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

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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

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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 re-oxygenated 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

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