Human umbilical cord MSC-derived hepatocyte growth factor enhances autophagy in AOPP-treated HK-2 cells

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Abstract. Mesenchymal stem cell (MSC) transplantation may serve as an important treatment modality in chronic kidney disease (CKD); however, the underlying mechanisms remain unclear. Advanced oxidation protein products (AOPP) have been demonstrated to induce renal tubular epithelial cell (RTEC) injury via autophagy inhibition. Therefore, the present study was performed to investigate the role of human umbilical cord-derived MSCs (hUC-MSCs) in RTEC autophagy. AOPP-treated HK-2 cells were co-cultured with hUC-MSCs or treated with recombinant humanized hepatocyte growth factor (HGF). Western blotting was used to detect the levels of autophagy-and PI3K/AKT/mTOR signaling pathway-related proteins, and immunofluorescence staining was used to detect the levels of autophagy-related proteins. The HGF protein levels in HK-2 cells and the hUC-MSC co-culture system were measured. The cells were subsequently treated with tivantinib, an HGF competitive inhibitor, and the levels of autophagy-related proteins were detected. Microtubule-associated protein 1 light chain 3B (LC3B) II/LC3B I (LC3II/LC3I) and beclin 1 protein levels were increased, while p62, PI3K, phosphorylated (p)-AKT and the p-mTOR protein levels were decreased in AOPP-treated HK-2 cells co-cultured with hUC-MSC, compared with the group treated with AOPP only. Furthermore, HGF expression was increased in AOPP-treated HK-2 cells co-cultured with hUC-MSC, compared with the group treated with AOPP alone. When HGF activity was inhibited using tivantinib, these effects on LC3II/LC3I, beclin 1, p62, PI3K, p-AKT, and p-mTOR expression were partially reversed. Furthermore, the effects of tivantinib were reversed by Ly294002. In conclusion, the present study revealed that hUC-MSCs partially reversed AOPP-mediated inhibition of autophagy in HK-2 cells via secretion of HGF, indicating that hUC-MSCs may serve as a potential therapy for preventing the progression of CKD.

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Introduction

Chronic kidney disease (CKD) is a leading cause of end-stage renal disease worldwide and is a global public health concern (1). A previous study has demonstrated that autophagy is associated with renal tubular epithelial cell (RTEC) damage, which eventually results in CKD (2). Dysregulated autophagy may occur in response to either intracellular or extracellular factors, such as endoplasmic reticulum stress, oxidative stress or pathogen infection (3,4). Consistent with those findings, our previous study revealed that advanced oxidation protein products (AOPP), a toxic protein product produced in patients with CKD, induced RTEC injury by inhibiting cell autophagy (5). Therefore, enhancing RTEC autophagy may suppress the progression of CKD.

Mesenchymal stem cells (MSCs) are mesodermal stem cells with self-renewal properties that can differentiate into a number of mesodermal cell lineages. Compared with MSCs from other sources, human umbilical cord-derived MSCs (hUC-MSCs) have received particular attention due to their abundant sources, simple extraction, good growth capacity, lower immunogenicity and decreased potential for harm to mothers and newborns (6). MSC transplantation may be an effective treatment modality in acute and chronic kidney disease (7); however, the underlying mechanisms remain unclear. Moreover, MSCs were reported to enhance autophagy in the nervous (8), digestive (9), respiratory (10) and endocrine (11) systems. However, whether hUC-MSCs increase renal cell autophagy to serve a protective role remains unknown.

Evidence suggests that MSCs promote the repair of damaged organs via a paracrine mechanism (12), including the secretion of growth factors such as hepatocyte growth factor (HGF), vascular endothelial growth factor and epidermal

growth factor. HGF is an antifibrotic cytokine and has been reported to attenuate organ fibrosis, including hepatic (13) and renal (14) fibrosis. Liu *et al* (15) found that MSCs promoted the regeneration of damaged neurons through the secretion of HGF in a model of Parkinson's disease. Lan *et al* (16) reported that HGF secreted from oncostatin M-preconditioned MSCs alleviated lung fibrosis in mice. Eom *et al* (17) demonstrated that HGF induced the expression microtubule-associated protein 1 light chain 3B (LC3B) II, an autophagy marker, in bone marrow-derived MSCs. However, whether HGF secreted from hUC-MSCs serves protective roles by enhancing RTEC autophagy in CKD requires further investigation.

During autophagy, the biosynthesis of LC3II/LC3I and beclin 1 increases, while upregulated expression of p62 inhibits autophagy (18). Studies have revealed that the PI3K/AKT/mTOR signaling pathway is an important negative modulator of autophagy (19,20). Our previous study revealed that AOPP inhibited HK-2 cell autophagy by activating the PI3K/AKT/mTOR signaling pathway (5).

The present study investigated the role of hUC-MSCs in AOPP-mediated inhibition of autophagy in human RTECs in CKD. Furthermore, the effect of HGF secreted from hUC-MSCs in hUC-MSC-enhanced autophagy, as well as the underlying mechanism in HK-2 cells, were examined.

Materials and methods

Materials and reagents. LC3B, Beclin 1, p62, phosphorylated (p)-mTOR, mTOR, p-AKT, AKT, PI3K antibodies and Ly294002, an inhibitor of the PI3K/AKT/mTOR signal pathway, were obtained from Cell Signaling Technology, Inc. GAPDH antibody was obtained from Bioworld Technology, Inc. BSA was obtained from Sigma-Aldrich; Merck KGaA. Hypochlorous acid (HOCl) was purchased from Fluka Chemie AG (Sigma-Aldrich; Merck KGaA). Tivantinib, a competitive inhibitor of HGF, and insulin-like growth factor 1 (IGF-1), an inducer of the PI3K/AKT/mTOR signal pathway, were acquired from APeXBIO Technology LLC. Recombinant human HGF (rhHGF, an analogs of HGF) was obtained from PeproTech, Inc., and the HGF ELISA kit was purchased from MultiSciences (Lianke) Biotech Co., Ltd. The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc.

AOPP preparation. AOPP was prepared as previously described (21). Briefly, HOCl (200 mmol/l) was added to a BSA solution for 30 min at room temperature and then dialyzed against PBS at 4˚C to remove free HOCl for 24 h. Native BSA was dissolved in PBS alone as the control. The AOPP content was measured at a wavelength of 340 nm to obtain the absorbance under acidic conditions and calibrated using chloramine-T in the presence of potassium iodide.

HK-2 cell culture and treatment. HK-2 cells were purchased from the American Type Culture Collection and cultured in DMEM/nutrient mixture F-12 (DMEM/F12; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and maintained at 37˚C in a humidified incubator containing a 5% CO_2 atmosphere. Cells were incubated in BSA (200 μg/ml), AOPP (200 μg/ml), rhHGF (343 pg/ml) conditions until they reached 70-80% confluence for 48 h at 37˚C. In subsequent experiments, cells were pretreated with 10 μM Ly294002, tivantinib, or 10 ng/ml IGF-1 for 1 h and then incubated with or without AOPP or co-cultured with hUC-MSCs for 48 h at 37˚C until the end of the experiments.

hUC-MSC isolation and co-culture with HK-2 cells. An adherent tissue method was used to isolate hUC-MSCs. A umbilical cord sample was obtained from the Department of Gynecology and Obstetrics, Zhujiang Hospital of Southern Medical University (Guangzhou, China). The sample was harvested with the mother's written informed consent. In the current study, hUC-MSCs were extracted from a newborn whose mother was 35 years old, hospitalized in March 2018. A large amount of hUC-MSCs could be extracted from a single individual, and the cells were frozen when the first generation reached 70-80% confluence and later thawed for use. Briefly, a 10 cm hUC from a full-term healthy newborn was cleaned with a PBS solution (containing 1% penicillin-streptomycin double-resistant solution). The hUC was subsequently cut into small pieces, the umbilical vein and artery were dislodged and stripped from the Wharton's jelly tissue. Wharton's jelly was then cut into 1x1x1 mm fragments at room temperature and cultured in DMEM/F12 medium containing 5% FBS at 37˚C; generations 3-6 were identified using flow cytometry, as previously described (22), and selected for follow-up experiments. When the hUC-MSC were co-cultured with HK-2 cells at 37˚C for 48 h, a co-culture chamber, including 6 wells, were used to block off the immediate contact between the hUC-MSC and HK-2 cells in order to explore the paracrine action of hUC-MSC. A total of $5x10^4$ HK-2 cells were seeded into the lower chamber compartment and $4x10^3$ hUC-MSC into the upper chamber compartment. Cells were co-cultured in DMEM/F12 medium containing 5% FBS at 37˚C for 48 h.

Western blotting. Total HK-2 cell protein was extracted from cells using pre-cooled radioimmunoprecipitation assay lysis buffer containing cocktail protease inhibitors (Biotool; Stratech Scientific, Ltd.). Protein concentrations were determined using a Micro Bicinchoninic Acid Assay kit (CoWin Biosciences), according to the manufacturer's protocol. According to the expression abundance and molecular weight of the proteins, 50 μg of LC3B and p62 were separated using 12% SDS-PAGE and 20 μg of the remaining proteins were separated using 10% SDS-PAGE and then transferred to PVDF membranes. Subsequently, the membranes were blocked in 5% non-fat milk powder at room temperature for 2 h, followed by primary antibody incubation at 4˚C overnight. The following primary antibodies were used: Anti-LC3B, beclin 1, p62, p-mTOR, mTOR, p-AKT, AKT, PI3K (dilution, 1:1,000) and anti-GAPDH (dilution, 1:5,000). After incubation with the horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 2 h, immunoreactive proteins were detected using an enhanced chemiluminescence system. Semiquantitative analysis was performed using the ImageJ system (National Institutes of Health). GAPDH was used as the internal control.

Immunofluorescence staining. A total of 10^3 HK-2 cells plated in 96-well plates were fixed with 4% paraformalde-

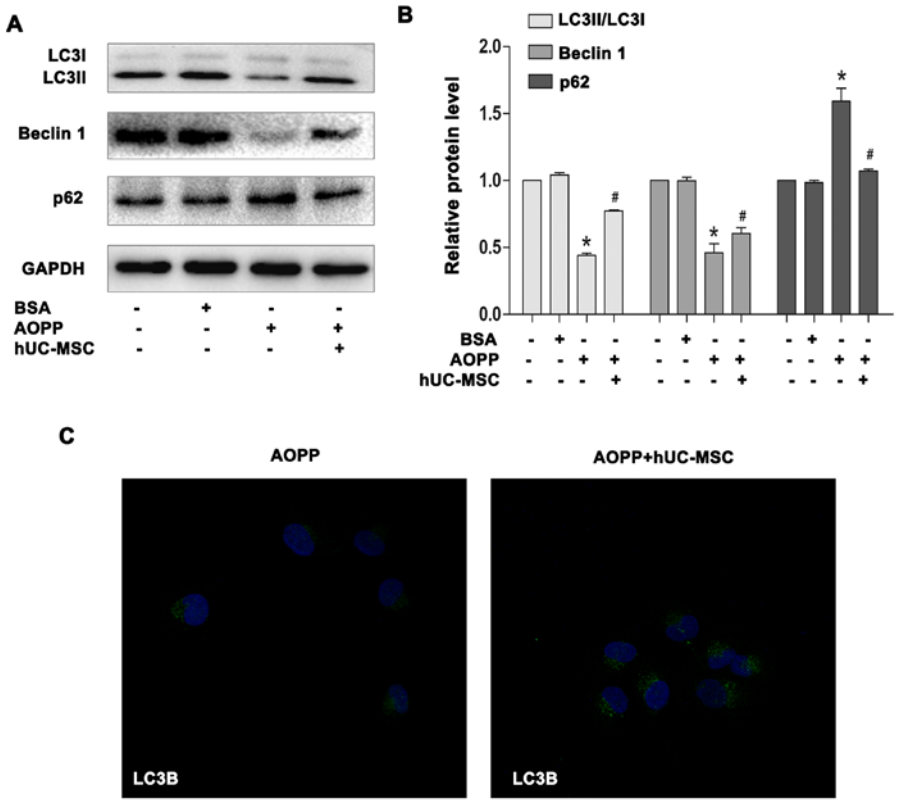

Figure 1. hUC-MSCs enhance autophagy in AOPP-treated HK-2 cells. AOPP-treated HK-2 cells were cultured alone or co-cultured with hUC-MSCs. (A and B) Western blotting revealed that the hUC-MSC co-culture group exhibited increased LC3II/LC3I and beclin 1 levels and decreased p62 levels compared with the AOPP-treated group. (C) Immunofluorescence staining revealed that LC3BII-positive staining was increased in the co-culture system. Data are presented as the mean ± SD. *P<0.05 vs. BSA control group; #P<0.05 vs. AOPP group. hUC-MSCs, human umbilical cord-derived mesenchymal stem cells; AOPP, advanced oxidation protein products; LC3B, microtubule-associated protein 1 light chain 3B.

hyde for 10 min at room temperature, permeabilized with 0.5% Triton X-100 for 10 min and incubated in 5% BSA for 1 h at room temperature, followed by incubation with LC3B antibodies (dilution, 1:50) overnight at 4˚C. Fluorescently-labeled secondary antibodies (Alexa Fluor® 488; dilution, 1:400) were applied for 1 h at room temperature while the samples were protected from light, followed by an incubation with 0.1% DAPI for 10 min at room temperature. The cells were observed and recorded using an inverted fluorescence microscope (magnification, x40).

CCK-8 assay. A total of 10^3 HK-2 cells were cultured under tivantinib treatment for 48 h. The growth medium was removed and the wells were washed twice with PBS. All the wells were filled with fresh medium containing 90 μl DMEM/F12 and 10 μl CCK-8 solution. After incubation for 30 min at 37˚C, cell viability was assessed via optical density (OD) detection at a wavelength of 450 nm with a microplate reader. The cell viability and IC_{50} were calculated using the OD values, according to the manufacturer's instructions.

ELISA. HGF ELISA kits were used to measure the expression of HGF (cat. no. 70-EK1H011) according to the manufacturer's protocol. HK-2 cells without rhHGF served as the normal control group. The supernatant of HK-2 cells was collected after centrifugation at a speed of 1,000 rpm for 5 min at room temperature and 100 μl standards or samples were added to the microplates in triplicate, followed by addition of 50 μl diluted detection antibody and incubation at room temperature for 2 h. A total of 100 μl diluted streptavidin-HRP was added and incubated for 45 min at room temperature. Finally, 100 μl of substrate solution protected from light was applied at room temperature for 20 min. The absorbance was read at 450 and 630 nm using a microplate reader.

Statistical analysis. All experiments were conducted in triplicate. The results are presented as the mean ± SD. Differences among the groups were determined using one-way ANOVA. The Least Significant Difference method or Bonferroni's test was used to compare two groups when the assumption of equal variances was met. Otherwise, the Dunnett T3 method was used. P<0.05 was considered to indicate a statistically significant difference. Analysis was performed using SPSS software (version 20.0; IBM Corp.).

Results

hUC-MSCs enhance autophagy in AOPP-treated HK-2 cells. The present study investigated whether hUC-MSCs enhanced autophagy in AOPP-treated HK-2 cells. The expression levels of the autophagy-related proteins LC3II/LC3I, beclin 1 and p62 were determined via western blotting. As indicated in Fig. 1A and B, AOPP significantly decreased the protein levels of LC3II/LC3I and beclin 1 and increased the protein level of p62. When AOPP-treated HK-2 cells were co-cultured with hUC-MSCs, this effect was partially

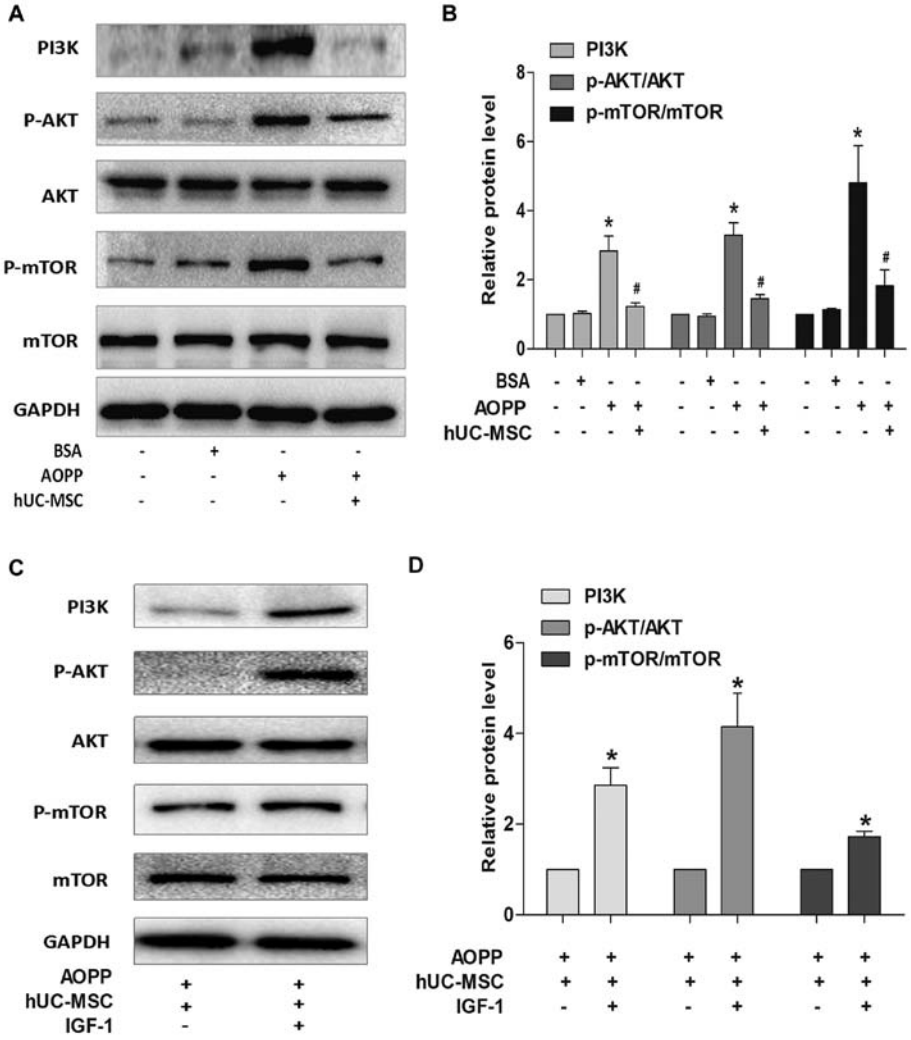

Figure 2. hUC-MSCs inhibit the PI3K/AKT/mTOR signaling pathway in HK-2 cells. AOPP-treated HK-2 cells were cultured alone or co-cultured with hUC-MSCs in the presence or absence of IGF-1, an inducer of the PI3K/AKT/mTOR signaling pathway. (A and B) Western blotting revealed that hUC-MSCs reduced the PI3K and phosphorylation of AKT and mTOR compared with AOPP-treated HK-2 cells. *P<0.05 vs. BSA control group; $^{\#}$P<0.05 vs. AOPP group. (C and D) IGF-1 partially reversed the effect of hUC-MSCs on the PI3K and the phosphorylation of, AKT and mTOR. *P<0.05 vs. AOPP + hUC-MSC group. Data are presented as the mean ± SD. hUC-MSC, human umbilical cord-derived mesenchymal stem cells; AOPP, advanced oxidation protein products; IGF-1, insulin-like growth factor 1; p, phosphorylated; hUC-MSC, human umbilical cord-derived mesenchymal stem cells.

reversed. Similarly, immunofluorescence staining revealed that LC3BII-positive staining was markedly increased in the hUC-MSC and HK-2 co-culture system compared with the HK-2 only group (Fig. 1C). These results indicated that hUC-MSCs may increase HK-2 cell autophagy in the presence of AOPP.

hUC-MSCs inhibit the PI3K/AKT/mTOR signaling pathway in AOPP-treated HK-2 cells. HK-2 cells, alone or in co-culture with hUC-MSC, were treated with AOPP and PI3K, AKT and mTOR levels were measured via western blotting. In the present study, hUC-MSCs decreased the PI3K and the phosphorylation of, AKT and mTOR in the AOPP-treated hUC-MSC and HK-2 cell co-culture system compared with HK-2 cells alone (Fig. 2A and B). However, IGF-1, an inducer of the PI3K/AKT/mTOR signaling pathway (23), partially abrogated this effect in the AOPP-treated hUC-MSC and HK-2 cell co-culture system (Fig. 2C and D). Therefore, hUC-MSCs inhibited the PI3K/AKT/mTOR signaling pathway in AOPP-treated HK-2 cells.

HGF enhances HK-2 cell autophagy. ELISA was used to detect the levels of HGF in rhHGF-treated HK-2 cells to investigate the role of HGF in HK-2 cell autophagy. It was revealed that the HGF level was increased in the rhHGF treatment group compared with the normal control group (Fig. 3A). Furthermore, western blotting revealed that rhHGF increased the protein expression levels of LC3II/LC3I and beclin 1 and decreased the p62 protein level, compared with the normal control group (Fig. 3B and C). In conclusion, these data suggest that HGF promotes HK-2 cell autophagy.

hUC-MSCs enhance AOPP-inhibited autophagy in HK-2 cells via the secretion of HGF via the PI3K/AKT/mTOR signaling pathway. To further confirm whether hUC-MSC-enhanced autophagy was mediated by the secretion of HGF, the effect of tivantinib, a competitive inhibitor of HGF (24), in HK-2 cells was investigated. HK-2 cells were cultured with 0.0003, 0.0200, 0.1150, 0.2100, 0.3050 and 0.4000 μM tivantinib and cell viability was measured via the CCK-8 assay. The results indicated that cell viability was gradually decreased with

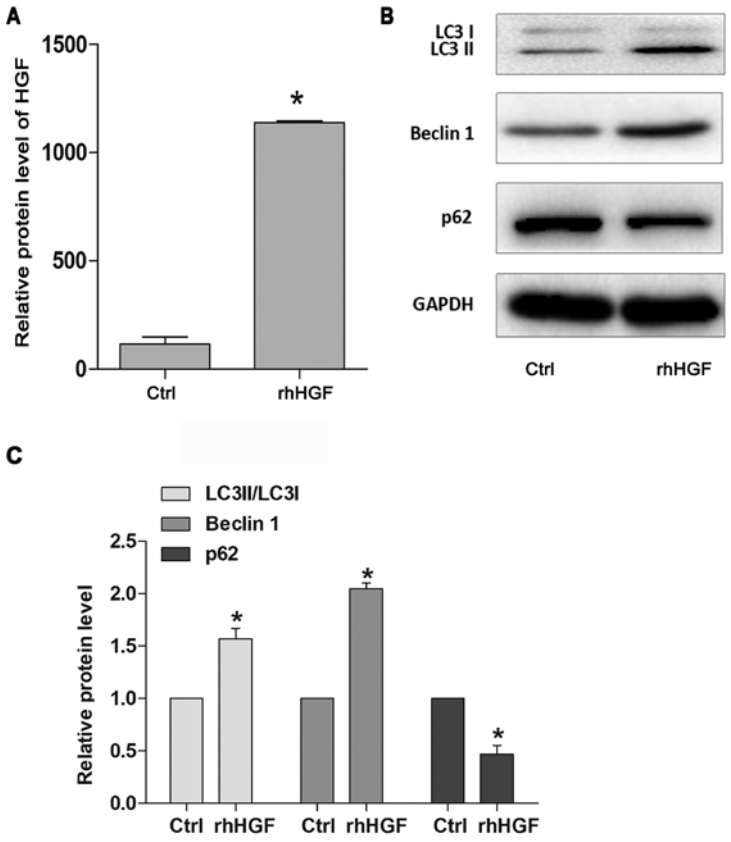

Figure 3. HGF enhances HK-2 cell autophagy. HK-2 cells were treated with rhHGF and autophagy-related proteins were detected. (A) ELISA results showed that the HGF level was increased in the rhHGF treatment group compared with the normal control group. (B and C) Western blotting indicated that rhHGF increased the protein expression levels of LC3II/LC3I and beclin 1 and decreased the p62 protein level compared with the normal control group. Data are presented as the mean ± SD. *P<0.05 vs. normal control group. HGF, hepatocyte growth factor; rh, recombinant human; LC3B, microtubule-associated protein 1 light chain 3B.

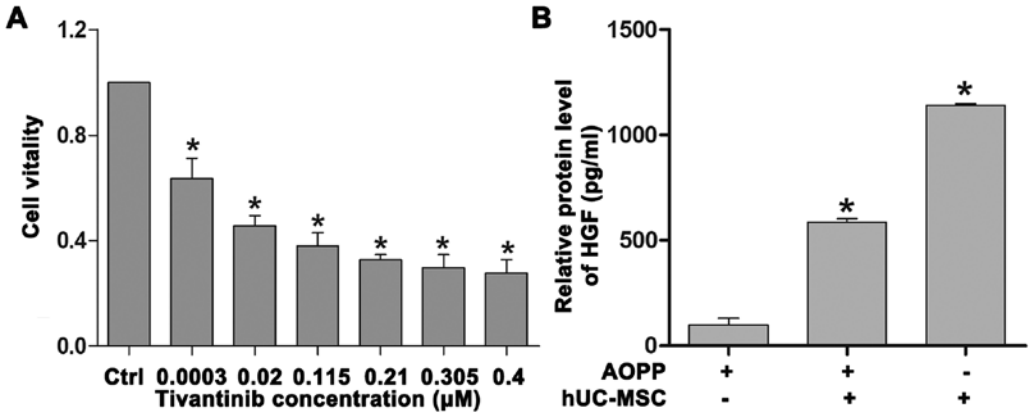

Figure 4. Effect of tivantinib on the viability of HK-2 cells co-cultured with hUC-MSCs. (A) Cell Counting Kit-8 assay results showed that cell viability decreased in a concentration-dependent manner following treatment with tivantinib and the IC_{50} was 0.006 μM (data not shown). *P<0.05 vs. normal control group. (B) ELISA results showed that HGF protein level was increased in both the hUC-MSC co-culture and hUC-MSC and AOPP co-culture groups compared with the AOPP only group. *P<0.05 vs. AOPP group. Data are presented as the mean ± SD. hUC-MSC, human umbilical cord-derived mesenchymal stem cells; HGF, hepatocyte growth factor; AOPP, advanced oxidation protein products.

increasing concentrations of tivantinib (Fig. 4A) and that the IC_{50} value was 0.006 μM (data not shown). Tivantinib was added to block the effect of hUC-MSCs on HK-2 cells. ELISA analysis revealed that the level of HGF was increased in both the hUC-MSC co-culture and hUC-MSC and AOPP co-culture groups compared with the AOPP only group (Fig. 4B). In addition, the HGF level in the hUC-MSC co-culture group was increased compared with the hUC-MSC and AOPP co-culture group, which indicated that AOPP may affect HGF expression.

In the AOPP-treated hUC-MSC and HK-2 cell co-culture system, tivantinib downregulated LC3II/LC3I and beclin 1, upregulated p62 and activated the PI3K and the phosphorylation of AKT and mTOR (Fig. 5A-C). In addition, immunofluorescence staining revealed that LC3BII-positive

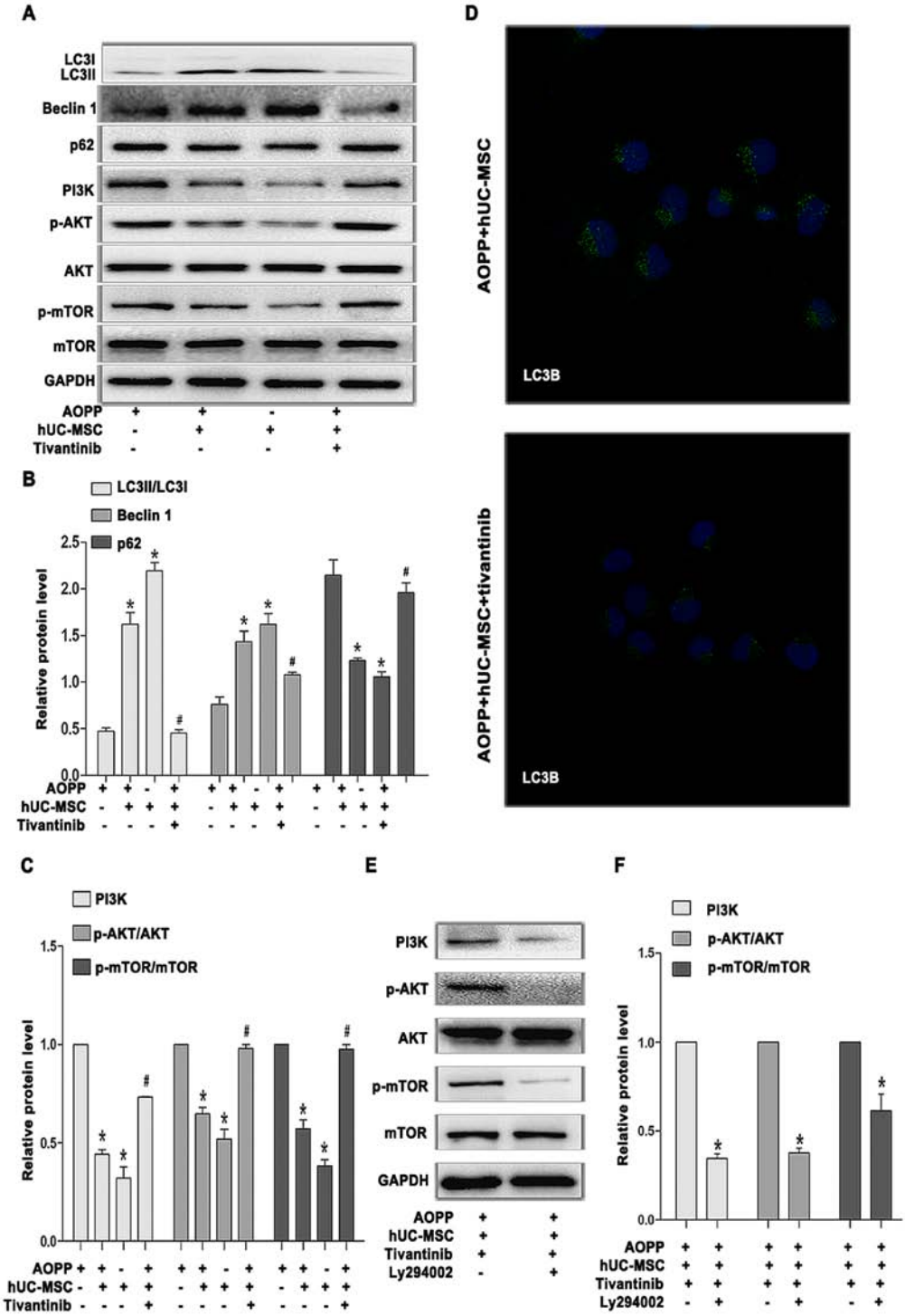

Figure 5. hUC-MSCs enhance HK-2 cell autophagy by secreting HGF. The AOPP-treated hUC-MSC co-culture system was incubated with tivantinib in the presence or absence of Ly294002. (A-C) Western blotting indicated that tivantinib decreased LC3II/LC3I and beclin 1 levels, increased p62 levels and re-activated the phosphorylation of PI3K, AKT and mTOR, reversing the effects of hUC-MSCs. *P<0.05 vs. AOPP group. #P<0.05 vs. AOPP + hUC-MSC group. (D) Immunofluorescence staining revealed that LC3B-positive staining was decreased in the presence of tivantinib in the hUC-MSC co-culture system. (E and F) Western blotting revealed that Ly294002 partially inhibited the PI3K and phosphorylation of, AKT and mTOR in the AOPP + hUC-MSC + tivantinib group. *P<0.05 vs. AOPP + hUC-MSC + tivantinib group. Data are presented as the mean ± SD. hUC-MSC, human umbilical cord-derived mesenchymal stem cells; LC3B, microtubule-associated protein 1 light chain 3B; AOPP, advanced oxidation protein products.

staining was decreased in the AOPP-treated hUC-MSC and HK-2 cell co-culture group incubated with tivantinib compared with the AOPP-treated hUC-MSC and HK-2 cell co-culture group alone (Fig. 5D). However, with the further addition of Ly294002, the PI3K and the phosphorylation of, AKT and mTOR was partially inhibited (Fig. 5E and F).

Discussion

The present study demonstrated the significant role of hUC-MSCs in HK-2 cell autophagy in CKD. hUC-MSCs promoted cell autophagy via inhibition of the PI3K/AKT/mTOR signaling pathway in AOPP-treated HK-2 cells. Moreover,

hUC-MSCs increased autophagy by secreting HGF, an antifibrotic factor. Collectively, the results suggested that hUC-MSCs may serve as a promising therapeutic strategy in CKD through their paracrine action.

Our previous study reported that AOPP inhibited RTEC autophagy and that autophagy inhibition induced RTEC injury (5). The present study revealed that hUC-MSCs enhanced AOPP-inhibited autophagy in HK-2 cells. A previous study revealed that MSCs increased autophagy, thereby protecting nerve cells in an Alzheimer's disease model (8). Furthermore, MSCs were demonstrated to increase α-synuclein removal in Parkinson's disease by increasing autophagy (25). In addition, Li *et al* (26) demonstrated that early intervention with MSCs prevented renal injury by ameliorating the inflammatory microenvironment in diabetic rats and Tang *et al* (27) reported that MSCs alleviate acute renal injury by suppressing the C5a/C5a anaphylatoxin chemotactic receptor-NF-κB signaling pathway. The results of the present study suggested that hUC-MSCs enhanced HK-2 cell autophagy and inhibited the PI3K/AKT/mTOR signaling pathway, which demonstrated a protective role of hUC-MSC in the aforementioned studies. Liu *et al* (28) demonstrated that MSC-derived exosomes inhibited H9C2 cell apoptosis by regulating autophagy via the PI3K/Akt/mTOR signaling pathway. Moreover, Zhu *et al* (29) revealed that MSCs affected autophagy via the PI3K/AKT/mTOR signaling pathway in the treatment of erectile dysfunction. To the best of our knowledge, the present study was the first to demonstrate that hUC-MSCs enhanced autophagy via inhibition of the PI3K/AKT/mTOR signaling pathway in HK-2 cells.

MSCs possess paracrine and endocrine functions and serve anti-inflammatory, antiapoptotic, antioxidative, proangiogenic, immunoregulatory and antifibrotic roles (30). Kennelly *et al* (31) reported that human MSC-derived HGF exerted an antiapoptotic effect in chronic obstructive pulmonary disease. Chang *et al* (32) determined that several angiogenic cytokines, including HGF, protected endothelial cells against radiation-induced apoptosis and accelerated the recovery of irradiated mice. To determine whether HGF enhanced autophagy, the present study investigated HK-2 cells treated with rhHGF and the results suggested that rhHGF increased HK-2 cell autophagy. It was previously reported that HGF activated autophagy in colorectal cancer cells (33). Furthermore, another study indicated that HGF protected cardiomyocytes from hypoxia-induced apoptosis by upregulating cell autophagy (34). To the best of our knowledge, the present study was the first to demonstrate that HGF enhanced autophagy in renal cells, indicating that HGF might exhibit therapeutic potential for renal diseases. Furthermore, the present study revealed that hUC-MSCs enhanced AOPP-inhibited HK-2 cell autophagy via the secretion of HGF.

Previous studies have demonstrated that MSCs prevented renal injury and promoted renal recovery in renal transplantation (35) and acute kidney injury (36). Additionally, clinical trials assessing the safety, feasibility and efficacy of MSC-based therapy in various kidney diseases have been registered with ClinicalTrials.Gov. However, the majority of these clinical trials are still in phase I or II, indicating the importance of exploring the mechanism of MSCs in kidney protection. The present study revealed that HGF protein levels were increased in the hUC-MSC and HK-2 cell co-culture system, indicating that hUC-MSCs secreted HGF, which had an effect on HK-2 cells. As hUC-MSCs and HGF enhanced HK-2 cell autophagy, tivantinib was added to the AOPP-treated hUC-MSC and HK-2 co-culture system to block the effect of HGF. Tivantinib inhibited hUC-MSC-upregulated autophagy via activating the PI3K/AKT/mTOR signaling pathway. Lee *et al* (37) revealed that HGF is more abundantly expressed in human embryonic stem cell-derived mesenchymal stem cells than in adult bone marrow-derived MSCs (hBM-MSCs). However, HGF-treated hBM-MSCs exhibited significantly improved therapeutic efficacy by promoting telomere lengthening and inducing mitochondrial DNA replication and function. Zhao *et al* (34) reported that MSCs overexpressing HGF were associated with decreased cardiomyocyte apoptosis, enhanced angiogenesis and increased proliferation of cardiomyocytes in myocardial infarction. These studies indicate that MSCs serve a favorable role via HGF upregulation. Similarly, the results of the present study demonstrated that hUC-MSCs increased HGF levels and inhibited the PI3K/AKT/mTOR signaling pathway and that this signaling pathway was re-activated by the HGF inhibitor, tivantinib. Therefore, HGF may regulate the PI3K/AKT/mTOR signaling pathway in HK-2 cells. Furthermore, following the addition of Ly294002, an inhibitor of the PI3K/AKT/mTOR signaling pathway, the PI3K/AKT/mTOR signaling pathway was significantly inhibited. These results indicated that hUC-MSCs enhanced HK-2 cell autophagy and inhibited the PI3K/AKT/mTOR signaling pathway by secreting HGF. Furthermore, hUC-MSCs enhanced HK-2 cell autophagy by secreting HGF, which provided a novel mechanism for the role of hUC-MSCs in the treatment of kidney diseases.

The present study did not explore how the hUC-MSC-secreted HGF was transported to HK-2 cells to enhance cell autophagy. Future in-depth studies will aim to explore the effect of hUC-MSCs on HK-2 cells. In a future study, the expression of HGF should be knocked out in hUC-MSCs prior to co-culture with HK-2 cells to confirm that hUC-MSC-secreted HGF affects HK-2 cells. In addition, further in vivo studies are necessary to validate the significance of hUC-MSCs and the secreted HGF in autophagy enhancement in HK-2 cells and in renal diseases.

In conclusion, the present study revealed that hUC-MSCs enhanced HK-2 cell autophagy by secreting HGF. MSCs may serve a therapeutic role in regenerative medicine and understanding the mechanisms of MSCs in renal protection may aid the development of novel therapeutic strategies in CKD.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ML designed the current study and involved in drafting the manuscript. TJ conducted the majority of the experiments, assisted in designing the present study and revising the manuscript. WZ assisted in isolating hUC-MSCs, co-culturing with HK-2 cells and western blotting. WX conducted immunofluorescence staining, CCK-8 assays and ELISA experiments. TG performed data analysis. XT took participation in the majority design of the work, in drafting the general content of the manuscript and revising it critically for important intellectual content, JZ made substantial contributions to conception, design, analysis and interpretation of data, and revised and submitted the manuscript. Moreover, XT and JZ both gave approval for the final manuscript and agreed to be accountable for any queries related to the accuracy or integrity of the current study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Medial Ethics Committee of Zhujiang Hospital, Southern Medical University (Guangzhou, China). Samples were harvested with the mother's written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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