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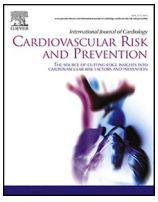

Resveratrol reinforces the therapeutic effect of mesenchymal stem cell (MSC)-derived exosomes against renal ischemia–reperfusion injury (RIRI)-associated fibrosis by suppressing TGF-β-induced epithelial-mesenchymal transition

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ABSTRACT

Resveratrol (RSV) has been shown to prevent epithelial-mesenchymal transition (EMT) in different diseases by modulating several signaling pathways, and RSV can prevent EMT by modulating the signaling of the TGF-β/Smad axis. In the development of renal ischemia–reperfusion injury (RIRI), RSV and MSC-derived exosomes could ameliorate RIRI via different signaling pathways. In this study, we aimed to investigate the effect of RSV plus MSC-derived exosomes on the prognosis of RIRI. Quantitative real-time polymerase chain reaction (PCR) was performed to measure the expression of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA in TCMK-1 cells and mice under various conditions. HE and Masson staining were used to evaluate kidney injury and fibrosis in mice under various conditions. RSV effectively maintained the TGF-β- and AA-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells. Moreover, MSC-derived exosomes effectively reinforced the effect of RSV on reducing the TGF-β- and AA-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells. Furthermore, MSC-derived exosomes enhanced the capability of RSV to maintain the RIRI-induced increases in Cr and BUN, as well as the upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in mice. In addition, MSC-derived exosomes enhanced the capability of RSV to decrease RIRI-induced kidney injury and fibrosis in mice. Our findings showed that the administration of MSC-derived exosomes and RSV could suppress the TGF-β-induced epithelial-mesenchymal transition. This suppressive effect was promoted by the coadministration of MSC-derived exosomes and RSV.

1. Introduction

Up to half of acute kidney injury (AKI) patients show a certain level of fibrosis. Chronic AKI is pathologically determined to be a considerable amount of extracellular matrix (ECM) in the glomeruli, which is the most significant cause of kidney failure. The ECM is associated with cellular signaling and therefore plays a necessary role in renal repair during ischemia–reperfusion (I/R) by modulating inflammation, cell expansion and fibroblast transdifferentiation. Thus, it is essential to fully recognize the mechanisms and signaling molecules involved in I/R-induced AKI [1,2]. Epithelial-mesenchymal transition (EMT) is a sophisticated reprogramming process that gives epithelial cells a mesenchymal phenotype. EMT occurs via several processes and plays a necessary role throughout cell repair, organogenesis, and fibrosis [3]. Studies have shown that EMT can occur in kidneys during persistent renal injury by participating in kidney fibrosis [4,5]. EMT may be the programmed proliferation of epithelial cells through which tubular cells lose their epithelial features and acquire mesenchymal phenotypes, giving them improved adaptation to injury [6]. However, these changes can result in the accumulation of extracellular matrix in the cortical interstitium, which may cause kidney fibrosis [7].

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Abbreviation

RIRI	renal ischemia–reperfusion injury
EMT	epithelial-mesenchymal transition
E-CAD	E-cadherin
VMT	vimentin
COL10A1	collagen10a1
AA	aristolochic acid
RSV	resveratrol
AD-MSCs	adipose-derived mesenchymal stem cells
EXOs	exosomes

Renal fibrosis is an outcome of chronic kidney disease (CKD) and may become end-stage renal disease (ESRD), and patients require dialysis or renal transplant to avoid death [8,9]. Excessive ECM deposition can lead to tubulointerstitial fibrosis and decreased kidney function [10]. Activated tubulointerstitial myofibroblasts are the primary cell type that triggers the excessive deposition of ECM [11]. Myofibroblasts are derived from tubular epithelial cells by means of EMT, and transforming growth factor-beta 1 (TGF-β1) has been shown to act as the key regulator of EMT induction and kidney fibrosis [12–14].

Bone marrow stromal cells (BMSCs) are multipotent cells derived from the mesoderm and have been extensively investigated [15]. It was previously discovered that exogenous BMSCs could easily alleviate AKI to improve renal function [16]. Moreover, BMSCs can easily promote heart function in the ischemic myocardium and protect the rodent hippocampus from transient ischemia–reperfusion injury (IRI) [17]. Thus, BMSCs exert protective effects against IRI. However, the clearance of transplanted BMSCs is extremely high [18]. Mesenchymal stem cells (MSCs) exert a number of biological effects, including repairing tissue damage, decreasing inflammation, and regulating the immune system [19,20].

MSCs function in a paracrine way [21]. Therefore, the soluble factors and exosomes derived from MSCs in the medium have been extensively analyzed. Exosomes were shown to trigger signaling processes by binding to their receptors [22,23]. Compared with MSCs, exosomes are highly stable without the risk of immune rejection and aneuploidy, providing an alternative treatment for certain diseases [24].

Resveratrol (RSV) is a polyphenol derived from grapes, mulberries, and peanuts. This compound has been shown to play a variety of pharmacological roles, including protecting against coronary heart disease [25,26]. In some studies, RSV was excellent in alleviating kidney injury and fibrosis, such as sepsis-induced renal injury, glycerol-induced kidney injury, and cisplatin-induced kidney injury [27,28].

In another study, TGF-β1 was used to induce EMT in glioblastoma (GBM) tissues. Resveratrol inhibited EMT, EMT-induced migration, and cell invasion. It was likewise shown that resveratrol decreased EMT in xenografts in vivo [29].

TGF-β is a major regulator of the induction of EMT and renal fibrosis, and EMT has been reported to play an essential role in the development of tubular atrophy in severe kidney injury [30,31]. Moreover, RSV has been shown to prevent EMT in different diseases by modulating several signaling pathways, and it is noteworthy that RSV can prevent EMT by modulating the signaling of the TGF-β/Smad axis [29,32]. In the development of renal ischemia–reperfusion injury (RIRI), RSV and MSC-derived exosomes could ameliorate RIRI via different signaling pathways [33–35]. We hypothesized that the administration of RSV and MSC-derived exosomes could suppress TGF-β-induced epithelial-mesenchymal transition. Therefore, this study aimed to investigate the effect of coadministration of RSV and MSC-derived exosomes on the prognosis of RIRI.

2. Materials and methods

2.1. Animals and treatment

Sixty male Balb/c mice aged 10–12 weeks with an average weight of 31.2 g were divided into 5 groups (12 mice in each group): 1. SHAM, 2. RIRI, 3. EXOs, 4. RSV, and 5. EXOs-RSV. All mice were purchased from the Shanghai Center of Laboratory Animals at the Chinese Academy of Sciences and were kept in the Animal Facility of our institution. The mice were kept under a 12 h/12 h light/dark cycle at 23 °C. The treatments were carried out according to the guidelines of the Animal Care Committee, and all methods were reviewed by the Animal Care Committee. The RIRI model was established according to published methods [36]. Briefly, a midline incision in the abdomen was made to expose the renal pedicles, and renal pedicles of right kidney were clamped for 30 min to induce ischemia. The clamps were then removed to allow renal reperfusion before the mice abdomen with sutures was closed for recovery. For the mice in the Sham group, the incision was made in the abdomen, and no other operations were made before the abdomen was closed. On D29, nephrectomy of the right kidney was carried out, and blood urea nitrogen and serum creatinine were tested to determine the functionality of the left kidney. For RSV treatment, the animals were given 20 mg/kg gavage of RSV on a daily basis according to previous publications [37]. For the EXOs treatment, a total of 50 μg MSCs-derived exosomes were injected intravenously during the ischemia. The average weight of the animals by the end of the experiment was 29.6 g.

2.2. RNA isolation and real-time polymerase chain reaction (PCR)

For the animal study, mouse kidney tissues were collected in small pieces and homogenized in TRIzol buffer (Thermo Fisher Scientific, Waltham, MA, USA). Chloroform was used to process the tissue mixture and prepare the RNA for subsequent isolation. For in vivo mRNA analysis, total RNA was isolated from cell or tissue samples with a miRNeasy assay kit (Catalog No. 74004, Qiagen, Germantown, MD, USA), and subsequently reverse transcribed following the below thermocycling conditions: 10 min at 25 °C for primer annealing, 60 min at 42 °C for reverse transcription, and 5 min at 85 °C for reverse transcriptase inactivation. Then, TaqMan real-time PCR (Thermo Fisher Scientific, Waltham, MA, USA) was performed following the below thermocycling conditions: 10 min at 95 °C for initial denaturation, 40 cycles of 15 s at 95 °C for denaturation and 1 min at 60 °C for annealing and extension. Finally, the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative mRNA expressions of E-CAD (Forward: 5′-GCCTCCTGAAAAGAGAGTGGAAG-3′; Reverse: 5′-TGGCAGTGTCTCTCCAAATCCG-3′), SMA (Forward: 5′-TGCTGACAGAGGCACCACTGAA-3′; Reverse: 5′-CAGTTGTACGTCCAGAGGCATAG-3′), COL10A1 (Forward: 5′-CGCTGAACGATACCAAATGCCC-3′; Reverse: 5′-TGGACCAGGAGTACCTTGCTCT-3′), VMT (Forward: 5′-AGGCAAAGCAGGAGTCCACTGA-3′; Reverse: 5′-ATCTGGCGTTCCAGGGACTCAT-3′) and MMP-7 (Forward: 5′-AGGTGTGGAGTGCCAGATGTTG-3′; Reverse: 5′-CCACTACGATCCGAGGTAAGTC-3′) in the samples, which were normalized to the expression of U6 and GAPDH.

2.3. Cell culture and experimental model establishment

AD-MSCs were extracted from mouse adipose tissue. In brief, adipose tissues were separated, washed with PBS, homogenized, hydrolyzed for 2 h at 37 °C and 5% CO_2 in 0.1% collagenase-I, and finally treated with 3–5 vol of complete medium containing collagenase I. Then, the cells were filtered with a 70 μm strainer and cultured in RPMI-1640 (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA). The cells were kept in a humidified environment at 5% CO_2 and 37 °C. In this study, TCMK-1 cells (purchased from ATCC, Manassas, VA, USA) were used to create several cellular models. In Model 1, TCMK-1 cells were divided into 3 groups: 1.

NC, 2. TGF-β, and 3. TGF-β+RSV. In Model 2, TCMK-1 cells were divided into 3 groups: 1. NC, 2. aristolochic acid (AA), 3. AA + RSV. In Model 3, TCMK-1 cells were divided into 4 groups: 1. NC, 2. TGF-β, 3. TGF-β + EXOs, and 4. TGF-β + EXOs - RSV. In Model 4, TCMK-1 cells were divided into 4 groups: 1. NC, 2. AA, 3. AA + EXOs, and 4. AA + EXOs – RSV. For exosomes treatment in each cell group, the TCMK-1 cells were treated with 10 μg exosomes.

3. H&E staining

The mice were divided into 5 groups: 1. SHAM, 2. RIRI, 3. EXOs, 4. RSV, and 5. EXO-RSV. Kidney tissues were collected and fixed in 10% neutral buffered formalin before immersion in fixative to preserve their structure for 48 h. Subsequently, the fixed tissues were dehydrated and embedded in paraffin wax before being sliced into 4 μm-thick sections. Then, the sliced sections were stained with hematoxylin and eosin using a commercial assay kit (Catalog No. NC1881153, Abcam, Cambridge, MA, USA) to examine renal tissue injury.

3.1. Masson assay

Mouse kidney tissues were fixed, dehydrated and embedded in paraffin wax. Subsequently, the fixed tissues were sliced into 4 μm-thick sections. Masson's trichrome staining was used to examine renal fibrosis by using a commercial Masson trichrome stain kit (Catalog No. NC1881153, Abcam). Morphometric dimensions of the tissue sections were calculated to determine the maximum level of fibrosis. The dimensions were determined using ImageJ.

3.2. Exosome isolation

Exosomes were isolated from the supernatant of AD-MSCs via ultracentrifugation. In brief, AD-MSCs were cultured in DMEM/F12 (Thermo Fisher Scientific, Waltham, MA, USA) with 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA) overnight, and the medium was collected. The supernatant was centrifuged for 30 min at 2000 g and then filtered with a 0.22 μm filter before the medium was centrifuged again at 100,000 g and 4 °C for 1 h (Beckman Coulter, San Jose, CA, USA). The supernatant was then discarded before the pellet was washed. Finally, the exosome suspension was diluted to 2 μg/μL, and 2 μL of this suspension was added onto a carbon-coated grid before the grid was stained with uranyl acetate for 60 s. Then, after the grid was dried, it was examined by transmission electron microscopy (TEM, Jeol USA Inc., MA, USA), estimating the exosomes size to be 50–100 nm (Supplementary Fig. 1).

3.3. Statistical analysis

The data were analyzed with one-way ANOVA and Tukey's post hoc test using SPSS software (version 25.0, IBM, NY, US). All data are expressed as the means ± SEs. Each experiment was repeated in triplicate. A value of $P < 0.05$ was considered significant.

4. Results

4.1. RSV effectively maintained the TGF-β-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells

TCMK-1 cells were treated with TGF-β and TGF-β+RSV. RT−PCR was performed to measure the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in TCMK-1 cells under various conditions. TGF-β treatment significantly upregulated the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in TCMK-1 cells. RSV effectively restored the TGF-β-induced upregulation of E-CAD (Fig. 1A), SMA (Fig. 1B), COL10A1 (Fig. 1C), VMT (Fig. 1D) and MMP-7 (Fig. 1E) mRNA expression in TCMK-1 cells.

4.2. RSV effectively maintained the AA-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells

TCMK-1 cells were treated with AA and AA + RSV. RT−PCR was performed to measure the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in TCMK-1 cells under various conditions. AA treatment significantly upregulated the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in TCMK-1 cells. RSV effectively restored AA-induced upregulation of E-CAD (Fig. 2A), SMA (Fig. 2B), COL10A1 (Fig. 2C), VMT (Fig. 2D) and MMP-7 (Fig. 2E) mRNA expression in TCMK-1 cells.

4.3. RSV and MSC-derived exosomes synergistically reduced the TGF-β-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells

TGF-β-treated TCMK-1 cells were administered MSC-derived exosomes or MSC-derived exosomes plus RSV. TGF-β treatment significantly upregulated the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in TCMK-1 cells. MSC-derived exosomes effectively restored the TGF-β-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells. Moreover, RSV treatment further reinforced the capability of MSC-derived exosomes to abrogate the TGF-β-induced upregulation of E-CAD (Fig. 3A), SMA (Fig. 3B), COL10A1 (Fig. 3C), VMT (Fig. 3D) and MMP-7 (Fig. 3E) mRNA expression in TCMK-1 cells.

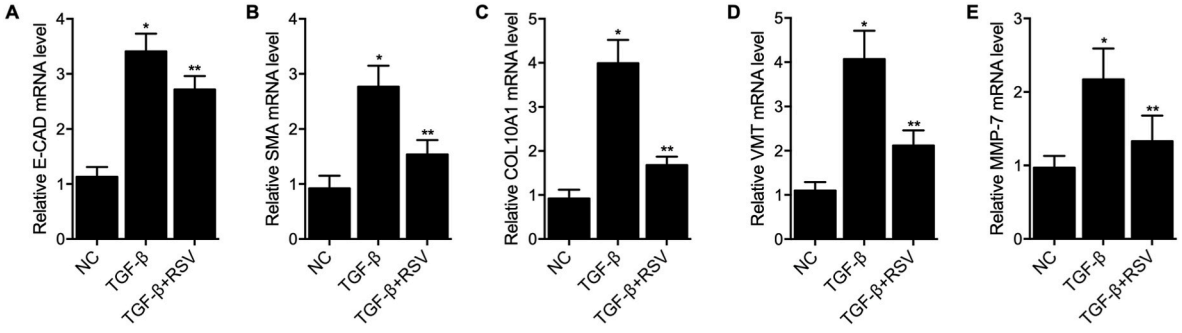

Fig. 1. The effect of RSV on TCMK-1 cells administrated of TGF-β was observed via measuring the changes in mRNA levels of E-CAD, SMA, COL10A1, VMT and MMP-7 by PCR analysis. Accordingly, RSV was demonstrated to effectively suppress the up-regulated mRNA level of E-CAD (A), SMA (B), COL10A1 (C), VMT (D) and MMP-7 (E) in TCMK-1 cells treated with TGF-β (*P value < 0.05 vs. NC group; **P value < 0.05 vs. TGF-β group; RSV: resveratrol).

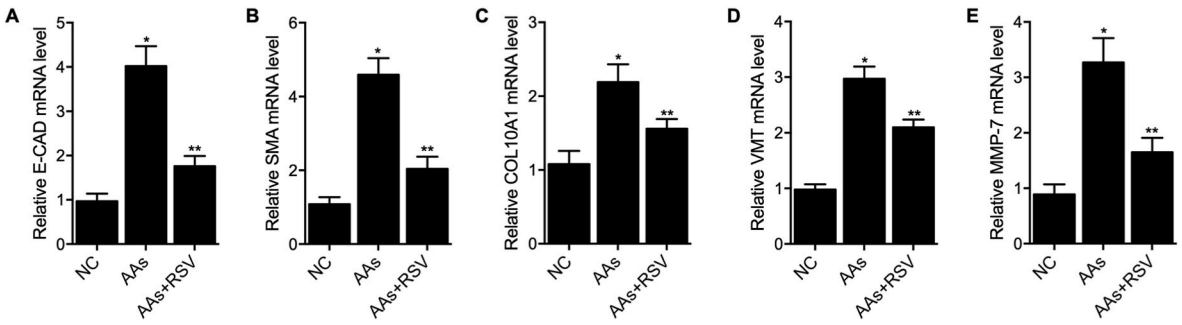

Fig. 2. The effect of RSV on TCMK-1 cells treated with AAs was investigated via observing the changes in mRNA levels of E-CAD, SMA, COL10A1, VMT and MMP-7 by PCR analysis. Accordingly, RSV was found to effectively offset to promotive effect of AAs on the mRNA levels of E-CAD (A), SMA (B), COL10A1 (C), VMT (D) and MMP-7 (E) in AA-treated TCMK-1 cells (*P value < 0.05 vs. NC group; **P value < 0.05 vs. AAs group; AA: aristolochic acid).

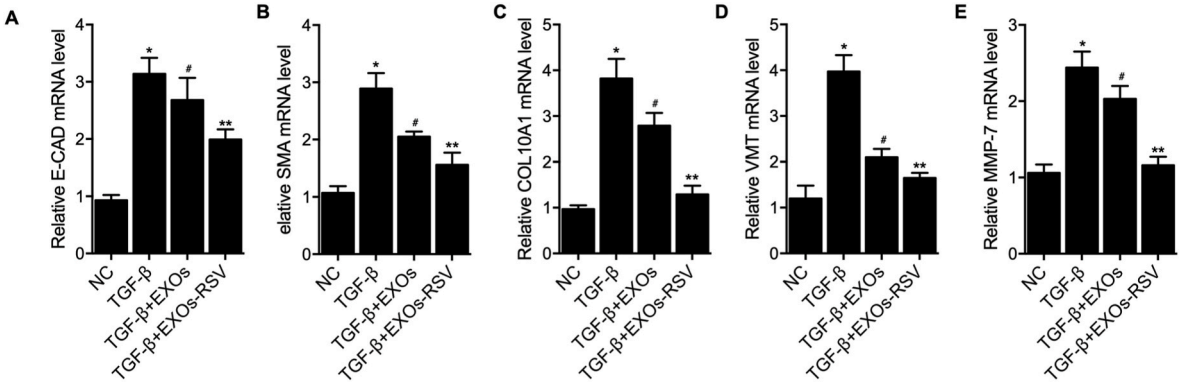

Fig. 3. The effect of administration of MSCs-derived exosomes, as well as the effect of co-administration of MSCs-derived exosomes and RSV, was observed via measuring the changes in mRNA levels in different TCMK-1 cell groups of E-CAD, SMA, COL10A1, VMT and MMP-7 by PCR analysis. Accordingly, both administration of MSCs-derived exosomes and co-administration of MSCs-derived exosomes and RSV significantly obstructed the TGF-β-induced up-regulation of the mRNA levels of E-CAD (A), SMA (B), COL10A1 (C), VMT (D) and MMP-7 (E) in TCMK-1 cells, with co-administration of MSCs-derived exosomes and RSV exhibiting a more significant effect (*P value < 0.05 vs. NC group; #P value < 0.05 vs. TGF-β group; **P value < 0.05 vs. TGF-β+EXOs group; EXOs: exosomes; RSV: resveratrol).

4.4. RSV and MSC-derived exosomes synergistically reduced the AA-induced upregulation of E-CAD, SMA, COL10A1, V MT and MMP-7 mRNA expression in TCMK-1 cells

AA-treated TCMK-1 cells were administered MSC-derived exosomes or MSC-derived exosomes plus RSV. AA treatment significantly upregulated the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in TCMK-1 cells. MSC-derived exosomes effectively restored the AA-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells. Moreover, RSV treatment further reinforced the capability of MSC-derived exosomes to decrease the AA-induced upregulation of E-CAD (Fig. 4A), SMA (Fig. 4B), COL10A1 (Fig. 4C), VMT (Fig. 4D) and MMP-7 (Fig. 4E) mRNA expression in TCMK-1 cells.

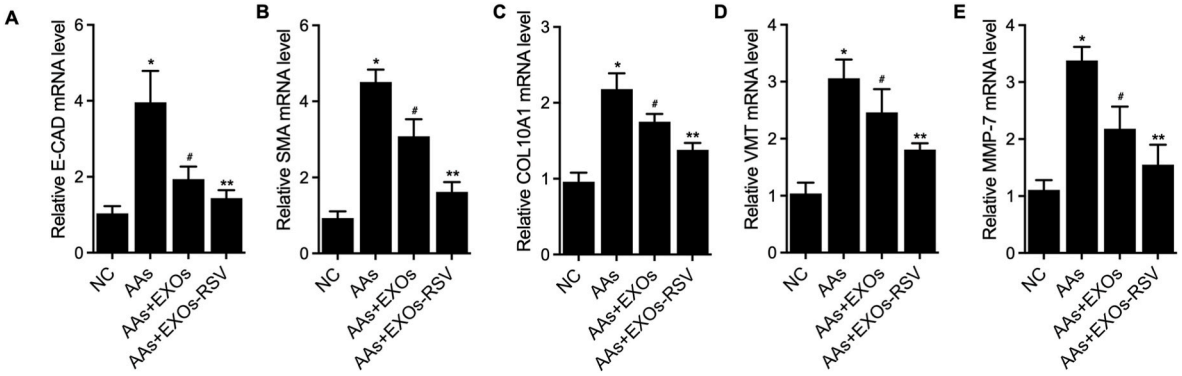

Fig. 4. The effect of administration of MSCs-derived exosomes, as well as the effect of co-administration of MSCs-derived exosomes and RSV, was observed via measuring the changes in mRNA levels in different TCMK-1 cell groups of E-CAD, SMA, COL10A1, VMT and MMP-7 by PCR analysis. Accordingly, both administration of MSCs-derived exosomes and co-administration of MSCs-derived exosomes and RSV significantly reduced AAs-induced up-regulation of the miRNA levels of E-CAD (A), SMA (B), COL10A1 (C), VMT (D) and MMP-7 (E) mRNAs in TCMK-1 cells (*P value < 0.05 vs. NC group; #P value < 0.05 vs. AAs group; **P value < 0.05 vs. AAs + EXOs group; EXOs: exosomes; AA: aristolochic acid).

4.5. EXO + RSV administration synergistically decreased RIRI-induced upregulation of Cr and BUN, as well as the increase in E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in mice

The mice were subjected to RIRI followed by EXO, RSV or EXO + RSV administration. BUN and Cr were analyzed in mice under various conditions. RIRI notably increased the levels of Cr and BUN in mice. EXOs and RSV showed considerable efficacy in restoring the levels of RIRI-induced Cr and BUN. EXOs + RSV administration synergistically abrogated the RIRI-induced Cr (Fig. 5A) and BUN (Fig. 5B) increase better than EXO or RSV administration. Moreover, RT−PCR was performed to examine the mRNA expression of E-CAD, SMA, COL10A1, VMT and MMP-7 in RIRI-induced mice under distinct therapeutic conditions. EXO and RSV treatment restored the RIRI-induced upregulation of E-CAD (Fig. 5C), SMA (Fig. 5D), COL10A1 (Fig. 5E), VMT (Fig. 5F) and MMP-7 (Fig. 5G) mRNA expression. Moreover, EXOs + RSV administration showed a significantly higher efficacy than monotherapy.

4.6. EXO + RSV administration synergistically decreased RIRI-induced kidney injury in the mouse model

HE staining was performed to evaluate RIRI-induced kidney injury in mice under distinct therapeutic conditions. EXOs and RSV treatment restored RIRI-induced kidney injury in the mouse model. EXO + RSV administration showed a significantly increased efficacy (Fig. 6A). Masson staining was carried out to examine RIRI-induced kidney fibrosis in mice under distinct therapeutic conditions. EXOs and RSV treatment restored RIRI-induced kidney fibrosis in the mouse model. EXO + RSV administration showed a significantly increased efficacy (Fig. 6B).

5. Discussion

Renal fibrosis is an adaptive repair process characterized by tubular degeneration, glomerulosclerosis, and interstitial fibrosis. Kidney fibrosis can trigger a helpful response to kidney injury [38]. However, depending on the severity and type of injury, this response might subsequently progress to fibroplasia or even fibrosis, during which functional cells are replaced by connective tissues that trigger permanent scar formation [39]. Epithelial-mesenchymal transition (EMT) plays an essential role in many processes, including cell growth, tissue regeneration, wound healing, cancer development, and fibrosis [40,41].

EMT is a conserved biological process that stimulates epithelial cells to sustain a number of biochemical modifications so that the cells lose the typical features of epithelial cells and become mesenchymal cells [42]. EMT includes a complicated series of activities that eventually cause modifications to gene expression [43]. In this study, we treated TCMK-1 cells with TGF-β and AA followed by RSV treatment. RSV treatment effectively maintained the TGF-β- and AA-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells.

Adipose-derived mesenchymal stem cells (ADMSCs) not only trigger angiogenesis to exacerbate organ disorders related to ischemia but also exert immunomodulatory effects to attenuate IR-induced organ disorders [44,45]. Exosomes are membrane fragments that contain specific subsets of microRNAs to induce angiogenesis and immunomodulation [46–48]. Exosomes are derived from endosomal parts and are released by most cells, including BMSCs [49,50]. The therapeutic effect of exosomes against I/R injury has been reported in many publications. For example, MSC-derived exosomes are demonstrated to promote proliferation of renal tubular cells and angiogenesis and down-regulate expression of pro-inflammatory cytokines in porcine models [51]. Also, MSCs-derived exosomes are found to attenuate the remarkably increased serum creatinine level, tubular necrosis, apoptosis, inflammatory cytokine production, and oxidative stress in I/R injured mice [52]. All these publications verified the renal-protective effect of exosomes on renal I/R injury, which is in consistence with the findings in our research. In this study, we not only treated the cells with

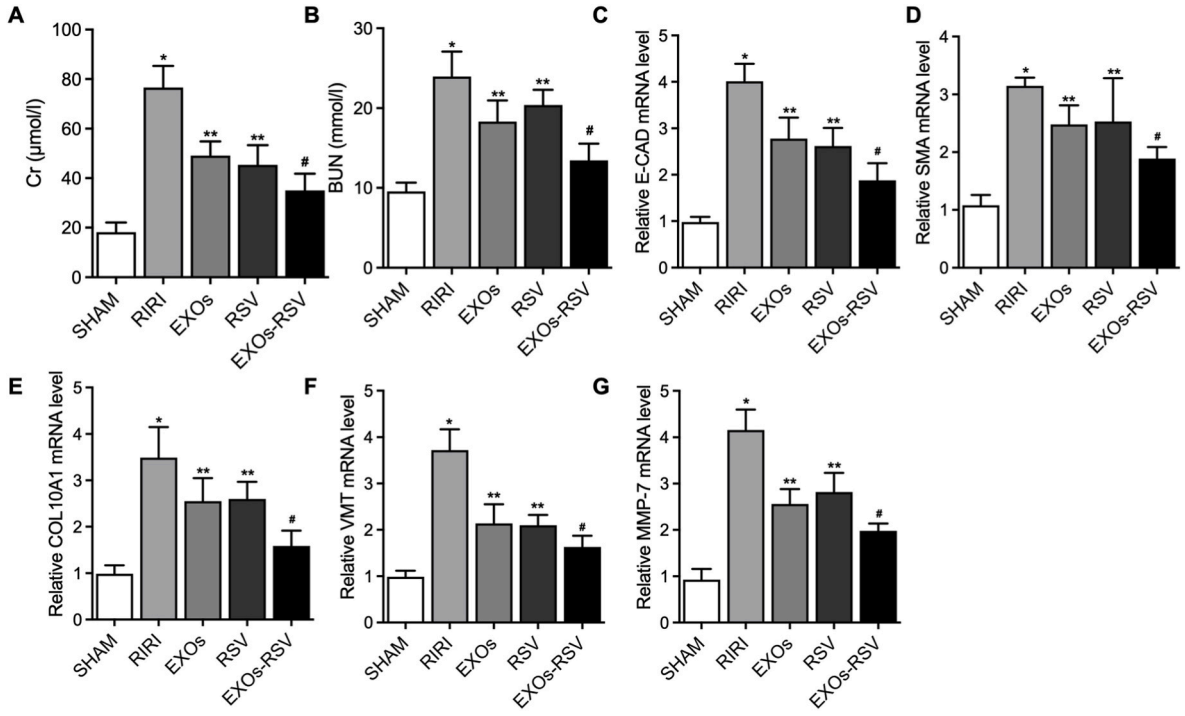

Fig. 5. The effect of co-administration of MSCs-derived exosomes and RSV on RIRI mice, we observed the changes of Cr and BUN level, as well as the E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA levels, in RIRI mice. Accordingly, we found that the co-administration of MSCs-derived exosomes and RSV synergistically decreased RIRI-induced Cr elevation (A) and BUN elevation (B), as well as the upregulation of the miRNA levels of E-CAD (C), SMA (D), COL10A1 (E), VMT (F) and MMP-7 (G), in RIRI mice (*P value < 0.05 vs. SHAM group; **P value < 0.05 vs. RIRI group; #P value < 0.05 vs. EXOs group; RIRI: renal ischemia-reperfusion injury; EXOs: exosomes; RSV: resveratrol).

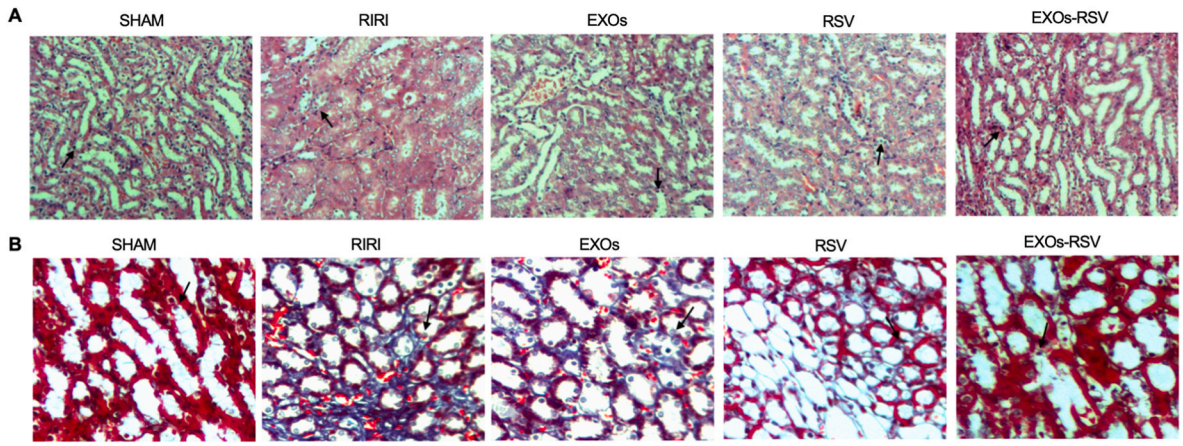

Fig. 6. The co-administration of MSCs-derived exosomes and RSV was shown to synergistically decreased RIRI-induced kidney injury in mice by observing the HE staining results (A) and MASSON staining (B) results (scale bar = 100 μm; EXOs: exosomes; RSV: resveratrol).

MSC-derived exosomes, but also investigated the effect of co-administration of MSC-derived exosomes and RSV to TCMK-1 cells treated with TGF-β and AA. Accordingly, we found that RSV and MSC-derived exosomes synergistically reduced the TGF-β- and AA-induced upregulation of E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression in TCMK-1 cells.

RVS is a natural phytoalexin present in grapes, mulberries, peanuts, and wine. RVS possesses a variety of pharmacological effect, including anti-inflammatory and anticancer activity [26]. Its anti-inflammatory effects are related to its role in inhibiting oxidation. Recently, RVS was shown to heal heart diseases and enhance microcirculation by strengthening the vascular endothelium and enhancing the release of cellular nitric oxide [53]. RVS also exhibits numerous bioactivities, such as anti-inflammatory, antioxidant, hepatoprotective, anticancer, and cardioprotective activities [54]. However, the effects of resveratrol on kidney fibrosis remain unclear. Recently, it was found that resveratrol could prevent glucose-induced EMT by inhibiting NOX/ROS signaling in tubular epithelial cells in the kidney [55]. RVS may decrease the deleterious effect of oxidative stress on live cells. Sun et al. showed that RVS protected cells against peroxidative stress [56]. In addition, Chanvitayapongs et al. revealed that RVS could reduce cell death induced by oxidized lipoproteins [57]. In another study, serum platelets exerted protective effects on the cell membrane by reducing ROS-induced DNA damage [58]. In this study, we subjected mice to RIRI followed by treatment with RSV and MSC-derived exosomes. RSV and MSC-derived exosomes synergistically reduced the RIRI-induced upregulation of Cr, BUN, E-CAD, SMA, COL10A1, VMT and MMP-7 mRNA expression. In addition, we examined kidney injury and fibrosis in mice under various conditions. RSV and MSC-derived exosomes synergistically reduced RIRI-induced kidney injury and fibrosis.

While the mechanism of renal fibrosis is still unclear, studies have shown that TGF-β plays a key role in controlling inflammation and fibrosis [59]. TGF-β can bind to type I and type II TGF-β receptors to activate serine/threonine kinases, which then initiate a signaling cascade that causes kidney fibrosis [60,61]. However, treatment with RSV dramatically reduced TGF-β expression. Increasing evidence shows that Smad3 plays an essential role in the pathogenesis of kidney fibrosis [62]. Li et al. discovered that Smad3 acetylation leads to renal fibrosis, but RSV can inhibit Smad3 acetylation [63].

Previous whole kidney studies have provided detailed insights into the role of ROS signaling in renal functions [64]. However, recent single-cell and single-nucleus RNA-sequencing of for kidney diseases have revealed that the kidney is more complex with respect to its cell-specific biological processes, and more than 20 different cell clusters are present in the kidney, many of which contain continuous intermediate transcriptional phenotypes [65]. The different cell clusters identified in the kidney also highlight the importance of investigating the function of specific cell types and the specific effects of ROS signaling on these cell types [66]. Moreover, since the signal from specific cell types can be diluted by the presence of other cell types [67], whole kidney studies can be limited due to the difficulty in identifying cell-specific changes in gene expression or protein activity. Therefore, in future research, the use of cell-specific approaches and the combination of data integration from multiple studies will help researchers to identify new mechanisms underlying the complex interaction between ROS signaling and renal functions.

6. Conclusion

In conclusion, we found that the administration of MSC-derived exosomes and RSV could suppress TGF-β-induced epithelial-mesenchymal transition by reducing the expression of E-CAD, SMA, COL10A1, VMT and MMP-7, thus improving the prognosis of RIRI-induced fibrosis. Moreover, when these agents were administered together, the suppressive effect was promoted.

Ethics approval

The institutional animal ethics committee has approved the protocol of this study.

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Availability of data and materials

Data is available upon reasonable request from corresponding author.

Patient consent for publication

Not applicable.

CRediT authorship contribution statement

Fuhe Liu: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft. **Jinlong Xu:** Formal analysis, Investigation, Resources, Validation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcrp.2024.200242.

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