Secretome of Adipose-Derived Stem Cells Protect Ischemia-Reperfusion and Partial Hepatectomy by Attenuating Autophagy

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Research

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Abstract

Background: The therapeutic effects of adipose-derived mesenchymal stem cells (ADSCs) may mainly come from their paracrine effects. ADSCs can ameliorate hepatic ischemia-reperfusion injury (IRI). We explored the therapeutic effect of ADSCs secretome from the perspective of excessive autophagy of hepatocytes induced by hepatic IRI.

Methods: In this study, we established a miniature pig model of hepatic ischemia-reperfusion (I/R) combined with hepatectomy using laparoscopic technique, and transplanted ADSCs and adipose-derived mesenchymal stem cell-conditioned medium (ADSC-CM) into the liver parenchyma immediately after surgery. Histopathological and TEM examinations were performed on liver tissue samples collected. We analyzed the roles of ADSC-CM and ADSCs in autophagy by RT-qPCR, western-blot and immunohistochemistry.

Results: The results showed that ADSCs and ADSC-CM all alleviated the pathological changes of liver tissue and the microstructural damage of hepatocytes after IRI. Moreover, the expression of the critical markers of autophagy including Beclin-1, ATG5, ATG12 and LC3II all decreased, whereas expression of p62 increased. And the data of autophagy regulation between ADSC-CM and ADSCs showed no significant difference. Finally, we found that ADSC-CM possibly inhibited autophagy by regulating the PI3K/Akt/mTOR pathway.

Conclusion: ADSC-CM can ameliorate excessive autophagy injury in hepatic I/R combined with partial hepatectomy, which is possibly involved with the modulation of the PI3K/Akt/mTOR signaling pathway. There was no significant difference between ADSCs and ADSC-CM in the regulation of hepatocyte autophagy. Therefore, ADSCs may improve the excessive autophagy injury of hepatocytes in hepatic I/R combined with hepatectomy through paracrine effect, thus protecting the liver and promoting the liver tissue repair.

Introduction

Liver IRI is a kind of pathophysiological stress state, which refers to the injury caused by interrupting the blood supply of the liver and resuming the blood perfusion of the liver after a certain period of time. Liver IRI is a serious complication, which has a negative impact on the prognosis of patients during hepatectomy and liver transplantation. [1]In the early stage of liver IRI, ischemia-induced deprivation of nutrition and oxygen will cause hepatocyte damage. With the decrease of ATP production, cell metabolism slows down, anaerobic glycolysis is activated, intracellular enzymes such as phospholipase C and protein kinase C are activated and induce liver necrosis and apoptosis. The imbalance of pH and the accumulation of ions caused by the dysfunction of ion channels lead to the change of mitochondrial permeability. After reperfusion, neutrophils and macrophages were activated and accumulated in the liver. These cells increase IRI by secreting paracrine and autocrine signals such as ROS and inflammatory

cytokines [2, 3]. The effect of IRI on liver and systemic is of great significance in the clinical application of liver transplantation[4].

Autophagy is a kind of intracellular self digestion pathway, which is responsible for the removal of long-lived proteins, damaged organelles and abnormal proteins during lysosomal biosynthesis [5]. Autophagy is a highly conserved biological process, which exists in eukaryotes and maintains cell homeostasis and viability by recycling and reusing energy[6, 7]. Autophagy is usually considered as a protective mechanism of cells, and over autophagy will lead to autophagy cell death[8, 9]. Studies have shown that autophagy can promote cell survival or death according to cell type, environmental conditions and specific stimuli [10]. The liver is largely dependent on pathological and physiological autophagy. Therefore, autophagy is of great significance in the pathogenesis of liver diseases and normal liver physiological processes [10]. In extreme cases, such as hepatic IRI, undisciplined autophagy leads to the accumulation of autophagic vacuoles, which can lead to cell death. Furthermore, these findings provide a potential new strategy for improving the effects of IRI, which can be achieved by regulating the level of autophagy [11–13]. PI3K/AKT/mTOR is one of the key signaling pathways regulating autophagy. Growth factors inhibit autophagy by stimulating the PI3K/AKT pathway and activating mTOR.

With the development of stem cell biology and technology, regenerative medicine therapy seeks to guide the inherent non-healing injury to the full recovery of tissue structure and function. A large number of studies have shown that mobilization of endogenous stem cells or exogenous injection of some stem cells into injured tissues can improve tissue structural regeneration and functional recovery[14–16]. However, many studies nowadays show that therapeutic potential for stem cell transplantation into host tissues may focus primarily on their paracrine effects rather than on cell replacement and differentiation alone [17–19]. Mesenchymal stem cells (MSCs) secrete a wide array of growth factors, chemokines, cytokines and extracellular vesicles, commonly referred to as the MSCs secretome. MSCs secretome control cell proliferation, migration and differentiation in the microenvironment and provide cellular protection to establish a regenerative environment.[20, 21]. In addition, MSCs secretome play roles in stimulating cell proliferation, inhibiting apoptosis, promoting angiogenesis, inhibiting inflammation and immune response. [22]. A study on myocardial infarction demonstrated that MSCs-exo could reduce the autophagic flux of H/SD (normoxic or hypoxic and serum deprivation) exposed cardiomyocytes[23]. ADSCs have been shown to ameliorate liver tissue injury by reducing excessive autophagy in hepatocytes in a model of hepatic I/R combined with hepatectomy[12]. To further explore the paracrine role of ADSCs in the treatment of excessive autophagic injury caused by hepatic I/R combined with hepatectomy, this study established a model of hepatic ischemia-reperfusion and partial hepatectomy in miniature pigs, and transplanted ADSC-CM as an intervention*into the liver tissue of animals. ADSC-CM significantly attenuated liver pathology and cell injury by inhibiting excessive autophagy injury. Our findings provide a new insight into the therapeutic potential of cell-free products instead of cell transplantation in liver diseases.

Materials And Methods

Animals

24 Guangxi Bama miniature pigs aged 4–6 months, weighing 20–30 kg, half male and half female, were provided by the College of College of Veterinary Medicine (Harbin, China). All of them were raised under the same conditions, and randomly divided into four groups with 6 animals in each group. IRI group (IRI), DMEM control group (DMEM), ADSC-CM treatment group (CM) and ADSCs treatment group (ADSCs). IRI group, DMEM group, ADSCs group and CM group were injected with saline, DMEM, ADSCs (1×10^6 cells/kg), ADSC-CM solution at different points around the liver section after the construction of hepatic I/R combined hepatectomy model. ADSC-CM is obtained by secreting various factors from ADSCs into the culture medium during the culture process, so the CM group was to collect the supernatant of ADSCs (1×10^6 cells/kg) during the culture process and concentrate the obtained. DMEM is the basic medium used to culture cells, as the control group of CM group in this experiment. All experiments were approved by the Animal Care and Use Committee of the Northeast Agricultural University (SQ-2016-389).

Culture of ADSCs and preparation of ADSC-CM

The adipose tissue of Bama miniature pig was obtained under sterile conditions, washed with PBS solution, removed fascia, blood vessel, cutted into pieces. digested with 0.01% collagenase type I, and re-suspended in L-DMEM supplemented with 10% FBS (Clark, United States), 100 μg/ml streptomycin, 1 μg/ml penicillin and 2 mM L-glutamine (Solarbio, China). Finally, the cells were cultured in an incubator at 37℃ with 5% CO2 [24]. The ADSCs from the fourth passages were seeded in T75 culture flasks (Corning, United States), and then filled, discarded the original culture medium, washed with PBS, and replaced with starvation culture medium. After 48 h of starvation, the supernatant was extracted, and dead cells were removed by centrifugation, filtered, concentrated with 3KD concentration tube, and centrifuged (5000 g, 1 h) to finally obtain the concentrated supernatant.

Surgical procedure

Establishment of a miniature pig model of hepatic I/R combined with hepatectomy [25],The miniature pigs were kept on the operating table in a supine position, and the temperature of the operating table was adjusted to maintain at 37℃. Respiratory anesthesia was used for intraoperative anesthesia, and then laparoscopic minimally invasive technique was used for model construction, right hemi-hepatic ischemia for 60 min, and left hemi-hepatectomy. Heart rate, blood pressure, body temperature and oxygen saturation were monitored throughout the operation.

Histological Analysis

The liver tissue samples collected in the experiment were fixed in 4% paraformaldehyde for 24 h, and stained with paraffin embedded, sectioned, hematoxylin and eosin (H&E). Finally, the morphological changes of liver tissues in each experimental group were observed under light microscopy, focusing on whether the structure of hepatic lobules was intact, whether hepatocytes had degeneration and necrosis, whether there was inflammatory cell infiltration and cytoplasmic vacuolization.

Transmission Electron Microscopy

The liver tissue samples were trimmed into 1 mm3 tissue blocks, double fixed with 2.5% glutaraldehyde and 1% osmic acid, dehydrated at 4℃, soaked at room temperature, embedded, sectioned (thickness 50–60 nm), placed on 200 mesh copper mesh, double stained with uranium acetate-lead citrate, and finally observed using an H-7650 electron microscope (Hitachi, Japan), and collected pictures.

Western Blotting

The protein lysate was prepared by adding PMSF (Beyotime, Shanghai, China) and phosphatase inhibitor (MCE, Monmouth Junction, United States) to the tissue protein extraction reagent at a volume ratio of 100:1.The liver tissue samples were put into the prepared protein lysate, homogenized by a tissue homogenizer, and lysed at 4℃ for 30 min. The supernatant was centrifuged to obtain the total protein of the samples. The total protein concentration of each experimental group was measured by Bicinchoninic Acid (BCA) protein quantitative method (Beyotime, China). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was prepared for electrophoretic separation of protein molecules with different molecular weights, and then nitrocellulose (NC) membranes (Biosharp, China) were used to transfer the target protein. The membranes was placed in 5% skim milk solution and sealed for 2 h at room temperature, and then removed, washed with TBST and incubated with primary antibodies against β-actin Beclin-1, p62 (Wanleibio, China), LC3Ⅱ (Novus Biologicals, USA), Akt, p-Akt, mTOR, p-mTOR (ABclonal Technology, USA) overnight at 4℃. The membranes were washed with TBST and incubated with horseradish peroxidase (HRP)-conjugated anti-species secondary antibody (1:7500, ImmunoWay, USA) for 2 h. Finally, the membranes were dripped with Western Bright ECL reagent (Advansta, USA), and imaged using a Tanon 5200 Imaging System (Tanon Science & Technology Co., Ltd., China). In this experiment, using β-actin as internal reference, the image processing application program ImageJ was used to analyze the gray level of strips.

Immunohistochemistry

The expression level of LC3Ⅱ in liver tissue was detected by immunohistochemistry. Liver tissue samples were cut into appropriate sizes, fixed in 4% paraformaldehyde for 24 h, and then embedded and sectioned. The sections were dewaxed in an 80 C oven overnight, and immersed in 3% H2O2 solution for 10 min in the dark for endogenous peroxidase blockade, followed by antigen repair in a pressure cooker using sodium citrate antigen repair solution. Sections were sealed for 20 min at room temperature with BSA, incubated with primary antibody (1:200, LC3Ⅱ, Novus Biologicals, USA) overnight at 4℃, and incubated with streptavidin-labeled HRP for 30 min at room temperature. Finally, the sections were stained with DAB and hematoxylin, sealed with neutral glue, and dried in an oven. The stained sections were placed under a microscope to observe the staining of tissue sections in each group, and analyzed using the Image-Pro Plus 6.0 software (Media Cybernetics, USA).

Real-Time Quantitative PCR

Total RNA was extracted from liver samples using TRIzol reagent (Invitrogen, China). The quality and concentration of the RNA were assessed by NanoDrop™ One/One (Thermo Fisher Scientific, USA). The total RNA was reverse transcribed into cDNA using the ReverTra Ace qPCR RT Master Mix (Toyobo,

Japan). Then, using cDNA as template, according to the manufacturer's instructions, RT-qPCR was carried out in a LightCycler 480 (Roche, Germany) to detect the hepatocyte-specific genes in liver tissue samples. The reaction program was as follows: 15 s at 95 °C for pre-denaturation, 40 cycles of 5 s at 95 °C for denaturation, 60 s at 60 min for annealing and elongation. The primers were synthesized by Sangon Biotech (Shanghai, China) and are listed in (Table 1).

Table 1
Gene-specific primers used in the RT-qPCR.

Gene	Primer sequences (5'-3')
Beclin-1	Forward 5'-TCATGCGATGGTGGCTTTCC-3'
	Reverse 5'-ATGGAATAGGAGCCGCCACT-3'
ATG5	Forward 5'-ACCTTTGCAGTGGCTGAGTG-3'
	Reverse 5'-TCAATCTGTTGGTTGCGGGA-3'
ATG12	Forward 5'-CAACTGCTGCTGAGGGCGATG-3'
	Reverse 5'-CACCGGCAGGTTCTTCTGTTCC-3'
P62	Forward 5'-CTGATGAAGGTGGCTGGCTGAC-3'
	Reverse 5'-CAAGGGCGGTGGGTGTTTCG-3'
PI3K	Forward 5'-ACGGAGGAGGTGCTCTGGAAC-3'
	Reverse 5'-GGACTCGGGACTGGGCATCTC-3'
Akt	Forward 5'-GACGGCACCTTCATCGGCTAC-3'
	Reverse 5'-CGCCACGGAGAAGTTGTTGAGG-3'
mTOR	Forward 5'-GCACGTCAGCACCATCAACCTC-3'
	Reverse 5'-GCCTCAGCCATTCCAACCAGTC-3'

Statistical Analysis

All the data was analyzed using GraphPad Prism 7.0 (GraphPad Software, USA). All values are expressed as the mean ± SD (standard deviation). Comparisons between groups were assessed by ANOVA (One-way analysis of variance). $P < 0.05$ were considered significant.

Results

ADSC-CM protects pig from Hepatic Ischemia-Reperfusion and hepatectomy injury.

The histological evaluation revealed that the structure of hepatocytes was intact and the morphology of hepatocytes was normal at preoperative(Fig. 1A-D). At 1d, severe liver tissue damage was caused by hepatic I/R and hepatectomy (Fig. 1E-F). A significant improvement was observed in the ADSCs and CM

groups（Fig. 1G-H）. The miniature pigs undergoing I/R and hepatectomy significantly inhibited the injury with stronger effects in the ADSCs group and CM group (Fig. 1A-H). The results of TEM observation showed that the overall microstructure of hepatocytes was normal, the structure of organelles was intact without abnormal damage, and the nucleus was intact without obvious lesions at preoperative (Fig. 1I-L). On the 1d, different degrees of hepatocyte damage were observed in each group. In addition, obvious autophagic structures were observed in all groups. As show in (Fig. 1M-P), the hepatocytes of IRI group and DMEM group had abnormal morphology with nucleus shrinkage and deformation, swelling of endoplasmic reticulum and mitochondria, even with the disappearance of mitochondrial cristae structure. The overall structure of ADSCs group and CM group (Fig. 1O-P) was relatively intact, the damage was mild, the swelling of mitochondria and endoplasmic reticulum was not serious, the nuclear structure was relatively normal.

ADSC-CM inhibites autophagy in Hepatic Ischemia-Reperfusion and hepatectomy injury.

As is shown in Fig. 2, hepatic I/R and hepatectomy injury enhance autophagy on 1d. On 1d, the protein expression levels of Beclin-1 and LC3Ⅱ and p62 in liver homogenates in the DMEM group was not significantly different from that in the IRI group (P＞0.05) (Fig. 2A-D), As expected, both Beclin-1 and LC3Ⅱ levels were significantly decreased and the level of p62 was significantly increased at 1d in ADSC-CM-treated when compared with DMEM control group(P＜0.01) (Fig. 2A-D). In addition, analysis of protein expression results showed that there was no significant difference in the expression of autophagy-related protein factors Beclin-1, LC3Ⅱ, and p62 in the ADSCs group and the CM group on 1d ($P > 0.05$) (Fig. 2A-D). Furthermore,as shown in (Fig. 3A-I). compared with preoperative control, the expression of LC3Ⅱ protein was significantly increased in all groups at 1d ($P < 0.01$) (Fig. A-I). The protein expression levels of LC3Ⅱin the DMEM group was not significantly different from that in the IRI group on 1d ($P > 0.05$) (Fig. 3A-I). However, following ADSC-CM treatment, LC3Ⅱ expression significantly decreased compared to the DMEM group at 1d ($P < 0.01$) (Fig. 3A-I). Similarly, there was no significant difference in the expression of LC3Ⅱin the ADSCs group and the CM group on 1d ($P > 0.05$) (Fig. 3A-I). The results of immunohistochemistry are consistent with the results of western-blotting.

We further detected autophagy by analyzing the formation of autophagy related gene expression by reverse-transcriptase quantitative PCR (RT-qPCR). The RT-qPCR assay analysis illustrated that there was a remarkably increased expression of Beclin-1 mRNA, ATG5 mRNA and ATG12 mRNA levels in all groups on 1d with Hepatic I/R and hepatectomy injury ($P < 0.01$) (Fig. 2E-G). In addition, autophagy factor p62 was markedly decreased ($P < 0.01$) (Fig. 2H). All detected factors were not significant difference between DMEM group and IRI group ($P > 0.05$) (Fig. 2E-H). Consistent with autophagy related protein expression results, autophagy related genes Beclin-1, ATG5, ATG12 were significantly downregulated and p62 was significantly upregulated in the CM group at 1d when compared with DMEM control group ($P < 0.01$) (Fig. 2E-H). Similarily, there was no significant difference in the ADSCs group and the CM group on 1d ($P > 0.05$) (Fig. 2E-H).

ADSC-CM activates PI3K/Akt/mTOR signaling in Hepatic Ischemia-Reperfusion and hepatectomy injury.

As is shown in Fig. 4. Western blot (Fig. 4A-C) and RT-qPCR (Fig. 4D-F) analysis identified that the PI3K/AKT/mTOR pathway was repressed at 1d under Hepatic I/R and hepatectomy injury. The results showed no significant changes in p-AKT/AKT and p-mTOR/mTOR protein expression (Fig. 4A-C) and mRNA expression of PI3K□AKT and mTOR (Fig. 4D-F) in the DMEM group at 1d compared with the IRI group ($P > 0.05$). In the CM group, ADSC-CM sharply elevated the data of p-AKT/AKT and p-mTOR/mTOR (Fig. 4A-C) and the genes expression of PI3K□AKT and mTOR compared with DMEM group at 1d ($P < 0.01$) (Fig. 4D-F). According to the results, there was no significant difference in the value of p-AKT/AKT and p-mTOR/mTOR in the ADSCs group and ADSCs group on 1d ($P > 0.05$) (Fig. 4A-C). Moreover,PI3K□AKT and mTOR mRNA levels were examined after ADSCs and ADSC-CM treatment by RT-qPCR and the results showed that the PI3K□AKT and mTOR mRNA expression levels were no significant difference in the two treatment groups at 1d ($P > 0.05$) (Fig. 4D-F).

Discussion

Recent studies have shown that ADSCs transplantation can alleviate hepatic I/R combined with hepatectomy injury by reducing the level of hepatocyte autophagy[12]. In addition, in hepatic I/R combined with hepatectomy injury, undisciplined autophagy leads to the accumulation of autophagy-related proteins, which leads to the death of hepatocytes[11, 12]. Consistent with previous studies, we observed that ADSC-CM improved the pathological and microscopic changes of liver parenchyma in porcine hepatic I/R combined with hepatectomy model by alleviating hepatocyte autophagy.

Autophagy is an intracellular degradation system that transports components of the cytoplasm into lysosomes for degradation[26]. Autophagy is also a highly conserved biological process that maintains cell homeostasis and viability by recycling and reusing energy[27]. The liver is largely dependent on pathological or physiological autophagy. However, in extreme cases, such as acute organ injury or reperfusion injury[11, 12], undisciplined autophagy can lead to the accumulation of autophagic vacuoles, which can lead to cell death[28].

Beclin-1, one of the first mammalian autophagy effectors, is involved in mammalian autophagy[29] Beclin-1 recruits PI3KC3 (Vps34) to form a protein complex[30, 31], which in turn regulates intracellular transport and autophagosome formation[32]. The formation of Beclin-1 complexes opens the production of autophagosome membranes. Beclin-1 also regulates autophagic activity[32, 33]. The execution of autophagy requires two ubiquitin-like conjugation systems: the ATG8 (LC3) conjugation system and the ATG12-ATG5 conjugation system. LC3 is the only mammalian protein known to bind stably to the autophagosome membrane. After the synthesis of LC3 protein, its carboxyl end is immediately sheared by ATG4 to produce cytosolically localized LC3I. In the process of autophagy, LC3I is modified by ubiquitination processing and binds to PE on the autophagosome membrane to form membrane-bound LC3II, which is localized in the autophagosome.LC3II present in autophagosomes is one of the molecular markers of autophagy, and the content of LC3II is proportional to the degree of autophagy.ATG7 activates ATG12 and binds to ATG5 under the action of ATG10 to form ATG12-ATG5 complex. In ubiquitin-binding reactions, the ATG5-ATG12 complex promotes the formation of ATG8-PE in a manner similar to the

function of E3 enzymes[34]. The formation of ATG8-PE and ATG16-ATG 5-ATG12 complexes is necessary for the formation of autophagosomes. The multifunctional protein p62 (also known as SQSTM1) plays an important role in signaling and selective autophagy. Therefore, we examined the gene and protein expression of autophagy-related factors Beclin-1, LC3Ⅱ, ATG5, ATG12, p62 to observe the changes of autophagy activity induced by hepatic I/R combined with hepatectomy. We observed an increase in the Beclin-1, ATG5, ATG12 mRNA and the Beclin-1, LC3Ⅱ protein levels, and an decrease in the p62 mRNA and protein levels in the liver tissues after IRI, which were significantly alleviated by the ADSC-CM. This clearly indicated that autophagy overexpression was induced in the hepatic I/R combined with hepatectomy and alleviated by injecting ADSC-CM.

Autophagy plays an important role in hepatic IRI[11, 12], and the mechanism target of PI3K/Akt/mTOR signaling pathway can regulate cell autophagy[35, 36]. PI3K-AKT controls cellular functions, including mTOR, by regulating the expression of many downstream molecules. mTOR is a molecular target of rapamycin in mammalian cells and an important factor regulating cell growth and metabolism[37]. Activation of mTOR can inhibit the expression of autophagy. Phosphorylated AKT activates mTOR, which negatively regulates autophagy by inhibiting the downstream molecule ULK1 complex. Here, hepatic I/R combined with hepatectomy injury inhibited the PI3K/Akt/mTOR pathway. ADSC-CM treatment activated the PI3K/Akt/mTOR pathway, upregulated the gene expression of PI3K, AKT and mTOR and the protein expression of p-AKT and p-mTOR in the liver. Therefore, combined with the above autophagy-related results, it is concluded that ADSC-CM may inhibit the excessive autophagy of hepatocytes after hepatic I/R combined with partial hepatectomy by regulating the PI3K/Akt/mTOR pathway to alleviate the autophagy injury of hepatocytes.

ADSC-CM is the supernatant concentrate of ADSCs collected at rest in serum-free culture medium, which contains a variety of growth factors, chemokines, cytokines and extracellular vesicles secreted by ADSCs in paracrine or autocrine ways[22, 38]. ADSCs secretome can stimulate cell proliferation, inhibit apoptosis, promote angiogenesis, inhibit inflammation and immune response. And it not only overcomes the limitations of cell therapy, but also maintains its advantages. Therefore, these cell-free products have the potential to replace cell transplantation therapies. The results of comparison of ADSC-CM and ADSCs in the regulation of hepatic IRI-induced excessive autophagy showed that after transplantation of ADSC-CM and ADSCs, the expression of excessive autophagy in hepatocytes decreased, and there was no significant difference between them. Therefore, both ADSC-CM and ADSCs have protective effects on hepatic autophagy injury, and ADSCs may regulate and treat autophagy injury caused by hepatic I/R combined with hepatectomy in miniature pigs through paracrine effect.

Conclusion

In conclusion, this study demonstrated that ADSC-CM could inhibit excessive autophagy of hepatocytes induced by hepatic IRI, and inhibit autophagy of hepatocytes possibly by upregulating the PI3K/Akt/mTOR signaling pathway, thereby alleviating hepatocyte pathological damage and ultrastructural changes. In addition, there was no significant difference between ADSC-CM and ADSCs in

hepatocyte autophagy, suggesting that the therapeutic effect of ADSCs on hepatocyte autophagy injury induced by hepatic IRI is possibly related to its paracrine effect. These findings provide a potential new strategy for improving hepatic IRI.

Abbreviations

IRI	Ischemia reperfusion injury
I/R	Ischemia reperfusion
ADSCs	Adipose-derived stem cells
ADSC-CM	Adipose mesenchymal stem cell-conditioned medium
MSCs	Mesenchymal stem cells

Declarations

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Ethics approval and consent to participate

The animal study was reviewed and approved by the Northeast Agricultural University Animal Care and Use Committee. The rules for care and use of experimental animals established by the Ministry of Science and Technology of the People's Republic of China (Approval number: 2006–398).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YJM, ZHJ , XNL, QZZ, CXP, JYX and HW performed the experiments. YJM and HBW conceived the study and designed the experiments. YJM, XNL, and ZHJ collected the data. QZZ, CXP and JYX provided the technical guidance. YJM analyzed the data and drafted the manuscript. All authors have read and approved the final manuscript.

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Figures

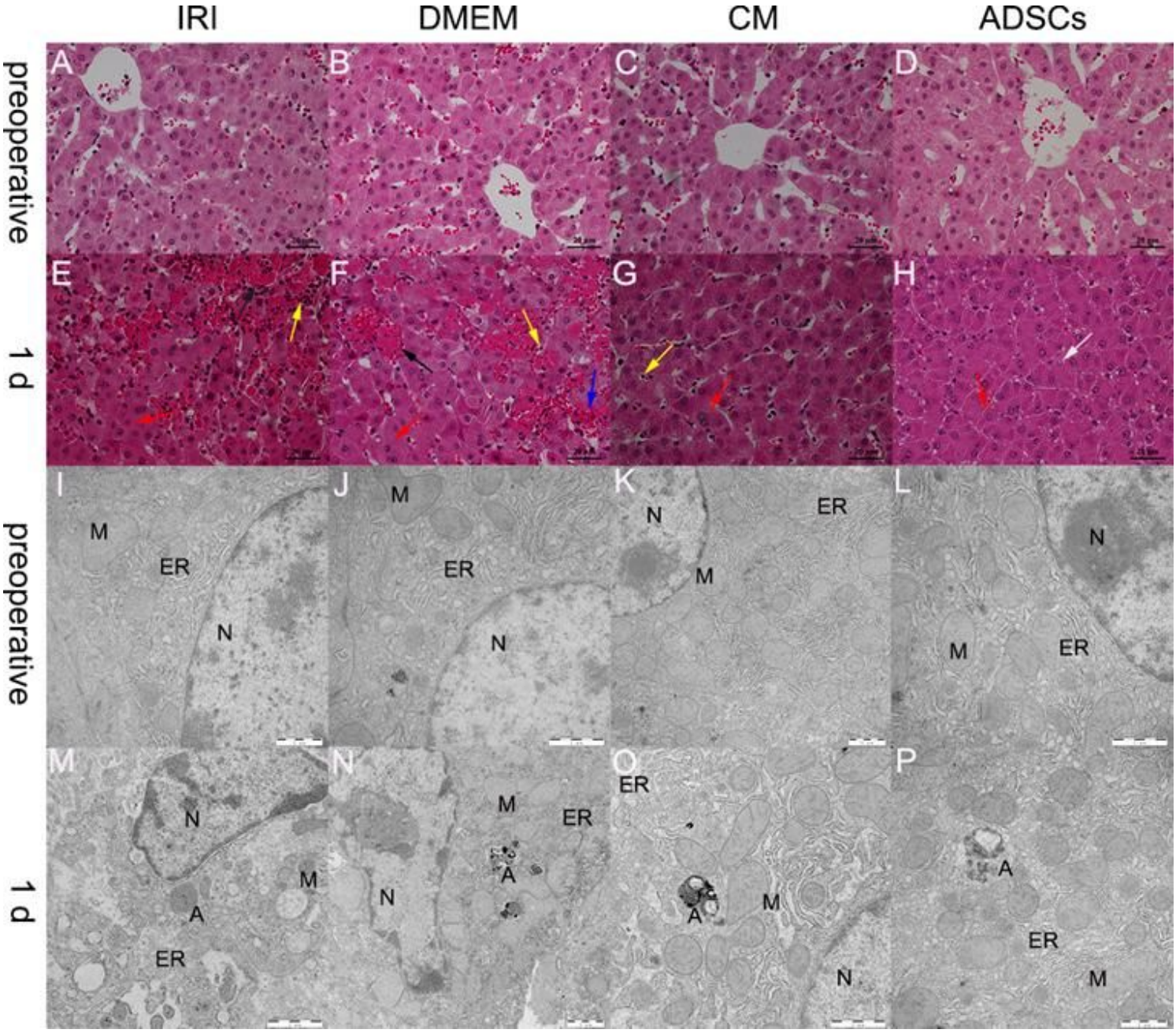

Figure 1

Histopathologic and ultrastructural changes in the liver. (A-H) Hematoxylin and cosin staining results. Black arrows indicate focal necrose, white arrow indicate hepatocyte vacuolar degeneration, blue arrow indicates hemorrhage, red arrows indicates swelling of hepatocytes and yellow arrows indicate

inflammatory cell infiltration (Magnification × 200). (I-P) Electron microscopy results. N, nuclei; M, mitochondria; ER, endoplasmic reticulum; A, Autophagy structure.

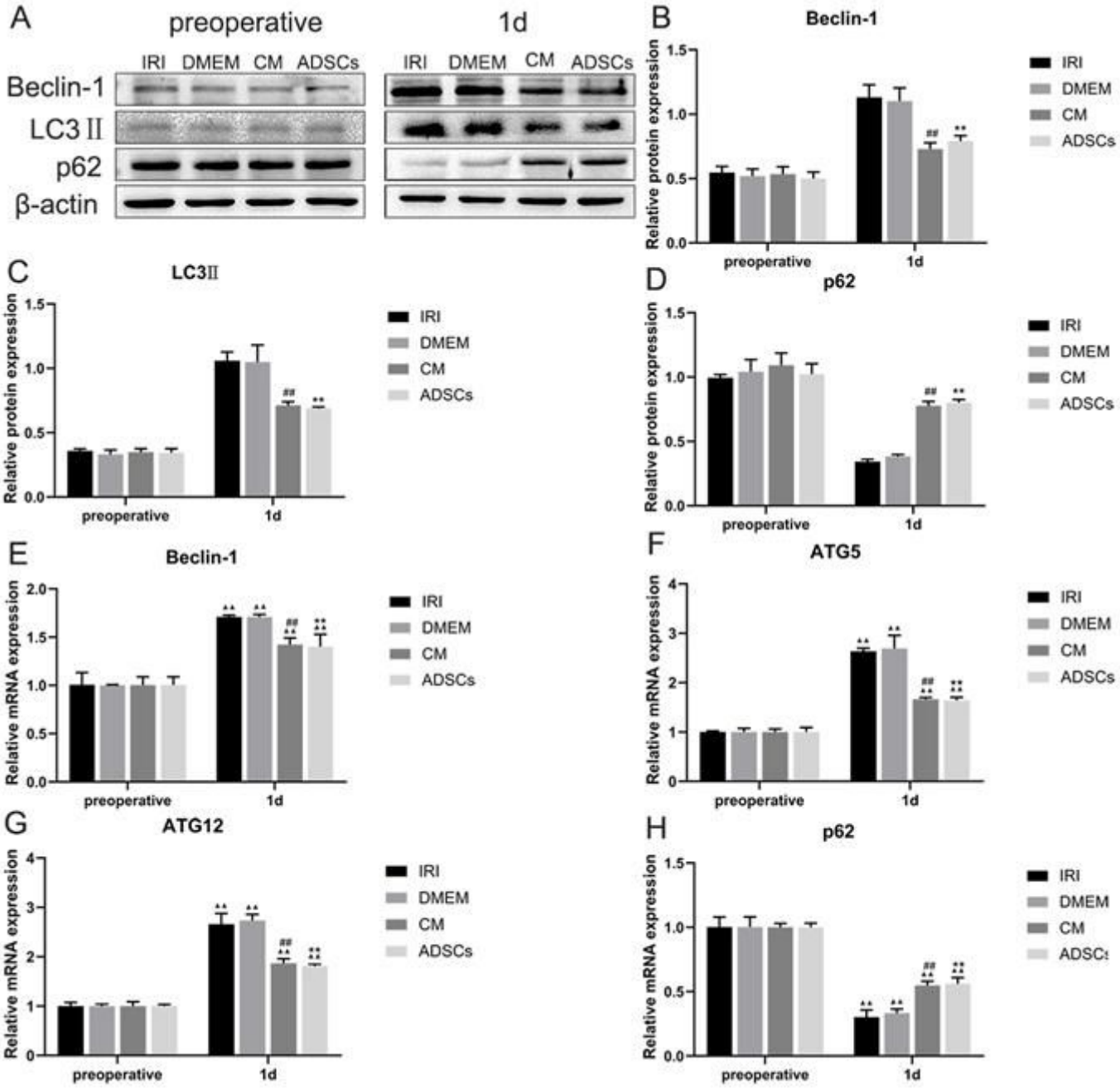

Figure 2

Expression of autophagy related factors in liver tissue. (A-D) Western blotting results showing Beclin-1, LC3Ⅱ,p62 proteins levels. (E-H) RT-qPCR results showing Beclin-1, LC3Ⅱ,p62 mRNAs levels. ##P < 0.01, vs DMEM group, **P < 0.01, vs IRI group, ⅡⅡP <0.01, vs ADSCs group.

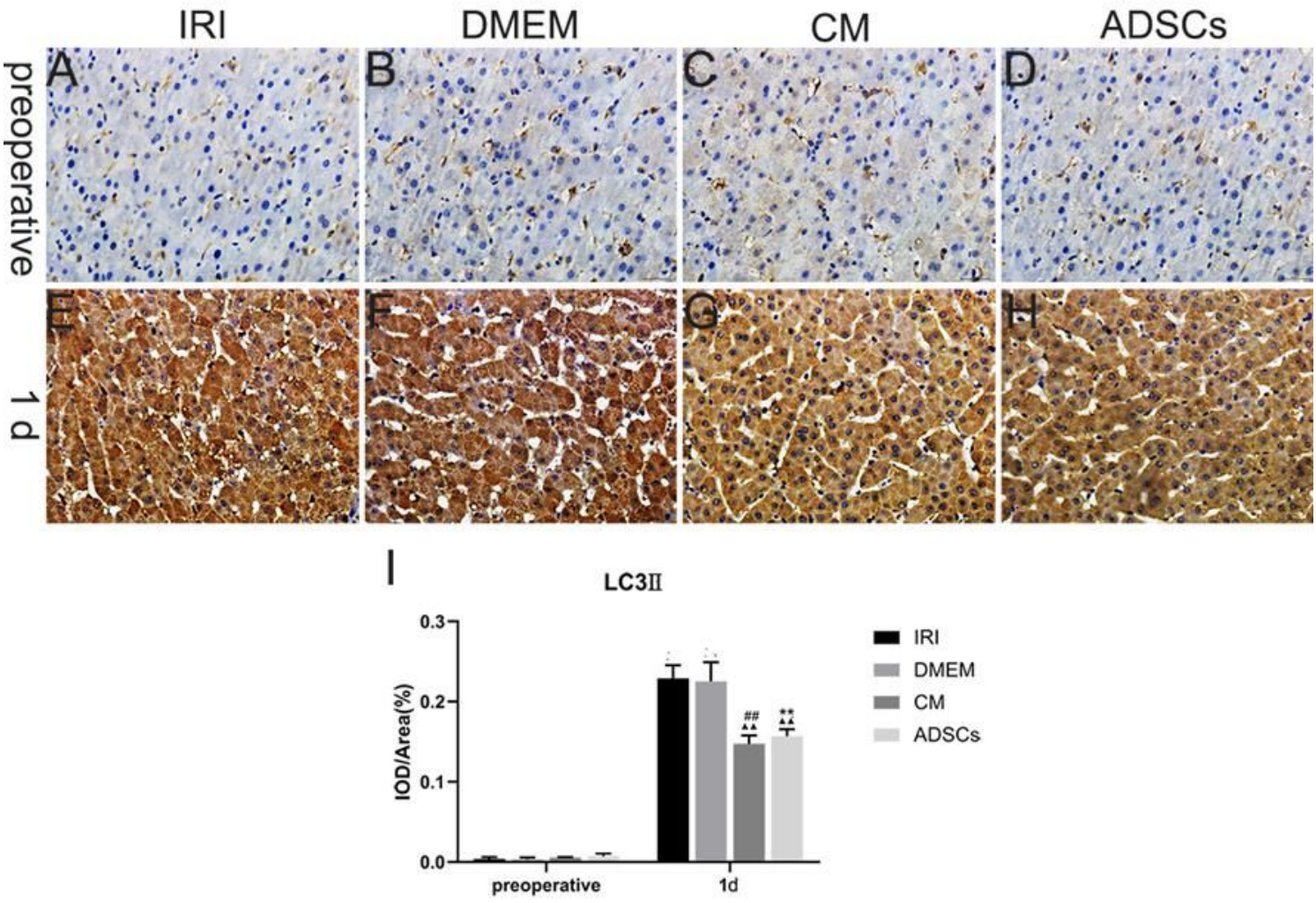

Figure 3

LC3Ⅱ immunohistochemistry staining of liver tissues. (A-H) LC3Ⅱ immunohistochemistry Staining results, (I) Analysis of LC3Ⅱ protein immunohistochemical results. IOD, integrated optical density. ▲▲P < 0.01, vs preoperative, ##P < 0.01, vs DMEM group, **P < 0.01, vs IRI group, ⅡⅡP <0.01, vs ADSCs group.

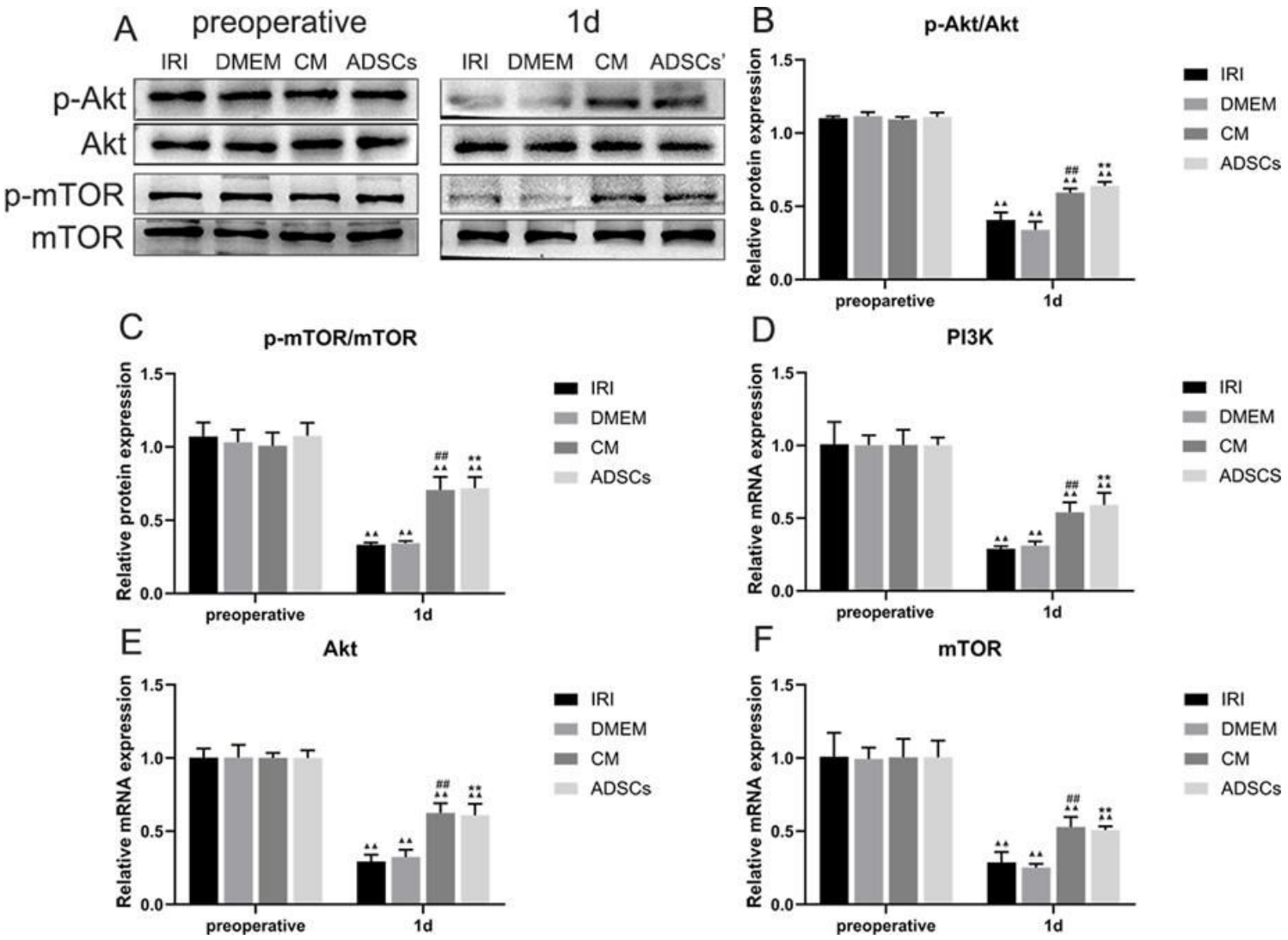

Figure 4

Expression of PI3K / Akt / mTOR related factors in autophagy related pathway of liver tissue. (A-C) Western blotting results showing Akt, p-Akt, mTOR, p-mTOR proteins levels. (D-F) RT-qPCR results showing PI3K, Akt, mTOR mRNAs levels. ##P < 0.01, vs DMEM group, **P < 0.01, vs IRI group, ▯▯P <0.01, vs ADSCs group.