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Caveolin induces cardioprotection through epigenetic regulation

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Abstract

Lipid rafts represent a sub-compartment of the plasma membrane that co-ordinate and regulate varieties of signalling processes, whereas caveolins are the integral membrane protein of the lipid raft. Recent evidence demonstrated the pivotal role of caveolins in cardioprotection against ischaemic injury, although their mechanism of action is not clear. However, new understanding of epigenetic modification during ischaemia reperfusion suggests additional targeted approaches that have not been explored before. To study the role of caveolin on epigenetic regulation, isolated mouse heart was prepared from wild-type (WT) and caveolin-1 knockout (Cav-1 KO) mouse and preconditioned them with four cyclic episodes of ischaemia/reperfusion followed by 30 min. global ischaemia and 120 min. reperfusion. We found that Cav-1 KO mouse abolished the acetylation of histone (H3 and H4) and increased the methylation of histone in the preconditioned heart. The increased histone methylation was significantly correlated with an increased level of histone methyl-tranferase G9a protein and increased the level of histone decaetylase (HDAC) activity. Cav-1 KO mouse also decreased the translocation of forkhead transcription factor (FOXO3a) to the nucleus and reduced the induction of the expression of SIRT-1 in the preconditioned heart. Cardioprotective property of Cav-1 was further confirmed by reduced ventricular function, increased cardiomyocyte apoptosis, increased expression of junas kinase (JNK) and Bax and decreased expression of phospho-adenosine monophosphate-activated protein kinase (AMPK), phospho-AKT and B cell lymphoma-2 (Bcl-2) in Cav-1 KO preconditioned heart. The results clearly indicate that Cav-1 induces cardioprotection through epigenetic regulation.

Keywords: caveolin • histone • HDAC • HAT • cardioprotection • epigenetics

Introduction

Limitation of ischaemic damage is a major goal of therapy for cardiovascular diseases. Over one million American die from myocardial infraction every year [1]. Acute coronary syndromes, congenital coronary abnormalities, congenital cardiomyopathies and cardiac surgery-all are encountered by ischaemic episodes in the heart. So far, ischaemic preconditioning (PC) has been proven to be most powerful mechanism for limiting infract size and/or ischaemic injury. Ischaemic PC is defined as an increased tolerance to ischaemia and reperfusion induced by previous sub-lethal periods of repeated ischaemia. Although initially it was believed that 'ischaemic PC' could be induced by short cyclic episodes of ischaemia and reperfusion, soon it became apparent that a similar phenotype could be elicited through a splendid array of stimuli. However, new understanding of ischaemia-induced epigenetic changes suggests additional targeted approach. For example, inhibition of HDAC by valporic acid or tributyrin can attenuate ventricular remodelling after infraction. This might provide a worthwhile therapeutic target [2]. Recent evidence also demonstrates that inhibition of HDACs triggers pharmacologic PC to protect the ischaemic heart, which involves p38 activation [3].

The regulation of gene expression by histone modification is well-known. Post-translational modifications of histone NH_2-terminal tails, such as acetylation, methylation, ubiquitination and phosphorylation, are important for chromatin organization and gene transcription [4]. To date, acetylation and methylation are among the best characterized histone modifications. Acetylation of histone residues results in unwinding of the DNA, which allows transcription factors and RNA polymerase II to bind more readily to DNA and thereby, increases gene transcription [5]. Histone methylation may have either positive or negative effects on transcription depending on the sites [6]. For instance, methylations on histone H3 lysine 4 (H3K4), histone H3 lysine 36 (H3K36) and histone H3 lysine 79 (H3K79) are often associated with transcriptional activation and elongation, whereas methylations on histone H3 lysine 9 (H3K9) and histone H3 lysine 27 (H3K27)

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correlate with transcriptional repression [6]. Adding to this complexity is the fact that lysine residues on histone can be monomethylated, dimethylated or trimethylated. Trimethylated H3K9 is typically associated with constitutive heterochromatin, whereas monomethylated H3K9 and dimethylated H3K9 are mainly found in euchromatin and are associated with repressed promoter regions [7].

Lipid rafts represent a sub-compartment of the plasma membrane that co-ordinate and regulate varieties of signalling processes whereas caveolins are the integral membrane protein of the lipid raft. Recent evidence demonstrated pivotal role of caveolins in cardioprotection against ischaemic injury, although their mechanism of action is not clear. To date, a few reports are available on caveolin-1 and caveolin-3 gene ablation. Cav-1 KO mice develop progressive cardiac hypertrophy as demonstrated by transthoracic echocardiography and magnetic resonance imaging (MRI) [8]. In contrast, caveolin-3 knockout mice develop cardiomyopathy characterized by hypertrophy, dilation and reduced contractility [9]. Caveolin-1 and caveolin-3 double knockout mice completely lacking caveolae are deficient in all three caveolin proteins because caveolin-2 is degraded in absence of caveolin-1. The double knockout mice developed a severe cardiomyopathic phenotype with cardiac hypertrophy and decreased contractility [10]. Earlier investigation from our laboratory demonstrated differential interaction and/or translocation of p38MAPKα and p38MAPKβ with caveolin-1 and caveolin-3, respectively, and such interaction functioned as a molecular switch for the conversion of ischaemia/reperfusion (I/R) induced death signal into PC-induced survival signal [11]. To date it is not known whether caveolin has any effect on epigenetic regulation and/or histone mofication, which can lead to cardioprotection. This study was constructed to examine the precise mechanism of action of caveolin and/or lipid raft in the generation of survival signal and their effect on histone modification. Our result confirmed strong influence of caveolin on epigenetic regulation and/or chromatin remodelling.

Materials and methods

Chemicals

Antibodies against H3, H4, Ac-H3, Ac- H4, H3K9, H3K27, G9a, FOXO3a, Sirt-1, AKT, AMPK, JNK, Bax and Bcl-2 were purchased from Cell Signaling Technology (Danvers, MA, USA).

Animals

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health (NIH Publication No. 85-23, revised 1996).

Experimental protocol

The study used isolated working mouse hearts subjected to ischaemia/reperfusion protocol. For this study, we used (1) WT mouse and (2) Cav-1 KO mouse. Isolated mouse hearts of each group of mice were randomly divided into three groups: perfused with KHB buffer only (group I); the hearts were subjected to 30 min. ischaemia and 120 min. reperfusion (I/R) (group II), the hearts were subjected to four cyclic episodes of 5 min. ischaemia and 10 min. reperfusion followed by 30 min. ischaemia and 120 min. reperfusion (PC) (group III). Control hearts (group I) after perfusion with KHB buffer for 15 min., were not exposed to ischaemia and reperfusion. They were only subjected to 2 hrs and 30 min. continuous perfusion.

Isolated working mouse heart preparation

The mice (25–34 g) were anesthetized with sodium pentobarbital (80 mg/kg b/w; Abbott Laboratories, North Chicago, IL, USA) i.p. and anti-coagulated with heparin sodium (500 IU/kg bow i.p.; Elkin-Sinn Inc., Cherry Hill, NJ, USA) injection. After ensuring sufficient depth of anaesthesia, thoracotomy was performed and aorta of the heart was identified. Excising of the heart from the chest by the aorta, the lung and fat tissues were removed and the whole heart transferred to ice-cold (4°C) modified Krebs–Henseleit bicarbonate solution (KHS), containing (in mM: NaCl 118; KCl 4.7; $CaCl_2$ 1.7; $NaHCO_3$ 24; KH_2PO_4 1.2; $MgSO_4$ 12; glucose 10) until contraction had ceased [12]. Both the aorta and pulmonary vein were cannulated as quickly as possible and perfused in retrograde Langendorff mode against constant perfusion pressure of 70 cm of water (7 kPa) for 10 min. period. Immediate initiation of retrograde perfusion helped to wash blood and its component from the vascular system. Perfusate (KHS) temperature was maintained at 37°C and saturated with 95% O_2 and 5% CO_2 gas mixture during the entire experiment. The duration of the retrograde perfusion was 10 min., after this procedure the heart was switched in an antegrade perfusion mode. In the antegrade perfusion mode, the buffer enters the cannulated left atrium at pressure equivalent to 10 cm water (1 kPa) and passed to the left ventricle, from which it is spontaneously ejected through the aortic cannula. Control measurements of heart rate, coronary flow, aortic flow, LV end-diastolic pressure and LV developed pressure and its first derivative were recorded in this period. After this period the antegrade perfusion line was closed, and the heart was subjected to 30 min. ischaemia. Before the initiation of 2 hrs reperfusion, the heart was perfused in retrograde mode to avoid the development of high incidence of ventricular fibrillation. The measurements of the cardiac function were carried out at 15, 30, 60 and 120 min. of the 2 hrs reperfusion. Any heart that showed any cardiac disturbance (ventricle arrhythmia and fibrillation) during the entire experiment was excluded from this study.

Estimation of apoptosis

The level of apoptosis was estimated by the degree of expression of caspase-3 and cytochrome *c* in cytosolic fraction of different groups of the hearts. Heart tissues were homogenized in ice cold buffer (25 mM Tris-HCl, 25 nM NaCl, 1 mM Na-orthovanadate, 10 mM NaF, 10 mM pyrophosphate, 10 nM okadoic acid, 0.5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM PMSF and protease inhibitor cocktail). Cytosolic fraction was prepared using standard graded centrifugation. Western blot analysis was then performed with antibodies against full length caspase-3, cleaved caspase-3 and cytochrome *c* according to established protocols.

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Nuclear protein isolation and acid extraction of histone proteins

Heart tissue (100 mg) was homogenized in 0.5 ml buffer A [10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 M EDTA, 0.2 mM NaF, 0.2 mM Na orthovandate, 1% (v/v) NP-40, 0.4 mM phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin] on ice. The homogenate was centrifuged at 2000 rpm in a benchtop centrifuge for 30 sec. at 4°C to remove cellular debris. The supernatant was then transferred to a 1.7-ml ice-cold microtube and further centrifuged for 30 sec. at 13,000 rpm at 4°C. The supernatant was collected as a cytoplasmic extract and kept frozen at −80°C for Western blotting. The pellet was resuspended in 200 μl of buffer C [50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 M EDTA, 1 mM DTT, 10% (v/v) glycerol, 0.2 mM NaF, 0.2 mM Na orthovandate and 0.6 mM phenylmethylsulfonyl fluoride] and placed on the rotator in the cold room for 30 min. After centrifugation at 13,000 rpm in a micro eppendorf tube for 5 min., the supernatant was collected as the nuclear extract. For extraction of histone protein, pellets from the nuclear extraction were resuspended in 150 μl deionized water, 0.2 N HCl and 0.36 N H_2SO_4. The histone proteins were precipitated from the supernatant, agitated overnight at 4°C, and then centrifuged at 13,000 rpm for 10 min., and the supernatant decanted into a fresh tube. Ice-cold acetone precipitation samples were incubated overnight at −20°C, centrifuged and the air-dried pellets were resuspended in 50 μl deionized water and kept frozen at −80°C for Western blotting [13].

HAT and HDAC activity assay

HAT assay

Histone acetyltransferases (HATs) have been implicated to play a crucial role in various cellular functions such as gene transcription, differentiation and proliferation. To study HAT activity we used HAT Activity Colorimetric Assay Kit (BioVision Research Product, Mountain View, CA, USA). Assay utilized active nuclear extract as a positive control and acetyl-coA as a cofactor. Acetylation of peptide substrate by active HAT releases the free form of coA, which then serves as an essential coenzyme for producing NADH. NADH can easily be detected spectrophotometrically (440 nm) upon reacting with a soluble tetrazolium dye.

HDAC assay

Inhibition of HDAC has been implicated to modulate transcription and to induce apoptosis or differentiation in cancer cells. To study HDAC activity, we used colorimetric HDAC Activity Assay Kit (BioVision Research Product). The kit used an acetylated lysine side chain as colorimetric substrate and used lysine developer to produce chromophore. The chromophore was analysed using an ELISA plate reader or spectrophotometer (400–405 nm).

Statistical analysis

The values for the functional parameters were all expressed as the mean ± standard error of mean (S.E.M.) for at least six animals per group. The Western blot analyses were performed with at least three animals per group. The statistical analysis was performed by analysis of variance followed by Bonferroni's correction for any differences between the mean values of all groups. Differences between data were analysed for significance by performing a Student's *t*-test. The results were considered significant if $P < 0.05$.

Results

Effects of caveolin on PC

As expected, PC-induced cardioprotective effect on WT mouse heart as demonstrated by its ability to enhance post-ischaemic LV function. But, PC-induced cardioprotection was abolished when PC was performed in Cav-1 KO mice. WT preconditioned group displayed better ventricular recovery as expected when perfusion progressed and LV developed pressure (LVDP), d*p*/d*t* and aortic flow were significantly improved ($P < 0.05$) in WT PC group compared to those of WT I/R group and Cav-1 KO PC group. No significant difference was observed in LVDP, d*p*/d*t* and aortic flow in between WT I/R, Cav-1 KO I/R and Cav-1 PC groups (Fig. 1).

Estimation of apoptosis

To study the level of apoptosis in Cav-1 KO mouse heart, caspase-3 and cytochrome *c* levels were examined by Western blot analysis. We have found that the level of full length caspase-3 is high in WT PC hearts compared Cav-1 I/R, Cav-1 PC and WT I/R heart. The level of cleaved caspase-3 was very low in WT PC heart compared to the other group of the heart. In case of cytochrome *c*, we tested the level of cytochrome *c* in cytosolic fractions of the heart and found that the level of myocardial cytochrome *c* was significantly higher in cytosolic fraction in WT I/R, Cav-1 KO I/R as well as in the Cav-1 KO PC heart compared to the WT PC group (Fig. 2).

Detection of histone protein acetylation and methylation

We then studied the histone modification histone acetylation and methylation were studied, which are the best characterized modification for epigenetics regulation. In this investigation, mainly we studied the methylation and acetylation of H3 and H4 histone after acid extraction of histone proteins. WT PC heart showed high level of expression of acetylated H3 and H4 histone proteins compared to the Cav-1 PC as well as Cav-1 I/R and WT I/R groups. For methylation of histone, two targets were selected (i) histone H3 lysine 9 (H3K9) and (ii) histone H3 lysine 27 (H3K27). Both H3K9 and H3K27 showed high level of expression in WT I/R and Cav-1 I/R group and also in Cav-1 PC group whereas WT PC group showed negligible level of expression. G9a, also known as Euchromatic histone lysine N-methyltransferase 2 (EHMT2), is a

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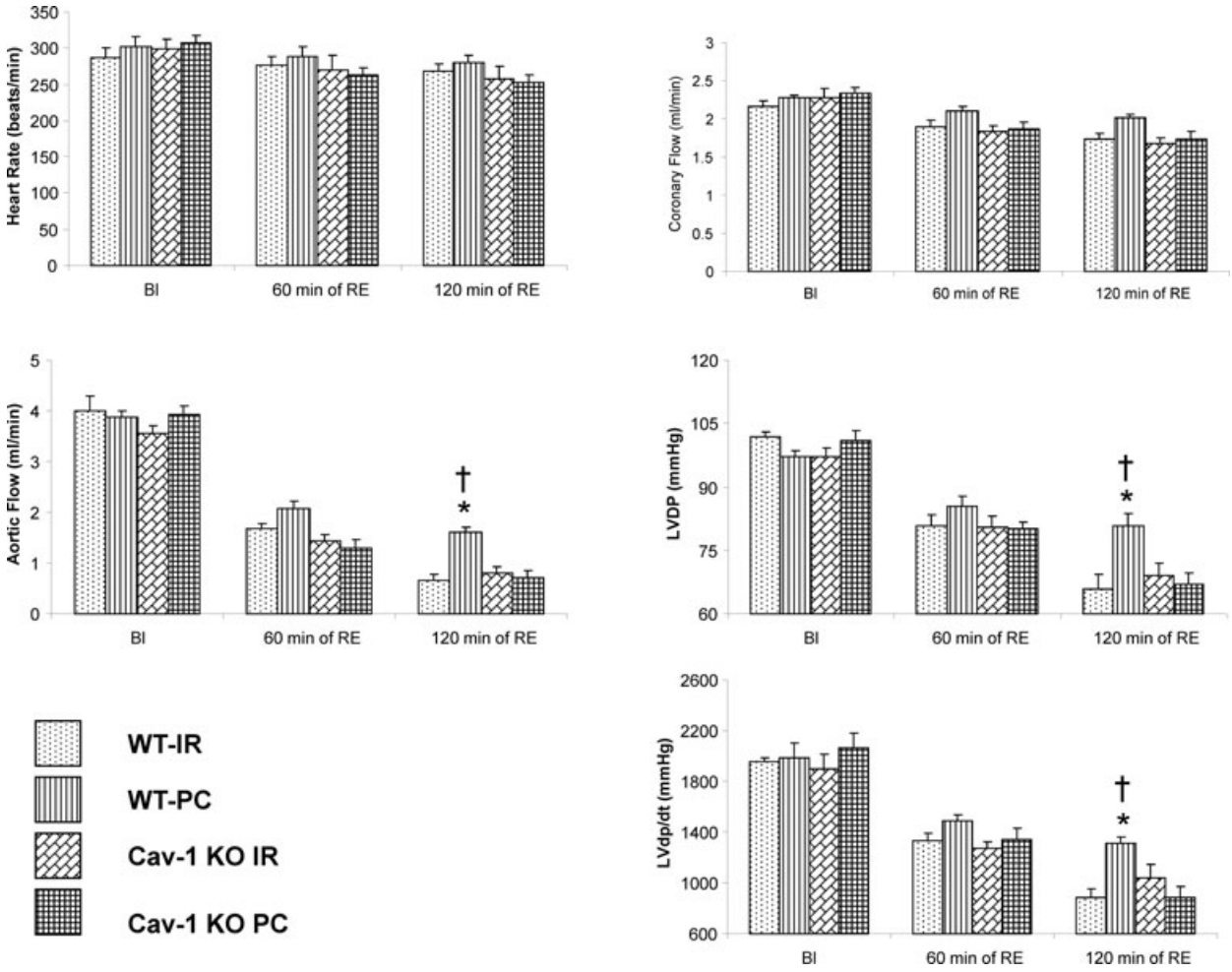

Fig. 1 Effect on caveolin-1 on preconditioning of the heart. Results are showing different haemodynamic parameters of the mouse heart. Data represent mean ± S.E.M. $^{*}P < 0.05$ *versus* WT I/R, $^{\dagger}P < 0.05$ *versus* Cav-1 KO PC. WT: wild-type mouse; Cav-1: caveolin-1 knockout mouse.

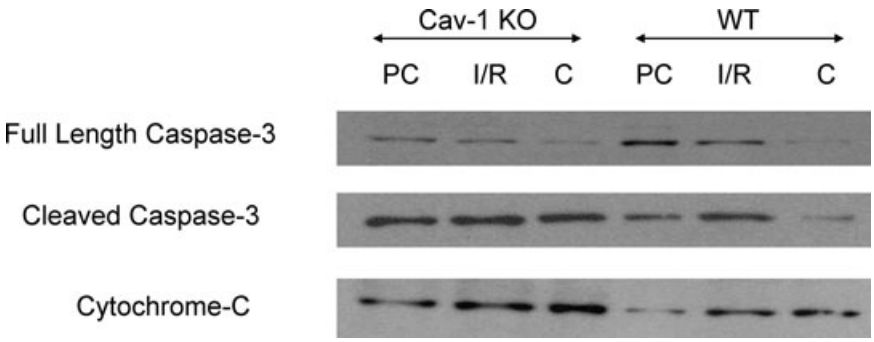

Fig. 2 Effect of caveolin-1 on caspase-3 and cytochrome *c*. C: control; I/R: ischaemia/reperfusion; PC: precondition; WT: wild-type mouse; Cav-1: caveolin-1 knockout mouse. (**A**) Representative picture of Western blot analysis. (**B**) Densitometric scanning of the blots. Results are average ± S.E.M. of three different sets of blots. $^{*}P < 0.05$ *versus* corresponding bots of Cav-1 KO.

member of the family histone lysine methyltransferase. G9a protein also showed high level of expression in both WT I/R and Cav-1 I/R group and also in Cav-1 PC group where as WT PC group showed negligible level of expression (Fig. 3).

HAT and HDAC activities

Both HAT and HDAC activities were measured by calorimetric method using the nuclear fraction of the heart tissues. WT PC heart

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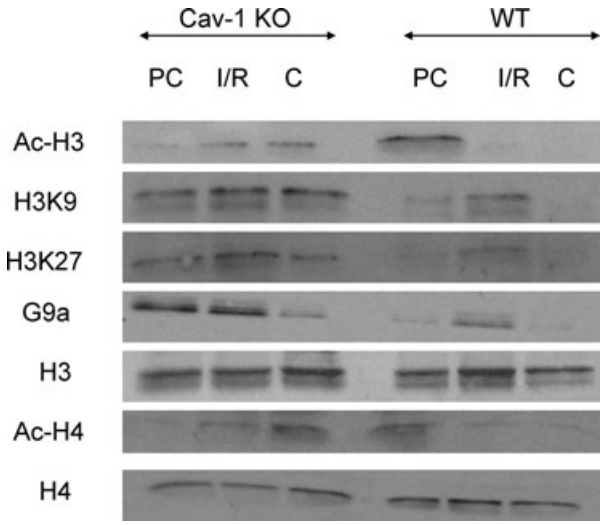

Fig. 3 Effect of caveolin-1 on acetylation and methylation of H3 and H4 histone protein. Ac-H3: acetylated H3 histone; Ac-H4: acetylated H4 histone; H3K9: histone H3 lysine 9; H3K27: histone H3 lysine 27; C: control; I/R: ischaemia/reperfusion; PC: precondition; WT: wild-type mouse; Cav-1: caveolin-1 knockout mouse.

showed significantly high HAT activity compared to WT I/R, Cav-1 I/R and Cav-1 PC groups. Whereas WT PC heart showed significantly lower HDAC activity compared to the other groups (Fig. 4).

Translocation of FOXO-1 and expression of Sirt 1

To study the translocation of FOXO-3a, Western blot was performed against FOXO antibody with both nuclear and cytosolic extract of WT and Cav-1 KO mouse heart. In WT PC heart, nuclear translocation of FOXO3a was higher compared to Cav-1 KO PC heart. Nuclear fraction showed high level of expression of FOXO3a in WT PC heart compared to the Cav-1 KO PC heart. Similarly, cytosolic fraction showed low level of expression of FOXO3a in WT PC heart compared to the Cav-1 KO PC heart. To study Sirt1 expression, nuclear fraction was used and found the expression of Sirt-1 only in WT PC heart not in Cav-1 KO PC heart or other groups (Fig. 5).

Expression of survival proteins

To confirm the cardioprotective role of caveolin-1, some pro-survival and death signal proteins were tested by Western blot from

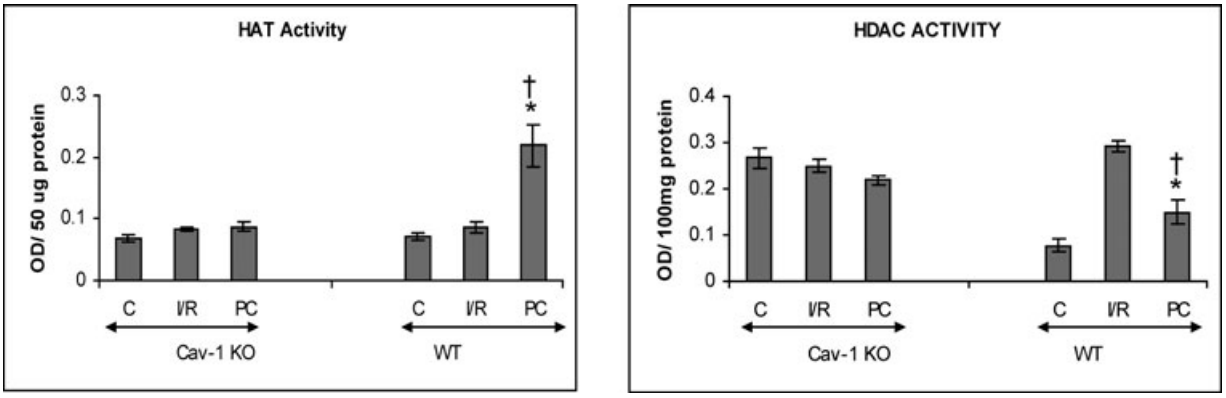

Fig. 4 Effect of caveolin-1 on HAT and HDAC activity. Results are mean ± S.E.M. $^{*}P < 0.05$ *versus* WT I/R, $^{\dagger}P < 0.05$ *versus* Cav-1 KO PC. C: control; I/R: ischaemia/reperfusion; PC: precondition; WT: wild-type mouse; Cav-1: caveolin-1 knockout mouse.

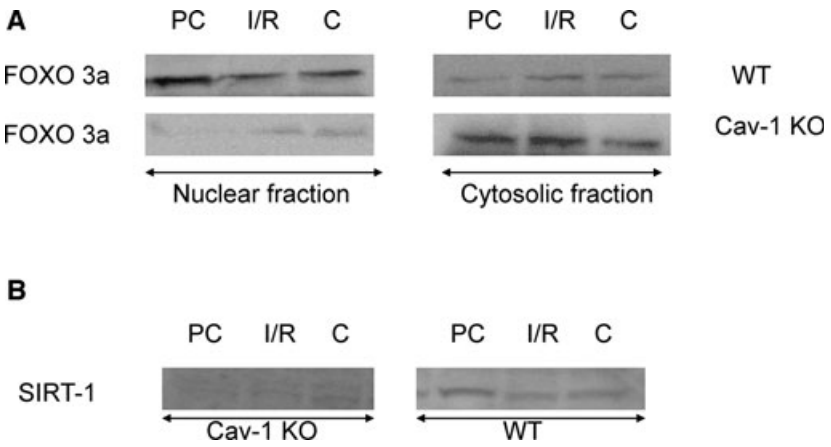

Fig. 5 Effect of caveolin-1 on (**A**) FOXO3a translocation and (**B**) SIRT-1 expression. C: control; I/R: ischaemia/reperfusion; PC: precondition; WT: wild-type mouse; Cav-1: caveolin-1 knockout mouse. (**A**) Representative picture of Western blot analysis. (**B**) Densitometric scanning of the blots. Results are average ± S.E.M. of three different sets of blots. $^{*}P < 0.05$ *versus* corresponding bots of Cav-1 KO.

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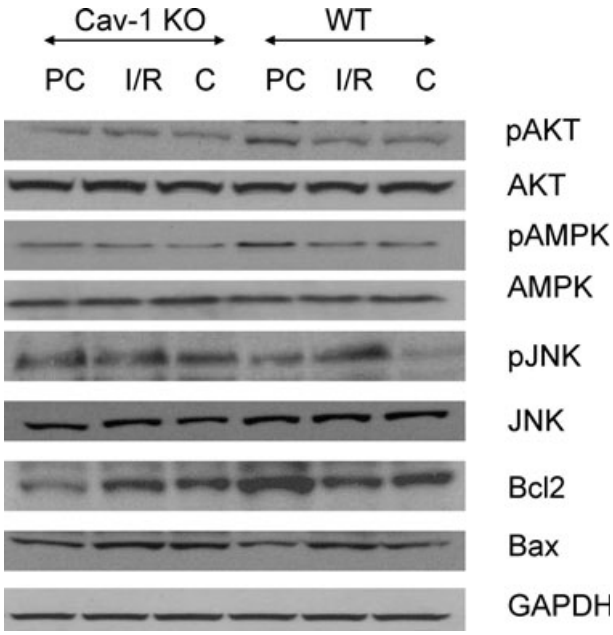

Fig. 6 Effect of caveolin-1 on different survival and non-survival proteins. C: control; I/R: ischaemia/reperfusion; PC: precondition; WT: wild-type mouse: Cav-1: caveolin-1 knockout mouse.

the heart homogenate. Pro-survival proteins phospho-AKT, phospho-AMPK, Bcl-2 expression were significantly high in WT PC group compared to Cav-1 KO PC group and other groups. Whereas death signal proteins, such as phospho-JNK, Bax expression were significantly low in WT PC group compared to Cav-1 KO PC group and other groups (Fig. 6).

Discussion

The present investigation clearly demonstrates the pivotal role of Cav-1 in PC-induced cardioprotection. Here we have seen that PC-induced cardioprotective effect on WT mouse heart as demonstrated by its ability to enhance post-ischaemic LV function and reduce cardiomyocyte apoptosis as expected. However, PC-induced cardioprotection was abolished when PC was performed in Cav-1 KO mice. WT PC group displayed better ventricular recovery and reduced apoptosis as expected. LVDP, d*p*/d*t* and aortic flow was significantly improved in WT PC group compared to those of WT I/R group and Cav-1 KO PC group. Although recent evidence demonstrated the importance of caveolins in cardioprotection against ischaemic injury, their mechanism of action is not clear.

Here in this investigation we reported for the first time that Cav-1 knockdown abolish cardioprotection by affecting the acetylation and methylation of histone proteins. Chen *et al.* (2006) reported that hypoxic stress induced dimethylation of H3 histone (H3K9) through histone methyltransferase G9a in mammalian cells [14]. Effect on ventricular remodelling also reported in infracted rats by inhibition of histone decaetylase [2]. It was also reported that inhibition of histone deacetylase trigger pharmacologic PC effects against myocardial ischaemic injury [3]. We have shown that PC induces acetylation of histone H3 and H4 in WT mouse, whereas acetylation of histone H3 and H4 are not observed in the preconditioned heart of Cav-1 KO mouse. Acetylation of histone residues results in unwinding of the DNA, which allows transcription factors and RNA polymerase II to bind more readily to DNA and, thereby, increase gene transcription.

Histone methylation is generally associated with transcriptional repression. Methylation of histone H3 lysine 9 (H3K9) and histone H3 lysine 27 (H3K27) were observed in I/R hearts of both WT and cav-1 KO mice, whereas methylation of H3K9 and H3K27 were negligible in the WT PC hearts. Cav-1 KO PC hearts showed significant methylation similar to I/R groups. The I/R groups and Cav-1 KO PC group also showed high level of expression of histone methyltranferase G9a protein, which facilitates the dimethylation of histone H3 during hypoxic stress.

HAT and HDAC have earned significant attention in recent years because these enzymes play a critical role in the regulation of wide variety of cellular processes. Histone acetylation is mediated by HAT. The resulting modification in the structure of chromatin leads to nucleosomal relaxation and altered transcriptional activation. This reverse reaction is mediated by HDAC, which induces deacetylation, chromatin condensation and transcriptional repression [15–17]. HDAC inhibition was shown to markedly decrease the infract size and reduce ischaemia-induced neurological deficit scores in focal cerebral ischaemia model of rats [18]. Inhibition of HDACs in myocyte silence the foetal gene activations, renders myocyte insensitive to hypertrophic agonists and blocks cardiac hypertrophy induced aortic banding [19, 20]. In this investigation, WT PC heart showed significantly high HAT activity compared to WT I/R, Cav-1 I/R and Cav-1 PC groups, whereas I/R groups and Cav-1 KO PC group showed significantly high HDAC activity compared to the WT PC group.

The present observation also indicates that PC stimulates histone acetylation and reduces histone methylation in WT mouse, which stimulates gene transcription and helps to produce cardioprotective proteins. In contrast, I/R heart abolish histone acetylation and stimulate histone methylation, which repress transcription and heart cannot produce survival proteins. In Cav-1 KO mouse, PC acetylation of histone was not observed rather there was significant induction of methylation of histone. PC-induced histone acetylation in WT mouse was correlated with increased HAT activity. HDAC activity was also correlated with histone methylation in I/R heart and Cav-1 KO hearts. Overall, Cav-1 knockdown appeared to abolish or reduce the PC-induced cardioprotection by inhibiting histone acetylation and stimulating histone methylation.

FOXO (Forkhead box O) proteins are a family of transcription factors that play important roles in regulating the expression of genes involved in cell growth, proliferation, differentiation and longevity. The first protein in the FOX family that was discovered

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was the *fork head* transcription factor in *Drosophila*. Since then a large number of family members have been discovered, especially in vertebrates. Originally they were given vastly different names (such as HFH, FREAC and fkh), but in 2000 a unified nomenclature was introduced that grouped the FOXO proteins into subclasses (FOXA-FOXS) based on sequence conservation.

In mammals, FOXO transcription factors promote longevity and tumour supression. FOXO transcription factors are directly phosphorylated in response to insulin/growth factor signalling by the protein kinase Akt, thereby causing their sequestration in the cytoplasm [21]. In the absence of insulin/growth factors, FOXO factors translocate to the nucleus where they trigger a range of cellular responses, including resistance to oxidative stress, a phenotype highly coupled with lifespan extension. FOXO factors integrate stress stimuli *via* phosphorylation, acetylation and mono-ubiquitination of a series of regulatory sites [21]. In particular, FOXO3a enhances expression of the antioxidant enzymes, mitochondrial MnSOD (manganese superoxide dismutase) and catalyse, which are scavenger of oxygen free radicals [22]. Enhanced expression of FOXO3a increases both hydrogen peroxide scavenging and oxidative stress resistance [22]. Oxidative stress has also been shown to facilitate SIRT1 binding and decaetylation of FOXO3a, which results in preferential activation of genes involved in cell cycle arrest, resistance to oxidative stress and reduced ability to activate pro-apoptotic target genes [23]. In response to stress, FOXO becomes dephosphorylated and translocate to the nucleus, where they are converted into hypoacetylated form by SIRT [24].

In this investigation, PC showed increased translocation of FOXO and induction of SIRT-1 expression in the nuclear fraction of WT mouse. But in Cav-1 KO mouse, PC demonstrated no increased translocation of FOXO and induction of SIRT-1 expression in the nuclear fraction. From this observation it can be concluded that Cav-1 knockdown abolishes stress resistance property of FOXO by inhibiting its translocation to nucleus and also by inhibiting the induction of SIRT1.

Several epigenetic markers, such as histone acetylation and methylation, and cytosine residue methylation in CpG dinucleotides, have been reported at the FOXP3 locus. FOXP3 (Forkhead box P3) is a gene involved in immune system responses. As member of the FOXO protein family, FOXP3 appears to function as the master regulator in the development and function of regulatory T cells. DNMT and HDAC inhibitors also enhance FOXP3 expression as well as inhibit the effect of pro-inflammatory cytokine Il-6 on FOXP3 regulation and function [25]. Cardioprotective role of caveolin-1 was further conformed by decreased expression of phospho-AKT, phospho-AMPK and Bcl-2 in Cav-1 knockout preconditioned mouse heart and increased expression phospho-AKT, phospho-AMPK and Bcl-2 in WT preconditioned mouse heart. This study thus reported for the first time that caveolin-1 regulates cardioprotection through epigenetic regulation, mainly through histone acetylation and FOXO translocation, which can be used in future as new therapeutic targets. Indeed, HDAC is known to induce anti-apoptotic signals such that many HDAC inhibitors induce apoptosis by down-regulating Bcl-2 [26]. Recently, it has been discovered that AMPK phosphorylates HDAC5 on Ser259 and Ser498 [27]. Vascular defects have also been observed in HDAC7 knockout animals [28]. Taken together, these findings suggest that AMPK phosphorylation of class IIa HDACs might contribute to many of the gene expression responses to AMPK activation in skeletal muscle and its surrounding vasculature. Another recent screen for potential class II HDAC kinases has potentially revealed why a number of AMPK loss-of-function transgenic models fail to show overt skeletal muscle phenotypes [27]. From these experiments, it is evident that there is considerable redundancy in class II HDAC signalling, with multiple kinases capable of phosphorylating Ser259 and Ser498 on HDAC5. AMPK is a key regulator of muscle oxidative genes through the class IIa HDACs, it is not strictly essential. The FOXO family proteins are primarily regulated by phosphorylation, with Akt being the most well-characterized kinase. Phosphorylation of FOXOs by Akt results in their nuclear export and transcriptional inactivation. FOXO transcriptional activity can also be regulated by acetylation [29]. The FOXO family members have been observed to regulate a broad programme of metabolic genes in a manner similar to AMPK in a number of experimental systems. This led to the hypothesis that the FOXOs might be transcriptional substrates of AMPK. However, to the best of our knowledge, there is no published report of the epigenetic regulation of FOXOs and AMPK by Cav-1.

Conflict of interest

The authors declare no conflict of interest.

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