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.

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% CO₂ 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 5x10⁴ HK-2 cells were seeded into the lower chamber compartment and 4x10³ 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 10⁴ HK-2 cells plated in 96-well plates were fixed with 4% paraformalde-
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-EK1H01) 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 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.
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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. 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), on 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...
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
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 anti-fibrotic 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, anti-apoptotic, antioxidative, proangiogenic, immunoregulatory and anti-fibrotic roles (30). Kennelly et al (31) reported that human MSC-derived HGF exerted an anti-apoptotic 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 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 HGF was transported to HK-2 cells to enhance cell autophagy. 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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Availabilty 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 reviewed the data. Moreover, XT and JZ both gave approval for the final manuscript and agreed to be accountable for the preparation of the manuscript. In conclusion, XT and JZ both performed data analysis. WX assisted in designing the present study and revising the manuscript. ML designed the current study and involved in drafting the general content of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Medical 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.

References

1. Woo KT, Choong HL, Wong KS, Tan HB and Chan CM: The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int 81: 1044-1045, 2012.
2. Ding Y, Kim S, Lee SY, Koo JK, Wang Z and Choi ME: Autophagy regulates TGF-β expression and suppresses kidney fibrosis induced by unilateral ureteral obstruction. J Am Soc Nephrol 25: 2835-2846, 2014.
3. Nijholt DA, de Graaf TR, van Haastert ES, Oliveira AO, Berkers CR, Zwart R, Ovaal H, Baas F, Hoozemans JJ and Schepers W: Endoplasmic reticulum stress activates autophagy but not the pro teaseosomes in neuronal cells: Implications for Alzheimer’s disease. Cell Death Differ 18: 1071-1081, 2011.
4. Majmundar AJ, Wong WJ and Simon MC: Hypoxia-inducible factors and the response to hypoxic stress. Mol Cell 40: 294-309, 2010.
5. Zhang J, Xiang X, Shi S, Zhang C, Liang Y, Jiang T, Zhang W, Guo T, Liang X and Tang X: Advanced oxidation protein products inhibit the autophagy of renal tubular epithelial cells. Exp Ther Med 15: 3908-3916, 2018.
6. Detamore MS: Human umbilical cord mesenchymal stromal cells in regenerative medicine. Stem Cell Res Ther 4: 142, 2013.
7. Morigli M and Benigni A: Mesenchymal stem cells and kidney repair. Nephrol Dial Transplant 28: 788-793, 2013.
8. Shin JY, Park HJ, Kim HN, Oh SH, Bae JS, Ha HJ and Lee PH: Mesenchymal stem cells enhance autophagy and increase β-amyloid clearance in Alzheimer disease models. Autophagy 10: 32-44, 2014.
9. Amiri F, Molaei S, Bahadori M, Nasiri F, Deyhim MR, Jalili MA, Nourani MR and Habibi Roudkkar M: Autophagy-modulated human bone marrow-derived mesenchymal stem cells accelerate liver restoration in mouse models of acute liver failure. Iran Biomed J 20: 135-144, 2016.
10. Luo D, Hu S, Tang C and Liu G: Mesenchymal stem cells promote cell invasion and migration and autophagy-induced epithelial-mesenchymal transition in A549 lung adenocarcinoma cells. Cell Biochem Funct 36: 88-94, 2018.
11. Zhou X, Hao H, Liu J, Tong C, Cheng Y, Xie Z, Zang L, Mu Y and Han W: Bone marrow-derived mesenchymal stem cells ameliorate chronic high glucose-induced β-cell injury through modulation of autophagy. Cell Death Dis 6: e1885, 2015.
12. Ionescu L, Byrne RN, van Haastten T, Badelone A, Alphson RS, Rey-Parrà GJ, Wustmann A, Hall A, Eaton F and Theubalt B: Stem cell conditioned medium improves acute lung injury in mice: In vivo evidence for stem cell paracrine action. Am J Physiol Lung Cell Mol Physiol 303: L967-L977, 2012.
13. Matsuno Y, Iwata H, Umeda Y, Takagi H, Mori Y, Kosugi A, Matsumoto K, Nakamura T and Hirose H: Hepatocyte growth factor gene transfer into the liver via the portal vein using electroporation attenuates liver cirrhosis. Gene Ther 10: 1559-1566, 2003.
14. Lv W, Booz GW, Wang Y, Fan F and Roman RJ: Inflammation and renal fibrosis: Recent developments on key signaling molecules as potential therapeutic targets. Eur J Pharmacol 820: 62-75, 2018.
15. Liu XS, Li JF, Wang SS, Wang YZ, Zhang YZ, Yin HL, Geng S, Gong HC, Han B and Wang YL: Human umbilical cord mesenchymal stem cells infected with adenovirus expressing HGF promote regeneration of damaged neuron cells in a Parkinson’s disease model. BioMed Res Int 2014: 909657, 2014.
16. Lan YW, Zheng SM, Huang TT, Choo KB, Chen CM, Kuo HP and Chong KY: Oncostatin M-preconditioned mesenchymal stem cells alleviate bleomycin-induced pulmonary fibrosis through paracrine effects of the hepatocyte growth factor. Stem Cells Transl Med 6: 1006-1017, 2017.
17. Eom YW, Oh JE, Li Ji, Baik SK, Rhee KJ, Shin HC, Kim YM, Ahn CM, Kong JH, Kim HS, et al: The role of growth factors in maintenance of stemness in bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun 445: 16-22, 2014.
18. Bjorkby G, Larmark T, Pankiv S, Ørväin A, Broch A and Johansen T: Monitoring autophagic degradation of p62/SQSTM1. Methods Enzymol 452: 181-197, 2009.
19. Heras-Sandoval D, Pérez-Rojas JM, Hernández-Damián J and Pedraza-Chaverri J: The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell Signal 26: 2694-2701, 2014.
20. Varshney P and Saini N: PI3K/AKT/mTOR activation and autophagy inhibition plays a key role in increased cholesterol during IL-17A mediated inflammatory response in psoriasis. Biochim Biophys Acta Mol Basis Dis 1864 (5 Pt A): 1795-1803, 2018.
21. Tang X, Song R, Gong B, Yu Y, Zhang S, Zhang M, Zhang J and Xiang J: Advanced oxidation protein products induce hypertrophy and epithelial-to-mesenchymal transition in human proximal tubular cells through induction of endoplasmic reticulum stress. Cell Physiol Biochem 35: 816-828, 2015.
22. Xiang J, Jiang T, Zhang W, Xue W, Tang X and Zhang H: Human umbilical cord-derided mesenchymal stem cells enhanced HK-2 cell autophagy through MicroRNA-145 by inhibiting the PI3K/AKT/mTOR signaling pathway. Exp Cell Res 15: 198-205, 2019.
23. Ronchi A, Liu Z, Leng X, Li H, Ma Y, Chen Y and Song F: Sialidase induces apoptosis and protective autophagy in human gastric cancer AGS cells through the PI3K/AKT/mTOR pathway. Biomed Pharmacother 122: 109726, 2020.
24. Ghanaatgar-Kasbi S, Khorrami S, Avan A, Aledavoud SA and Ferns GA: Targeting the C-MET/HGF Signaling Pathway in Pancreatic Ductal Adenocarcinoma. Curr Pharm Des 24: 4619-4625, 2018.
25. Park HJ, Shin JY, Kim HN, Oh SH and Lee PH: Neuroprotective effects of mesenchymal stem cells through autophagy modulation in a Parkinsonian model. Neurobiol Aging 35: 1920-1928, 2014.
26. Li Y, Liu J, Liao G, Zhang J, Chen Y, Li L, Li L, Liu F, Chen B, Guo G, et al: Early intervention with mesenchymal stem cells prevents nephropathy in diabetic rats by ameliorating the inflammatory microenvironment. Int J Mol Med 41: 2629-2639, 2018.
27. Tang M, Zhang K, Li Y, He QH, Li GQ, Zheng QY and Zhang KQ: Mesenchymal stem cells alleviate acute kidney injury by down-regulating C5a/C5aR pathway activation. Int Urol Nephrol 50: 1545-1553, 2018.
28. Liu H, Sun X, Gong X and Wang G: Human umbilical cord mesenchymal stem cells derived exosomes exert antiapoptosis effect via activating PI3K/Akt/mTOR pathway on H9C2 cells. Cell Biochem 120: 14455-14464, 2019.

29. Zhu GQ, Jeon SH, Bae WJ, Choi SW, Jeong HC, Kim KS, Kim SJ, Cho HJ, Ha US, Hong SH, et al: Efficient promotion of autophagy and angiogenesis using mesenchymal stem cell therapy enhanced by the low-energy shock waves in the treatment of erectile dysfunction. Stem Cells Int 2018: 1302672, 2018.

30. Khubatiya MS, Vagabov AV, Tenmov AA and Sklifas AN: Paracrine mechanisms of proliferative, anti-apoptotic and anti-inflammatory effects of mesenchymal stromal cells in models of acute organ injury. Cytotherapy 16: 579-585, 2014.

31. Kennelly H, Mahon BP and English K: Human mesenchymal stromal cells exert HGF dependent cytoprotective effects in a human relevant pre-clinical model of COPD. Sci Rep 6: 38207, 2016.

32. Chang PY, Zhang BY, Cui S, Qu C, Shao LH, Xu TK, Qu YQ, Dong LH and Wang J: MSC-derived cytokines repair radiation-induced intra-villi microvascular injury. Oncotarget 8: 87821-87836, 2017.

33. Mira A, Morello V, Céspedes MV, Perera T, Comoglio PM, Mangues R and Michieli P: Stroma-derived HGF drives metabolic adaptation of colorectal cancer to angiogenesis inhibitors. Oncotarget 8: 38193-38213, 2017.

34. Zhao L, Liu X, Zhang Y, Liang X, Ding Y, Xu Y, Fang Z and Zhang F: Enhanced cell survival and paracrine effects of mesenchymal stem cells overexpressing hepatocyte growth factor promote cardioprotection in myocardial infarction. Exp Cell Res 344: 30-39, 2016.

35. Casiraghi F, Perico N, Cortinovis M and Remuzzi G: Mesenchymal stromal cells in renal transplantation: Opportunities and challenges. Nat Rev Nephrol 12: 241-253, 2016.

36. Morigi M and De Coppi P: Cell therapy for kidney injury: Different options and mechanisms - mesenchymal and amniotic fluid stem cells. Nephron, Exp Nephrol 126: 59-63, 2014.

37. Lee EJ, Hwang I, Lee JY, Park JN, Kim KC, Kim GH, Kang CM, Kim I, Lee SY and Kim HS: Hepatocyte growth factor improves the therapeutic efficacy of human bone marrow mesenchymal stem cells via RAD51. Mol Ther 26: 845-859, 2018.

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