Biochemical and Biophysical Research Communications 447 (2014) 649–654

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Ablation of *cereblon* attenuates myocardial ischemia–reperfusion injury

Jooyeon Kim [a,b], Kwang Min Lee [b], Chul-Seung Park [b], Woo Jin Park [a,b,*]

[a] Global Research Laboratory, Gwangju Institute of Science and Technology (GIST), Gwangju 500-712, Republic of Korea
[b] School of Life Sciences, Gwangju Institute of Science and Technology (GIST), Gwangju 500-712, Republic of Korea

ARTICLE INFO

Article history:
Received 8 April 2014
Available online 19 April 2014

Keywords:
AMPK
Cereblon
Ischemia–reperfusion injury
Heart

ABSTRACT

Cereblon (CRBN) was originally identified as a target protein for a mild type of mental retardation in humans. However, recent studies showed that CRBN acts as a negative regulator of AMP-activated protein kinase (AMPK) by binding directly to the AMPK catalytic subunit. Because AMPK is implicated in myocardial ischemia–reperfusion (I–R) injury, we reasoned that CRBN might play a role in the pathology of myocardial I–R through regulation of AMPK activity. To test this hypothesis, wild-type (WT) and *crbn* knockout (KO) mice were subjected to I–R (complete ligation of the coronary artery for 30 min followed by 24 h of reperfusion). We found significantly smaller infarct sizes and less fibrosis in the hearts of KO mice than in those of WT mice. Apoptosis was also significantly reduced in the KO mice compared with that in WT mice, as shown by the reduced numbers of TUNEL-positive cells. In parallel, AMPK activity remained at normal levels in KO mice undergoing I–R, whereas it was significantly reduced in WT mice under the same conditions. In rat neonatal cardiomyocytes, overexpression of CRBN significantly reduced AMPK activity, as demonstrated by reductions in both phosphorylation levels of AMPK and the expression of its downstream target genes. Collectively, these data demonstrate that CRBN plays an important role in myocardial I–R injury through modulation of AMPK activity.

1. Introduction

The AMP-activated protein kinase (AMPK), a cellular fuel gauge, plays an important role in the signaling pathways that regulate cellular energy status [1]. For example, AMPK promotes energy production by activating glucose transport and glycolysis [2,3], as well as mitochondrial fatty acid uptake and oxidation [4]. In addition, AMPK is involved in a variety of other cellular processes, such as promoting cell survival by regulating apoptosis [5], autophagy [6,7], and the generation of reactive oxygen species [8].

Myocardial ischemia–reperfusion (I–R) injury is a major health problem in developed countries and is mediated by an increased generation of reactive oxygen species in ischemic cardiomyocytes upon abrupt resupply of oxygen. A number of previous studies have suggested a cardio-protective role for AMPK against I–R injury. In mice lacking the catalytic subunit of AMPK or expressing a dominant negative mutant of this subunit in the heart, the ischemia-induced stimulation of glucose uptake and glycolysis is inhibited, leading to ATP depletion and ischemic contracture [9]. Similarly, the infarct size following coronary ligation is larger in mice expressing a dominant negative form of AMPK than in controls [10,11]. By contrast, activation of AMPK reduces myocardial I–R injury in mice [9–11]. Moreover, AMPK activators, such as 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) [12], A-769662 (a non-nucleoside thienopyridone) [13], and metformin [14], protect hearts against I–R injury.

Cereblon (CRBN), a candidate protein linked with mild mental retardation, is a primary target of thalidomide teratogenicity. CRBN forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 and cullin 4A to play important roles for proper limb development. Thalidomide exerts its teratogenic effects by binding to CRBN and inhibiting its associated ligase activity [15]. Recently, CRBN was shown to inhibit AMPK activity by binding directly to

Abbreviations: CRBN, cereblon; I–R, ischemia–reperfusion; WT, wild-type; KO, knockout; AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; PARP1, poly (ADP-ribose) polymerase 1; AICAR, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; A-769662, a non-nucleoside thienopyridone; TTC, triphenyltetrazolium; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; AAR, areas at risk; IA, infarcted area; LV, left ventricular; PGC1α, peroxisome proliferator-activated receptor γ coactivator1 α; SREBP1, sterol regulatory element-binding protein 1; eNOS, endothelial nitric oxide synthase; CPT1, carnitine palmitoyltransferase 1.

* Corresponding author at: School of Life Sciences, Gwangju Institute of Science and Technology (GIST), 123 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Republic of Korea. Fax: +82 62 715 2484.
E-mail address: woojinpark@me.com (W.J. Park).

http://dx.doi.org/10.1016/j.bbrc.2014.04.061
0006-291X/

the catalytic subunit of AMPK [16]. The ablation of the *crbn* gene prevents high fat diet-induced obesity and insulin resistance in mice through elevation of AMPK activity [17]. These data suggest that CRBN is an endogenous inhibitor of AMPK.

In the present study, we found that ablation of *crbn* significantly reduced myocardial I–R injury in mice and that this cardio-protective effect was paralleled by an increase in AMPK activity. CRBN modulated AMPK activity in cardiomyocytes, as has been shown in other tissues. Therefore, down-regulating CRBN or disrupting CRBN–AMPK interactions may provide therapeutic protection for hearts against I–R injury.

2. Materials and methods

2.1. Animal care

Male CRBN knockout (KO) mice (8–10 weeks old, weighing 20–25 g) were generously donated by Dr. Chul-Seung Park (Gwangju Institute Science and Technology). Mice were maintained under controlled conditions at 22 °C with 55–56% humidity and a 12 h light:dark cycle. All procedures were conducted in accordance with protocols approved by the Gwangju Institute Science and Technology Animal Care Committee and national guidelines.

2.2. Ischemia–reperfusion protocol

Myocardial I–R was induced as previously described [18]. Briefly, the left anterior descending coronary artery was ligated using 7–0 silk sutured approximately 2 mm below the level of the tip of the normally positioned left auricle. Polyethylene (PE) 10 tubing with a diameter of 1 mm was placed on top of the vessel, and the suture was tied. After 30 min of occlusion, reperfusion was established by cutting the knot and removing the PE10 tubing. The chest wall was closed using 5–0 suture. Mice were sacrificed, and their hearts were removed after 24 h of reperfusion.

2.3. Determination of cardiac infarct size

Evan's blue dye (0.1%) was injected retrogradely via the abdominal aorta. The heart was removed and sectioned transversely into five slices from the level of the coronary ligature to the apex. Each slice was placed in 1.5% triphenyltetrazolium (TTC) blue dye. The slices of the heart were viewed using a dissecting microscope (400ES, Nikon). Images underwent computer enhancement using Adobe Photoshop, and the areas at risk or infarcted areas were calculated using MetaMorph software.

2.4. Masson's trichrome staining

Hearts were fixed with 4% paraformaldehyde, embedded in paraffin, and sliced into 6 μm thick sections (RM2135 microtome, Leica). Masson's trichrome staining was conducted as directed in the manufacturer's instructions (HT15 kit, Sigma–Aldrich). Images were captured and the fibrotic areas were quantified using MetaMorph software.

2.5. TUNEL assay

Apoptotic changes in DNA were identified with a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using fluorescein-labeled dUTP (Roche Diagnostics). TUNEL staining was performed on 8 μm-thick cryosections from hearts fixed with 4% paraformaldehyde perfusions and processed according to the manufacturer's instructions. Slides were examined for apoptotic nuclei using a fluorescence microscope. The number of TUNEL-positive nuclei was counted in three or four fields for each section.

2.6. Neonatal cardiomyocyte culture and transfection

Primary cardiomyocyte cultures were generated from 1-day-old Sprague–Dawley rats. The whole heart was prepared and digested

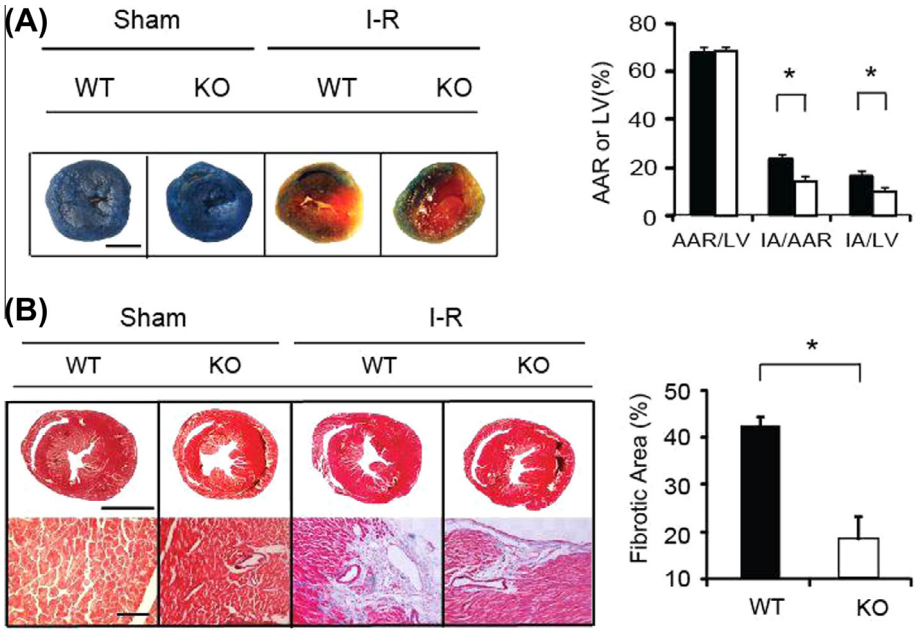

Fig. 1. Ablation of *crbn* attenuates myocardial infarction and fibrosis following I–R. (A) Wild-type (WT) and *crbn* knockout (KO) mice were subjected to 30 min of ischemia followed by 24 h of reperfusion. Heart sections were stained with Evan's blue and triphenyltetrazolium (TTC) blue dye. Healthy areas, areas at risk (AAR), and infarcted areas (IA) are distinguished in blue, red, and whitish colors, respectively. AAR and IA are normalized to whole left ventricular (LV) areas or AAR. Black bar, WT; white bar, KO. Scale bar = 5 mm. $n = 3–7$. $^{*}p < 0.05$. (B) Trichrome staining was performed 4 weeks after I–R. Fibrotic area is blue. Scale bars = 5 mm (upper row) and 5 μm (bottom row). $n = 3$. $^{*}p < 0.05$.

with collagenase type II (GIBCO BRL). Cardiomyocytes were enriched using a Percoll (Amersham Pharmacia) gradient centrifugation. Isolated cardiomyocytes were plated onto collagen-coated culture dishes and cultured in Dulbecco's modified Eagle medium (DMEM, GIBCO BRL) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 μM bromodeoxyuridine. Cardiomyocytes were transfected with plasmids using Opti-MEM (GIBCO) and Lipofectamine 2000 (Invitrogen).

2.7. Western blotting

The heart tissue homogenates (50 μg) and cell lysates were subjected to SDS–PAGE and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with primary antibodies against poly (ADP-ribose) polymerase 1 (PARP1) (Cell signaling), caspase 3 (Abcam), AMPK, phospho-AMPK, acetyl-CoA carboxylase (ACC), phospho-ACC, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Cell Signaling Technology). The membranes were then incubated with a secondary antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch) and developed using a chemiluminescent substrate (PerkinElmer).

2.8. Quantitative RT-PCR

The total RNA was isolated using TRI reagent (Sigma). The reverse transcription was performed using ImProm II reverse-transcriptase (Promega) with oligo-dT priming. The polymerase chain reaction (PCR) was performed using Takara with SYBR Green (Takara, Japan) as the fluorescent dye. The following PCR primers were used: Adiponectin, 5′-GCA CTG GCA AGT TCT ACT GCA A-3′ and 5′-GTA GGT GAA GAG AAC GGC CTT GT-3′; PGC1α, 5′-CCC TGC CAT TGT TAA GAC C-3′ and 5′-TGC TGC TGT TCC TGT TTT

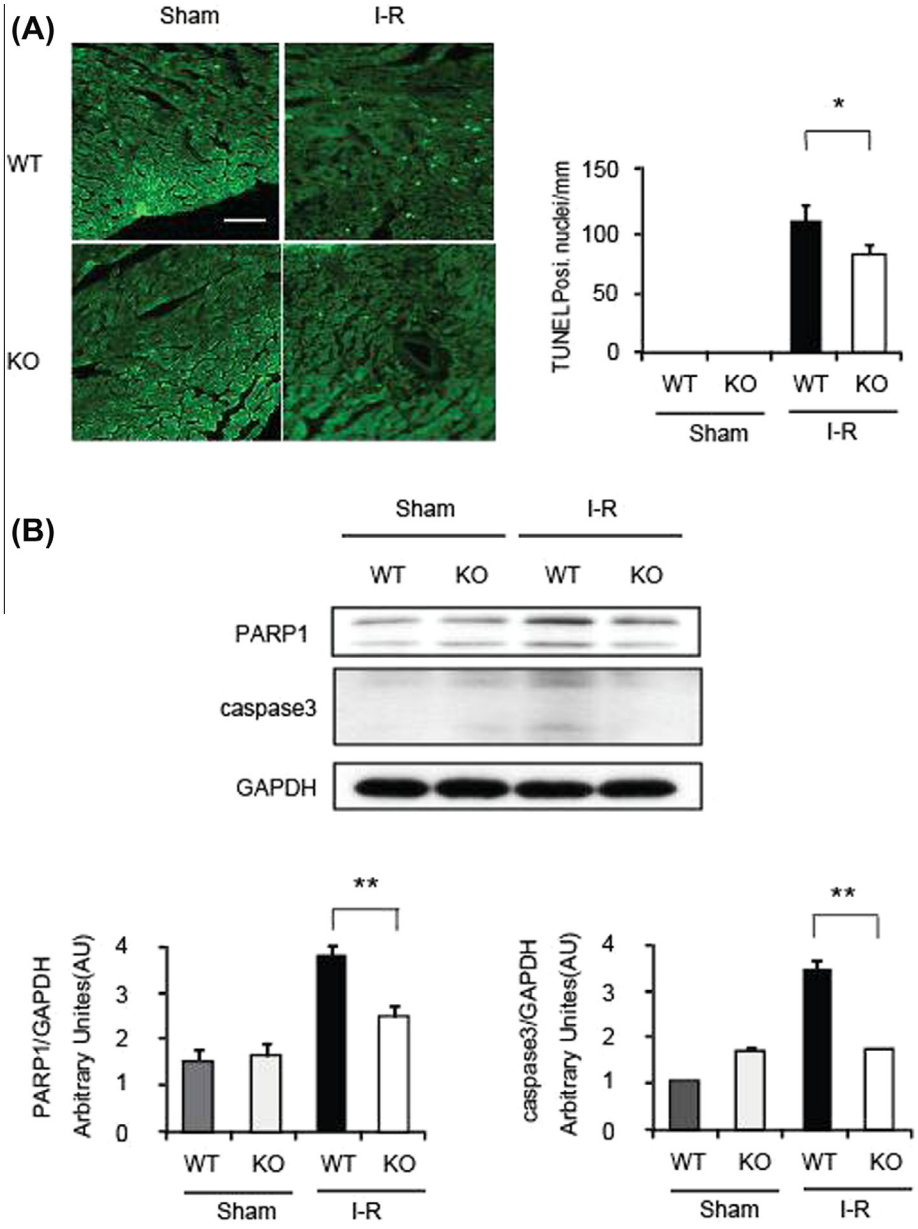

Fig. 2. Ablation of *crbn* attenuates cardiomyocyte apoptosis following I–R. (A) TUNEL assay was performed with heart sections. Green fluorescent dots represent apoptotic cells. Average numbers of apoptotic cells in 1 mm^2 are plotted. $n = 3–4$. $^{*}p < 0.05$. (B) Western blot analyses were performed with whole heart lysates (50 μg). Protein bands for poly (ADP-ribose) polymerase 1 (PARP1) and caspase 3 are quantified and plotted. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

C-3′; SERBP1, 5′-ATG GAC GAG FTG GCC TTC GGT GAG GCG GC-3′ and 5′-CAG GAA GGC TTC CAG AGA GGA-3′; eNOS, 5′-GGC TGG GTT TAG GGC TGT-3′ and 5′-GCT GTG GTC TGG TGC TGG T-3′; and CPT1, 5′-GCA TCA TCA CTG GTG TGT TC-3′ and 5′-GTG TTG CCA GCT CTC GCT G-3′.

2.9. Statistics

Where appropriate, the data were expressed as means ± SDs. Comparisons of the group means were made with Student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Ablation of crbn attenuates myocardial infarction and fibrosis following I–R

To test the role of CRBN in myocardial I–R injury, wild-type (WT) and *crbn* KO mice were subjected to 30 min of complete coronary artery occlusions followed by 24 h of reperfusion. The combined Evan's blue and TTC staining of the heart sections revealed healthy areas, areas at risk (AAR), and infarcted areas (IA). The percentages of the AAR per whole left ventricular (LV) area in WT and KO mice were indistinguishable (WT, 68.5 ± 1.4%; KO, 70.2 ± 1.8%), ensuring that the I–R was comparably executed in both groups. The IA was significantly smaller in KO mice than in WT mice, as calculated against both the AAR (WT, 23.4 ± 2.3%; KO, 14.9 ± 2.4%) and LV areas (WT, 16.1 ± 1.7%; KO, 10.4 ± 1.7%) (Fig. 1A). The fibrotic areas in the heart sections were evaluated with trichrome staining four weeks after I–R. The severe fibrosis observed in WT mice following I–R was significantly blunted in KO mice (WT, 43.3 ± 2.5%; KO, 19.2 ± 10.9%) (Fig. 1B). These data suggested that CRBN negatively regulates myocardial infarction and fibrosis following I–R.

3.2. Ablation of crbn attenuates cardiomyocyte apoptosis following I–R

Myocardial infarction is associated with apoptosis in cardiomyocytes. The apoptosis in heart sections was evaluated using a TUNEL assay. The number of TUNEL-positive nuclei was markedly increased in WT mice following I–R, which was significantly decreased in KO mice (WT, 105.6 ± 12.7; KO, 76.0 ± 6.5) (Fig. 2A). Western blotting revealed that the expression levels of two pro-apoptotic molecules, PARP-1 and caspase 3, that were significantly induced in WT mice following I–R compared with those in WT sham controls were significantly blocked in KO mice (Fig. 2B). These data suggested that CRBN is a negative regulator of apoptosis following I–R.

3.3. Ablation of crbn increases AMPK activity

Because CRBN negatively modulates AMPK, we tested whether AMPK activity was altered in the hearts of KO mice. Phosphorylation in the catalytic α subunit of AMPK (AMPKα) is critical for activation of AMPK. At baseline (Sham), the phosphorylation level of AMPKα was significantly higher in KO mice than in WT mice. Following I–R, the phosphorylation level of AMPKα was markedly reduced in WT mice but not in KO mice. ACC is an immediate downstream target molecule of AMPK. The phosphorylation level of ACC was higher in KO mice than in WT mice at baseline. The phosphorylation level of ACC was significantly reduced in WT mice upon I–R, whereas it remained unchanged in KO mice upon I–R (Fig. 3). These data indicated that CRBN acts as a negative regulator of AMPK in cardiomyocytes, as has been observed in other tissues.

3.4. CRBN inhibits AMPK activity in neonatal cardiomyocytes

To examine the interaction of CRBN with AMPKα directly in cardiomyocytes, we performed co-immunoprecipitation experiments. Neonatal cardiomyocytes were transiently transfected with pcDNA-CRBN-HA that expressed mouse CRBN tagged with HA. Immunoprecipitation was performed using control IgG or anti-AMPKα antibodies, and then the precipitates were probed with anti-AMPKα or anti-HA antibodies. CRBN-HA was co-precipitated with AMPKα (Fig. 4A, left panel). Neonatal cardiomyocytes were transiently transfected with pcDNA control plasmids or with pcDNA-CRBN-HA, and the lysates were analyzed using Western blot assays. The expression of CRBN-HA significantly reduced the

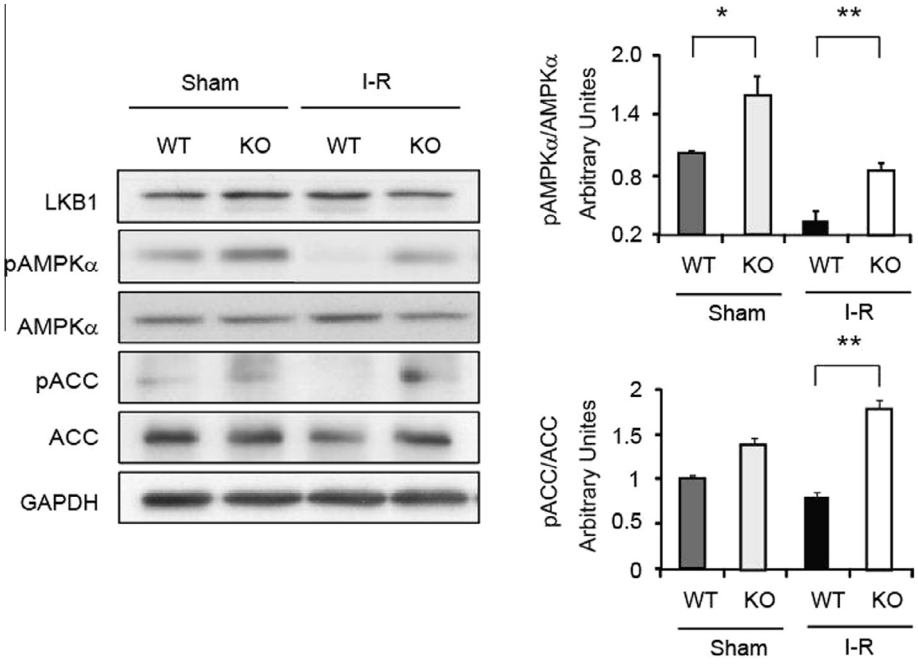

Fig. 3. Ablation of *crbn* increases AMPK activity. Whole heart lysates (50 μg) were subjected to Western blotting for AMPKα, phospho-AMPKα, acetyl-CoA carboxylase (ACC), and phospho-ACC. Protein band intensities were quantified and are plotted. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$.

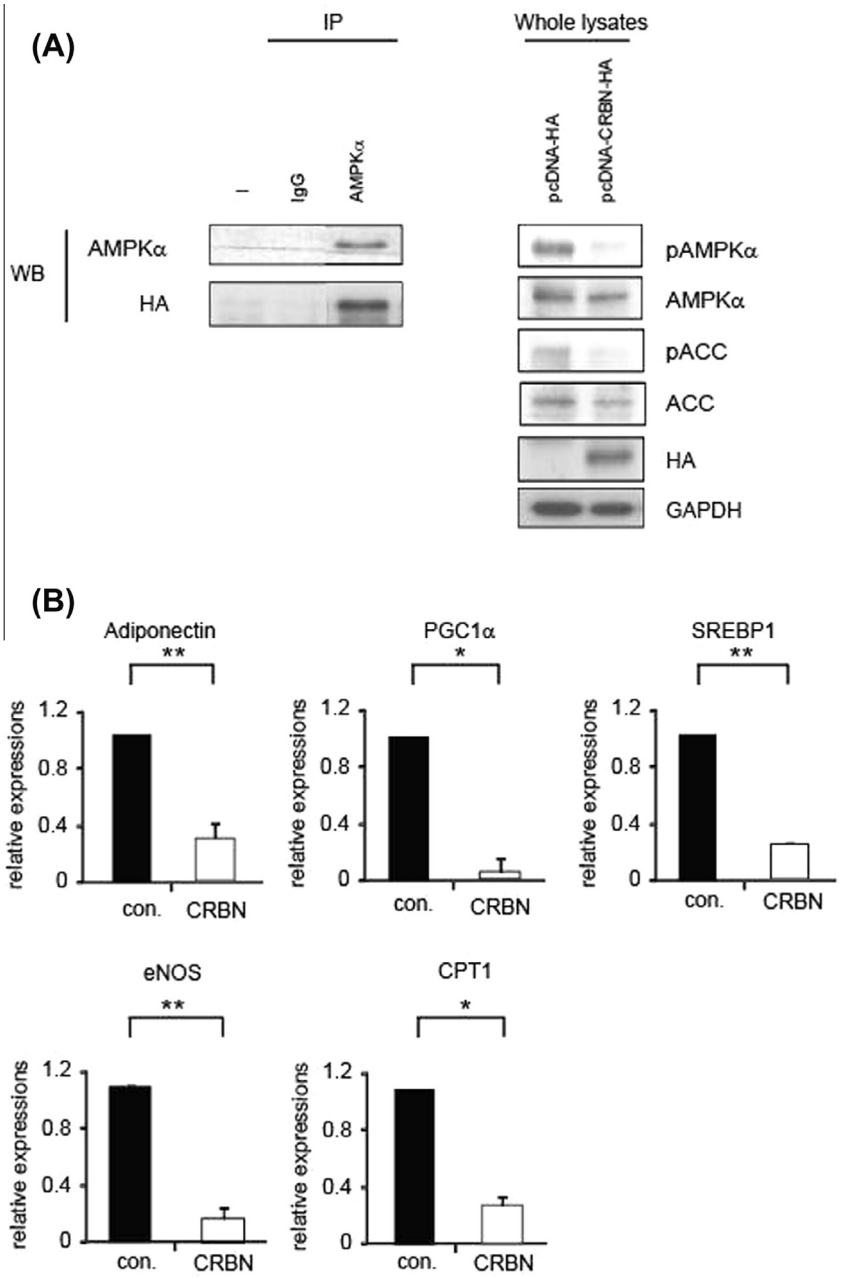

Fig. 4. CRBN inhibits AMPK activity in neonatal cardiomyocytes. (A) Neonatal cardiomyocytes were transfected with pcDNA-CRBN-HA. Whole cell lysates (50 μg) were immunoprecipitated using anti-AMPKα1 antibody, and the precipitates were probed with anti-AMPKα1 and anti-HA antibodies (left panel). Whole heart lysates (50 μg) were subjected to Western blotting for AMPKα, phospho-AMPKα, acetyl-CoA carboxylase (ACC), phospho-ACC, and HA. (B) Neonatal cardiomyocytes were transfected with pcDNA (control) or pcDNA-CRBN-HA (CRBN). Expression levels of several genes downstream of AMPK were analyzed by qRT-PCR, including adiponectin, peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1α), sterol regulatory element-binding protein 1 (SREBP1), endothelial nitric oxide synthase (eNOS), and carnitine palmitoyltransferase 1 (CPT1). Relative expression levels are plotted. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$.

phosphorylation of AMPKα and ACC (Fig. 4A, right panel). Quantitative real-time PCR (qRT-PCR) was performed to examine the expression levels of several molecules downstream of AMPK, including adiponectin, peroxisome proliferator-activated receptor γ coactivator1 α (PGC1α), sterol regulatory element-binding protein 1 (SREBP1), endothelial nitric oxide synthase (eNOS), and carnitine palmitoyltransferase 1 (CPT1). The expression levels of all these molecules were reduced by 5- to 10-fold in cardiomyocytes transfected with pcDNA-CRBN-HA (Fig. 4B). Collectively, these data suggested that CRBN negatively modulates AMPK activity in cardiomyocytes.

4. Discussion

AMPK targets many metabolic processes, such as glucose uptake, glycolysis, and glucose and fatty acid oxidation. The roles of AMPK during myocardial I–R are controversial; however, a number of previous studies showed that AMPK is cardio-protective under conditions of I–R. For example, myocardial I–R injury was significantly exacerbated in mice expressing the dominant negative form of AMPKα or lacking the functional AMPKα subunit [9].

The *crbn* gene was linked to autosomal recessive nonsyndromic mental retardation (ARNSMR) in humans and was later shown to

be the primary target of thalidomide-induced teratogenicity [16]. Surprisingly, CRBN was also shown to bind directly to and negatively modulate AMPK activity [16,17]. A series of biochemical analyses revealed that CRBN directly interacts with the catalytic α subunit of AMPK and interferes with the phosphorylation of this subunit, a critical activation mechanism of AMPK [16]. It was also shown that *crbn* deficiency protects mice from obesity, fatty liver, and insulin resistance caused by a high fat diet, and that this protection is mediated through the elevation of AMPK activity. Therefore, it is evident that CRBN acts as an endogenous negative regulator of AMPK, although how this function is related to the pathogenesis of ARNSMR and thalidomide-induced teratogenicity remains uncertain [16,17].

We initially observed that the expression of *crbn* was up-regulated in the hearts of mice with pressure overload-induced heart failure or I–R injury, suggesting that CRBN plays an important role during pathogenesis of these cardiac conditions (Supplementary Fig. 1). We indeed found that the array of I–R injuries, including infarction, fibrosis, and apoptosis, was significantly reduced in *crbn* KO mice (Figs. 1 and 2). In parallel, AMPK activity was significantly preserved in KO mice (Fig. 3) and inhibited by overexpression of CRBN in neonatal cardiomyocytes (Fig. 4).

The data from the present study support the hypothesis that AMPK is cardio-protective under I–R conditions. In addition, this study provides the first evidence that CRBN acts as a negative modulator of AMPK in the heart, as has been observed in other tissues. We suggest that CRBN is a potential therapeutic target for myocardial I–R injury.

Acknowledgments

W.J.P. was supported by a grant (M6-0605-00-0001) from the Global Research Laboratory Program of the Korean Ministry of Education, Science and Technology and a grant from the Systems Biology Infrastructure Establishment Program of GIST.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.04.061.

References

[1] D.G. Hardie, D. Carling, The AMP-activated protein kinase – fuel gauge of the mammalian cell?, Eur J. Biochem. 246 (1997) 259–273.
[2] J. Li, X. Hu, P. Selvakumar, R.R. Russell 3rd, S.W. Cushman, G.D. Holman, L.H. Young, Role of the nitric oxide pathway in AMPK-mediated glucose uptake and GLUT4 translocation in heart muscle, Am. J. Physiol. Endocrinol. Metab. 287 (2004) E834–E841.
[3] A.S. Marsin, L. Bertrand, M.H. Rider, J. Deprez, C. Beauloye, M.F. Vincent, G. Van den Berghe, D. Carling, L. Hue, Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia, Curr. Biol. 10 (2000) 1247–1255.
[4] J.J. Luiken, S.L. Coort, J. Willems, W.A. Coumans, A. Bonen, G.J. van der Vusse, J.F. Glatz, Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling, Diabetes 52 (2003) 1627–1634.
[5] S. Horman, G. Browne, U. Krause, J. Patel, D. Vertommen, L. Bertrand, A. Lavoinne, L. Hue, C. Proud, M. Rider, Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis, Curr. Biol. 12 (2002) 1419–1423.
[6] Y. Matsui, H. Takagi, X. Qu, M. Abdellatif, H. Sakoda, T. Asano, B. Levine, J. Sadoshima, 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 (2007) 914–922.
[7] K. Imahashi, M.D. Schneider, C. Steenbergen, E. Murphy, Transgenic expression of Bcl-2 modulates energy metabolism, prevents cytosolic acidification during ischemia, and reduces ischemia/reperfusion injury, Circ. Res. 95 (2004) 734–741.
[8] E. Murphy, C. Steenbergen, Mechanisms underlying acute protection from cardiac ischemia–reperfusion injury, Physiol. Rev. 88 (2008) 581–609.
[9] C. Beauloye, L. Bertrand, S. Horman, L. Hue, AMPK activation, a preventive therapeutic target in the transition from cardiac injury to heart failure, Cardiovasc. Res. 90 (2011) 224–233.
[10] R.R. Russell 3rd, J. Li, D.L. Coven, M. Pypaert, C. Zechner, M. Palmeri, F.J. Giordano, J. Mu, M.J. Birnbaum, L.H. Young, AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury, J. Clin. Invest. 114 (2004) 495–503.
[11] M.A. Paiva, Z. Rutter-Locher, L.M. Goncalves, L.A. Providencia, S.M. Davidson, D.M. Yellon, M.M. Mocanu, Enhancing AMPK activation during ischemia protects the diabetic heart against reperfusion injury, Am. J. Physiol. Heart Circ. Physiol. 300 (2011) H2123–H2134.
[12] X.N. Li, J. Song, L. Zhang, S.A. LeMaire, X. Hou, C. Zhang, J.S. Coselli, L. Chen, X.L. Wang, Y. Zhang, Y.H. Shen, Activation of the AMPK-FOXO3 pathway reduces fatty acid-induced increase in intracellular reactive oxygen species by upregulating thioredoxin, Diabetes 58 (2009) 2246–2257.
[13] A.S. Kim, E.J. Miller, T.M. Wright, J. Li, D. Qi, K. Atsina, V. Zaha, K. Sakamoto, L.H. Young, A small molecule AMPK activator protects the heart against ischemia reperfusion injury, J. Mol. Cell. Cardiol. 51 (2011) 24–32.
[14] J.W. Calvert, S. Gundewar, S. Jha, J.J. Greer, W.H. Bestermann, R. Tian, D.J. Lefer, Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK eNOS-mediated signaling, Diabetes 57 (2008) 696–705.
[15] T. Ito, H. Ando, T. Suzuki, T. Ogura, K. Hotta, Y. Imamura, Y. Yamaguchi, H. Handa, Identification of a primary target of thalidomide teratogenicity, Science 327 (2010) 1345–1350.
[16] K.M. Lee, S. Jo, H. Kim, J. Lee, C.S. Park, Functional modulation of AMP-activated protein kinase by cereblon, Biochim. Biophys. Acta 2011 (1813) 448–455.
[17] K.M. Lee, S.J. Yang, Y.D. Kim, Y.D. Choi, J.H. Nam, C.S. Choi, H.S. Choi, C.S. Park, Disruption of the cereblon gene enhances hepatic AMPK activity and prevents high-fat diet-induced obesity and insulin resistance in mice, Diabetes 62 (2013) 1855–1864.
[18] R. Shibata, K. Sato, D.R. Pimentel, Y. Takemura, S. Kihara, K. Ohashi, T. Funahashi, N. Ouchi, K. Walsh, Adiponectin protects against myocardial ischemia–reperfusion injury through AMPK- and COX-2-dependent mechanisms, Nat. Med. 11 (2005) 1096–1103.