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Metformin promotes autophagy in ischemia/reperfusion myocardium via cytoplasmic AMPKα1 and nuclear AMPKα2 pathways

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ABSTRACT

Aims: In myocardial ischemia-reperfusion (MI/R) injury, impaired autophagy function worsens cardiomyocyte death. AMP-activated protein kinase (AMPK) is a heterotrimeric protein that plays an important role in cardioprotection and myocardial autophagic function. AMPKα1 and α2 are localized primarily in the cytoplasm and nucleus, respectively, in cardiomyocytes, but the isoform-specific autophagy regulation of AMPK during MI/R remains unclear.

Materials and methods: An MI/R model was built, and the protein expression of AMPKα1/α2, p-AMPK, mTOR, p-mTOR, TFEB, p-FoxO3a, SKP2, CARM1, TBP, Atg5, LAMP2, LC3B, and p62 during ischemia and reperfusion was determined by western blotting. Recombinant adeno-associated virus (serotype 9) vectors carrying tandem fluorescent-tagged LC3 or mRFP-GFP-LC3/GFP-LC3 were used to evaluate the autophagy status. AMPKα2 knockout mice were used for in vivo studies.

Key findings: Both cytoplasmic AMPKα1 and nuclear α2 subunit expression decreased during the reperfusion period, which led to AMPKα1-mTOR-TFEB and AMPKα2-Skp2-CARM1-TFEB signaling inhibition, respectively. The decreased TFEB level during reperfusion suppressed autophagy. Metformin could activate both the AMPKα1- and α2- mediated pathways, thus restoring autophagy flux during reperfusion. Nevertheless, in AMPKα2 knockout mice, nuclear α2-regulated Skp2-CARM1-TFEB signaling was inhibited, while α1-related signaling was comparatively unaffected, which partially impaired metformin-enhanced autophagy.

Significance: Our study suggests that metformin had the dual effects of promoting both cytoplasmic AMPKα1- and nuclear AMPKα2-related signaling to improve autophagic flux and restore cardiac function during MI/R.

1. Introduction

Autophagy is a crucial process in the degradation of damaged or unnecessary proteins and organelles. In the myocardium, autophagy remains at a basal level under normal conditions and regulates energy salvage, nutrient homeostasis, and malfunctional organelle degradation. In contrast to these salutary functions at the basal level, dysfunctional autophagy is a programmed cell death process that induces cellular damage attributed to many disorders and diseases, such as ischemic heart disease, cardiac hypertrophy and heart failure [1–3]. Autophagic flux, which initiates the formation of autophagosomes and autophagosome-lysosome fusion for ultimate degradation, begins in the ischemic period during myocardial ischemia-reperfusion (MI/R) [4]. Autophagy induction in myocardial ischemia is generally regarded as a protective mechanism due to its compensation for energy loss [5]. Autophagy was reported to be further enhanced during cardiac reperfusion and associated with the accumulation of autophagosomes. It has also been reported that autophagic flux is "impaired" rather than "excessive" autophagosome clearance, as evidenced by decreased autophagosome clearance rather than increased autophagosome formation [6,7]. Restoring autophagic flux to prevent cardiomyocyte death is a possible way to inhibit MI/R injury.

In MI/R injury, AMPK activation inhibits downstream mammalian target of rapamycin (mTOR) by phosphorylating tuberous sclerosis complex 2 (TSC2) and induces eukaryotic elongation factor 2 (eEF2) phosphorylation in the cytoplasm, which is required for autophagy induction during the ischemia phase [8]. Recent research has revealed that coactivator-associated arginine methyltransferase 1 (CARM1), a methyltransferase, acts as a co-activator of TFEB in the nucleus [7]. S-phase kinase-associated protein 2 (SKP2) is known as an E3 ubiquitin

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ligase that has been reported to regulate CARM1 degradation in the nucleus [9]. Previous studies indicate that SKP2 is transcriptionally suppressed via FoxO3a phosphorylation [10,11]. In addition, FoxO3a phosphorylation, which regulates its transcriptional activity, is modulated by AMPKα2 [11]. The AMPK catalytic subunit α1 and α2 isoforms are located in the cytoplasm and nucleus, respectively. However, whether AMPK regulates autophagic flux during MI/R injury via the cytoplasmic AMPKα1-mTOR or nuclear AMPKα2-CARM1 pathway remains unclear. Metformin, a first-line therapeutic medicine for the treatment of type 2 diabetes, has cardioprotective effects against MI/R injury [12]. Additionally, metformin has proven to be an AMPK agonist and has cardioprotective effects through the activation of AMPK [13]. However, the underlying mechanism of how metformin modulates autophagic flux through AMPKα1 and α2 during MI/R injury has rarely been studied.

Therefore, the aim of this study was to identify the state of autophagic flux in the ischemia and reperfusion phases and determine the role of the cytoplasmic AMPKα1-mTOR and nuclear AMPKα2-CARM1 pathways in autophagic flux during MI/R injury.

2. Materials and methods

2.1. Animals

All animal experiments were reviewed and approved by the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University. Male C57BL/6 mice aged 6–8 months and weighing 22–25 g were obtained from the animal center of Fourth Military Medical University. AMPKα2 knockout mice (AMPK KO) were purchased from The Jackson Laboratory. Metformin dissolved in saline at 125 μg/kg was administered to the mice intraperitoneally daily for 4 weeks. Saline was used as the vehicle. All animals were housed under a 12:12-h light/dark cycle with ad libitum access to a regular pellet diet.

2.2. Materials

Metformin (1,1-dimethylbiguanide hydrochloride), triphenyltetrazolium chloride (TTC), and Evans blue were purchased from Sigma-Aldrich (St. Louis, MO, USA). An NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (No. 78835, Thermo Fisher Scientific) was used to extract nuclear and cytoplasmic proteins. Serum lactate dehydrogenase (LDH) levels were detected by a kit (MAK066) purchased from Sigma-Aldrich. MG132 was purchased from Selleck (TX, USA).

2.3. Western blotting and coimmunoprecipitation

Western blotting was conducted as previously described [14]. Coimmunoprecipitation analysis was performed according to the manufacturer's protocol (No. 26149, Thermo Fisher Scientific). Antibodies against AMPKα1 (2795), α2 (2757), p-AMPK (Thr172) (50081), mTOR (2972), phosphor-mTOR (2971), TFEB (4240), p-FoxO3a (Ser413) (8174), SKP2 (4358), CARM1 (4438), TBP (8515), Atg5 (2630), and LAMP2 (49067) were purchased from Cell Signaling Technology (MA, USA). LC3B (ab51520) and p62 (ab56416) antibodies were purchased from Abcam (MA, USA).

2.4. MI/R injury and measurement of infarct size

An MI/R model was built as previously described [15]. Briefly, animals were anesthetized with pentobarbital (65 mg/kg, i.p.). A Harvard rodent respirator was used to sustain ventilation after tracheostomy. After a left thoracic incision was performed, a 7-0 silk suture slipknot was placed to block the blood flow of the left anterior descending coronary artery for 30 min. Reperfusion was sustained for 4 h (for western blotting, coimmunoprecipitation, infarct size measurement, and LDH detection) or for 8 weeks (for cardiac function and survival statistics). The mice were injected intraperitoneally with metformin or PBS (as a control) for 4 weeks before MI/R surgery.

2.5. In vivo gene interference

Recombinant adeno-associated virus (serotype 9) vectors carrying tandem fluorescent-tagged LC3 or mRFP-GFP-LC3/GFP-LC3 (Hanbio Inc., Shanghai, China) were used. The left ventricular wall of the mice was injected with a total of 30 μl (1×10^{12} IFU/ml) of mRFP-GFP-LC3/GFP-LC3 over 6 injections (each injection was 5 μl) through a 29G catheter. Four weeks after injection, MI/R surgery was performed. After 4 h of reperfusion, the hearts were excised, embedded in optimal cutting temperature (OCT) compound and frozen immediately. The frozen tissue was cut into 5-μm cryosections. DAPI (4′,6-diamidino-2-phenylindole) staining was performed to label the nuclei. The GFP signal is attenuated in an acidic lysosomal environment and degraded by lysosomal hydrolases, while the mRFP signal is comparatively stable after autophagosome and lysosome fusion. Because of the difference between the pKa value of GFP and mRFP (pKaGFP = 6.0, pKamRFP = 4.5), mRFP-GFP-LC3/GFP-LC3 shows merged GFP and mRFP fluorescence in autophagosomes, whereas mRFP fluorescence appears in lysosomes only (17). Images were taken with a Fluoreview FV300 laser scanning confocal microscope (Olympus, Tokyo, Japan).

2.6. Statistical analysis

The values are represented as the mean ± SEM. GraphPad Prism 7 software was used to analyze the data. Log-rank testing was used to evaluate the equality of the survival curves. Significance was analyzed by ANOVA or 2-tailed, unpaired Student's *t*-test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Metformin treatment ameliorates MI/R injury

As autophagy is an important process in ischemic heart disease, our above results have shown that autophagic flux was partly impaired and that autophagosome clearance was dramatically inhibited in the reperfusion phase. However, metformin treatment could enhance autophagic flux and autophagosome clearance. We also observed that metformin treatment markedly minimized MI/R injury-induced myocardial infarct size compared with vehicle treatment (Fig. 1A). The reduction in lactate dehydrogenase (LDH) also showed that metformin alleviated MI/R injury (Fig. 1B). Furthermore, MI/R induced cardiac contractile dysfunction, and left ventricular dilation was restored in the metformin treatment group (Fig. 1C). Chronic MI/R-induced mortality was also lower in the metformin treatment group than in the vehicle control group (Fig. 1D).

3.2. Autophagy was impaired in the reperfusion phase, and both AMPKα1-mTOR and AMPKα2-p-Foxo3a-Skp2 were involved in the TFEB decrease

To investigate the discrepancy in autophagic flux in response to the separate ischemia and reperfusion phases in the myocardium, we established an MI/R model (ischemia for 30 min, followed by reperfusion for 4 h). Compared with the sham group, autophagosome levels of Atg5 and LC3-II were increased in the ischemia group and markedly decreased in the reperfusion group (Fig. 2A and B). The lysosome membrane protein LAMP2 was obviously reduced in the reperfusion group (Fig. 2A and B), indicating dysfunctional autophagic flux with the accumulation of p62 (Fig. 2A and B). These results shed light on the different state of autophagic flux during the ischemia and reperfusion phases and the apparent autophagy impairment during reperfusion.

To determine the underlying mechanism of impaired autophagy in

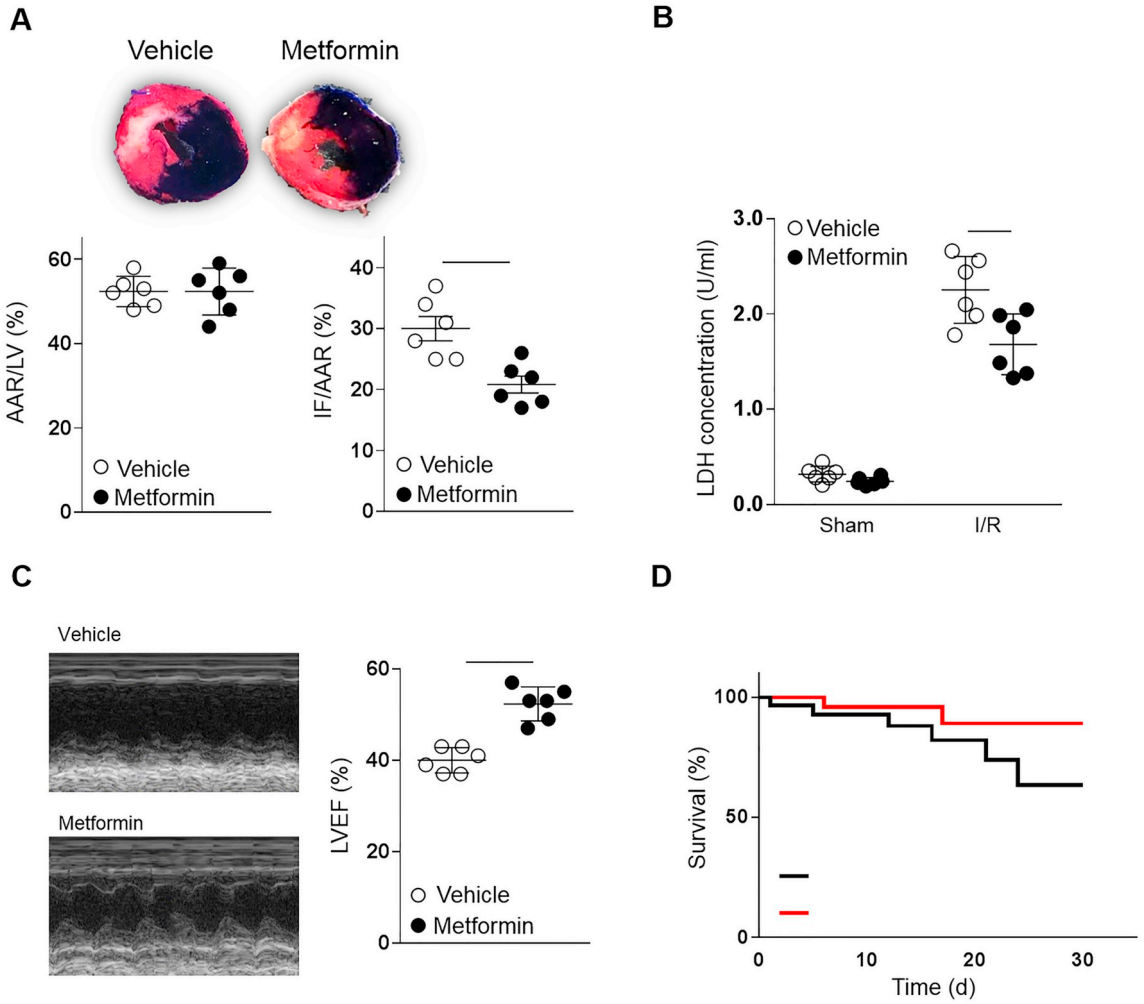

Fig. 1. Autophagy was impaired in the reperfusion phase, and both AMPKα1-mTOR and AMPKα2-p-Foxo3a-Skp2 were involved in the decrease in TFEB. (A) Representative western blot data for myocardial Atg5, LC3-I/II, LAMP2, and p62 expression in hearts from the sham, ischemia, and reperfusion mouse groups. (B) Quantitative analysis of panel (A). (C) Representative western blot data for cytoplasmic AMPKα1, p-AMPK, and p-mTOR expression in the myocardium. (D) Quantitative analysis of panel (C). (E) Representative western blot data for myocardial AMPKα2, p-FoxO3a, SKP2, CARM1, and TFEB in the nucleus. (F) Quantitative analysis of panel (E). (G) Representative coimmunoprecipitation (Co-IP) analysis of nuclear CARM1 and TFEB in the sham, ischemia and reperfusion groups. The data are presented as the mean ± SEM, n = 6 mice per group. $^{*}P < 0.05$ vs. sham group.

the reperfusion phase, we detected AMPKα1 signaling in the cytoplasm. The expression of AMPKα1 after 30 min of ischemia did not change noticeably (Fig. 2C and D). After 4 h of reperfusion, AMPKα1 expression decreased with the phosphor-AMPK levels, which increased mTOR phosphorylation (Fig. 2C and D). mTOR-dependent TFEB phosphorylation is essential for TFEB nuclear transport, and TFEB phosphorylation prevents its translocation to the nucleus [16]. We found that TFEB levels were weakened in the nucleus during reperfusion compared with those in the sham and ischemia groups (Fig. 2E and F).

Once TFEB shuttles to the nucleus, TFEB and CARM1 form a co-activated complex that recruits autophagic genes [17]. The nuclear TFEB-CARM1 complex was markedly impaired during the reperfusion phase, which is consistent with the decrease in autophagy (Fig. 2G) and the suppression of nuclear TFEB. In addition, CARM1 expression also decreased during reperfusion (Fig. 2E and F). S-phase kinase-associated protein 2 (SKP2), acting as an E3 ubiquitin ligase, was reported to regulate CARM1 degradation in the nucleus [9]. A recent study also revealed that the SKP2 upstream transcriptional repressor FoxO3a is regulated by nuclear AMPKα2 [10,11]. Therefore, to explore the modulation of CARM1 during MI/R injury, we measured the AMPKα2-FoxO3a-SKP2 signaling pathway upstream of CARM1.

According to our results, AMPKα2 and p-FoxO3a decreased during reperfusion, which led to SKP2 inhibition and then increased CARM1 degradation. These data agreed with the inhibition of TFEB nuclear translocation by AMPKα1-mTOR signaling in the cytoplasm, resulting in decreased TFEB-CARM1 interaction and autophagic dysfunction.

3.3. Metformin activates both the AMPKα1 and α2 pathways and restores autophagic flux

Metformin has been reported to have cardioprotective effects through the stimulation of AMPK catalytic activity [18], while the influence of metformin on AMPKα1 and α2 isoforms is unclear. To determine the effect of metformin on AMPKα1 and α2, groups of mice treated with or without metformin for 4 weeks (125 μg/kg, i.p.) were subjected to MI/R surgery in vivo. Notably, after metformin treatment, AMPKα1 in the cytoplasm was increased concomitant with AMPK phosphorylation and sustained during reperfusion, and AMPK activation regulated mTOR inhibition (Fig. 3A and B), which led to TFEB nuclear translocation (Fig. 3C and D). We also verified that AMPKα2 expression was higher after metformin treatment than vehicle treatment during reperfusion. AMPKα2-dependent FoxO3a phosphorylation also increased in the nucleus, which induced a reduction in SKP2 and an increase in CARM1 (Fig. 3C and D). Accordingly, metformin treatment

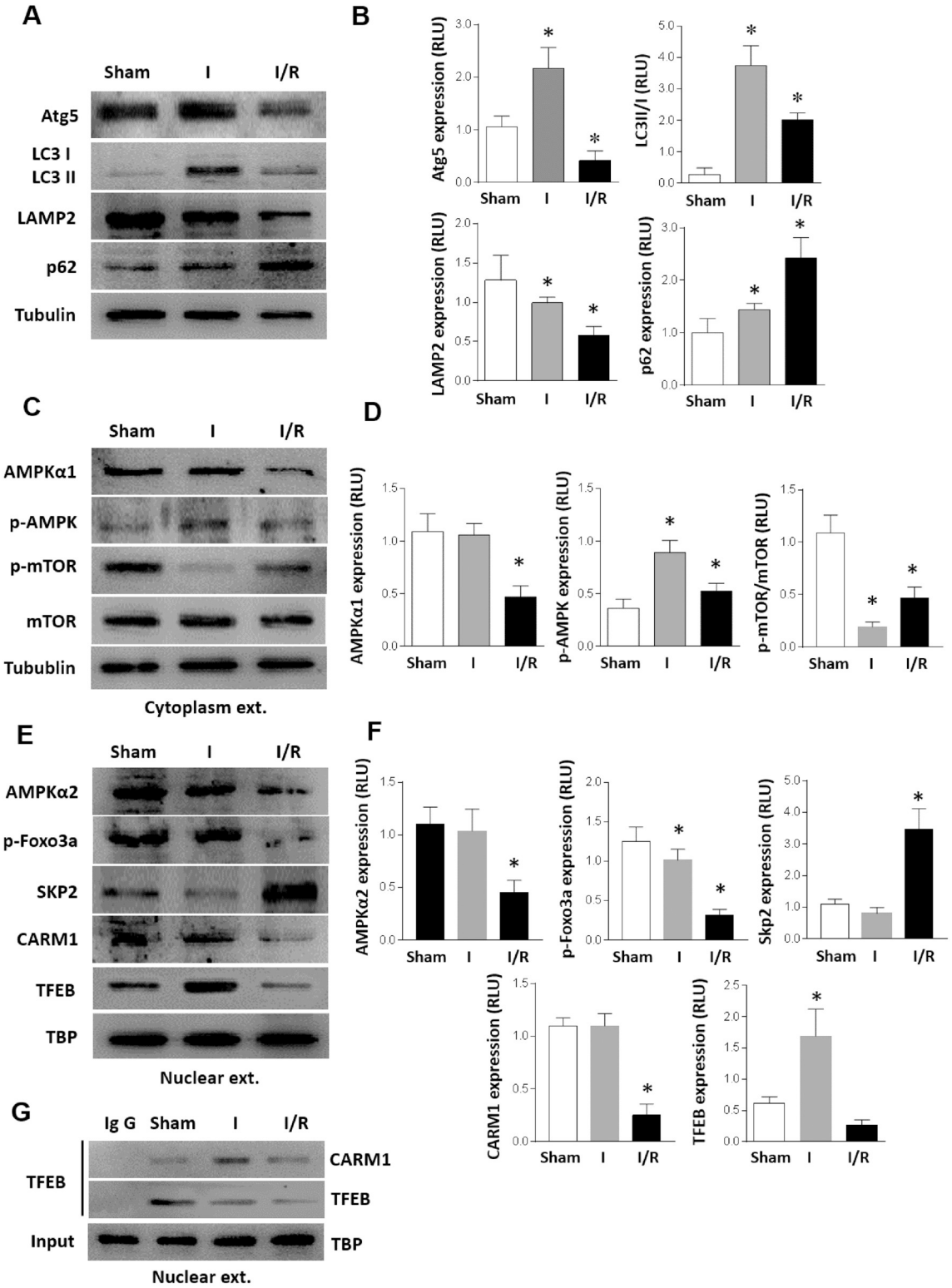

Fig. 2. Metformin activates both the AMPKα1 and α2 pathways and restores autophagy flux. (A) Representative western blot data for cytoplasmic AMPKα1, p-AMPK, and p-mTOR expression in hearts from the sham, ischemia, and reperfusion groups treated with or without metformin. (B) Quantitative analysis of panel (A). (C) Representative western blot data for nuclear AMPKα2, p-FoxO3a, SKP2, CARM1, and TFEB. (D) Quantitative analysis of panel (C). (E) Representative Co-IP analysis of nuclear CARM1 and TFEB levels with or without metformin treatment. (F) Representative western blot data for Atg5, LC3-II, LAMP2, and p62 levels in the myocardium. (G) Representative fluorescent confocal microscope images of mRFP-GFP-LC3. mRFP-GFP-LC3 was injected into mice 4 weeks before they were subjected to MI/R injury and treated with or without metformin. The data are presented as the mean ± SEM, n = 6 mice per group. *$P < 0.05$ vs. vehicle-sham group.

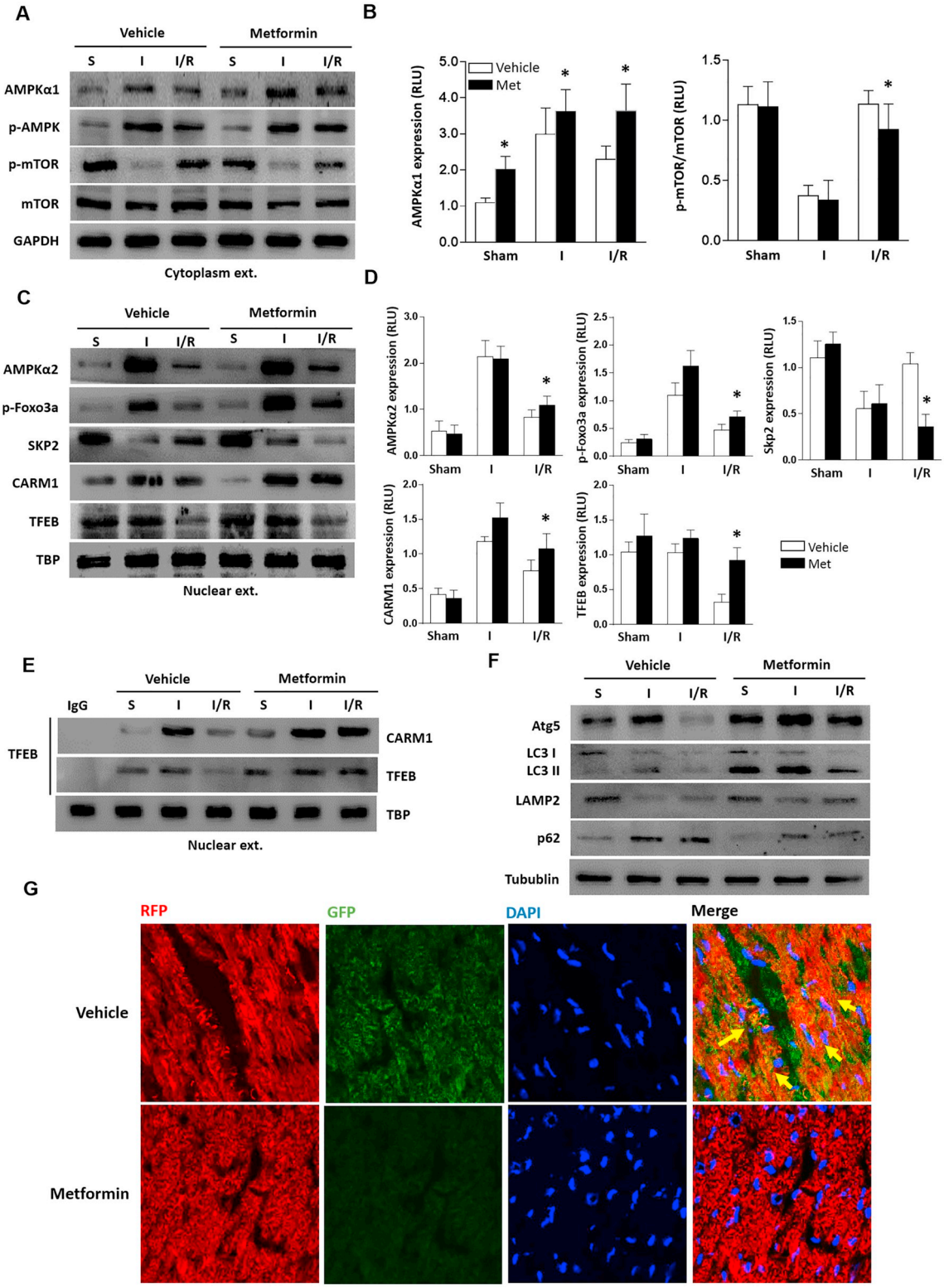

Fig. 3. The cardioprotective effect of metformin was partly inhibited in AMPKα2 knockout mice. (A) AMPKα2 knockout and wild-type mice were subjected to sham, ischemia and reperfusion procedures (30 min ischemia/4 h reperfusion). Representative western blots for AMPKα1, p-AMPK, and p-mTOR in cardiomyocytic cytoplasm. (B) Quantitative analysis of panel (A). (C) Representative western blots for AMPKα2, p-FoxO3a, SKP2, CARM1 and TFEB in the cardiomyocytic nucleus. (D) Quantitative analysis of panel (C). (E) and (F) AMPKα2 knockout and wild-type mice were treated with or without metformin for 4 weeks before being subjected to sham, ischemia and reperfusion procedures. Representative western blots for Atg5, LC3, LAMP2 and p62 in cardiomyocytes. (G) AMPKα2 knockout and wild-type mice treated with or without metformin for 4 weeks before MI/R injury. Representative fluorescent confocal microscope images of mRFP-GFP-LC3. The data are presented as the mean ± SEM, n = 6 mice per group. $^{*}P < 0.05$ vs. WT-sham group.

increased the nuclear TFEB-CARM1 complex levels (Fig. 3E).

We next evaluated the state of autophagic flux, and the data implied that Atg5, LC3II and LAMP2 increased, and p62 accumulation was alleviated during reperfusion after metformin treatment compared with vehicle treatment (Fig. 3F and Supplementary Fig. S1). Mouse hearts were transfected with AAV9-mRFP-GFP-LC3 4 weeks before MI/R surgery. mRFP-GFP-LC3 was dyed and imaged by immunofluorescence. Autophagosomes marked by mRFP-GFP-LC3 showed both mRFP and GFP signals; however, GFP signals were weakened, and only mRFP signals could be observed after the fusion of autophagosomes with lysosomes [19]. These results showed that autophagosome fusion with lysosomes and lysosome degradation were inhibited during reperfusion; however, metformin treatment could restore autophagic flux (Fig. 3G).

Taken together, these results indicated that impairment of cytoplasmic AMPKα1-mTOR and nuclear AMPK-SKP2-CARM1 signaling during the reperfusion phase was activated by metformin treatment, which rejuvenated autophagic flux during MI/R injury.

3.4. The cardioprotective effect of metformin was partly inhibited in AMPKα2 knockout mice

To examine the importance of AMPK-mediated signaling in the cardioprotective effect of metformin, AMPKα2 KO mice were subjected to MI/R surgery. Compared with that in WT mice, the AMPKα1-mTOR pathway was partly influenced by AMPKα2 deficiency, as indicated by decreased AMPK phosphorylation, slightly increased mTOR phosphorylation (Fig. 4A and B), and, consequently, further decreased TFEB levels in the nucleus during reperfusion (Fig. 4C and D). In the nucleus, AMPKα2 deficiency also induced further p-FoxO3a inhibition during the reperfusion phase, leading to increased SKP2 and decreased CARM1 (Fig. 4C and D). Then, we treated KO mice with metformin and measured the autophagic flux. The results showed that autophagic flux during reperfusion was partly blocked in the KO + Met group, as evidenced by lower Atg5, LC3II, and LAMP2 and higher p62 (Fig. 4E and F) levels than those in the WT + Met group. RFP-GFP-LC3 was dyed and imaged by immunofluorescence, and the results showed that metformin-enhanced autophagic flux was suppressed in the KO + Met group (Fig. 4G).

These results demonstrated that AMPKα2 deficiency inhibited nuclear FoxO3a-SKP2-CARM1 signaling and influenced cytoplasmic AMPKα1-mTOR-TFEB signaling. These effects, in turn, rendered metformin-enhanced autophagy dysfunctional. The data above shed light on the AMPK-dependent cardioprotective effect of metformin treatment (Fig. 5).

4. Discussion

Autophagy dysfunction is involved in various cardiovascular diseases, such as heart failure, dilated cardiomyopathy and ischemic heart disease [1]. Myocardial ischemia is characterized by an imbalance between blood supply and energy requirements. AMPK acts as an energy sensor activated by a decrease in ATP levels and an increase in the AMP/ATP ratio during the ischemic period, which consequently inhibits mTOR [20]. The AMPK-mTOR pathway is regarded as an important regulator of autophagy in response to MI/R injury [21,22]. However, AMPK is no longer activated during the reperfusion phase [23], and our results showed that Atg5, LC3II and LAMP2 were reduced upon the accumulation of p62, which indicated autophagy impairment.

Subunit α is the catalytic subunit of AMPK, and location restrictions and functional distinctions of AMPKα1 and α2 have been reported [24]. In cardiomyocytes, AMPKα1 is located in the cytoplasm, while AMPKα2 is located in the nucleus. Therefore, to gain insight into the function of AMPKα1 and α2, we separated cytoplasmic and nuclear proteins. We found that AMPK phosphorylation was inhibited and that AMPKα1 was decreased during reperfusion, resulting in AMPK-competitive mTOR activation. Recent studies have demonstrated that the mTOR-dependent phosphorylation of TFEB at Ser211 prevents the translocation of TFEB into the nucleus, where it stimulates genes involved in autophagy [16]. Consistently, during reperfusion, a lower level of TFEB in the nucleus was found in parallel with mTOR upregulation.

CARM1 is an important arginine methyltransferase that is often recruited by various transcription factors as an activator. Previous research found that CARM1 could bind to the TFEB transcriptional activation domain as a co-activator of autophagic genes [25]. According to our results, during reperfusion, the level of CARM1 was reduced, as well as that of the nuclear CARM1-TFEB complex, as shown by coimmunoprecipitation. SKP2-dependent CARM1 ubiquitylation and degradation has been reported [10]. The SKP2 upstream transcriptional repressor FoxO3a was reported to be regulated by nuclear AMPKα2 [11]. Our data demonstrated that AMPKα2 decreased during reperfusion. AMPKα2 suppression led to p-FoxO3a inhibition, the loss of SKP2 repression, and reduced CARM1, which contributed to impairing the CARM1-TFEB interaction. AMPKα2 knockout was used to verify the importance of AMPK-regulated signaling in autophagy. Our results showed that cytoplasmic AMPKα1-mTOR-TFEB signaling was partly affected by AMPKα2 deficiency via weakened p-AMPK activation; nuclear AMPKα2-mediated FoxO3a-SKP2-CARM1 signaling was obviously suppressed.

Metformin, one of the oldest but widely used glucose-controlling drugs, has an effective role in cardiac and vascular disease prevention [26]. Despite its use in diabetes, the pharmacodynamics of metformin in MI/R injury have been reported recently. Both in vitro and in vivo experiments support that metformin administration reduces infarct size and attenuates left ventricular post-MI/R injury dysfunction [27–32]. These beneficial effects were regarded to be mediated mainly through the stimulation of AMPK catalytic activity [18], which affects a number of metabolic pathway-related energy-consuming biosynthetics [33]. Recent studies have demonstrated that metformin-induced AMPK-mediated autophagy conferred protection against cardiac dysfunction following MI/R injury [12]. However, whether metformin has a protective effect via AMPKα1 and α2 has rarely been studied.

Our data showed that metformin augmented AMPKα1 levels and upregulated AMPK phosphorylation during the reperfusion phase compared with vehicle treatment, and these effects alleviated mTOR phosphorylation and allowed TFEB mobilization to the nucleus. Metformin administration also stimulated AMPKα2 in the nucleus, which activated FoxO3a phosphorylation and SKP2-CARM1 signaling. These factors contributed to the increase in the autophagic co-activator TFEB-CARM1 interaction, which improved autophagic flux and cardiac function.

AMPKα2-deficient mice were used, and we found that the cardioprotective effects of metformin were blunted partially via the AMPKα1 pathway and inhibiting the AMPKα2 pathway. Metformin administration to AMPKα2 KO mice inactivated autophagic flux compared with metformin administration to WT mice. These data shed light on the importance of metformin's cardioprotective effect through AMPKα1 and α2.

5. Conclusion

Our present study reveals the underlying mechanism of metformin against MI/R injury via cytoplasmic AMPKα1- and nuclear α2-mediated autophagic flux, which might be a potential therapeutic target for autophagy dysfunction.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.04.002.

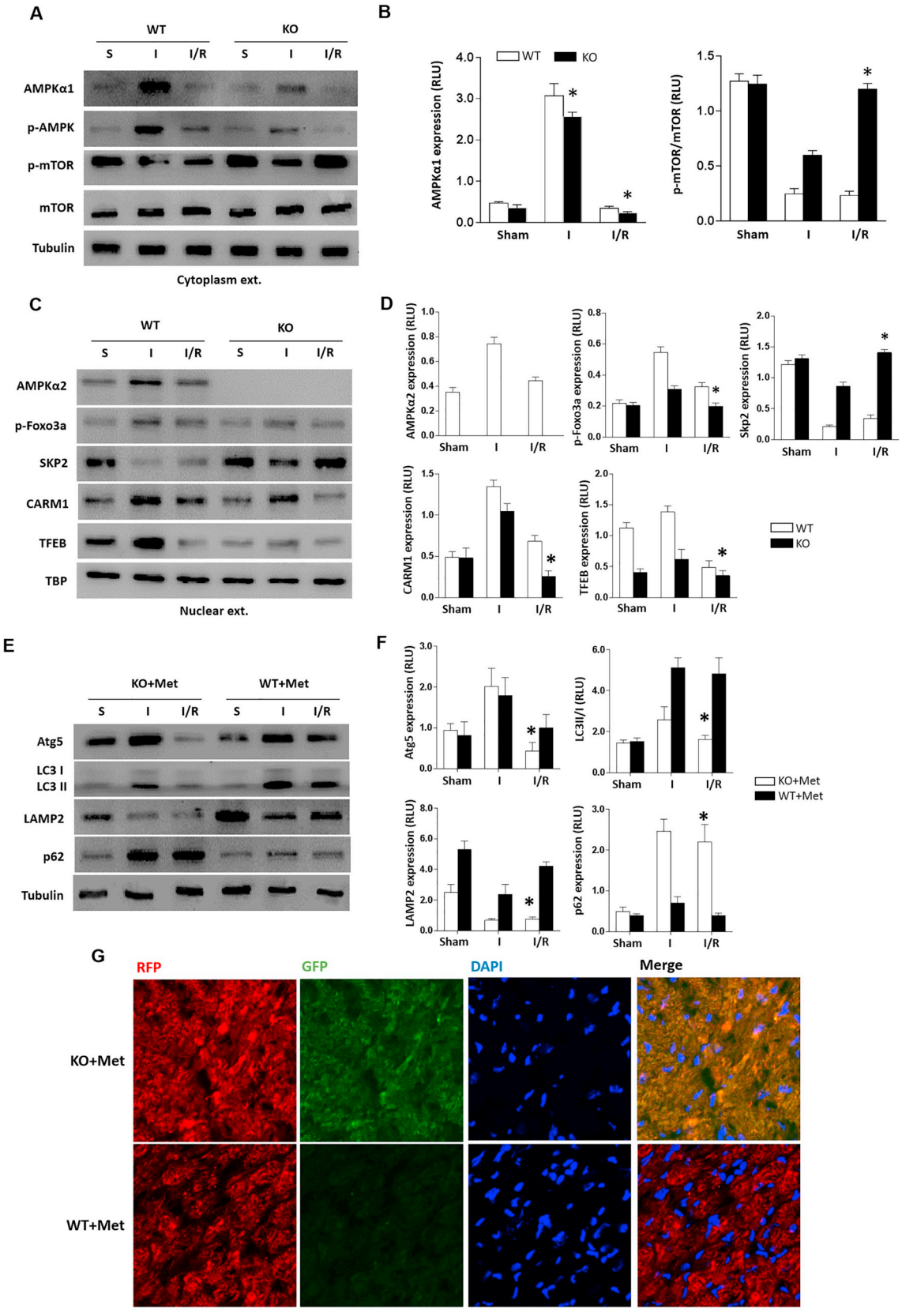

Fig. 4. Metformin treatment ameliorates MI/R injury. (A) Representative photographs and quantitative data of infarct size (INF) and area at risk (AAR) in hearts from mice treated with or without metformin before MI/R injury. (B) Serum LDH levels in the vehicle and metformin groups of mice with sham or MI/R injury. (C) Left ventricular ejection fraction (LVEF) was assessed by echocardiography in the vehicle and metformin groups of mice subjected to sham procedure in vivo. (D) Survival curves of the vehicle and metformin groups of mice subjected to sham procedure in vivo. The data are presented as the mean ± SEM, n = 6 mice per group. *$P < 0.05$ vs. WT-Met group.

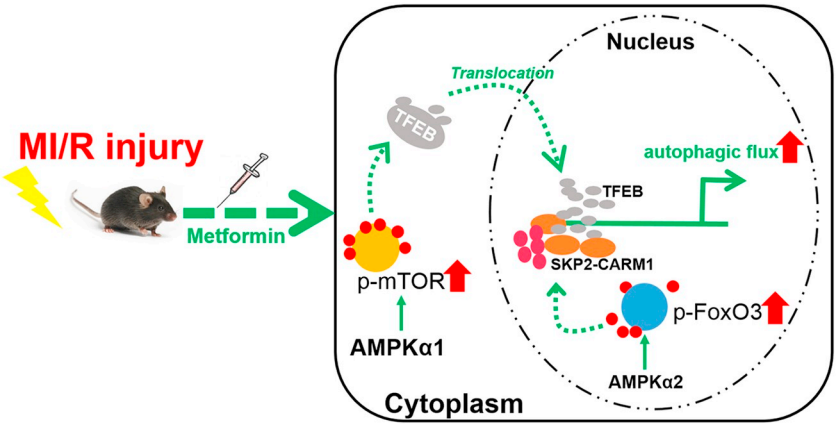

Fig. 5. The schematic diagram of the metformin signaling events involving cytoplasmic AMPKα1 and nuclear AMPKα2 in MI/R injury. Metformin augmented AMPKα1 levels and upregulated AMPK phosphorylation during reperfusion, and these effects alleviated mTOR phosphorylation and allowed TFEB mobilization to the nucleus. Metformin also stimulated AMPKα2 in the nucleus, which activated FoxO3a phosphorylation and SKP2-CARM1 signaling. These factors contributed to the increase in the autophagic co-activator TFEB-CARM1 interaction, which improved autophagic flux and cardiac function. These data shed light on the importance of metformin's cardioprotective effect through AMPKα1 and α2.

Abbreviations

MI/R	myocardial ischemia-reperfusion
AMPK	AMP-activated protein kinase
mTOR	mammalian target of rapamycin
CARM1	coactivator-associated arginine methyltransferase 1
SKP2	S-phase kinase-associated protein 2
TFEB	transcription factor EB

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

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