

Research article

**MAINTENANCE OF RAT HEPATOCYTES UNDER INFLAMMATION  
BY COCULTURE WITH HUMAN ORBITAL FAT-DERIVED  
STEM CELLS**XIA CHEN<sup>1,2</sup>, SHICHANG ZHANG<sup>1</sup>, TAO LIU<sup>1</sup>, YONG LIU<sup>1</sup>  
and YINGJIE WANG<sup>1,\*</sup><sup>1</sup>Department of Infectious Disease, Southwest Hospital Affiliated to Third  
Military Medical University, Chongqing 400038, China, <sup>2</sup>Department  
of Pediatrics, the 324th Hospital of PLA, Chongqing 400020, China

**Abstract:** Preservation of hepatocyte functions *in vitro* will undoubtedly help the management of acute liver failure. The coculture system may be able to prevent functional decline of hepatocytes. It has already been shown that hepatocytes, when cocultured with bone marrow mesenchymal stem cells, could undergo long-term culture *in vitro* without loss of functions. In this study, human orbital fat-derived stem cells were isolated and cocultured with rat hepatocytes. When treated with serum from an acute liver failure patient, rat hepatocyte monoculture showed reduction of cell viability and loss of liver-specific functions. However, rat hepatocytes in the coculture system were still able to secrete albumin and synthesize urea. IL-6 was significantly elevated in the coculture of rat hepatocyte with orbital fat-derived stem cells, and it might be the key immunoregulator which protects rat hepatocytes against inflammation. Our data confirmed that orbital fat-derived stem cells, or other adipose tissue-derived stem cells, are an ideal candidate to support rat hepatocyte functions *in vitro*.

**Key words:** Acute liver failure, Coculture, Differentiation, Fat tissue, Hepatocytes, Inflammation, Interleukin-6, Mesenchymal stem cells, Orbital fat-derived stem cells, Serum

---

\* Author for correspondence. e-mail: wangyj2012@126.com, tel.: 86-023-68765218, fax: 86-023-68754007

Abbreviations used: ALF – acute liver failure; ADSCs – adipose tissue-derived stem cells; ALB – albumin; CK32 – connexin 32; CYP3A4 – cytochrome P450 subtype 3A4; IDO – indoleamine 2,3-dioxygenase; IFN – interferon; IL – interleukin; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; MSCs – mesenchymal stem cells; OFSCs – orbital fat-derived stem cells; PAS – periodic acid-Schiff; TDO2 – tryptophan 2,3-dioxygenase; TNF – tumor necrotic factor

## INTRODUCTION

The introduction of orthotopic liver transplantation has been proved to reduce mortality in acute liver failure (ALF) [1]. Because of the severe shortfall of donor livers, patients with ALF have to seek aid from alternative treatments before an appropriate donor organ is available for transplantation or recovery by themselves. At present, several novel approaches are being developed to replace the non-functional liver during ALF, such as hepatocyte transplantation [2] and extracorporeal bioartificial liver supportive devices [3]. Isolated and cultured hepatocytes *in vitro* play a central role in those remedies; however, they are unavoidably exposed to the inflammatory circumstance of ALF which would suppress their viability and functions. In addition, loss of liver-specific functions of isolated hepatocytes was reported due to spontaneous dedifferentiation [4]. An optimized method is required to preserve the hepatocyte functions *in vitro*.

Coculture of hepatocytes with nonparenchymal cells may be able to prevent functional decline of hepatocytes [5]. Recently, bone marrow mesenchymal stem cells (MSCs) as a novel partner for coculture with hepatocytes have been proposed [6-9]. Moreover, treatment of liver supportive devices carrying cocultures of MSCs and hepatocytes greatly improved hepatic functional and histological parameters in ALF animals [10, 11].

Adult adipose tissue is developed from embryonic mesenchyme, and a stem cell population within the adipose stromal vascular compartment has been identified and termed adipose tissue-derived stem cells (ADSCs) [12]. ADSCs are characterized by their distinct accessibility compared to bone marrow MSCs. Human orbital fat tissue is a new source of ADSCs [13]. The morphology, growth kinetics and surface immuno-phenotype of orbital fat-derived stem cells (OFSCs) are similar, although not identical, to MSCs from bone marrow. These cells express CD105 and CD90 but lack hematopoietic and epithelial cell surface markers. Thus OFSCs are classified as mesenchymal cells in nature, and possess pluripotent differentiation ability as they are able to differentiate into osteoblasts, chondrocytes and adipocytes under certain inductions [13].

So far, the interaction between ADSCs and hepatocytes in their coculture system remains unclear. In the present study, we tested whether coculture of rat hepatocytes with OFSCs could maintain rat hepatocyte functions *in vitro*, especially under the stimulation of serum from an ALF patient. We also investigated the changes of cytokine levels, which might contribute to the beneficial effects of OFSCs.

## MATERIALS AND METHODS

### Isolation and culture of rat hepatocytes

6-week-old male SD rats were purchased from the Third Military Medical University Laboratories. Hepatocytes were isolated as previously described [14]. Typically,  $1.0-1.5 \times 10^8$  rat hepatocytes could be obtained from a single liver with viability more than 90%. Isolated rat hepatocytes were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal

bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin under humidified air at 37°C. Rat hepatocyte viability was measured by trypan blue dye exclusion assay. All procedures were approved by the Animal Ethic Committees of the Third Military Medical University.

#### **Isolation and culture of human OFSCs**

Orbital fat tissues were collected during blepharoplasty surgery from the intraorbital cavity of six healthy donors with a mean age of  $35 \pm 6$  years. Informed consent was provided and the study was approved by the Ethics Committee of the 324th hospital of PLA. OFSCs were isolated using a method described previously [13]. Briefly, the orbital fat tissue was rinsed with phosphate buffered saline (PBS), cut into small pieces and then incubated in a solution containing 1 mg/ml collagenase type I (Worthington Biochemical, Lakewood, NY) for 4 hours at 37°C with shaking. Digested tissue was filtered through a 200 µm nylon mesh and the filtrate was washed twice with PBS. The cells were then resuspended in DMEM supplemented with 10% FBS, and plated at a density of  $1 \times 10^5$ /ml in a six-well plate. Cells were allowed to adhere overnight. Nonadherent cells were washed out with medium. After reaching 80% confluence, the cells were subcultured and the fourth passage cells were harvested and stored in liquid nitrogen at a density of  $5 \times 10^5$  cells/ml. The frozen vials were thawed later for experimental needs.

#### **Flow cytometry analysis**

The phenotype of isolated OFSCs was analyzed by flow cytometry. The cells were incubated with primary antibody for 30 min on ice, followed by washing and incubation with FITC- or PE-conjugated secondary antibody. The cells were then rinsed twice with PBS, fixed with 1% paraformaldehyde and analyzed by flow cytometry (BD Bioscience, Sparks, MD). Antibodies against human CD13, CD14, CD29, CD34, CD44, CD45, CD54, CD105 and CD117 were purchased from eBiosciences (San Diego, CA). Antibodies against human CD166 were purchased from Ancell (Bayport, MN).

#### **Collection of serum from acute liver failure patient**

Peripheral blood was collected from one woman undergoing ALF. The coagulated blood was centrifuged and serum was collected and stored at -20°C until use. Informed consent was provided.

#### **Coculture of rat hepatocyte and human OFSCs**

For direct coculture, approximately  $7.5 \times 10^5$  primary rat hepatocytes were seeded into a six-well plate pretreated with rat tail collagen type I (Sigma, St. Louis, MO). After 24 hours,  $7.5 \times 10^4$  human OFSCs were seeded. Indirect coculture study was conducted in transwell plates with 0.4 µm pore filters (Millipore, Billerica, MA). Approximately  $1 \times 10^5$  rat hepatocytes were seeded in the lower chamber. After 24 hours,  $1 \times 10^4$  OFSCs were added in the upper chamber. To study the protective effects of OFSCs on rat hepatocytes in the

acute phase of inflammation, the cultures were treated with ALF serum for 3 days. For neutralization experiments, IL-6 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was added to the coculture to a final concentration of 1 µg/ml together with ALF serum for 3 days. Then the medium was replaced with fresh DMEM and cultures were further maintained for 24 hours. Next, medium samples were collected and stored at -80°C until subsequent analysis.

#### **Measurement of albumin, urea nitrogen and cytokine concentration in culture supernatant**

Albumin secretion of rat hepatocyte was analyzed with an ELISA kit according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI). The urea nitrogen concentration was measured using a urea nitrogen diagnostic kit (Jancheng Bioengineering, Nanjing, China). The absorbance was read using a spectrophotometer at a wavelength of 640 nm. Interleukin (IL)-6, interferon (IFN)-γ, tumor necrotic factor (TNF)-α ELISA kit (R&D, Minneapolis, MN), and indoleamine 2,3-dioxygenase (IDO) ELISA kit (EIAab Science, Wuhan, China) were used to measure the levels of cytokines.

#### **Periodic acid-Schiff (PAS) staining for glycogen**

OFSCs in the upper chamber were monitored with PAS staining for glycogen content. Cells were fixed in 95% alcohol for 10 min and oxidized in 1% periodic acid for 5 min. After rinsing three times, cells were treated with Schiff's reagent for 15 min. Finally, the preparations were observed under a light microscope, and the glycogen deposition was visualized by the magenta stain.

#### **RNA isolation and RT-PCR for hepatic gene expression profile**

Total RNA was extracted from OFSCs using Trizol reagent (Invitrogen, Carlsbad, CA). First-strand cDNA was generated using the random hexamer primer. Specific primers for each gene (Table 1) were used. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. PCR amplifications were carried out by 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, followed by a final extension at 72°C for 7 min, using Taq platinum (Roche Diagnostics, Indianapolis, IN). The PCR products were then separated by electrophoresis in 2% agarose gels.

#### **Measurement of cytochrome P450 subtype 3A4 (CYP3A4) activity**

P450-Glo CYP 3A4 assay kit (Promega, Madison, WI) was used to examine the CYP activity. A luminogenic CYP substrate was incubated with OFSCs for 1 hour. Next, luciferin detection reagent was added to initiate a luminescent reaction and incubate for 20 minutes at room temperature. The luminescence was read using a luminometer.

#### **Statistical analysis**

Data are expressed as means ± SD. Statistical analyses were performed using one-way ANOVA for the comparison of data from different groups. Differences with a  $p < 0.05$  were considered statistically significant.

Table 1. Primer sequences used for RT-PCR (For: forward; Rev: reverse)

Gene	Primer sequences	Product length	Accession
ALB	For: GACAGGGCGGACCTTGCCAA Rev: TGGCAAGTCTCAGCAGCAGCA	286 bp	NM_000477
CK32	For: CAGCTTGTTGATCTCATTCTGC Rev: GCATCATCCTCAATGTGGC	150 bp	NM_000166
CYP3A4	For: CACTGCTGTGCAGGGCAGGA Rev: AAGGCAGAGGTGTGGGCCCT	240 bp	NM_017460
TDO2	For: TCGGATGCACCGAGTGTCAAGTGA Rev: GGAGTTCTTTCCAGCCATGCCTCC	300 bp	NM_005651
GAPDH	For: CCATGTTTCGTCATGGGTGTGAACCA Rev: GCCAGTAGAGGCAGGGATGATGTTTC	251 bp	NM_002046

## RESULTS

### OFSCs protect rat hepatocytes against cytotoxicity induced by serum from ALF patient

First, we isolated human OFSCs and assessed their surface markers by flow cytometry. As shown in Fig. 1, OFSCs were negative for hematopoietic stem cell markers CD34 and CD117, monocyte and granulocyte marker CD13, antigen-presenting cell activation marker CD54, leukocyte common antigen CD45, and monocyte marker CD14. In contrast, OFSCs were highly positive for MSCs markers, including CD29, CD44, CD105 and CD166, suggesting their mesenchymal origin, rather than hematopoietic or epithelial origin. The fourth passage of OFSCs, primary hepatocyte monoculture and the direct coculture of OFSCs and hepatocytes at a ratio of 1:10 are shown in Fig. 2A.

Elevated inflammatory cytokines have been demonstrated in serum from ALF patients [15]. To evaluate whether OFSCs can support rat hepatocyte survival against inflammation, both hepatocyte monoculture and direct coculture were exposed in ALF serum. In monoculture, increased round and floating hepatocytes were observed after 3 days of ALF serum treatment (Fig. 2B). However, the rat hepatocytes in the coculture treated with ALF serum still showed a typical cuboidal morphology and formed aggregative clusters surrounded by OFSCs. Using the indirect coculture method, we were able to detect the survival rat hepatocytes by trypan blue staining. As shown in Fig. 2C, live hepatocytes decreased to less than 30% after 3 days exposure of ALF serum. But there was no significant reduction of the percentage of live cells in the coculture, suggesting that OFSCs protected rat hepatocytes from ALF serum induced cytotoxicity. Among different ratios of OFSCs and rat hepatocytes, from 1:5 to 1:50, the ratio of 1:10 exhibited the optimal rat hepatocyte preservation. Thus, the following experiments were performed using the 1:10 ratio.

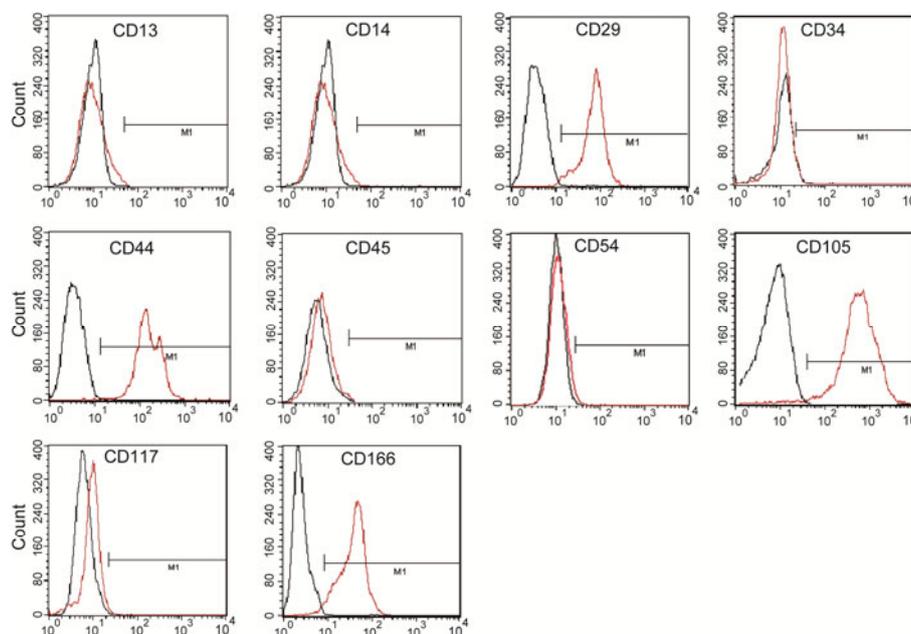


Fig. 1. Cell surface markers of OFSCs. Isolated OFSCs were stained with different antibodies as described in Materials and Methods and analyzed using flow cytometry. The black curve is the corresponding control, and the OFSCs are shown as a red curve.

### **OFSCs preserve rat hepatocyte functions during an acute phase response to ALF serum stimulation**

Albumin secretion and urea synthesis are important functions of hepatocytes. We examined the concentrations of albumin and urea nitrogen in the culture medium. After treatment with ALF serum for 3 days, the fresh medium was changed and harvested after 24 h. Before ALF serum stimulation, the levels of albumin and urea nitrogen were almost the same as the hepatocyte monoculture (Fig. 3A and 3B). After ALF serum stimulation, rat hepatocytes released a significantly higher amount of albumin and urea when cocultured with OFSCs compared to hepatocytes alone. ALF serum reduced rat hepatocyte functions in monoculture, whereas hepatocytes in coculture showed enhanced functions even under inflammatory conditions for 3 days.

### **Alteration in cytokine levels of coculture of rat hepatocytes with OFSCs**

To examine the potential immunomodulatory factors in the coculture system, we measured the production of IL-6, IDO, IFN- $\gamma$  and TNF- $\alpha$ . As shown in Table 2, IL-6 was below the detection limit in the monoculture of hepatocytes. Nevertheless, IL-6 concentrations are dramatically high in the coculture both before and after the ALF serum treatment. Other cytokines were either undetectable or showed no difference between the two groups. To further confirm the role of IL-6, IL-6 neutralizing antibody was added in the coculture and the proportion of live cells, as well as the hepatic functions, was analyzed.

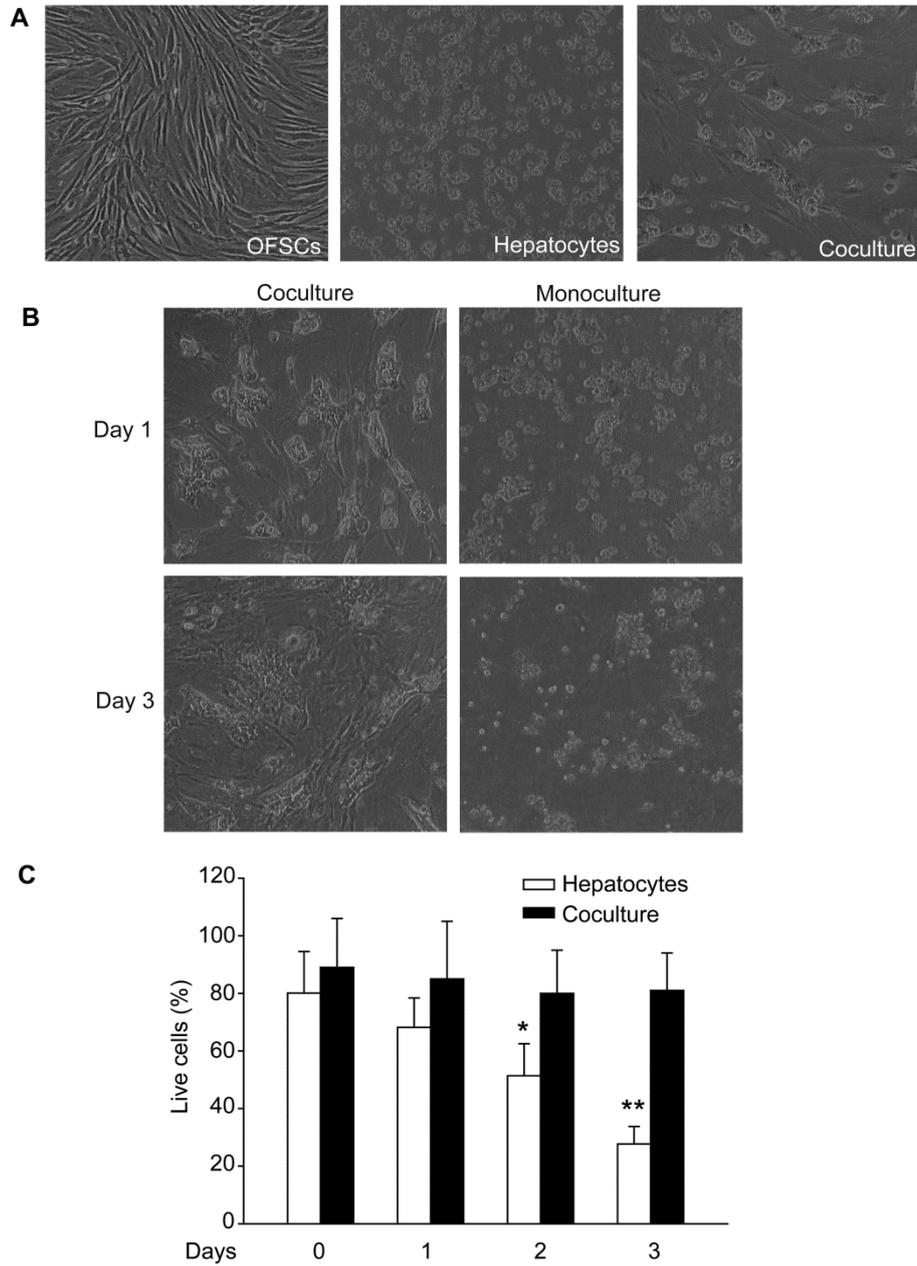


Fig. 2. Coculture of rat hepatocytes with OFSCs prevents ALF serum cytotoxicity. A – Photos of OFSCs, rat hepatocytes and the coculture of them at the ratio of 1:10 (OFSCs:hepatocytes). B – Rat hepatocyte monoculture and coculture with OFSCs were treated with ALF serum, photos were taken at day 1 and day 3, respectively. C – Trypan blue staining was performed to examine the percentage of live hepatocytes in the indirect coculture. \*  $p < 0.05$ ; \*\*  $p < 0.01$  vs. day 0,  $n = 5$ .

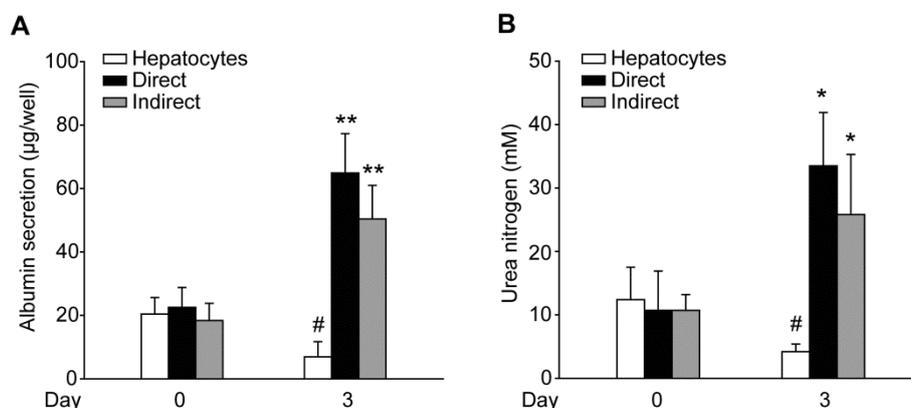


Fig. 3. Preservation of rat hepatocyte function by coculturing with OFSCs. Both monoculture and coculture were treated with ALF serum for 3 days, and then the cultures were maintained in fresh medium for another 24 h. The concentrations of albumin (A) and urea nitrogen (B) were measured. \*  $p < 0.05$ ; \*\*  $p < 0.01$  vs. rat hepatocyte monoculture; #  $p < 0.05$  vs. day 0,  $n = 5$ .

Table 2. Cytokine concentrations in the culture supernatant by ALF-serum treatment in the hepatocyte alone culture, OFSCs alone culture, and OFSCs and hepatocytes coculture. Values are expressed as means  $\pm$  SD,  $n = 4-5$ .

	Hepatocyte		OFSCs		Coculture	
	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
IL-6 (pg/ml)	N.A.	N.A.	990 $\pm$ 420	846 $\pm$ 312	1248 $\pm$ 368	984 $\pm$ 129
IDO (IU/ml)	8.53 $\pm$ 6.44	4.80 $\pm$ 3.27	2.01 $\pm$ 1.18	3.66 $\pm$ 2.60	7.09 $\pm$ 5.14	5.93 $\pm$ 3.09
IFN $\gamma$ (pg/ml)	2.77 $\pm$ 1.30	N.A.	2.52 $\pm$ 0.55	N.A.	3.40 $\pm$ 2.76	N.A.
TNF- $\alpha$ (pg/ml)	0.91 $\pm$ 0.64	0.29 $\pm$ 0.23	N.A.	0.09 $\pm$ 0.05	0.13 $\pm$ 0.09	0.38 $\pm$ 0.28

As shown in Fig. 4A, neutralization of IL-6 restrained the protective effects of OFSCs on rat hepatocytes. In addition, the albumin secretion and urea synthesis were significantly decreased by IL-6 antibody treatment (Fig. 4B and 4C). These data suggested that OFSCs-derived IL-6 is the critical cytokine that preserved rat hepatocytes.

#### Protective effects of OFSCs on rat hepatocytes are independent of OFSCs differentiation

As a member of MSCs, ADSCs can be induced to differentiate into hepatocyte-like cells which possess similar metabolic capabilities as hepatocytes [16]. It is possible that the beneficial effects were due to the differentiation of OFSCs into hepatocytes rather than direct protection of hepatocytes. In order to verify this hypothesis, PAS staining was used to show the glycogen deposition in the upper chamber of OFSCs in the indirect coculture. As shown in Fig. 5A, after

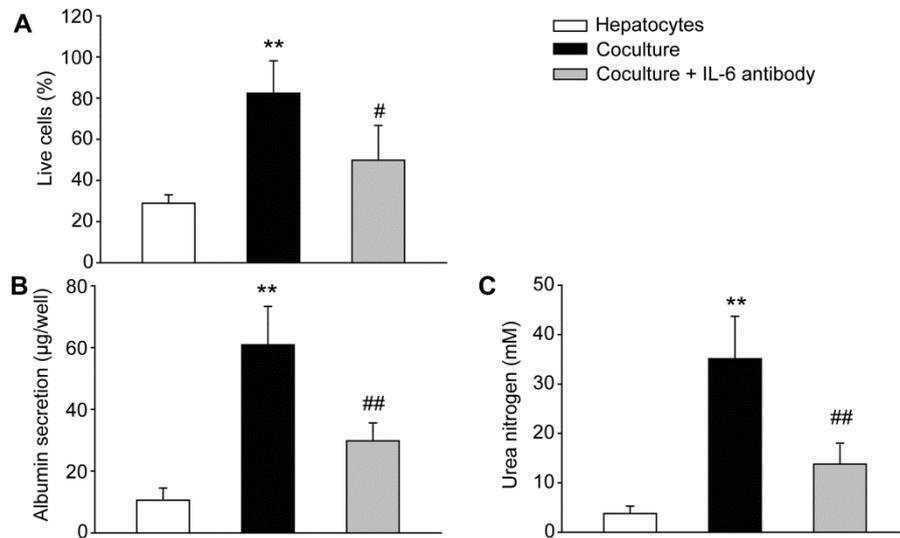


Fig. 4. Neutralization of IL-6 inhibits the protective effects of OFSCs. IL-6 antibody was treated together with ALF serum for 3 days. The percentage of live hepatocytes was evaluated by trypan blue staining (A). The concentration of albumin (B) and urea nitrogen (C) were measured. \*\* $p < 0.01$  vs. rat hepatocyte monoculture, # $p < 0.05$ , ## $p < 0.01$  vs. coculture,  $n = 3-4$ .

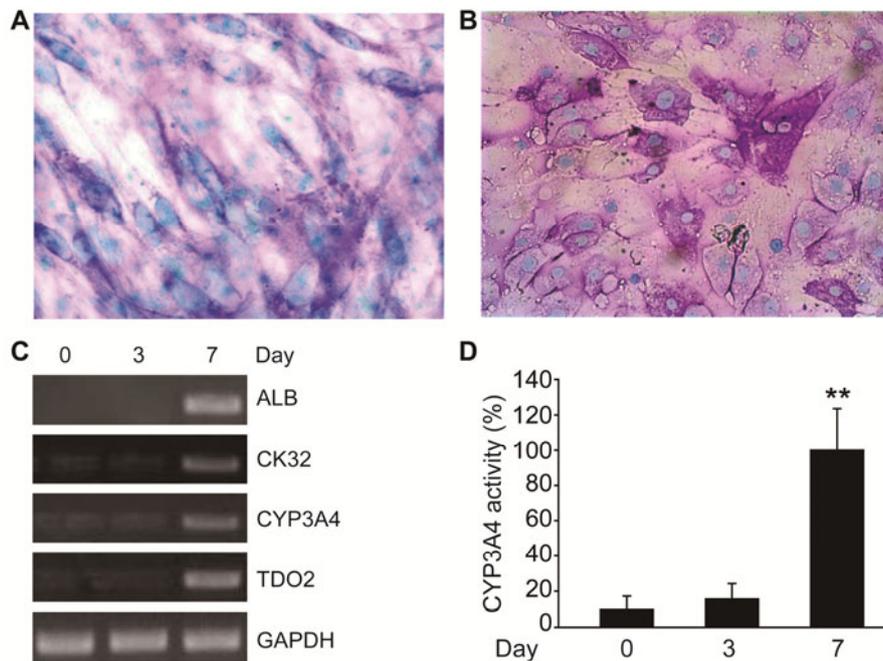


Fig. 5. OFSCs became hepatocyte-like cells in longer term coculture with rat hepatocytes. PAS staining was performed at day 3 (A) and day 7 (B) on the upper chamber of OFSCs in the indirect coculture. mRNA levels of hepatic specific genes were analyzed by RT-PCR (C). CYP3A4 activity was measured by a luminescent method (D). \*\* $p < 0.01$  vs. day 3.

coculture with rat hepatocytes for 3 days, there is no positive magenta staining, but clear glycogen accumulation could be observed after coculture with rat hepatocytes for 7 days (Fig. 5B). The morphology of OFSCs also transformed from a spindle shape to a polygonal shape following 7 days coculture with rat hepatocytes.

Total RNA was also extracted from the OFSCs in the indirect coculture, and RT-PCR was performed to evaluate the liver-specific gene expression, such as albumin (ALB), connexin 32 (CK32), CYP3A4 and tryptophan 2,3-dioxygenase (TDO2). As shown in Fig. 5C, none of these hepatic genes were expressed in OFSCs after coculture with rat hepatocytes for 3 days. OFSCs acquired liver-specific gene expression from 7 days. Further functional assay revealed the CYP3A4 activity was greatly increased in the OFSCs cocultured with rat hepatocytes (Fig. 5D). It is suggested that OFSCs could differentiate into hepatocyte-like cells in a longer time of coculture, and the supportive effects on rat hepatocyte function is through immunomodulation rather than differentiation during an acute phase response to ALF serum stimulation.

## DISCUSSION

In this study, we provided evidence that human OFSCs could be an effective candidate to support rat hepatocyte functions *in vitro*. Human OFSCs were isolated, validated and subcultured, and the fourth passage OFSCs were cocultured with rat hepatocytes. The coculture was subjected to the serum from an ALF patient containing various inflammatory cytokines. OFSCs assisted rat hepatocytes to keep their cell viability, morphology and functions under the stimulation of ALF serum. IL-6 might be the key bioactive molecule through which OFSCs exert their protective effects. The pluripotent potential of OFSCs is not involved in the protective effects in the early phase of ALF serum treatment. Previous studies focused on the coculture system of hepatocytes with bone marrow MSCs and found increased albumin secretion and urea synthesis activities of the hepatocytes [6-8, 11]. Our data proved that ADSCs could play the same roles as bone marrow MSCs when cocultured with rat hepatocytes. In regard to the isolation technique, ADSCs are much more convenient to obtain than bone marrow MSCs. A high yield of human ADSCs can be isolated even from redundant adipose tissue through surgical operation, like the OFSCs used in this study [13]. Another advantage of human ADSCs is their inherent safety, since they are free of animal-origin pathogens. Interestingly, ADSCs were reported to control graft-versus-host disease [17] and rheumatoid arthritis [18], suggesting their nonspecific immunoregulatory roles.

Since there was no significant difference between direct and indirect coculture, it is unlikely that heterotypic cell-cell contact of rat hepatocytes and OFSCs is involved in the preservation of hepatocytes. It seems that some soluble factors secreted by OFSCs keep the rat hepatocyte functions under the inflammatory condition. Indeed, the secretome of ADSCs has been shown to possess the

capability of immunomodulation [19]. It is well known that IL-6, IDO, IFN $\gamma$  and TNF- $\alpha$  are modulators of the hepatocyte immune response and regeneration [20-23]. We examined production of the four cytokines in the supernatant and found a high level of IL-6 in the coculture medium, while in the rat hepatocyte monoculture, IL-6 concentration was too low to be measured by ELISA. The pattern of the other three cytokines was similar between the two cultures. Treatment of IL-6 neutralizing antibody in the coculture system suppressed the beneficial effects of OFSCs on rat hepatocytes. As a pleiotropic cytokine, IL-6 is related to the immune response, support of hematopoiesis, induction of inflammation and oncogenesis [24]. It has been documented that IL-6 could maintain hepatocyte functions under ischemia reperfusion injury [25]. Consistently, an elevated IL-6 level was also found in the coculture of bone MSCs with hepatocytes [9]. In *in vivo* experiments, the serum level of IL-6 was also increased by the transplantation of three-dimensional coculture of bone MSCs with hepatocytes [10]. Thus, IL-6 may be a critical cytokine secreted in the co-culture of OFSCs and rat hepatocytes. It can immunomodulate and protect rat hepatocytes when exposed in ALF serum, along with increased production of albumin and urea.

ADSCs can be induced to differentiate into hepatocyte-like cells, and could provide a novel source for hepatic regeneration and support hepatic functions [16, 26, 27]. However, the process of differentiation requires a certain period of time. Two weeks might be enough to induce hepatogenic characteristics in ADSCs when they are treated with activin A and fibroblast growth factor on collagen type I-coated dishes, followed by treatment of many other growth factors and chemicals [28]. And these ADSC-derived hepatocytes can be transplanted into mice with acute liver failure to alleviate liver damage. Interestingly, just transplantation of ADSCs into nude mice with CCl<sub>4</sub>-caused liver injury can improve liver functions; further investigation revealed that ADSCs produce high levels of many kinds of cytokines and growth factors which could stimulate hepatocyte proliferation [29]. The rapid protective effects strongly indicate that the paracrine secretions of OFSCs play a more important part than hepatic differentiation of OFSCs in the present experimental settings. Our data proved the differentiation ability of OFSCs in the coculture system. But during the first 3 days of ALF serum treatment, OFSCs did not exhibit any hepatic characteristics, evidenced by glycogen deposition and hepatic gene expressions. If the culture time was prolonged, the newly formed hepatocyte-like cells from OFSCs might undertake the hepatocyte functions as well.

In conclusion, we demonstrated that human OFSCs, when cocultured with rat hepatocytes, preserve rat hepatocyte functions in the setting of inflammation. According to our data, the immunoregulatory effects of OFSCs were identical to bone marrow MSCs. It seems that IL-6 is the common effective cytokine in coculture of rat hepatocytes with OFSCs or bone marrow MSCs. OFSCs, or other ADSCs, can be an alternative cell source for the cell-based therapeutic strategies for maintenance of liver functions *in vitro*.

**Acknowledgements.** Supported by the National Natural Science Foundation of China (31070880).

## REFERENCES

1. Bernal, W., Auzinger, G., Dhawan, A. and Wendon, J. Acute liver failure. **Lancet** 376 (2010) 190-201.
2. Dhawan, A., Puppi, J., Hughes, R.D. and Mitry, R.R. Human hepatocyte transplantation: current experience and future challenges. **Nat. Rev. Gastroenterol. Hepatol.** 7 (2010) 288-298.
3. Phua, J. and Lee, K.H. Liver support devices. **Curr. Opin. Crit. Care** 14 (2008) 208-215.
4. Elaut, G., Henkens, T., Papeleu, P., Snykers, S., Vinken, M., Vanhaecke, T. and Rogiers, V. Molecular mechanisms underlying the dedifferentiation process of isolated hepatocytes and their cultures. **Curr. Drug Metab.** 7 (2006) 629-660.
5. Kaihara, S., Kim, S., Kim, B.S., Mooney, D.J., Tanaka, K. and Vacanti, J.P. Survival and function of rat hepatocytes cocultured with nonparenchymal cells or sinusoidal endothelial cells on biodegradable polymers under flow conditions. **J. Pediatr. Surg.** 35 (2000) 1287-1290.
6. Mizuguchi, T., Palm, K., Hui, T., Aoki, T., Mochizuki, Y., Mitaka, T., Demetriou, A.A. and Rozga, J. Effects of bone marrow stromal cells on the structural and functional polarity of primary rat hepatocytes. **In Vitro Cell Dev. Biol. Anim.** 38 (2002) 62-65.
7. Isoda, K., Kojima, M., Takeda, M., Higashiyama, S., Kawase, M. and Yagi, K. Maintenance of hepatocyte functions by coculture with bone marrow stromal cells. **J. Biosci. Bioeng.** 97 (2004) 343-346.
8. Ijima, H., Murakami, S., Matsuo, T., Takei, T., Ono, T. and Kawakami, K. Enhancement of liver-specific functions of primary rat hepatocytes cocultured with bone marrow cells on tissue culture-treated polystyrene surfaces. **J. Artif. Organs** 8 (2005) 104-109.
9. Gu, J., Shi, X., Chu, X., Zhang, Y. and Ding, Y. Contribution of bone marrow mesenchymal stem cells to porcine hepatocyte culture in vitro. **Biochem. Cell Biol.** 87 (2009) 595-604.
10. Takeda, M., Yamamoto, M., Isoda, K., Higashiyama, S., Hirose, M., Ohgushi, H., Kawase, M. and Yagi, K. Availability of bone marrow stromal cells in three-dimensional coculture with hepatocytes and transplantation into liver-damaged mice. **J. Biosci. Bioeng.** 100 (2005) 77-81.
11. Yagi, H., Parekkadan, B., Suganuma, K., Soto-Gutierrez, A., Tompkins, R.G., Tilles, A.W. and Yarmush, M.L. Long-term superior performance of a stem cell/hepatocyte device for the treatment of acute liver failure. **Tissue Eng. Part A** 15 (2009) 3377-3388.
12. Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J., Benhaim, P., Lorenz, H.P. and Hedrick, M.H. Multilineage cells from

- human adipose tissue: implications for cell-based therapies. **Tissue Eng.** 7 (2001) 211-228.
13. Ho, J.H., Ma, W.H., Tseng, T.C., Chen, Y.F., Chen, M.H. and Lee, O.K. Isolation and characterization of multi-potent stem cells from human orbital fat tissues. **Tissue Eng. Part A** 17 (2011) 255-266.
  14. Tanaka, K., Soto-Gutierrez, A., Navarro-Alvarez, N., Rivas-Carrillo, J.D., Jun, H.S. and Kobayashi, N. Functional hepatocyte culture and its application to cell therapies. **Cell Transplant.** 15 (2006) 855-864.
  15. Wu, Z., Han, M., Chen, T., Yan, W. and Ning, Q. Acute liver failure: mechanisms of immune-mediated liver injury. **Liver Int.** 30 (2010) 782-794.
  16. Ishikawa, T., Banas, A., Hagiwara, K., Iwaguro, H. and Ochiya, T. Stem cells for hepatic regeneration: the role of adipose tissue derived mesenchymal stem cells. **Curr. Stem Cell Res. Ther.** 5 (2010) 182-189.
  17. Yanez, R., Lamana, M.L., Garcia-Castro, J., Colmenero, I., Ramirez, M. and Bueren, J.A. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. **Stem Cells** 24 (2006) 2582-2591.
  18. Gonzalez, M.A., Gonzalez-Rey, E., Rico, L., Buscher, D. and Delgado, M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. **Arthritis Rheum.** 60 (2009) 1006-1019.
  19. Salgado, A.J., Reis, R.L., Sousa, N.J. and Gimble, J.M. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. **Curr. Stem Cell Res. Ther.** 5 (2010) 103-110.
  20. Horras, C.J., Lamb, C.L. and Mitchell, K.A. Regulation of hepatocyte fate by interferon-gamma. **Cytokine Growth Factor Rev.** 22 (2011) 35-43.
  21. Mao, R., Zhang, J., Jiang, D., Cai, D., Levy, J.M., Cuconati, A., Block, T.M., Guo, J.T. and Guo, H. Indoleamine 2,3-dioxygenase mediates the antiviral effect of gamma interferon against hepatitis B virus in human hepatocyte-derived cells. **J. Virol.** 85 (2011) 1048-1057.
  22. Yuceturk, H., Yagmurdur, M.C., Gur, G., Demirbilek, M., Bilezikci, B., Turan, M., Karakayali, H. and Haberal, M. Role of heparin on TNF- $\alpha$  and IL-6 levels in liver regeneration after partial hepatic resection. **Eur. Surg. Res.** 39 (2007) 216-221.
  23. Lin, Y.C., Goto, S., Tateno, C., Nakano, T., Cheng, Y.F., Javan, B., Kao, Y.H., Hsu, L.W., Lai, C.Y., Yoshizato, K. and Chen, C.L. Induction of indoleamine 2,3-dioxygenase in livers following hepatectomy prolongs survival of allogeneic hepatocytes after transplantation. **Transplant. Proc.** 40 (2008) 2706-2708.
  24. Kishimoto, T. IL-6: from its discovery to clinical applications. **Int. Immunol.** 22 (2010) 347-352.
  25. Tiberio, L., Tiberio, G.A., Bardella, L., Cervi, E., Cerea, K., Dreano, M., Garotta, G., Fra, A., Montani, N., Ferrari-Bravo, A., Callea, F., Grigolato, P., Giuliani, S.M. and Schiaffonati, L. Mechanisms of interleukin-6

- protection against ischemia-reperfusion injury in rat liver. **Cytokine** 34 (2006) 131-142.
26. Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Quinn, G., Okochi, H. and Ochiya, T. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. **Hepatology** 46 (2007) 219-228.
  27. Talens-Visconti, R., Bonora, A., Jover, R., Mirabet, V., Carbonell, F., Castell, J.V. and Gómez-Lechón, M.J. Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells. **World J. Gastroenterol.** 12 (2006) 5834-5845.
  28. Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Osaki, M., Kato, T., Okochi, H. and Ochiya, T. Rapid hepatic fate specification of adipose-derived stem cells and their therapeutic potential for liver failure. **J. Gastroenterol. Hepatol.** 24 (2009) 70-77.
  29. Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Osaki, M., Kawamata, M., Kato, T., Okochi, H. and Ochiya, T. IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. **Stem Cells** 26 (2008) 2705-2712.