These data make modifying PP1β expression in order to improve cardiac contractility in the failing heart an attractive concept.

However, AAV9-PP1βshRNA treated mice failed to show an improved survival throughout the 7 month observation period, even though PP1βshRNA treated mice initially

showed a better tendency to survive. In this regard, we may have to take consideration into the recent findings. Liu et al. reported that cardiac specific deletion of PP1β did not only enhanced cardiomyocyte contractility by changing myofilament protein phosphorylation, it also induced pathological hypertrophy and cardiac fibrosis. They documented a remarkable increase in phosphorylation in MLC2v, but not PLN.[20] Although we did observe an increase in the phosphorylation of PLN and did not see any change in MLC2v phosphorylation at 1 month after TAC, the effect of long term BNP promoter-mediated PP1β inhibition on those phosphoproteins was not assessed, as the second study was intended to assess animal survival for a period of 7 months. Therefore, we cannot rule out the possibility that PP1β suppression affected myofilament phosphorylation dynamics during the long term follow-up period, as we were unable to further analyze changes in the phosphorylation status of MLC2v and PLN. In addition, discrepancies in the phosphorylation levels of PLN and MLC2v may also be attributed to the different degree of the suppression of PP1β between these studies and also due to the different experimental approaches employed. They completely deleted PP1β gene expression by using NKX2.5-Cre recombinase or tamoxifen-inducible αMHC-MerCreMer recombinase, whereas we utilized a cardiac-specific and heart failure-specific gene transfer approach, using a BNP promoter-dependent shRNA that reduced the PP1β expression level by approximately 15% at 1 month. On the other hand, it is also intriguing that PP1β is the only isoform of the three PP1 catalytic subunits that significantly modified cardiomyocyte contractility in both studies. Further investigation is clearly needed.[20]

There is a limitation in the present study with regard to extending it to the clinical setting. We performed AAV9-mediated gene transfer prior to the onset of pressure overload, namely TAC. The reasons are as follows: therapeutic gene expression takes at least 2 weeks after AAV9 tail vain injection according to the previous study, whereas TAC-induced pressure overload using a 27 gauge induces severe pressure overload of the left ventricle, resulting in hypertrophy and adverse chamber remodeling. Therefore, to maximize the therapeutic gene transfer effect and clarify the molecular targeting effect of PP1β suppression, the above described pretreatment approach using AAV9 tail vein injection, even though pretreatment gene therapy may be criticized as not clinically relevant.

In conclusion, the BNP promoter-mediated suppression of PP1β in pressure overload induced heart failure suggests a potential new molecular strategy for ameliorating disease progression, at least for the short term of up to 1 month, in this pressure-overload induced mouse model.

Conflict of Interest

The authors declare no conflict of interest.

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