Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function

Feihong Yang [a, b, c, d, 1], Li Xue [a, b, c, d, 1], Ziqi Han [a, b, c, d], Feng Xu [a, b, c, d], Shengchuan Cao [a, b, c, d], Shuai Dai [a, b, c, d], Baoshan Liu [a, b, c, d], Qiuhuan Yuan [a, b, c, d], Zheng Wang [a, b, c, d], Ping Guo [e, **], Yuguo Chen [a, b, c, d, *]

[a] Department of Emergency Medicine and Chest Pain Center, Qilu Hospital of Shandong University, Jinan, China
[b] Clinical Research Center for Emergency and Critical Care Medicine of Shandong Province, Institute of Emergency and Critical Care Medicine of Shandong University, Qilu Hospital of Shandong University, Jinan, China
[c] Key Laboratory of Emergency and Critical Care Medicine of Shandong Province, Key Laboratory of Cardiopulmonary-Cerebral Resuscitation Research of Shandong Province, Qilu Hospital of Shandong University, Jinan, China
[d] Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education, Chinese Ministry of Health and Chinese Academy of Medical Sciences, State and Shandong Province Joint Key Laboratory of Translational Cardiovascular Medicine, Qilu Hospital of Shandong University, Jinan, China
[e] Medical Insurance Office, Qilu Hospital of Shandong University, Jinan, China

ARTICLE INFO

Article history:
Received 28 April 2018
Accepted 2 May 2018
Available online xxx

Keywords:
Vaspin
Myocardial ischaemia/reperfusion injury
Autophagic flux
Cardiomyocytes
AMPK

ABSTRACT

Visceral adipose tissue-derived serine protease inhibitor (vaspin), as a secretory adipokine, was reported to exert a protective role on insulin resistance. Recent studies showed that serum vaspin level was downregulated in patients with coronary artery disease. However, whether vaspin exerted specific effects on myocardial injury remains unknown. In this study, we determined to explore the role of vaspin overexpression in myocardial ischaemia/reperfusion (I/R) injury and the underlying mechanisms. Our results showed that the systemic delivery of adeno-associated virus-vaspin to mice reduced myocardial infarct size and apoptosis, and improved cardiac function after reperfusion, accompanied with upregulated autophagic flux and restored lysosomal function in the ischaemic heart. Blockage of the autophagic flux with choroquine mitigated the protection of vaspin on myocardial I/R injury. Moreover, we testified that administration of exogenous recombinant human vaspin on cultured cardiomyocytes suppressed hypoxia/reoxygenation-induced apoptosis, through the AMPK-mTOR-upregulated autophagic flux. Overall, we verified that vaspin functions as an adipokine which can alleviate I/R injury in the heart by suppressing myocardial apoptosis through AMPK-mTOR-dependent activation of autophagic flux, and then provided a potential breakthrough in the treatment of myocardial I/R injury and other ischaemic diseases.

1. Introduction

Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide [1]. The application of modern pharmacological interventions and early reperfusion therapies have greatly improved life quality and prognosis in AMI patients [2], however, these treatments can also bring about irreversible damage, such as deteriorative cardiac function and reperfusion arrhythmias, termed myocardial ischaemia/reperfusion (I/R) injury [3,4]. Thus, it is urgent to explore effective therapeutic interventions to alleviate myocardial I/R injury.

Visceral adipose tissue-derived serine protease inhibitor (vaspin), an adipokine firstly isolated from the visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats in 2000 [5], was later found in visceral and subcutaneous adipose tissues in obese humans [6]. It has been already confirmed that vaspin could

* Corresponding author. Qilu Hospital, Shandong University, No. 107 Wenhua Xi Road, Jinan, 250012, China.
** Corresponding author. Qilu Hospital, Shandong University, No. 107 Wenhua Xi Road, Jinan, 250012, China.
E-mail addresses: 13953100732@163.com (P. Guo), chen919085@sdu.edu.cn (Y. Chen).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.05.004
0006-291X/

mitigate insulin resistance and obesity through autocrine and paracrine [6,7]. Recently, it is reported that lower levels of circulating vaspin are positively related with the prevalence and severity of coronary artery diseases [8]. The serum concentration of vaspin is lower in atherosclerotic patients and the lowest in ST-segment elevation myocardial infarction (STEMI) patients [9,10].

To clarify the functional role of vaspin in coronary artery diseases (CAD), researchers have gained some achievements. Vaspin can exert its anti-atherosclerotic properties by reducing endothelial apoptosis as a result of activating insulin signal pathway to increase insulin sensitivity [11] and increasing endothelial nitric oxide synthase (eNOS) activity [12]. It also inhibits nuclear factor-kappa B (NF-κB) activation-induced vascular inflammation through activating the AMP-activated protein kinase (AMPK) pathway [13]. Furthermore, vaspin participates in regulating glucose and lipid metabolism to lower the risks of metabolic syndrome-associated cardiovascular complications [6,14]. Thus, these results may help explain why vaspin affects the initiation and development of CAD. However, the role of vaspin in the regulation of I/R injury remains unclear.

In this study, we evaluated the role of vaspin in myocardial I/R injury and explored the mechanisms by which vaspin modulated I/R damages in vivo and in vitro.

2. Materials and methods

2.1. Animals

C57BL/6 male mice (8–10 weeks old) were purchased from Huafukang company (Beijing, China) and fed in the experimental animal center of Qilu Hospital, Shandong University (Jinan, China). The mice were kept in cages with free access to food and water and at a constant temperature with 12-h light/dark cycles. All animal procedures were performed in accordance with the National Institute of Health guide for the care and use of Laboratory animals and were approved by the Institutional Animal Care and Use Committee of Shandong University.

2.2. I/R surgery and assessment of infarct area

Mice were volatilely anesthetized with 1% isoflurane and then subjected to reversible ligation of left anterior descending coronary (LAD) for 30 min, followed by reperfusion for 23.5 h as previously described [15,16]. The sham group was subjected to the same procedures without LAD ligation. 100 μL of AAV-vaspin or AAV-GFP (ViGene Biosciences Company) containing 1×10^8 viral particles were delivered via tail vein 4 weeks before surgery. Chloroquine (CQ) (10 mg/kg, HY-17589, MedChem Express) was intraperitoneally injected 1 h before LAD ligation [17]. After reperfusion, the mice were euthanized for subsequent experiments. To assess the infarct area, the hearts were quickly frozen after isolation and then sectioned into 5 mm slices before being submerged in 2% 2,3,5-triphenyltetrazolium (TTC) dye (DK0005, Leagene). Photographs were taken immediately after TTC incubation at 37 °C for 30 min in the dark.

2.3. Cell culture and H/R treatment

The H9C2 rat cardiac cell line was firstly subjected to 4-h' hypoxia in a hypoxic workstation (H35, Don Whitley Scientific) containing 94% N_2, 5% CO_2 and 1% O_2 at 37 °C, and then was reoxygenated for 2 h in a CO_2 incubator (3543, Thermo Fisher Scientific) containing 95% air and 5% CO_2. Human recombinant vaspin (40 μg/L, 130-11, Peprotech), Compound C (10 μM, T1977, Targetmol), CQ (10 μM, HY-17589, MedChem Express), rapamycin (100 μM, abs810030a, Absin) or vehicle were added 1 h before H/R.

2.4. Echocardiography

Measurement of mice cardiac function was carried out with a transthoracic ultrasound machine (Vevo2100, Visualsonics). Left ventricular M-mode images and related parameters (left ventricular ejection fraction, LVEF and fractional shortening, FS) in end-systole and end-diastole of both the long and short axes were recorded. The depth of anaesthesia was set to maintain the heart rate at approximately 550 beats per minute.

2.5. Immunostaining

After fixation, permeabilization and blocking, 5 μm heart slices or H9C2 slides were stained with LC3B or LAMP2 antibody overnight at 4 °C. Then, the samples were incubated with secondary antibody at room temperature for 1 h. Hematoxylin (DH0002, Leagene) or DAPI (AR1176, Boster) was added for nuclear counterstaining. Images were recorded under a microscope (IX53 + DP73, Olympus) and were analysed with Image-Pro Plus 6.0.

2.6. TUNEL assay

Myocardial apoptosis was detected by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) (11684795910, Roche) following the manufacturer's instructions. Briefly, 8 μm-thick frozen sections or H9C2 slides were fixed in 4% paraformaldehyde before permeabilization. Then, samples were incubated with reaction mixtures for 60 min at 37 °C in a humidified atmosphere in the dark. After DAPI staining, images were recorded using a fluorescence microscope.

2.7. Western blot analysis

The concentration of total proteins extracted from the frozen hearts or collected cells was measured with a BCA protein assay (AR0146, Boster). 20 μg protein samples were electrophoresed in SDS-PAGE gels and then transferred onto 22 μm PVDF membranes (Millipore). After blocking with 5% nonfat dried milk, the bands were incubated with primary antibodies overnight at 4 °C, and then were incubated in secondary antibody for 1 h at room temperature. Images were recorded using a chemiluminescence instrument (AI600 RGB, General Electric Company) and were analysed with ImageJ.

2.8. Evaluation of fluorescent LC3 puncta

The method used to evaluate the changing fluorescent LC3 puncta with a tandem RFP-GFP-LC3 construct was described previously [18]. H9C2 cells were transfected with Ad-RFP-GFP-LC3 (ViGene Biosciences Company) at 50 MOI before subsequent treatment. The green and red fluorescence intensity was analysed with ImageJ.

2.9. Antibodies

The primary antibodies used were as follows: β-actin (1/1000, 60008-1-Ig, Proteintech), GRP78 (1/1000, ab21685, Abcam), vaspin (1/1000, 17451-1-AP, Proteintech), vaspin (1/200, bs-7536R, Bioss), AMPK (1/1000, 2532S, Cell Signaling Technology, CST), and p-AMPK (Thr172) (1/1000, 2535S, CST). The autophagy-related primary antibodies used were as follows: LC3B (1/1000, 3868S, CST), p62 (1/1000, 5114S, CST), LAMP2 (1/1000, ab203224, Abcam), mTOR (1/1000, 2983P, CST). The apoptosis-related primary antibodies used

Please cite this article in press as: F. Yang, et al., Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.004

were as follows: bcl-2 (1/1000, GTX100064, Genetex), bax (1/1000,2772 T, CST), cleaved caspase-3 (1/1000, 9661S, CST). The secondary antibodies used in immunological experiments were purchased from Jackson Immunoresearch.

2.10. Statistical analysis

All data were expressed as means ± SEM. Evaluation was performed using Student's *t*-test for 2 groups and one-way ANOVA for multiple groups. $P < 0.05$ was considered significant.

3. Results

3.1. Vaspin overexpression reduced myocardial infarct size and improved cardiac function in mice after I/R

Considering the lower vaspin levels in AMI patients, male C57BL/6 J mice were injected with adeno-associated virus (AAV)-vaspin via tail vein 4 weeks before myocardial I/R to explore the protective role of vaspin (Fig. 1A). It was shown that circulating vaspin level was mildly increased 1.3-fold at 4 weeks after transfecting AAV-vaspin compared to the control group (Fig. 1B). Similar changes were detected in visceral adipose tissue (Fig. 1C). TTC staining showed that vaspin in mice with I/R injury significantly reduced the mean infarct size up to 32% compared with the control group (Fig. 1D). Furthermore, vaspin increased LVEF from 46% to 59% and FS from 30% to 33% (Fig. 1E).

In addition, we investigated the influence of vaspin on myocardial apoptosis. As shown in Fig. 1F, vaspin obviously reduced cleaved caspase-3 expression and increased bcl-2 expression, thus verifying the anti-apoptotic role of vaspin in myocardial I/R injury. Furthermore, accumulated ROS after I/R were reduced greatly by vaspin overexpression (Fig. 1G). In brief, these data suggest that vaspin plays a protective role against myocardial I/R injury and apoptosis.

3.2. Vaspin protected H9C2 cells from H/R-induced apoptosis

To examine whether vaspin affected apoptosis in vitro, H9C2 cells were treated with normoxia or H/R in the presence/absence of recombinant human vaspin. Exogenous vaspin administration significantly increased bcl-2 expression and decreased bax and cleaved caspase-3 expressions under H/R (Fig. 2A). Vaspin also reduced the number of TUNEL-positive cells (Fig. 2B). Furthermore, we found that ROS accumulation induced by H/R was suppressed by vaspin (Fig. 2C). Therefore, we consider vaspin to have a protective effect against H/R-induced cardiomyocyte injury.

3.3. Vaspin ameliorated I/R or H/R-induced myocardial apoptosis by activating autophagic flux and restoring lysosomal function

Since autophagy is a conserved cellular process which acts an important role in modulating myocardial apoptosis, we determined to evaluate the effect of vaspin on autophagy in mice heart suffering from I/R injury. It was shown that the expressions of LC3-II/LC3-I and p62 were obviously increased after myocardial I/R injury,

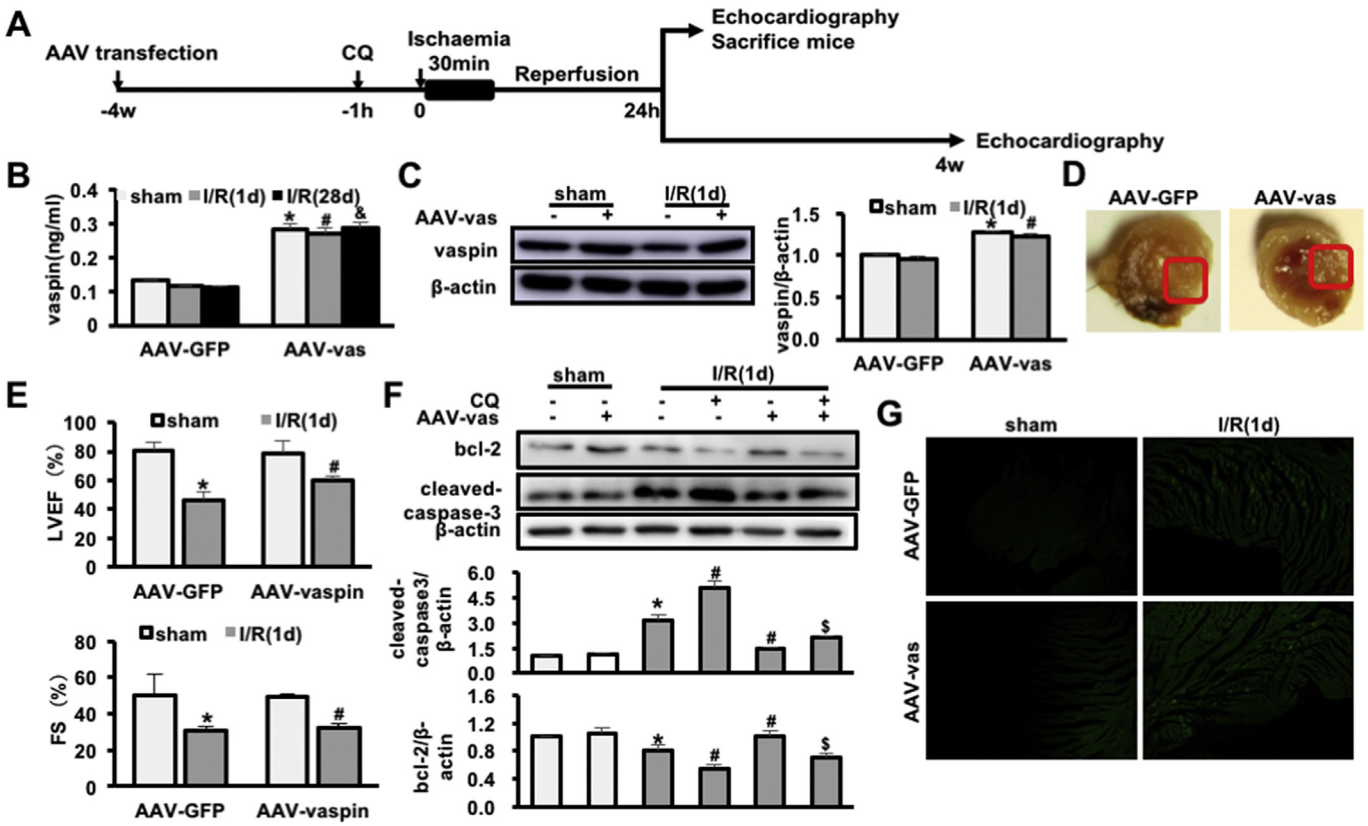

Fig. 1. Systemic delivery of AAV-vaspin improved myocardial injury in mice after I/R. (A) Experimental design assessing the cardioprotective effects of vaspin. (B) Detection of plasma vaspin levels in mice with an ELISA assay. (C) Western blot analysis of vaspin in the visceral adipose tissues. (D) Representative cross-sections stained with 2% TTC dye to determine the myocardial infact size after I/R (1 d). (E) The cardiac function after I/R (1 d) as shown by LVEF and FS obtained from echocardiography. (F) Western blot analysis of myocardial apoptosis as shown by bcl-2 and cleaved caspase-3. (G) Representative cross-sections (× 200) stained with H2DCFDA (green) to determine ROS levels in myocardium after I/R (1 d). $^{*}P < 0.05$ vs AAV-GFP sham, $^{\#}P < 0.05$ vs AAV-GFP I/R (1 d), $^{\&}P < 0.05$ vs AAV-GFP I/R (28 d), $^{\$}P < 0.05$ vs AAV-vas I/R (1 d). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Please cite this article in press as: F. Yang, et al., Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.004

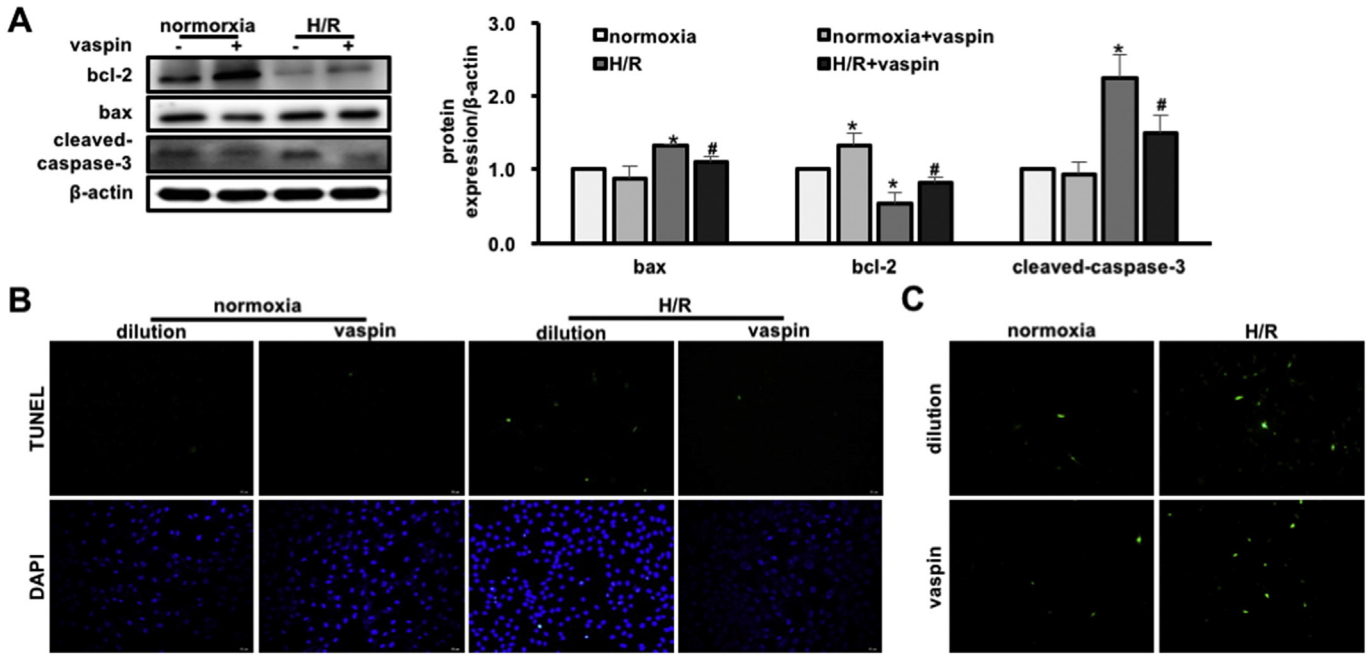

Fig. 2. Recombinant human vaspin reduced apoptosis in H9C2 cells after H/R. (A) Western blot analysis of apoptosis-associated markers in H9C2 cells. $^{*}P < 0.05$ vs normoxia, $^{\#}P < 0.05$ vs H/R group. (B) Representative images of TUNEL-positive cells (× 200) with vaspin pretreatment. Apoptotic nuclei and total nuclei were respectively identified by TUNEL staining (green) and DAPI staining (blue). (C) Representative images of ROS staining (green) in H9C2 cells (× 200). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

which were further enhanced with vaspin overexpression (Fig. 3A and Fig. S1A). The lysosomal-associated membrane protein 2 (LAMP2), a lysosomal membrane glycoprotein, is associated with lysosomal function ensuring the effective operation of the autophagic flux. Injection of AAV-vaspin significantly rescued the decreased LAMP2 expression caused by myocardial I/R injury (Fig. 3B and Fig. S1B). Immunohistochemical staining for LC3 and LAMP2 presented similar results (Fig. 3C and D).

To verify the protective role of vaspin on autophagy, we employed an autophagy inhibitor chloroquine (CQ) in vivo and in vitro. It was shown that the application of CQ increased myocardial infarct size which restrained the protective effects of vaspin in reducing I//R damage (Fig. 3E). Moreover, CQ aggravated myocardial apoptosis though in the presence of vaspin (Fig. 1F), illustrating the mediatory role of autophagy in the anti-apoptotic protection provided by vaspin. In addition, CQ eliminated the protective effects of vaspin on cardiac function after I/R, as shown by the changes in LVEF from 59% to 43% and in FS from 32% to 23% (Fig. 3F). CQ also decreased LVEF from 71% to 55% and FS from 50% to 34% 4 weeks after I/R (Fig. 3G).

Similar to changes in vivo, vaspin significantly increased LC3-II/LC3-I expression, determined by western blot and immunostaining, and restored LAMP2 expression to repair H/R-triggered lysosome dysfunction (Fig. 3H, I and Fig. S1C). However, CQ inhibited the degradation of autophagosomes as shown by LC3-II/LC3-I and p62 accumulation in the presence of vaspin (Fig. 3J and Fig. S1D). To further demonstrate the effect of vaspin on autophagic flux, H9C2 cells were transfected with RFP-GFP-LC3B virus. The autophagic flux was then examined, including the biogenesis and maturation of autophagosomes (GFP-LC3, green puncta) and acidic autolysosomes (RFP-LC3, red puncta), in which GFP was degraded by lysosomes. As shown in Fig. 3K, puncta representing autophagosomes and autolysosomes both increased significantly under vaspin treatment after H/R, indicating that vaspin exerted promotive and reparative effects on autophagic flux, which were blocked by CQ. The anti-apoptotic and anti-oxidant effects of vaspin were also suppressed by CQ (Figs. 3J and 4D, E and Fig. S1D).

3.4. The protective effects of vaspin on autophagic flux depended on activation of AMPK/mTOR signal pathway

We then detected the upstream signal pathway regulating autophagy. As shown in Fig. 4A and Fig. S2A, vaspin upregulated the phosphorylation of AMPK at Thr-172 in a time-dependent manner. AMPK/mammalian target of rapamycin (mTOR) pathway was further activated with vaspin pretreatment in H9C2 cells under H/R (Fig. 4B and Fig. S2B). Compound C, an AMPK inhibitor, was applied to assess the regulatory role of AMPK in the protection by vaspin. As shown in Fig. 4C and Fig. S2C, Compound C inhibited expressions of LC3-II/LC3-I and p62 to downregulate the autophagic flux, and then to abolish the anti-apoptotic effect of vaspin. It also increased the number of TUNEL-positive cells and ROS production after H/R in the presence of vaspin (Fig. 4D and E). Therefore, vaspin can activate autophagy through AMPK/mTOR pathway to reduce H/R-induced cardiomyocyte damage.

4. Discussion

In the present study, we provide the first evidence that increased levels of circulating vaspin achieved by AAV transfection reduced the infarct size and improved the cardiac function in mice with myocardial I/R injury. Moreover, in vitro, the administration of exogenous recombinant human vaspin alleviated cardiomyocyte apoptosis and ROS levels after H/R treatment. Mechanically, we verified that vaspin protected the heart against I/R impair through AMPK-mTOR-dependent autophagy activation, suggesting that enhancing circulating vaspin levels may represent a new therapeutic approach to improving cardiac function after acute myocardial I/R damage.

In recent years, some achievements clarifying the functions of

Please cite this article in press as: F. Yang, et al., Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.004

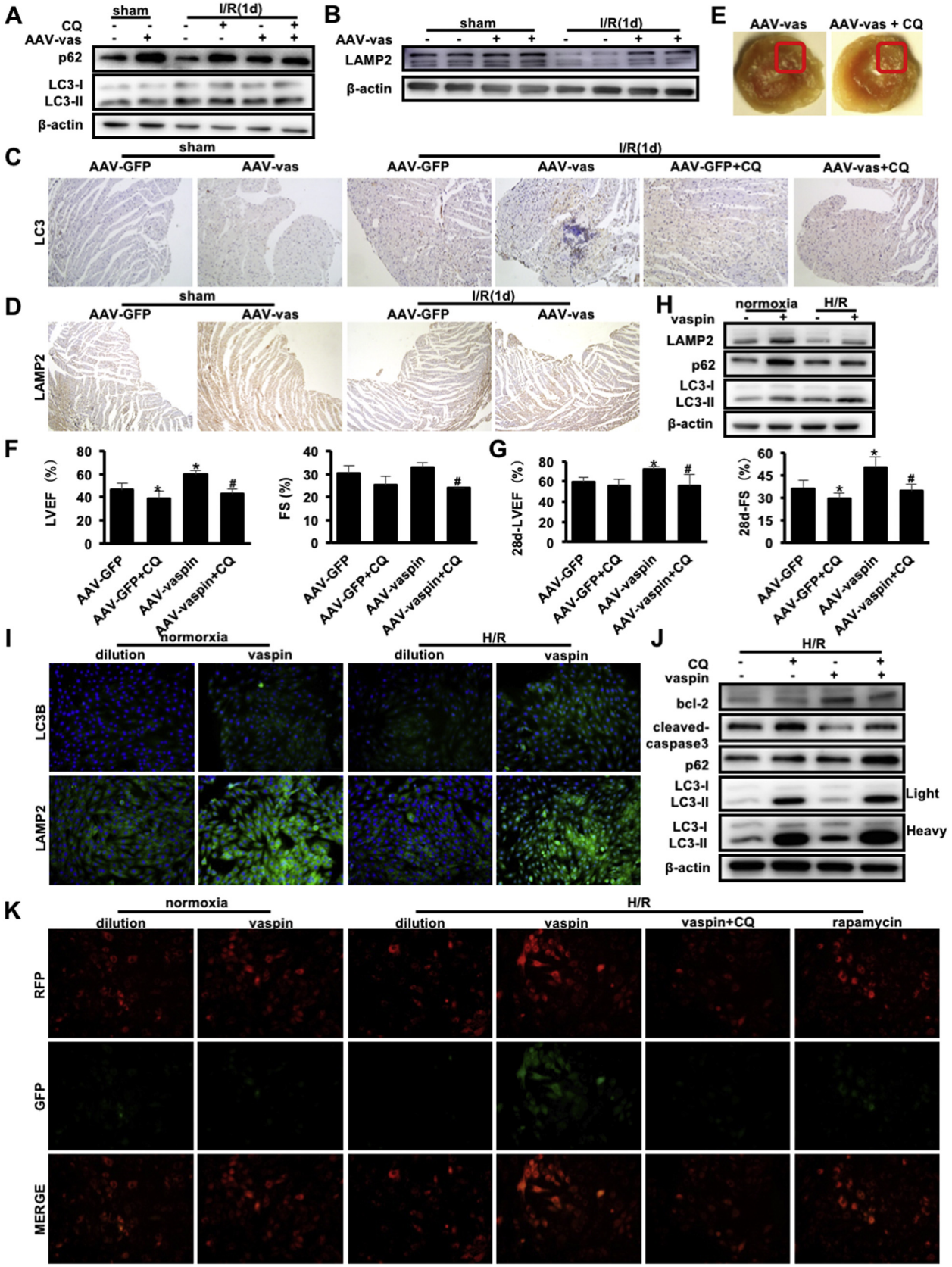

Fig. 3. The cardioprotection of vaspin was mediated by enhanced autophagic flux and restored lysosomal function. Western blot analysis of autophagy markers (A) and LAMP2 (B) in mice with AAV-vaspin and CQ pretreatment after I/R (1 d). (C and D) Representative images of heart sections (× 200) stained for LC3B and LAMP2 in mice after I/R (1 d). (E) Representative images of TTC staining to determine myocardial infarct size with AAV-vaspin and CQ pretreatment after I/R (1 d). The cardiac function in mice with AAV-vaspin and CQ pretreatment 1 day (F) and 28 days (G) after I/R. $^{*}P < 0.05$ vs AAV-GFP I/R group, $^{\#}P < 0.05$ vs AAV-vas I/R group. (H) Western blot analysis of LC3-II/LC3-I, p62 and LAMP2 in H9C2 cells after H/R. (I) Representative images of H9C2 cells (× 200) stained for LC3B and LAMP2 (green) after H/R. Nuclei were identified by DAPI counterstaining (blue). (J) Western blot analysis of autophagy and apoptosis-associated markers in H9C2 cells with vaspin and CQ pretreatment after H/R. (K) Assessment of autophagic flux in H9C2 cells (× 200) transfected with Ad-RFP-GFP-LC3. Rapamycin is shown as a positive control. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Please cite this article in press as: F. Yang, et al., Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.004

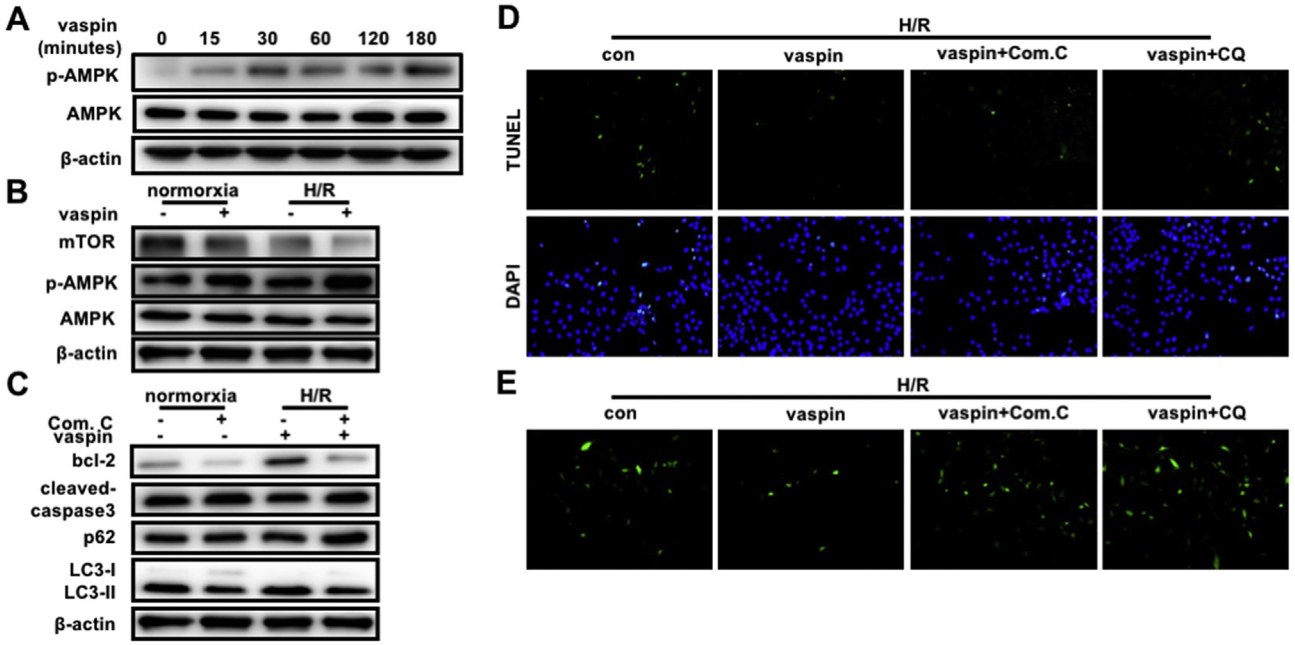

Fig. 4. Vaspin activated autophagy through AMPK/mTOR pathway. (A) Western blot analysis of time-dependent changes in p-AMPK (at Thr172) in H9C2 cells after vaspin treatment. Western blot analysis of AMPK, p-AMPK and mTOR (B), markers of autophagy and apoptosis (C) in H9C2 cells with vaspin pretreatment under H/R. Representative images of TUNEL-positive cells (D) and ROS staining (green) (E) in H9C2 cells (× 200) with vaspin, Compound C or CQ under H/R. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

adipokines in cardiovascular diseases have been gained [19–23]. Compared to previous studies, our data provided the new evidence on the protective role of vaspin in the recovery of cardiac function after I/R. We experimentally verified that upregulating vaspin levels alleviated myocardial apoptosis and infarct size after I/R. Restricting myocyte apoptosis has been an important therapeutic target for reducing ischaemic heart damage [24,25]. Thus, the ability of vaspin to attenuate the infarct size and improve heart function after I/R may be dependent, at least in part, on inhibiting myocardial apoptosis. Similarly, vaspin has also been proved to protect against LPS-induced apoptosis in pulmonary endothelial cells [26].

Since being found in OLETF rats in 2000 [5], vaspin has gained much attention. Vaspin has been reported to ameliorate ER stress and insulin resistance through interacting with cell-surface GRP78 on endothelial cells and HepG2 cells [27,28]. However, we regrettably found that the expression of GRP78 did not change with vaspin levels both in mice and in H9C2 cells (Fig. S3). Identifying the specific receptor expressed on cardiomyocytes that directly responds to vaspin requires further study.

Autophagy acts as a double-edged sword on myocardial apoptosis in the ischaemia and reperfusion stages [25]. In the ischaemia stage, autophagy activation can eliminate damaged mitochondria or other organelles to rescue dying myocardium [25,29]. In the reperfusion stage, inhibition of the hyper-activation of autophagy with partial beclin1 knockdown can improve myocardial survival [25]. However, our results showed that in the reperfusion phase, vaspin enhanced autophagy activation to exert anti-apoptotic protection in mice and in H9C2 cells. The explanation for this discrepancy may be that vaspin can also increase myocardial LAMP2 expression and subsequently restore the injured lysosomal function during reperfusion. In other words, an increase in LAMP2 can mediate the protection of vaspin by eliminating over-accumulated damaged proteins and organelles which were wrapped in the autophagosomes in the reperfusion period, suggesting that vaspin can accelerate both the initiation and degradation of autophagy, thus ensuring the effective operation of autophagic flux. These data are consistent with previous results showing the important role of LAMP2 in the regulation of autophagic flux [30].

It has been known that AMPK/mTOR functions as an important signaling pathway to regulate cellular autophagy and apoptosis [25,30,31]. AMPK activation inhibits mTOR activity to initiate autophagy [30–32]. Consistent with these findings, the present study found that vaspin induced AMPK activation in a time-dependent manner. Importantly, in cardiomyocytes after H/R, the inhibition of AMPK activity suppressed the protective effects of vaspin on activating autophagy and inhibiting apoptosis.

In conclusion, vaspin can protect against myocardial I/R injury via AMPK-mTOR-dependent activation of autophagic flux and restoration of lysosomal function. Prospectively, vaspin may act as a novel target for the treatment of ischaemic heart diseases.

Conflicts of interest

None.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (81570401, 81772036, 81571934, 81671952, 81300219, 81671951), Taishan Scholar Program of Shandong Province (ts20130911), Taishan Young Scholar Program of Shandong Province (tsqn20161065), Key R&D Program of Shandong Province (2016GSF201235, 2016ZDJS07A14, 2018GSF118003), department of Science and Technology of Shandong Province (ZR2013HQ058), Fundamental Research Funds of Shandong University (2014QLKY04), Natural Science Foundation of Shandong Province (BS2014YY032) and China Postdoctoral Science Foundation (2016M602149).

Please cite this article in press as: F. Yang, et al., Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.004

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.05.004.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bbrc.2018.05.004.

References

[1] G. Yang, Y. Wang, Y. Zeng, et al., Rapid health transition in China, 1990-2010: findings from the global burden of disease study 2010, Lancet 381 (9882) (2013) 1987–2015.
[2] Z. Chen, Launch of the health-care reform plan in China, Lancet 373 (9672) (2009) 1322–1324.
[3] R.B. Jennings, H.M. Sommers, G.A. Smyth, et al., Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog, Arch. Pathol. 70 (1960) 68–78.
[4] E. Castedo, J. Segovia, C. Escudero, et al., Ischemia-reperfusion injury during experimental heart transplantation. Evaluation of trimetazidine's cytoprotective effect, Rev. Esp. Cardiol. 58 (8) (2005) 941–950.
[5] K. Hida, J. Wada, H. Zhang, et al., Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats, J. Lipid Res. 41 (10) (2000) 1615–1622.
[6] N. Kloting, J. Berndt, S. Kralisch, et al., Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes, Biochem. Biophys. Res. Commun. 339 (1) (2006) 430–436.
[7] B.S. Youn, N. Kloting, J. Kratzsch, et al., Serum vaspin concentrations in human obesity and type 2 diabetes, Diabetes 57 (2) (2008) 372–377.
[8] H.L. Li, W.H. Peng, S.T. Cui, et al., Vaspin plasma concentrations and mRNA expressions in patients with stable and unstable angina pectoris, Clin. Chem. Lab. Med. 49 (9) (2011) 1547–1554.
[9] M.A. Kobat, A. Celik, M. Balin, et al., The investigation of serum vaspin level in atherosclerotic coronary artery disease, J. Clin. Med. Res. 4 (2) (2012) 110–113.
[10] B. Zhang, W. Peng, H. Li, et al., Plasma vaspin concentrations are decreased in acute coronary syndrome, but unchanged in patients without coronary lesions, Clin. Biochem. 46 (15) (2013) 1520–1525.
[11] C.H. Jung, W.J. Lee, J.Y. Hwang, et al., Vaspin protects vascular endothelial cells against free fatty acid-induced apoptosis through a phosphatidylinositol 3-kinase/Akt pathway, Biochem. Biophys. Res. Commun. 413 (2) (2011) 264–269.
[12] C.H. Jung, W.J. Lee, J.Y. Hwang, et al., Vaspin increases nitric oxide bioavailability through the reduction of asymmetric dimethylarginine in vascular endothelial cells, PLoS One 7 (12) (2012), e52346.
[13] C.H. Jung, M.J. Lee, Y.M. Kang, et al., Vaspin inhibits cytokine-induced nuclear factor-kappa B activation and adhesion molecule expression via AMP-activated protein kinase activation in vascular endothelial cells, Cardiovasc. Diabetol. 13 (2014) 41.
[14] H.O. El-Mesallarmy, D.H. Kassem, E. El-Demerdash, et al., The xanthine oxidase inhibitor oxypurinol does not limit infarct size in a canine model of 40 minutes of ischemia with reperfusion, J. Am. Coll. Cardiol. 12 (1) (1988) 209–217.
[15] R. Shibata, K. Sato, D.R. Pimentel, et al., Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms, Nat. Med. 11 (10) (2005) 1096–1103.
[16] A. Diwan, M. Krenz, F.M. Syed, et al., Inhibition of ischemic cardiomyocyte apoptosis through targeted ablation of Bnip3 restrains postinfarction remodeling in mice, J. Clin. Invest. 117 (10) (2007) 2825–2833.
[17] X. Ma, H. Liu, S.R. Foyil, et al., Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury, Circulation 125 (25) (2012) 3170–3181.
[18] S. Kimura, T. Noda, T. Yoshimori, Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3, Autophagy 3 (5) (2007) 452–460.
[19] S. Bhandari, P. Gupta, P. Quinn, et al., Pleiotropic effects of statins in hypercholesterolaemia: a prospective observational study using a lipoproteomic based approach, Lancet 385 (Suppl 1) (2015) S21.
[20] A.J. Kennedy, A.P. Davenport, International union of basic and clinical pharmacology CIII: chemerin receptors CMKLR1 (Chemerin1) and GPR1 (Chemerin2) nomenclature, pharmacology, and function, Pharmacol. Rev. 70 (1) (2018) 174–196.
[21] Y. Wang, D. Chen, Y. Zhang, et al., A novel adipokine, FAM19A5, inhibits postinjury neointima formation through sphingosine-1-phosphate receptor 2, Circulation (2018). Doi:10.1161.
[22] J.J. Fuster, N. Ouchi, N. Gokce, et al., Obesity-induced changes in adipose tissue microenvironment and their impact on cardiovascular disease, Circ. Res. 118 (11) (2016) 1786–1807.
[23] L. Lu, R.Y. Zhang, X.Q. Wang, et al., C1q/TNF-related protein-1: an adipokine marking and promoting atherosclerosis, Eur. Heart J. 37 (22) (2016) 1762–1771.
[24] K. Nishida, O. Yamaguchi, K. Otsu, Crosstalk between autophagy and apoptosis in heart disease, Circ. Res. 103 (4) (2008) 343–351.
[25] Y. Matsui, H. Takagi, X. Qu, et al., Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy, Circ. Res. 100 (6) (2007) 914–922.
[26] D. Qi, D. Wang, C. Zhang, et al., Vaspin protects against LPSinduced ARDS by inhibiting inflammation, apoptosis and reactive oxygen species generation in pulmonary endothelial cells via the Akt/GSK3beta pathway, Int. J. Mol. Med. 40 (6) (2017) 1803–1817.
[27] A. Nakatsuka, J. Wada, I. Iseda, et al., Visceral adipose tissue-derived serine proteinase inhibitor inhibits apoptosis of endothelial cells as a ligand for the cell-surface GRP78/voltage-dependent anion channel complex, Circ. Res. 112 (5) (2013) 771–780.
[28] A. Nakatsuka, J. Wada, I. Iseda, et al., Vaspin is an adipokine ameliorating ER stress in obesity as a ligand for cell-surface GRP78/MTJ-1 complex, Diabetes 61 (11) (2012) 2823–2832.
[29] W. Zhang, C. Chen, J. Wang, et al., Mitophagy in cardiomyocytes and in platelets: a major mechanism of cardioprotection against ischemia/reperfusion injury, Physiology 33 (2) (2018) 86–98.
[30] Y. Wang, B. Liang, W.B. Lau, et al., Restoring diabetes-induced autophagic flux arrest in ischemic/reperfused heart by ADIPOR (adiponectin receptor) activation involves both AMPK-dependent and AMPK-independent signaling, Autophagy 13 (11) (2017) 1855–1869.
[31] J. Li, P.W. Wu, Y. Zhou, et al., Rage induces hepatocellular carcinoma proliferation and sorafenib resistance by modulating autophagy, Cell Death Dis. 9 (2) (2018) 225.
[32] S. Horman, G. Browne, U. Krause, et al., Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis, Curr. Biol. 12 (16) (2002) 1419–1423.

Please cite this article in press as: F. Yang, et al., Vaspin alleviates myocardial ischaemia/reperfusion injury via activating autophagic flux and restoring lysosomal function, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.004