Biochemical and Biophysical Research Communications 461 (2015) 321–328

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

PDCD5 protects against cardiac remodeling by regulating autophagy and apoptosis

Shu Zhang [a,1], Ge Li [c,1], Xin Fu [a], Yanchao Qi [a], Mengtao Li [b], Guang Lu [b], Jia Hu [c], Nan Wang [a], Yingyu Chen [c], Yun Bai [b], Ming Cui [a,*]

[a] Department of Cardiology, Peking University Third Hospital and Key Laboratory of Cardiovascular Molecular Biology and Regulatory Peptides, Ministry of Health, Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education and Beijing Key Laboratory of Cardiovasicular Receptors Research, Beijing 100191, China
[b] Department of Cell Biology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China
[c] Key Laboratory of Medical Immunology, Ministry of Health, Peking University Health Science Center, Beijing 100191, China

ARTICLE INFO

Article history:
Received 2 April 2015
Available online 13 April 2015

Keywords:
PDCD5
Autophagy
Cardiac remodeling

ABSTRACT

Cardiac remodeling, including cardiac hypertrophy and fibrosis, is an important pathological process that can lead to heart failure. A previous study demonstrated that autophagy could represent an active adaptive response in cardiomyocytes that affords protection from cardiac remodeling. In the present study, we investigated the role of an autophagy-related gene, *PDCD5* (Programmed cell death 5), in cardiac remodeling induced by β-adrenergic stimulation *in vivo*. We report for the first time that mice systemically overexpressing *PDCD5* (*PDCD5tg*) were protected from cardiac remodeling. In addition, cardiac function was preserved in *PDCD5tg* mice in response to isoproterenol (ISO) stimulation. Importantly, basal autophagy was significantly higher in *PDCD5tg* mice than in the wild-type (WT) mice. Moreover, apoptosis was significantly lower in *PDCD5tg* mice than in WT mice, among the ISO-induced animals. In summary, our findings reveal that *PDCD5* overexpression improves cardiac function and inhibits cardiac remodeling induced by ISO via induction of autophagy and inhibition of apoptosis.

1. Introduction

Cardiac remodeling is a pivotal pathological phenomenon that occurs during the clinical course of stress-induced heart failure and represents an independent risk factor for subsequent cardiac morbidity and mortality [1]. Specifically, cardiac hypertrophy is characterized by an abnormal enlargement of the heart muscle originating from increased myocyte cell size and abnormal proliferation of non-muscle cells [2,3]. Cardiac fibrosis is characterized by excessive extracellular matrix accumulation and fibroblast deposition, which eventually destroys organ architecture and abolishes normal function [4–6]. Cardiac remodeling is a major biological determinant of fatal events including heart failure, severe arrhythmias, and sudden cardiac death [7,8]. Thus, elucidation of the mechanisms implicated in cardiac protection against remodeling is of great significance.

Autophagy, a highly conserved catabolic process involved in the delivery of cytoplasmic components to lysosomes for degradation, plays a pivotal role in the maintenance of the cellular environment of the heart [9]. Effective autophagy in cardiomyocytes is necessary for normal metabolism and cellular survival. The inability of autophagy to completely remove damaged structures results in the progressive accumulation of cellular debris, including cytosolic protein aggregates and defective mitochondria. Previous studies have demonstrated that dysregulation of autophagy can promote the development of many forms of heart disease [10–14], such as cardiac remodeling. Moreover, inhibition of autophagy has been observed during the progression of cardiac hypertrophy, an important process of cardiac remodeling [9]. Consistent with this, cardiac remodeling was attenuated through the facilitation of autophagy [15,16]. However, the precise role of autophagy in cardiac remodeling remains to be elucidated.

Programmed cell death 5 (*PDCD5*) has been proposed to act as an autophagy-regulated factor [17,18], a tumor suppressor, and pro-apoptotic factor [19–22]. It is also involved in paraptosis [23], cell cycle regulation [24], ischemia/reperfusion [25], immunoregulation

* Corresponding author.
E-mail address: mingcui@bjmu.edu.cn (M. Cui).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.04.032
0006-291X/

[26], and viral infection [27]. Clinical studies suggest that abnormal expression of *PDCD5* is associated with many diseases, such as tumor, autoimmune diseases, heart disease [18,26,28,29], and various inflammatory processes [30]. However, the role of *PDCD5* in catecholamine-induced (e.g. isoproterenol) cardiac remodeling is still unknown.

In the present study, we aimed to determine the role of *PDCD5* in cardiac remodeling induced by isoproterenol (ISO). Therefore, we created transgenic mice with systemic overexpression of human *PDCD5 (PDCD5tg)*. We found that transgenic overexpression of *PDCD5* did not result in obvious abnormalities related to either survival or cardiac function. However, transgenic mice were protected from cardiac remodeling in association with increased autophagy and attenuated apoptosis. Our findings support the potential of *PDCD5* as a therapeutic target for heart disease intervention.

2. Materials and methods

2.1. Experimental animals

PDCD5 transgene mice with the C57BL/6J background were constructed by Chinese Academy of Medical Sciences and bred at the Experimental Animal Center, Peking University Health Sciences Center (Beijing, China). The integration of the human PDCD5 gene was determined by PCR on genomic DNA of the mice. The animal experiment protocol was approved by the Biomedical Research Ethics Committee of Peking University (LA 2010-048) and strictly adhered to the American Physiological Society's "Guiding Principles in the Care and Use of Vertebrate Animals in Research and Training". Male C57BL/6J mice of 8 weeks were provided from the Animal Department of Peking University Health Science Center (Beijing, China). Mice were housed in groups of four and maintained on a 12 h dark/light cycle in a room with controlled temperature (25 ± 2 °C), with free access to food and water.

2.2. Establishment of cardiac remodeling model

Cardiac remodeling model was established by subcutaneous injection of isoproterenol (ISO, 7.5 mg/kg/day, dissolved in PBS, Sigma–Aldrich, St. Louis, USA) for 14 consecutive days. Mice were randomly divided into four groups to receive different treatments: (i) WT + PBS group (WT group): wild-type mice with daily administration of PBS for 14 consecutive days as control; (ii) PDCD5tg + PBS group (PDCD5tg group): PDCD5 transgene mice with daily subcutaneous administration of PBS for 14 consecutive days; (iii) WT + ISO group: wild-type mice with daily subcutaneous administration of ISO for 14 consecutive days; (iv) PDCD5tg + ISO group: PDCD5 transgene mice with daily subcutaneous administration of ISO for 14 consecutive days. Echocardiography analysis was performed on 1 day before the first injection for the baseline, the 3rd day after the first injection, the 7th day after the first injection and 1 day after the last injection as the final examination. Then the mice were anaesthetized and euthanized.

2.3. Western blot analysis

Mice organs were collected and disrupted in lysis buffer containing protease inhibitors (Roche Diagnostics, Germany). After centrifugation, the supernatant was collected and equivalent amounts of protein were subjected to SDS-PAGE and transferred to nitrocellulose membrane. The membranes were incubated with antibodies to GAPDH (KM9002, Sungene), LC3 (SAB4200361, Sigma) and PDCD5, and protein bands were visualized using DyLight 800/DyLight 680-conjugated secondary antibodies. The infrared fluorescence image was obtained using an Odyssey infrared imaging system (LI-COR Biosciences, USA).

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was prepared from mice tissues using TRIZOL reagent (Invitrogen), and cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (K1622, Fermenta). mRNA expression was analyzed by RT-PCR and were normalized to GAPDH housekeeping gene. RT-PCR was performed in triplicate for each sample. The primers used for RT-PCR were as follows: mouse PDCD5 (mPDCD5) AAGCGATTCCAACCGAGTGT and ACCGCGCCATCTGTATAAGG; human PDCD5 (hPDCD5) CGGAATTCACCATGGCGGACGAGGAGC and CGGAATTCAATAATCGTCATCTTCATC; GAPDH CAAGGTCATCCATGACAACTTTG and GTCCACCACCCTGTTGCTGTAG.

2.5. Echocardiographic analysis

Mice were anaesthetized with 1% isoflurane (Baxter Healthcare Corporation, New Providence, USA). Echocardiographic images were obtained by the Visualsonics high-resolution Vevo 770 system (VisualSonics, Incorporated, Toronto, Canada). Two-dimensional parasternal long-axis views and short-axis views were obtained at the level of the papillary muscle. Diastolic left ventricular posterior wall thickness (LVPW; d) and systolic left ventricular posterior wall thickness (LVPW; s) were measured to calculate the ejection fraction (EF) and fractional shortening (FS). All measurements were averaged from three consecutive cardiac cycles.

2.6. Histological analyses

Following sacrifice, the hearts were harvested and perfused in retrograde with cold phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 8 h, dehydrated in 20% sucrose for 24 h and then embedded in paraffin. Serial sections (5 μm thick) were stained with hematoxylin and eosin (H & E) for morphological analysis, and/or picrosirius red for the detection of fibrosis. For morphometrical analysis, photographs of left ventricular sections cut from the same location of each heart were observed under 400 × magnification (Leica Microsystems Imaging Solutions Ltd., Cambridge, UK). Interstitial fibrosis was evaluated by picrosirius red staining, and the cardiac fibrosis volume fraction was calculated as the ratio of the stained fibrotic area to total myocardial area. For TUNEL staining, the hearts were embedded in freezing matrix and cut as serial 7 μm cryostat sections. TUNEL assay was performed with the In Situ Cell Death Detection Kit (Roche Applied Science) according to the manufacturer's instructions. The sections were counterstained with Hoechst33342.

2.7. Statistical analysis

Data were presented as means ± SEM. Differences between groups were compared using Prism 5 (GraphPad Software Incorporae, La Jolla, CA, USA) with Student's unpaired two-tailed t-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Generation of human PDCD5 transgenic (PDCD5tg) mice

Consistent with previous reports [17,22], RT-PCR results showed that *PDCD5* mRNA was highly expressed in adult heart tissue (Fig. 1A). To determine whether *Pdcd5* may be involved in cardiac remodeling, we first examined gene expression in heart extracts from adult mice subjected to a 2-week isoproterenol (ISO)

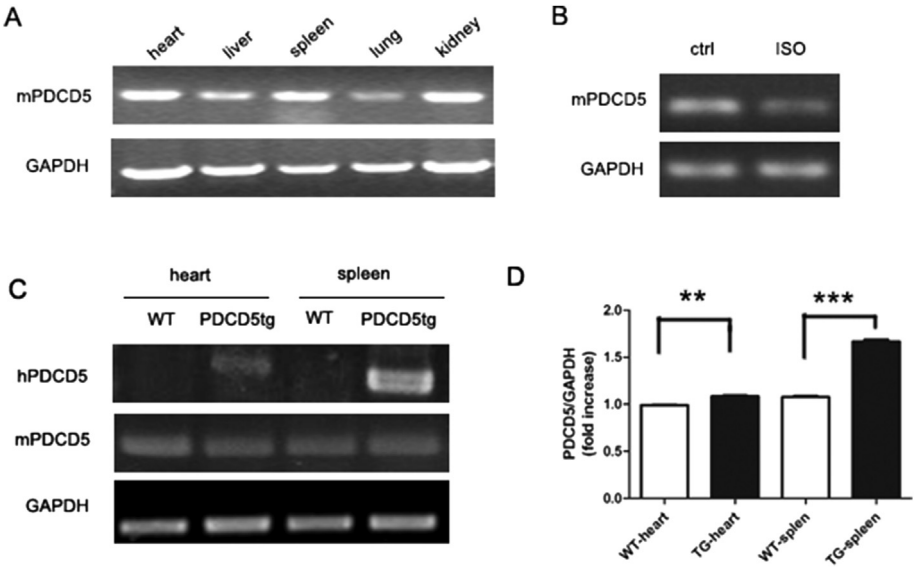

Fig. 1. Characterization of *PDCD5* expression in WT and *PDCD5tg* mice. (A) Representative RT-PCR results showing endogenous *Pdcd5* expression in different tissues from WT mice. (B) Representative RT-PCR results showing endogenous *Pdcd5* expression in ISO-induced cardiac remodeling. (C) Representative RT-PCR results showing endogenous and transgenic human *PDCD5* expression in different tissues from WT and *PDCD5tg* mice. (D) Densitometric analysis of *PDCD5*. Results were expressed as the ratio of collagen area to heart area (n = 3).

treatment. There was a significant decrease in *Pdcd5* expression in ISO-induced hypertrophied hearts (Fig. 1B). These data suggest PDCD5 is potentially involved in cardiac remodeling. To determine the role of PDCD5 in cardiac remodeling, we generated transgenic mice with systemic overexpression of human *PDCD5* (*PDCD5tg* mice). *PDCD5tg* mice were identified by PCR and RT-PCR analyses and showed significantly increased *hPDCD5* expression in heart and lung tissues (Fig. 1C, D). No difference in survival was observed between *PDCD5tg* mice and WT mice. *PDCD5tg* mice did not display pathological alterations in ventricular chamber dimensions, ventricular wall thickness, fractional shortening, or heart weight normalized to body weight (data not shown). Extensive histological analysis did not reveal any gross morphological alterations or fibrosis (data not shown).

3.2. Effects of PDCD5 overexpression on cardiac hypertrophy

To investigate the role of *PDCD5* in biomechanical stress in the heart, we performed a β-adrenergic stimulation in 8-week-old *PDCD5tg* and WT mice for two weeks. We examined cardiac hypertrophy by echocardiography and found that diastolic left ventricular wall thickness (LVPW; d) was not significantly different between *PDCD5tg* + ISO mice and their WT + ISO counterparts (Fig. 2A). Compared with WT + ISO mice, the heart weight/body weight (HW/BW) ratio of *PDCD5tg* + ISO mice was not significantly different (Fig. 2B), whereas the lung weight/body weight (LW/BW) ratio was dramatically lower (Fig. 2C). No significant differences were observed between *PDCD5tg* and WT mice without β-adrenergic stimulation. The increase in left ventricle (LV) chamber dimensions and thickness induced by ISO were also not significantly different during either systole or diastole between the *PDCD5tg* + ISO group and the WT + ISO group. The WT + ISO group displayed a larger cross-sectional cardiomyocyte area than the WT mice. However, there was no significant difference between *PDCD5tg* + ISO and WT + ISO mice (Fig. 2D, E). Collectively, these results indicated that *PDCD5* overexpression in mice had no beneficial effects on cardiac hypertrophy associated with β-adrenergic stimulation.

3.3. Overexpression of PDCD5 attenuates cardiac fibrosis in vivo

Cardiac fibrosis is an important process in pathological cardiac remodeling and can lead to heart failure. Therefore, we investigated the effect of *PDCD5* on cardiac fibrosis induced by β-adrenergic stimulation. Cardiac fibrosis was examined by Sirius Red staining. In the WT + ISO group, collagen continued to accumulate in the heart after 2 weeks of β-adrenergic stimulation. Increased collagen deposition was observed in the WT + ISO group. However, collagen accumulation was absent in the *PDCD5tg* + ISO group (Fig. 2F). Quantitative analysis showed lower collagen volume in the myocardium of *PDCD5tg* + ISO group than that observed in the WT + ISO group (Fig. 2G). Decreased fibrosis in the *PDCD5tg* + ISO group may represent increased collagen degradation or decreased collagen synthesis in response to tissue damage. Thus, these results indicate that *PDCD5* overexpression in mice attenuates ISO-induced cardiac fibrosis.

3.4. Overexpression of PDCD5 protects cardiac function in vivo

To determine the alteration of cardiac structure and function of WT and *PDCD5tg* mice in response to β-adrenergic stimuli, we performed an echocardiography. LV end-diastolic diameter (LVID; d) and LV end-systolic diameter (LVID; s) were greater in the WT + ISO group than in the *PDCD5tg* + ISO group (Fig. 3A, B). LV function was severely impaired in the WT + ISO group, as indicated by a significant decline in percent fractional shortening (FS) and a significant decrease in ejection fraction (EF) (Fig. 3C, D), whereas such declines were markedly attenuated in the *PDCD5tg* + ISO group. Consistent with these findings, lung weight normalized to body weight was significantly higher in the WT + ISO group than in the *PDCD5tg* + ISO group after β-adrenergic stimuli, which was indicative of lung congestion (Fig. 2C). Moreover, mice were analyzed for several cardiac remodeling-related indicators 1 day before ISO stimulation as well as 3 d, 7 d, and 14 d post-ISO stimulation. At 3 d post-ISO, the LVID; d was greater in the WT + ISO group than in the *PDCD5tg* + ISO group; there was also a marked difference at 14 d (Fig. 3E). In addition, ejection fraction (EF) and

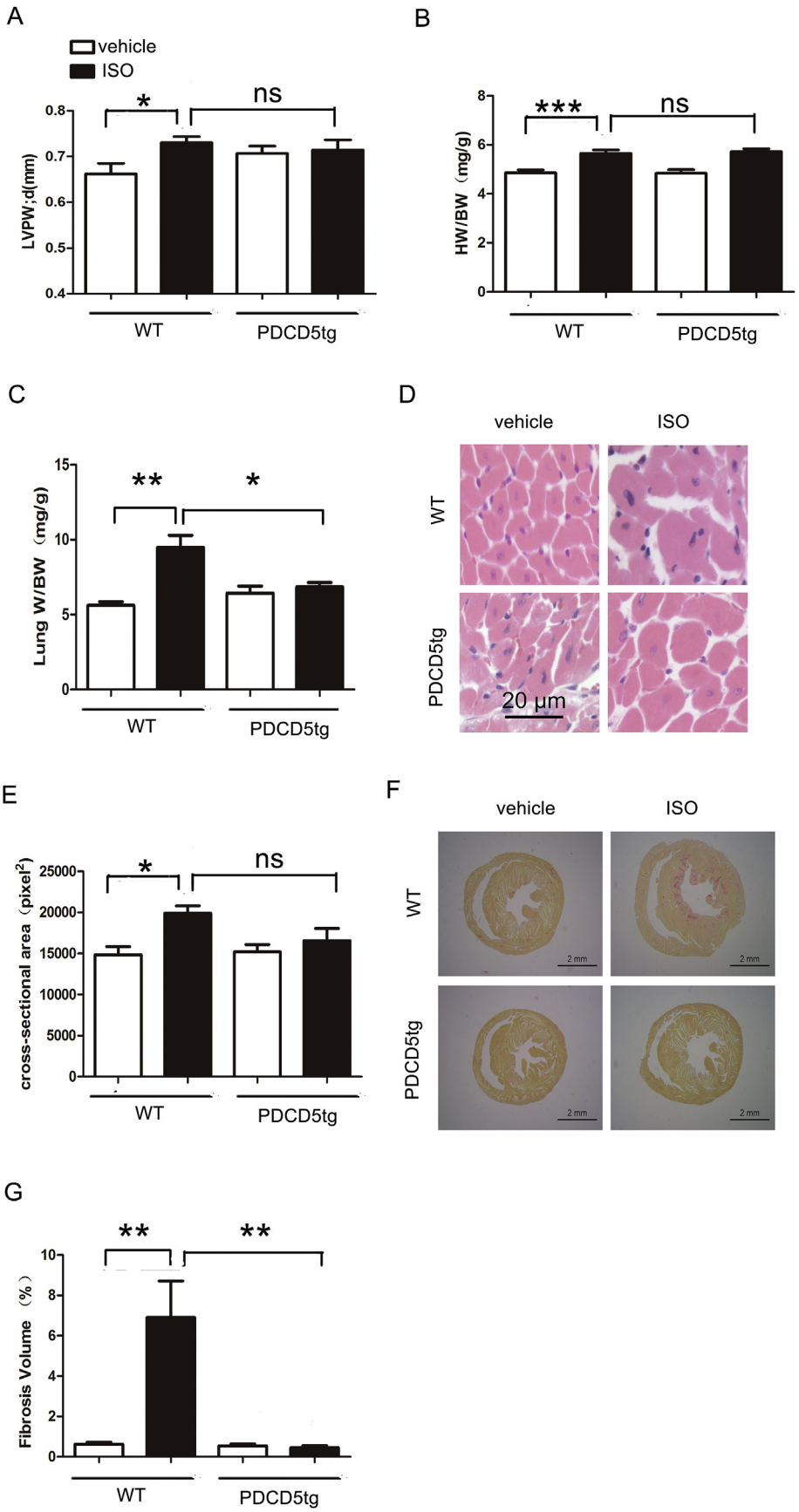

Fig. 2. Effects of *PDCD5* overexpression on cardiac hypertrophy and fibrosis. (A) Representative M-mode echocardiography images. Diastolic left ventricular wall thickness (LVPW; d) was not significantly different between WT + ISO and *PDCD5tg* + ISO groups (10 weeks old). (B) The ratio of heart weight to body weight (HW/BW) was not significantly different

fractional shortening (FS) were significantly lower in the WT + ISO group than in the *PDCD5tg* + ISO group from 3 d after ISO stimulation (Fig. 3F, G). In summary, these results indicate that *PDCD5tg* mice displayed better protection of cardiac function than the WT mice following β-adrenergic stimulation.

3.5. Effects of PDCD5 overexpression on autophagy

Previous studies have shown that autophagy increases protein turnover during cardiac remodeling [21]. Therefore, we examined LC3II/LC3I and P62, two molecular markers for autophagy. Western blot analysis showed that the LC3II/LC3I level was significantly higher in *PDCD5tg* mice than in WT mice (Fig. 4A, B), whereas the LC3II/LC3I level in *PDCD5tg* + ISO mice were not significantly different from the levels in WT + ISO mice (data not shown). P62 expression was low and there were no marked differences between the vehicle- and ISO-treated groups (data not shown). Collectively, these data suggest that autophagy likely plays a beneficial role in the heart in response to β-adrenergic stress.

3.6. Effects of PDCD5 overexpression on apoptosis

Since apoptosis and autophagy have simultaneously been observed in the failing human heart and *PDCD5* plays an important role in the regulation of apoptosis, we asked whether cardiac remodeling is associated with apoptosis. We performed a TUNEL assay using heart sections from *PDCD5tg* and WT mice post-β-adrenergic stimulation. Apoptotic cells were detected in *PDCD5tg* + ISO mice and WT + ISO mice. However, the fraction of apoptotic cells relative to total cells was significantly lower in *PDCD5tg* + ISO mice than in WT + ISO mice (Fig. 4C, D). However, there was no significant difference in the proportion of apoptotic cells between the *PDCD5tg* mice and the WT mice in the vehicle-treated groups. These data suggest that apoptosis likely resulted in exacerbation of cardiac remodeling in the WT + ISO group.

4. Discussion

In the current study, we demonstrated that (1) transgenic mice with systemic overexpression of *PDCD5* showed no obvious abnormalities in either cardiac function or survival; (2) transgenic systemic overexpression of *PDCD5* provided protection against cardiac fibrosis and cardiac function following ISO-induced cardiac remodeling; (3) the cardiac protection afforded by *PDCD5* overexpression was associated with increased autophagy and attenuated apoptosis. Therefore, the ability of *PDCD5* to prevent cardiac dysfunction and cardiac fibrosis mediated by ISO suggests that *PDCD5* is a promising therapeutic candidate for heart disease.

Autophagy plays a beneficial role in the heart in response to pressure overload, β-adrenergic stress, and other forms of stress. In particular, autophagy seems to increase protein turnover in remodeling hearts and to prevent the accumulation of abnormal proteins or damaged organelles, which could disrupt cardiac function [9,15,16,31]. In the absence of autophagy, the accumulation of polyubiquitinated proteins may be responsible for increased endoplasmic reticulum stress, resulting in apoptosis [9]. Moreover, a previous study demonstrated that cardiac autophagy increased in the early phase of adrenergic overload and acted as a cytoprotective response against apoptosis in cardiac myocytes [32]. However, the role of *PDCD5* in ISO-induced cardiac remodeling was unknown. Therefore, we hypothesized that the upregulation of the autophagy-related gene *PDCD5* could represent a potential adaptive response to protect cardiomyocytes under stress by regulating their survival and function. We found that *PDCD5tg* mice displayed enhanced cardiac function and were protected from cardiac fibrosis following β-adrenergic stimulation. Our results indicate that *PDCD5tg* + ISO mice remained at the early stage of cardiac remodeling, whereas WT + ISO mice progressed to the late stage. Moreover, our study showed that the LC3II/LC3I level was significantly higher in *PDCD5tg* mice than in WT mice (Fig. 4A, B), whereas no difference was observed in the levels between *PDCD5tg* + ISO mice and their WT + ISO counterparts (data not shown). We also showed that attenuation of cardiac function was associated with upregulated basal autophagy. Increased basal autophagy enabled mice to better respond to adverse stimuli. *PDCD5tg* mice had systemic overexpression of *PDCD5*, which can have effects on different types of cells and organs and could lead to compensatory survival. Consistent with a previous report, upregulation of autophagy could be protective and compensatory by removing damaged mitochondria and proteins at the early stages of myocardial stress [9,32]. Moreover, activation of autophagy by rapamycin has been reported to prevent cardiac hypertrophy induced by thyroid hormone treatments [32,33]. Pressure overload due to transverse aortic constriction also revealed that autophagic activity was decreased during hypertrophic responses [9]. The potential for autophagy as a therapeutic target should be considered in future studies and the mechanism of autophagy should be elucidated. In addition, our results showed that the fraction of apoptotic cells was significantly lower in *PDCD5tg* + ISO mice than in WT + ISO mice. These findings are likely related to the pro-apoptotic β-adrenergic agonists and compensatory autophagy. Autophagy may be an active adaptive response for protecting cardiomyocytes by removing damaged mitochondria and proteins at early stages of myocardial stress [9,15,16]. However, once cellular homeostasis is sufficiently altered, these cells may undergo apoptosis [32,34]. Moreover, a previous study using the *Atg5* knockout mouse demonstrated that autophagy inhibited β-adrenergic stress-induced cardiac cell death [9].

The underlying mechanisms of the inhibitory effects of *PDCD5* on cardiac remodeling are unknown. Different regulation networks likely produce varied biological effects in diverse cell types and organs. Thus, elucidation of the specific network will allow a greater understanding of the protective role of *PDCD5* in the heart and provide potential drug targets for heart disease treatment. A previous study demonstrated that *PDCD5* was strongly induced upon angiotensin II treatment or transverse aortic constriction, consistent with a potential role for *PDCD5* in cardiac remodeling and cardiac function [18]. Although these results are different from our findings, the differences could be attributed to the expression level and region of *PDCD5*. In this study, *PDCD5tg* mice had systemic overexpression of *PDCD5*, which can influence different cell types and organs, potentially leading to compensatory survival. Another key difference is that we induced cardiac remodeling using ISO rather than angiotensin II, suggesting that *PDCD5* function could depend on the nature of the stimulus.

between WT + ISO mice and *PDCD5tg* + ISO mice. (C) The ratio of lung weight to body weight (LW/BW) was significantly different between WT + ISO mice and *PDCD5tg* + ISO mice. (D) H&E staining of heart sections. Scale bar = 20 μm. (E) Measurements of two-dimensional cardiomyocyte cross-sectional areas. (F) Representative micrographs of picrosirius red-stained sections of the ventricle. Red areas represent collagen. Scale bar = 2 mm. (G) Quantification of cardiac interstitial collagen content from picrosirius red-stained sections. Results are expressed as the ratio of collagen area to heart area (n = 4–12, 10 weeks old). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

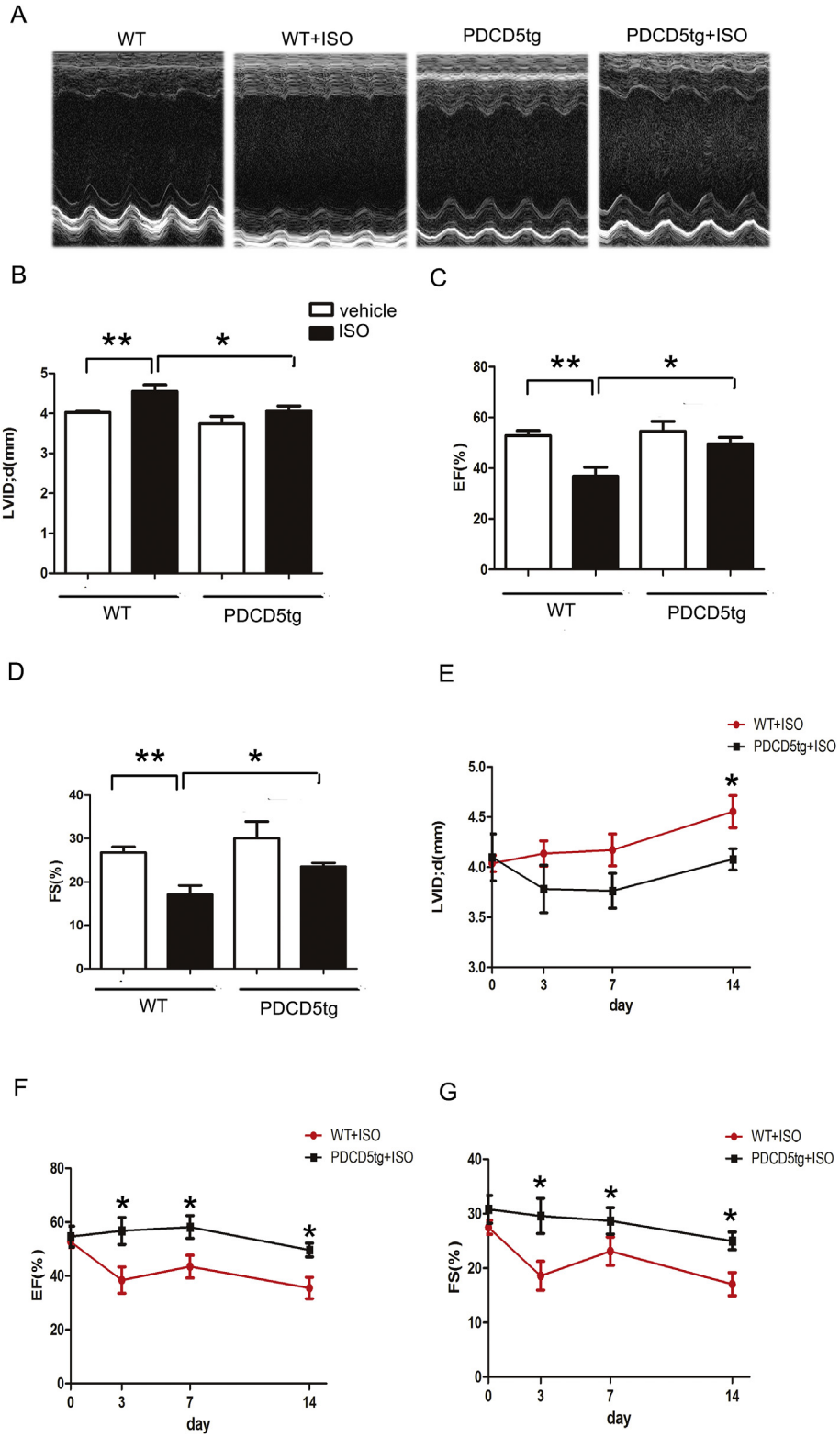

Fig. 3. Effects of *PDCD5* overexpression on cardiac function. (A) Representative M-mode echocardiography images showing left ventricular wall thickness and systolic function in 10-week-old mice. (B) LV end-diastolic diameter (LVID; d) was significantly higher in WT + ISO mice than in *PDCD5tg* + ISO mice. (C–D) Ejection fraction (EF) and fractional shortening (FS) were significantly lower in WT + ISO mice than in *PDCD5tg* + ISO mice. (E–G) Echocardiographic analysis revealed enlarged left ventricular diastolic dimension (LVID; d) and decreased ejection fraction (EF) and fractional shortening (FS) in WT + ISO mice (n = 4–9, 10 weeks old).

In conclusion, the present work demonstrates for the first time that *PDCD5* protects mice against cardiac remodeling in response to ISO. The mechanism underlying the protective effects of *PDCD5* appears to involve increased basal autophagy and inhibition of apoptosis. The potential for *PDCD5* as a therapeutic target should be considered in future studies.

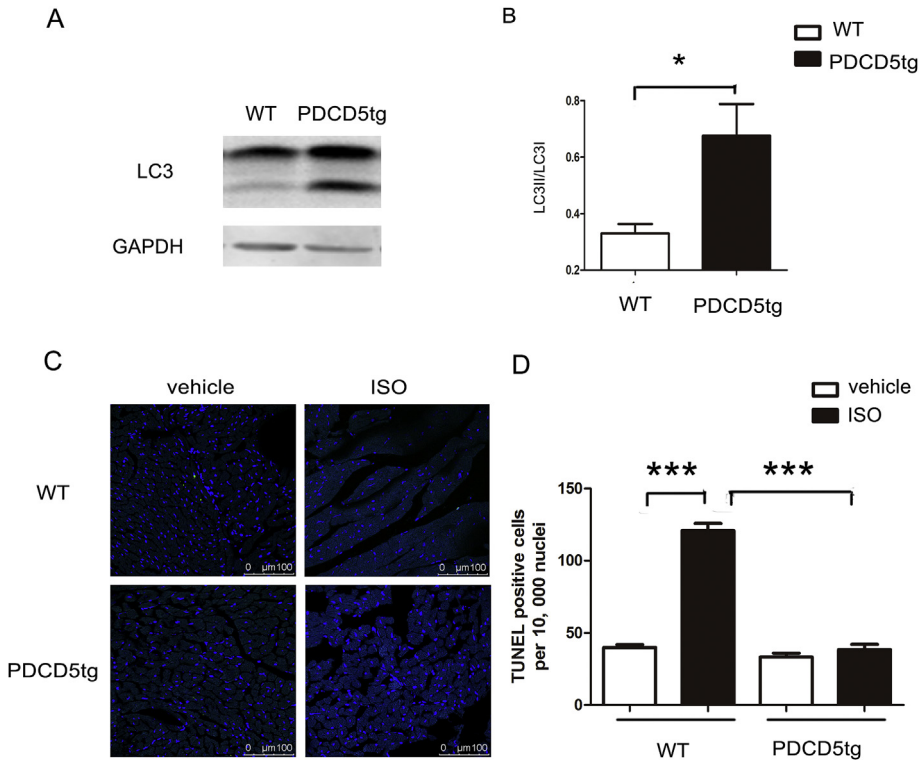

Fig. 4. Assessment of autophagy and apoptosis in *PDCD5tg and* WT mice. (A) Representative western blot of autophagy-related proteins LC3 in heart extracts obtained from 10-week-old mice. (B) Densitometric analysis of LC3 immunoblots. (C) Representative images of TUNEL staining (green) and Hoechst staining (blue) of nuclei on cryosectioned hearts. (D) Quantification of positive cells displaying TUNEL staining (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Conflict of interest

None.

Acknowledgments

This work is supported by National Key Basic Research Program of China (973, 2011CB910103) and National Natural Science Foundation of China (81272432, 81070260).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.032.

References

[1] D. Levy, R.J. Garrison, D.D. Savage, et al., Prognostic implications of echocardiographically determined left ventricular mass in the Framingham heart study, N. Engl. J. Med. 322 (1990) 1561–1566.
[2] N. Frey, E.N. Olson, Cardiac hypertrophy: the good, the bad, and the ugly, Annu Rev. Physiol. 65 (2003) 45–79.
[3] S. Oparil, Pathogenesis of ventricular hypertrophy, J. Am. Coll. Cardiol. 5 (1985) 57B–65B.
[4] M. Minnaard-Huiban, J.M. Emmen, L. Roumen, et al., Fadrozole reverses cardiac fibrosis in spontaneously hypertensive heart failure rats: discordant enantioselectivity versus reduction of plasma aldosterone, Endocrinology 149 (2008) 28–31.
[5] H. Tao, K.H. Shi, J.J. Yang, et al., Epigenetic regulation of cardiac fibrosis, Cell. Signal 25 (2013) 1932–1938.
[6] F. Ma, Y. Li, L. Jia, et al., Macrophage-stimulated cardiac fibroblast production of IL-6 is essential for TGF beta/Smad activation and cardiac fibrosis induced by angiotensin II, PLoS One 7 (2012) e35144.
[7] Y.E. Koshman, N. Patel, M. Chu, et al., Regulation of connective tissue growth factor gene expression and fibrosis in human heart failure, J. Card. Fail 19 (2013) 283–294.
[8] P. Sirish, N. Li, J.Y. Liu, et al., Unique mechanistic insights into the beneficial effects of soluble epoxide hydrolase inhibitors in the prevention of cardiac fibrosis, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 5618–5623.
[9] A. Nakai, O. Yamaguchi, T. Takeda, et al., The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress, Nat. Med. 13 (2007) 619–624.
[10] S. Kostin, L. Pool, A. Elsasser, et al., Myocytes die by multiple mechanisms in failing human hearts, Circ. Res. 92 (2003) 715–724.
[11] Z.V. Wang, B.A. Rothermel, J.A. Hill, Autophagy in hypertensive heart disease, J. Biol. Chem. 285 (2010) 8509–8514.
[12] A.B. Gustafsson, R.A. Gottlieb, Autophagy in ischemic heart disease, Circ. Res. 104 (2009) 150–158.
[13] G.R. De Meyer, W. Martinet, Autophagy in the cardiovascular system, Biochim. Biophys. Acta 1793 (2009) 1485–1495.
[14] K.M. Mellor, M.E. Reichelt, L.M. Delbridge, Autophagy anomalies in the diabetic myocardium, Autophagy 7 (2011) 1263–1267.
[15] R.L. Thomas, D.J. Roberts, D.A. Kubli, et al., Loss of MCL-1 leads to impaired autophagy and rapid development of heart failure, Genes. Dev. 27 (2013) 1365–1377.
[16] M. Sun, M. Ouzounian, G. de Couto, et al., Cathepsin-L ameliorates cardiac hypertrophy through activation of the autophagy-lysosomal dependent protein processing pathways, J. Am. Heart Assoc. 2 (2013) e000191.
[17] Z. Jiang, C.H. Chen, Y.Y. Chen, et al., Autophagic effect of programmed cell death 5 (PDCD5) after focal cerebral ischemic reperfusion injury in rats, Neurosci. Lett. 566 (2014) 298–303.
[18] L. An, X. Zhao, J. Wu, et al., Involvement of autophagy in cardiac remodeling in transgenic mice with cardiac specific over-expression of human programmed cell death 5, PLoS One 7 (2012) e30097.
[19] M. Xie, J.H. Niu, Y. Chang, et al., A novel triple-regulated oncolytic adenovirus carrying PDCD5 gene exerts potent antitumor efficacy on common human leukemic cell lines, Apoptosis 14 (2009) 1086–1094.
[20] G.R. Ruan, H.S. Zhao, Y. Chang, et al., Adenovirus-mediated PDCD5 gene transfer sensitizes K562 cells to apoptosis induced by idarubicin in vitro and in vivo, Apoptosis 13 (2008) 641–648.
[21] Y.H. Yang, M. Zhao, W.M. Li, et al., Expression of programmed cell death 5 gene involves in regulation of apoptosis in gastric tumor cells, Apoptosis 11 (2006) 993–1001.
[22] H. Liu, Y. Wang, Y. Zhang, et al., TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1, could enhance apoptosis of some tumor cells induced by growth factor withdrawal, Biochem. Biophys. Res. Commun. 254 (1999) 203–210.

[23] Y. Wang, X. Li, L. Wang, et al., An alternative form of paraptosis-like cell death, triggered by TAJ/TROY and enhanced by PDCD5 overexpression, J. Cell. Sci. 117 (2004) 1525–1532.
[24] L. Xu, J. Hu, Y. Zhao, et al., PDCD5 interacts with p53 and functions as a positive regulator in the p53 pathway, Apoptosis 17 (2012) 1235–1245.
[25] C.H. Chen, Z. Jiang, J.H. Yan, et al., The involvement of programmed cell death 5 (PDCD5) in the regulation of apoptosis in cerebral ischemia/reperfusion injury, CNS Neurosci. Ther. 19 (2013) 566–576.
[26] J. Xiao, C. Liu, G. Li, et al., PDCD5 negatively regulates autoimmunity by upregulating FOXP3(+) regulatory T cells and suppressing Th17 and Th1 responses, J. Autoimmun. 47 (2013) 34–44.
[27] K. Li, Z. Zhou, Y.O. Wang, et al., Pretreatment of mice with oligonucleotide prop5 protects them from influenza virus infections, Viruses 6 (2014) 573–581.
[28] N. Wang, H.S. Lu, Z.P. Guan, et al., Involvement of PDCD5 in the regulation of apoptosis in fibroblast-like synoviocytes of rheumatoid arthritis, Apoptosis 12 (2007) 1433–1441.
[29] P. Zhang, M. Zhao, G. Liang, et al., Whole-genome DNA methylation in skin lesions from patients with psoriasis vulgaris, J. Autoimmun. 41 (2013) 17–24.
[30] Y. Chen, Z. Zou, A. Xu, et al., Serum programmed cell death protein 5 (PDCD5) levels is upregulated in liver diseases, J. Immunoass. Immunochem. 34 (2013) 294–304.
[31] A.F. Ceylan-Isik, M. Dong, Y. Zhang, et al., Cardiomyocyte-specific deletion of endothelin receptor A rescues aging-associated cardiac hypertrophy and contractile dysfunction: role of autophagy, Basic Res. Cardiol. 108 (2013) 335.
[32] M. Nakaoka, E. Iwai-Kanai, M. Katamura, et al., An alpha-adrenergic agonist protects hearts by inducing Akt1-mediated autophagy, Biochem. Biophys. Res. Commun. 456 (2015) 250–256.
[33] J.A. Kuzman, T.D. O'Connell, A.M. Gerdes, Rapamycin prevents thyroid hormone-induced cardiac hypertrophy, Endocrinology 148 (2007) 3477–3484.
[34] K. Nishida, O. Yamaguchi, K. Otsu, Crosstalk between autophagy and apoptosis in heart disease, Circ. Res. 103 (2008) 343–351.