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Full Length Research Paper

Amniotic fluid-derived mesenchymal stem cells as a novel therapeutic approach in the treatment of fulminant hepatic failure in rats

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As a potential alternative treatment for terminal liver diseases, amniotic fluid derived mesenchymal stem cells (AFMSCs) have many advantages over other stem cells: avoiding much ethical controversy and decrease in both quantity and differentiation potential with age. However, the therapeutic role of AFMSC for fulminant hepatic failure (FHF) has not yet been clearly elucidated. Therefore, we investigated the reparation effects of transplanted AFMSCs in rats with FHF. AFMSCs were transplanted into injured liver via the portal vein in the rat FHF model. Therapeutic effect was evaluated after cell transfusion by histologic pathology, hepatic enzyme levels and animal survival. Cryostat sections were prepared and directly assessed for green fluorescent protein (GFP) expression and localization, and *in vivo* differentiation of AFMSC was confirmed by double-immunostaining analyses. Our results show that AFMSCs prevented liver failure and reduced mortality in rats with FHF. These animals also exhibited improved liver function and animals survival after injection with AFMSCs using GFP, we demonstrated that the engrafted cells and their progeny incorporated into injured livers and produced albumin. We found that AFMSCs transplantation modestly promoted the repair of FHF in rats. AFMSCs implanted in the injured liver may be a novel therapeutic approach in the treatment of FHF.

Key words: Amniotic fluid-derived mesenchymal stem cells, fulminant hepatic failure, cell transplantation, treatment, hepatogenic differentiation.

INTRODUCTION

Fulminant hepatic failure is a serious clinical condition that is associated with a high mortality rate. Despite a combination of all available treatments including medical treatment and bioartificial liver (BAL) device, the mortality of fulminant hepatic failure (FHF) is more than 70%. To date Orthotopic liver transplantation is the only effectively treatment of choice for FHF and end-stage liver disease (Farmer et al., 2009), however, it has limitations primarily due to a lack of readily available donors, associated high costs, and the requirement for lifelong immunosuppression (Zheng et al., 2011). And BAL device could ideally be regarded as a bridge to transplantation or liver regeneration for the patients with FHF. According to recent clinical results, BAL systems containing animal hepatocytes have proven to be safe, but immunological rejection and zoonosis still remain as major problem (Park and Lee, 2005). Considering the potential of pluripotency and differentiation into tridermal lineages, stem cells may serve as an alternative of cell-based therapy (Cipriani et al., 2007). Multipotent stem cells, referring mainly to embryonic stem cells (ESCs) and bone marrow-derived mesenchymal stem cells (BM- MSCs) (Lodi et al., 2011), have been isolated from tissues outside

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the liver, and in some cases hepatocytic differentiation has been reported (Duncan etal.,2009). However, ESCs derived from blastocysts grow as teratocarcinomas when implanted *in vivo* (Cowan et al., 2004), which raises significant ethical concerns. And the difficulties with bone marrow is obtaining tissue samples from donors, and finding a significant decrease in both quantity and differentiation potential of BM-MSCs with age (Stenderup et al., 2003). Meanwhile, the derivation of autologous MSCs from appropriate tissue cannot be readily obtained or expanded in culture from patients with recent acute liver disease (Atala et al., 2006).

Recent observations also indicate that the hepatic differentiation capability of BM-MSC can be impaired to some extent as a result of exposure to environmental pollution such as insecticides, heavy metals and antiputrefactives (Ghen et al., 2006). Recent study showed that induced pluripotent stem cells (iPSCs) have been regarded as the most promising way to create stem cells. However, the use of iPSCs has raised concerns. The iPSCs are easily created by modulating the human genome to ectopically express transcriptional factors. Since their over-expression has been associated with tumorigenesis (Foster et al., 2005; Hochedlinger et al., 2005), there is a risk that the differentiated cells might also be tumorigenic when transplanted into patients. The insertion of transgenes into functional genes of the human genome can be detrimental (Nair 2008). Furthermore, although the transcription factors are mostly silenced following reprogramming, it has been reported that residual transgene expression may be responsible for some of the differences between ESCs and iPSCs such as the altered differentiation potential of iPSCs into functional cell types (Soldner et al., 2009). These disadvantages of ESCs, BM-MSCs and iPSCs have restricted their use in tissue engineering and organogenesis, which has accelerated the search for alternative sources of stem cells. Recently, it has been reported that stem cells derived from second-trimester amniocentesis were pluripotent stem cells with the capability to differentiate into multiple lineages, including representatives of all three embryonic germ layers (De Coppi et al., 2007). Many recent reports have shown that AFMSCs have the capability of differentiating into osteoblasts, hepatocyte-like cells (De Coppi et al., 2007), chondrocytes (Kolambkar., 2007), adipocytes (Kim et al., 2007), neurons (Chen et al., 2011), cardiomyocytes (Chiavegato et al., 2007) and renal cells (Perin et al., 2007), when exposed to specific conditions. Furthermore, our previous study (Zheng et al., 2008) also showed that AFMSCs exhibited higher capacity for cell proliferation, self-renewal and hepatic differentiation compared to bone marrow-derived mesenchymal stem cells. And Mesenchymal stem cells also have been a cell type of interest because of their accessibility and amenability to transfection with exogenous genes (Yu et al., 2007). So AFMSCs may provide an ethically uncontroversial and easily accessible source of human hepatocytes for future clinical applications. Based on this concept, in this study, we explored a rat FHF model, and tested whether transfusion of AFMSCs could repair damaged livers, promote regeneration after cell transplantation and improve short-term functional recovery and long-term survival rate in FHF.

MATERIALS AND METHODS

Experimental animals

Syngenic male S-D rats (8 to 12-weeks of age, weighing approximately 250 g; the Animal Center of Sun Yat-Sen University, Guangzhou, China) were used in all of the experiments, in order to exclude any effects of environmental field interference. The animals were kept in the animal facilities at Sun Yat-Sen University, and the experiments were conducted in accordance with the guidelines approved by the China Association of Laboratory Animal Care.

Preparation of mesenchymal stem cells (MSCs)

Amniotic fluid MSCs were harvested from a pregnant Sprague-Dawley rats at gestation day 14 ± 1d, as previously described by our study (Zheng et al., 2008). MSCs between the third and sixth passage were used in the experiments. At least 2×10^5 MSCs were harvested and re-suspended in 0.1 ml phosphate-buffered saline containing 1% bovine serum albumin (Gibco). The cell suspension was incubated with 0.2 µg fluorescein isothiocyanate-or phycoerythrin- conjugated primary antibody (1:100 dilution), mouse monoclonal anti-rat CD34 (Santa Cruz Biotechnology, CA), anti-CD117, anti-CD29 (BD PharMingen, USA), anti-CD44(Immunotech, Coulter Company, Marseille, France) anti-CD45 (eBiosciences, USA) and anti-CD133 (Miltenyi Biotech) for 40 min at 4°C. The mouse IgG1 kappa antibody (Catag Laboratories, Burlingame, CA) was used as an isotype control. MSC surface markers were analyzed by fluorescence-activated cell sorter (FACS Calibur, BD biosciences company, USA).

Lentiviral vectors transduction

GFP lentivirus were established as previously described (Wang et al., 2009). Briefly, to obtain lentiviral particles, HEK-293T cells were plated at 7 to 8 × 10^5 cells per T-175 tissue culture flask in the growth medium (10% high-glucose DMEM) without antibiotics, 24 h before transfection. Forty micrograms of plasmid DNA was used for the transfection, which consisted of 20 µg of the transfer vector plasmid pCS-CDF-CG-PRE, 12 µg of the packaging plasmid pMDLg/pRRE, 3 µg of the Rev plasmid pRSV-Rev, and 5 µg of the envelope plasmid pMD.G (RIKEN, Tsukuba, Ibaraki, Japan).To achieve precipitation, the plasmids were diluted with 100 µL Opti-MEM and 120 µL Lipofectamine 2000 to a final volume of 220 µL and incubated together at room temperature. The DNA/Lipofectamine 2000 complex was added dropwise to the culture flask and incubated at 37°C in a carbon dioxide incubator overnight. After 12 h, the medium was replaced with 25 mL of 10% high-glucose DMEM. This conditioned medium was collected after an additional 48 h of incubation and filtered through 0.45 µm cellulose acetate filters. The conditioned medium was then ultracentrifuged at 4°C at 20,000 rpm for 2 h, and the virus pellet was resuspended in phosphate-buffered saline and frozen at −80°C. The multiplicity of infection (MOI) was determined by the infection of HEK-293T cells with serial dilutions of the vector stocks. AFMSCs were seeded at 5×10^5 cells per 25 cm^2 flasks one day before transduction. AFMSCs were transduced with Lentiviral

vectors containing GFP (Lv-GFP). The medium was replaced with virus-containing supernatant supplemented with 8 µg/ml polybrene (Sigma-Aldrich), and incubated for 48 h. After 96 h of transfecction, the GFP positive AFMSCs were sorted by FACS according to the low and high intensity of GFP expression. The MSCs transduced with Lv-GFP were designated as GFP/MSCs.

Experimental design and animal groups

Fulminant hepatic failure was induced by an intraperitioneal administration of 1.5 g/kg galactosamine (D-GalN) and 200 ug/kg lipopolysaccharides (LPS). On day 1 after the induction, the rats with FHF were anesthetized with intraperitioneal injections of 6ml/kg 5%chloral hydrate (Sigma-Aldrich) and 0.4 mg/kg xylazine (Sangon Biotech (Shanghai) Co., Ltd). The rats undergoing FHF induction were divided into two groups according to the material they would receive by transfusion: (i) physiological saline (PS), (ii) GFP/MSCs, as a positive control. One day after FHF induction, PS, 1×10^6 cells in a volume of 0.8 ml were transfused into the portal vein of each recipient rat with 28-gauge needle over a time period of 5 min. Rats were evaluated every 12 h and killed if they appeared moribund.

Survival study

Fifteen rats in each group were used for the survival study. Rats that had lived for more than 21 days after transplantation were considered to be survivors.

Collection of serum samples and hepatic tissue specimens

Rats were killed on days 4, 6, 8, 12 and 15 post-transfusion. To detect serum hepatic enzyme levels on days 3, 5, 7, 11 and 14 after transfusion, blood samples were collected from tail vein 24 h before sacrificed on days 4, 6, 8, 12 and 15 after cells transfusion. Liver tissues were excised and fixed, and processed for histology and immunohistochemistry.

Measurement of hepatic enzyme levels after cell transfusion

The two commonly measured serum transaminases which act as sensitive indicators of hepatocellular damage are ALT and AST as previously described by Collier and Bassendine (2002). So the plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) after cell transfusion were measured in a biochemistry laboratory (AEROSET, Abbott, USA).

Liver histology

Tissue was fixed in 10% buffered formalin, embedded in paraffin, sectioned to 6 mm thickness, and stained with hematoylin and eosin.

Determination of extent of retention of transfused MSCs in livers

Cryostat sections of livers were prepared and directly estimated for GFP expression and localization, with 4′, 6-diamidino-2-phenylindole(1:10000, DAPI, Sigma-Aldrich,China) counterstaining, and utilizing a fluorescence microscope (BX51, Olympus, Japan). The number of GFP-labeled hepatocytes per 1000 hepatocellular nuclei (200-fold magnification) was also detected. In order to measure the presence of albumin by immunofluorescent staining, cryostat sections were fixed in acetone and then incubated with primary rabbit anti-rat albumin monoclonal antibody (1:200, R&D Systems,USA) overnight at 4°C. Sections were then incubated at 37°C for 1 h with Cy3-conjugated goat anti-rabbit secondary antibody (Sigma-Aldrich,USA) at a dilution of 1:200 in phosphate-buffered saline.

Statistical analyses

All data were expressed as means ± SD. Comparisons involving one independent factor (for example, treatment) or two independent factors (for example, treatment and time) were analyzed using one-way and two-way ANOVA, respectively followed by Bonferroni post-hoc testing. Results from survival experiments were analyzed using the log-rank test and expressed as Kaplan-Meier survival curves. P-values less than 0.05 were considered to be statistically significant. Statistical analysis was performed using SPSSv16.0 software.

RESULTS

Characteristics and Lentivirus transfer in AFMSCs

AF-MSCs retained a fibroblastic morphology after repeated passages (Figure 1A), and their immunophenotypical characterization was confirmed by flow cytometry. Over 92% of the isolated AFMSCs expressed CD29 and CD44, but not CD34, CD45, CD133 and CD117 (Figure 1B). These results are consistent with well-established markers of bone marrow derived MSCs (Zheng et al., 2008; Peng et al., 2011).

Ninety-six hour after transduction, 92.53% of GFP/MSCs were green fluorescent protein-positive (Figure 2 A, B and D). Lentivirus-mediated stable expression of GFP by MSCs was maintained for at least 14 days *in vitro.*

AFMSCs decreased mortality rates with FHF

There was no significant difference in the survival rates in any of the rodent groups until 96 h after cell transplantation. However, the survival rates of the GFP/MSCs groups were better than the PS groups after 96 h after cell perfusion. The 21-day survival rates of the PS and GFP/MSCs groups were 19.3 and 38.1%, respectively ($P < 0.05$; Figure 2C). These results show that the survival rates of animals transplanted with GFP/MSCs are markedly higher than those of the PS groups.

AFMSCs improved liver function by extenuating hepatocytes necrosis and attenuating intrahepatic inflammatory responses

The degree of hepatic injury of rat FHF mode was assessed by histological analyses (Figure 3A to D). Histological manifestation of injured liver demonstrated marked infiltration of mononuclear cells and destruction of the liver architecture in periportal areas on day 14 after induction. Evident dilation of the sinusoids and some areas of necrosis were also determined. And in PS

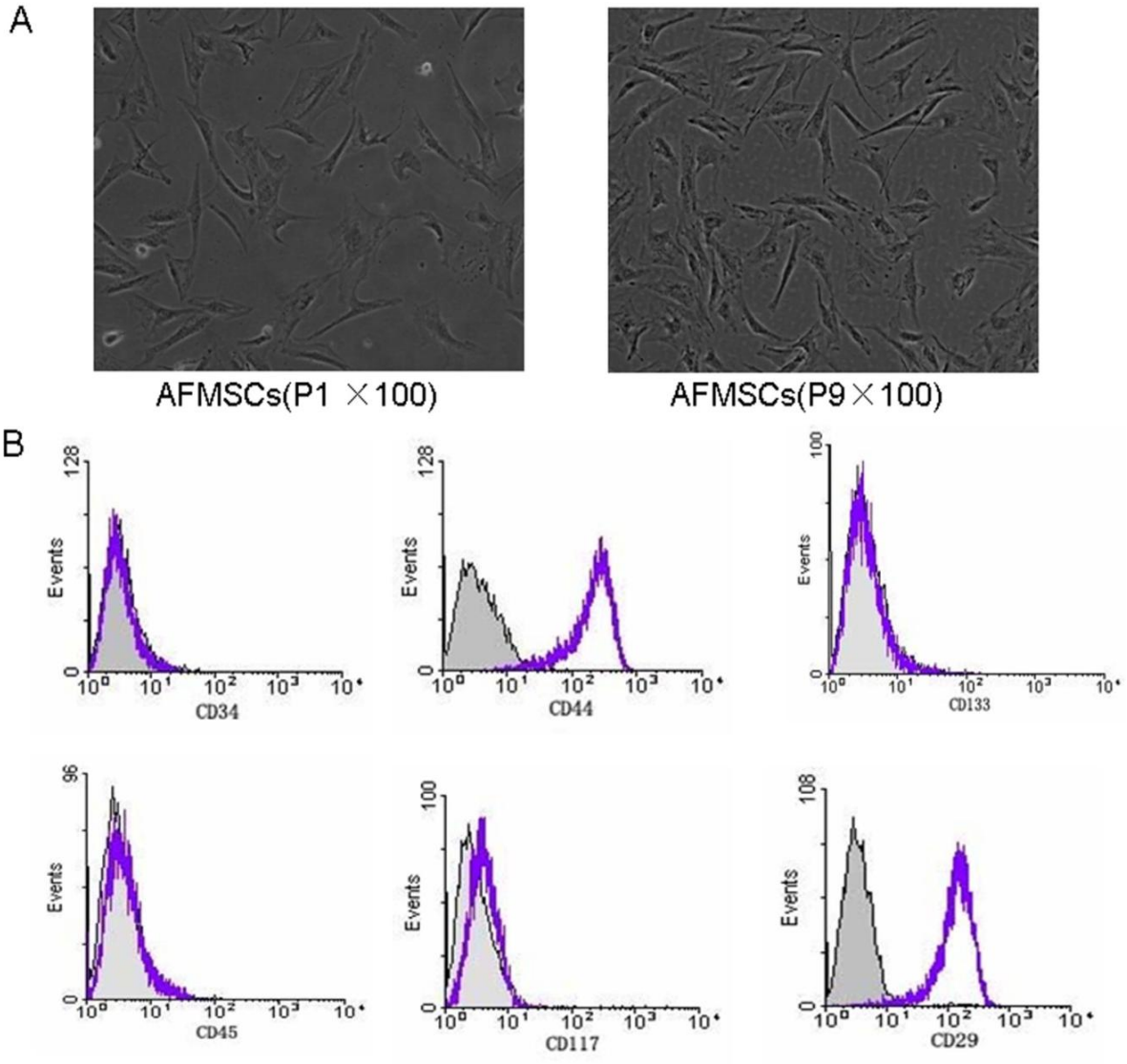

Figure 1. Characterization of amniotic-fluid–derived mesenchymal stem cells (AFMSCs) at passage 3. (**A**) Morphological characterization of AFMSCs. (**B**) Fluorescence-activated cell sorting analysis of rat AFMSCs.

group, major perivascular infiltration of mononuclear cells, and focal and small areas of hepatocyte necrosis was also detected (Figure 3A and B) while the slight histological injuries were observed in the GFP/MSCs groups (Figure 3C and D). Notably, focal area of hepatic necrosis occupied by conspicuous infiltration of mononuclear cells were detected in the PS group four weeks after cell transplantation, however very few mononuclear cell in the GFP/MSCs group. And the frequency of appearance of the mononuclear cells per high-power field was significantly higher in PS rats (32.1 ± 4.5%) than in AFMSC rats (8.6 ± 3.4%; n = 5, P < 0.05) (Figure 3 A to D). However, normal rat showed normal architecture of liver tissue and almost no mononuclear cell was observed in the livers of normal rats (Figure 3 E and F).

AFMSCs improved liver function by incorporating into injured livers and producing albumin (ALB)

Serum levels of hepatic enzyme including ALT and AST were measured on days 3, 5, 7, 11 and 14 after cell transplantation in order to evaluate the degree of hepatocellular lesion. Both PS and GFP/MSCs groups after cell perfusion had significantly higher levels of ALT and AST than normal group on days 3, 5, 7 and 11. On day 5, the GFP/MSCs and PS group had obviously higher levels of AST than the normal group (116.1±14.7 IU/L, P<0.01; n=5), and the GFP/MSCs group had significantly lower levels of AST (656.5±108.3 IU/L), than the PS group (797.3 ±125.3 IU/L) (P < 0.05; n = 5), however, there was no significant difference in serum levels of ALT between the GFP/MSCs and PS group. On

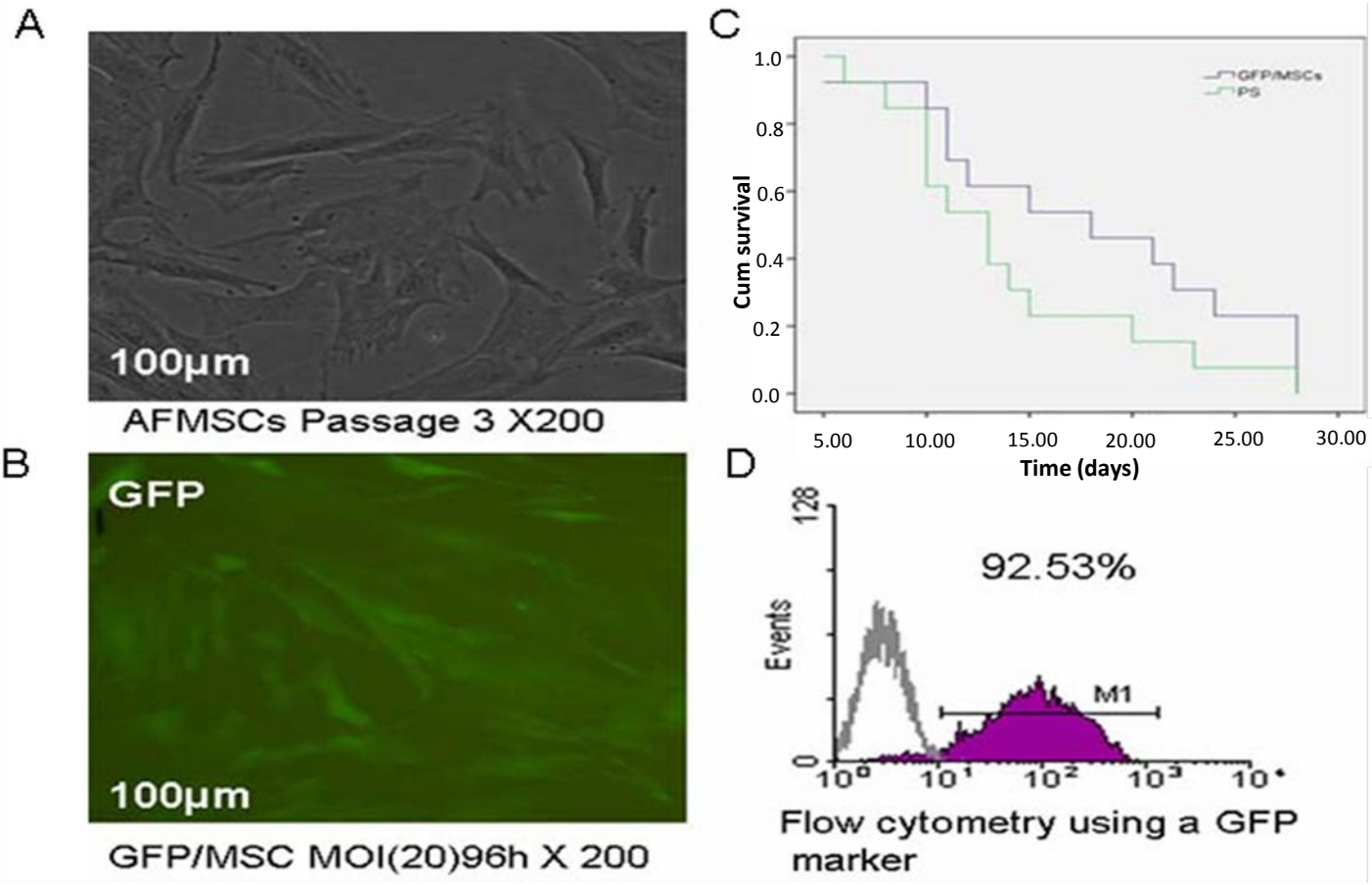

Figure 2. Characterization of genetically-modified amniotic-fluid–derived mesenchymal stem cells (AFMSCs) and Kaplan-Meier plot showing the pattern of survival. (**A**) Morphological characterization of AFMSCs. (**B**,**D**) AFMSCs were transduced with lentivirus green fluorescence protein (LV-GFP) and the transduction efficiency of AFMSCs was detected by flow cytometry using a GFP marker. (**C**) Kaplan-Meier plot showing the pattern of survival of fulminant hepatic failure (n = 15/group) after various treatments. MSCs, mesenchymal stem cells; PS, physiological saline; GFP, green fluorescence protein.

day 7, the serum ALT levels of the GFP/MSCs group (323.3 ± 86.5 IU/L) decreased to Two-thirds that of the PS group (495.8 ±126.8 IU/L) ($P < 0.05$; $n = 5$), and both GFP/MSCs and PS group had visibly higher ALT levels than normal group (98.4 ± 13.6, $P < 0.01$; $n = 5$). All of the rats with high AST levels in PS and GFP/MSCs group died between 5 and 7 days, however, the recovery of the ALT levels were detected in the GFP/MSCs-transfused rats despite a mild transient increase in ALT (Figure 4A to B).

In order to detect hepatic differentiation of graft MSCs, double-fluorescent immunohistochemistry for expression of albumin (red) in the GFP-positive (green) cells was applied. Among the GFP-labeled cells, the percentages of the double-positive cells were approximately 37.2 ± 3.5% in the GFP/MSCs groups, while there were no double-positive cells in PS groups, thereby suggesting that the resident MSCs were producing albumin and had differentiated into hepatocyte like cells *in vivo* (Figure 4C). So we extrapolated AFMSCs improved liver function by incorporating into injured livers and producing ALB.

DISCUSSION

Our results show that delivering AFMSCs into a FHF rat is possible to attenuate hepatic inflammatory responses, enhance the regeneration of liver cells, attenuate necrosis in hepatocytes and resulting in significant improvement of survival, and liver function.

Our previous studies have indicated that human MSCs from second trimester amniotic fluid are able to differentiate into functional hepatocyte-like cells and, hence, may serve as a cell source for tissue engineering and cell therapy of hepatic tissues *in vitro* (Zheng et al., 2008). However, fulminant hepatic failure is characterized by massive hepatocyte necrosis and inflammation. Endotoxin and inflammatory cytokines are important mediators in acute liver failure, and IL-1βand TNF-α are involved in its pathogenesis (Yumoto et al., 2002). Popp et al. (2007) reported that these inflammatory cytokines

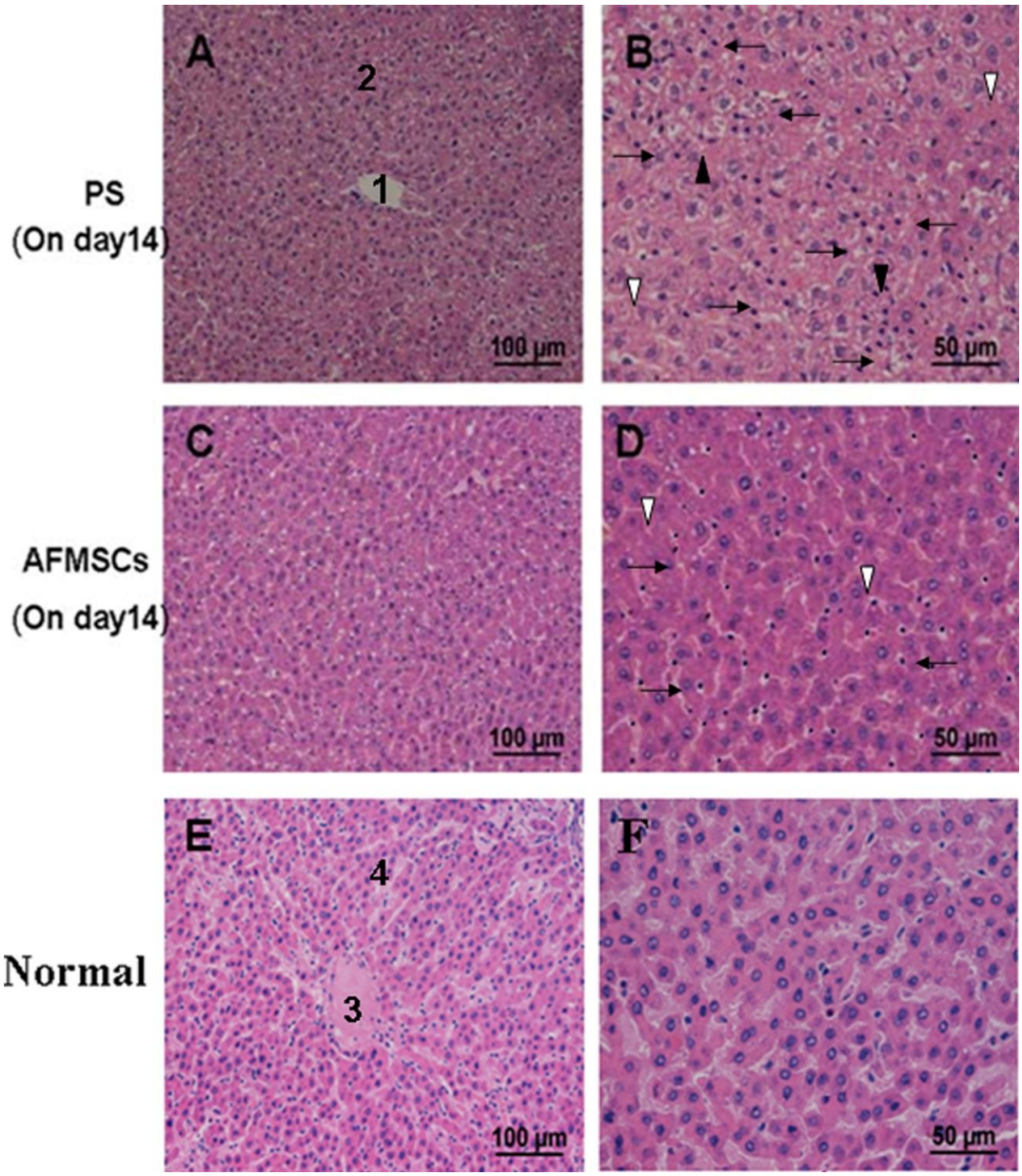

Figure 3. Histology of hepatic injury in rat fulminant hepatic failure after cell perfusion and normal liver: (**A**) Apparent structural disorder of liver lobules was detected in the physiological saline (PS) group, disorder hepatic strands (2) and central vein (1). (**B**) Increased infiltration of mononuclear cells, vacuolar degeneration, and necrosis were observed in the PS group, focal area of hepatic necrosis with dark pyknotic nuclei (▲) occupied by leucocytic cells infiltration (↑) and sporadic necrosis of hepatocytes with whilte triangle (∆), as compared to the amniotic-fluid–derived mesenchymal stem cell (AFMSCs) group (**C**, **D**). (**E, F**) Liver of control, normal rat showing normal architecture of liver tissue, normal hepatic strands (4) and central vein (3), almost no hepatic necrosis and mononuclear cell was observed in the livers of normal rats. (Hx and E stain original magnification ×200 (**A, C, E**), ×400 (**B, D, F**)).

of acute phase response in the liver are attributed to be cytotoxic for both the liver regeneration and the hepatic differentiation of engrafted stem cells. In this study, we found that engrafted AFMSCs can differentiate into hepatocyte-like cells in failure liver tissue of rat FHF model. And the 21-day survival rates of the PS and GFP/MSCs groups were 38.1%, and while in PS group the 21-day survival rates was 19.3%. On the basis of these findings, it appears likely that prevention of the inflammatory effects of proinflammatory cytokines increase hepatocyte proliferation, and ultimately increased survival rates in earlier period of hepatic

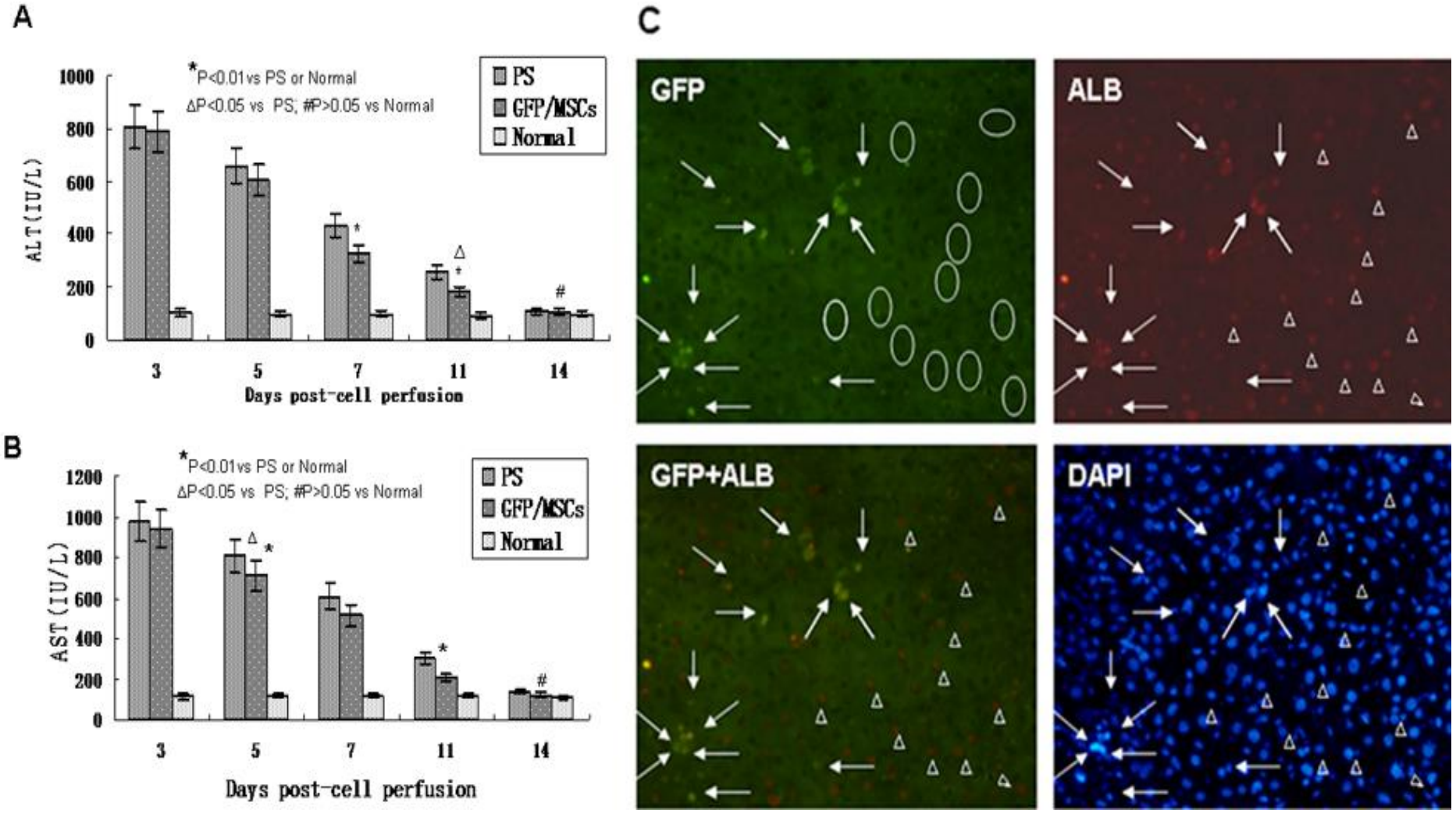

Figure 4. Hepatic function in rat fulminant hepatic failure (FHF) and normal group was assessed by serum liver enzymes after cell perfusion , the incorporation of MSCs into injured livers and differentiation of hepatic MSCs *in vivo*. (**A**,**B**) There were significantly lower alanine aminotransferase (ALT) levels and aspartate aminotransferase(AST) in the normal and green fluorescent protein (GFP)/MSC rats than those in the PS groups by day 5 after cell transplantation. * $P < 0.01$, when compared to the normal and the PS group. ΔP < 0.05 when compared to the PS group. #P > 0.05 when compared to the the normal group. (**C**) Expression of albumin in GFP-labeled MSCs engrafted in liver tissues, detected by immunofluorescence histochemistry of tissues from GFP/MSC rats at day15 after cell perfusion. A arrow(↑) refers to albumin expression in GFP-labeled MSCs in GFP/MSC rats. A triangle(Δ) refers to albumin expression in hosted liver cells, A ring(O) refers to no GFP expression in hosted liver. (**C**)Original magnification ×200; Alb, albumin; GFP, green fluorescent protein; DAPI, 4′,6-diamidino-2-phenylindole.

failure.

Besides down regulating hepatic inflammatory responses, AFMSCs has higher capacity for hepatic differentiation (detecting the expression of albumin in AFMSCs) and repair impaired hepatic cells resulted from various etiological factors the same as our previous study *in vitro* (Zheng et al., 2008; Peng et al., 2011). And there were no differences in alanine aminotransferase (ALT) levels between GFP/MSC and PS groups at day 3, day 5 and day 14, and in aspartate aminotransferase (AST) at day 3, day 7and day 14. And maybe because there is some compensated repairing or stimulating by other endogenous growth factor (p.e. HGF) produced in PS group and GFP/MSCs groups. However, there were significantly lower ALT levels in GFP/MSC rats than those in the PS group at day 7 and day11 after cell transplantation, the same pattern as AST levels at day 5 and day 11. The serum transaminases including ALT and AST were usually regarded as sensitive indicators of hepatocellular damage as previously describ Collier and Bassendine, 2002). Moreover, all of the rats with high ALT levels in the PS and GFP/MSC groups died first week after liver failure. Our study has also demonstrated that AFMSCs with expression GFP were transfused into the injured liver and contributed to extenuating hepatocytes necrosis which was supported by histological analyses. Recently, some study reports also suggested bone marrow derived stem cells migrated into the injured liver and contributed to liver regeneration (Gehling et al., 2005). However, there were insufficient hepatocyte regeneration and hepatic differentiation effects of mesenchymal stem cells under hepatic injuries conditions ((Popp et al.(2007). In our study, necrotic hepatocytes counts in histological analyses was also significantly higher in PS groups than that in GFP/MSCs groups on days 14 after cell perfusion. Siegel et al. (2007) reported that AFMSCs were used as a new tools to study human genetic diseases and are suitable for transfection with exogenous genes. On the basis of these findings, we decided to utilize AFMSCs in our experiments.

In the present study, we first expanded AFMSCs *ex vivo* and then transfused these stem cells into the portal vein of rats with FHF. Our findings also indicate that transfusion of GFP/MSCs into rat with FHF significantly improved liver function and reduce hepatocellular damage, ultimately ameliorate the 21-day survival rate.

Other studies (Moriscot et al., 2005) have shown that transfused MSCs were observed in the sinusoid for the first 1 week before, migrated into liver parenchyma 1 week after engraftment, and further differentiated into hepatocyte-like phenotypes at least two weeks. Our results show that GFP/MSCs enhanced the regeneration of hepatocyte by attenuating intrahepatic inflammatory responses, ultimately leading to a significant improvement in the survival rate of rats, may through enhanced liver cell proliferation and decreased cell death.

Although, our previous study showed that bone marrow-derived mesenchymal stem cells (BM-MSCs) represent a promising source of autologous cells of cell therapies for liver failure (Peng et al., 2011). However, therapeutic efficacy were also limited to some extent, that is to say, BM-MSC therapy is limited mainly by poor cell and significant decrease in both quantity and differentiation potential of BM-MSCs with age (Stenderup et al., 2003). However, recent study showed that AFMSCs can be used as new tools to investigate and treat human different disease (Siegel et al., 2007). Herein, human AFMSCs were perfused to liver which may be AFMSCs a valuable and promising therapy for human diverse terminal liver diseases.

At the same time, we showed that AFMSCs could be efficiently infected by lentiviral vectors and retained a high hepatic differentiation potential, a genetically modified with a therapeutic gene, and reserved the ability to differentiate into different lineages (Grisafi et al., 2008).

The major risks to be considered for research with HIV-1 based lentivirus vectors are potential for generation of replication-competent lentivirus (RCL), and potential for oncogenesis. These risks all can be mitigated by the nature of our vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector. In this context, it should be noted that our single-injection gene delivery system using a lentiviral vector was proven to be a safe approach, and no tumor formations or other lesions were observed in the relevant organs (data not shown).

In conclusion, our studies showed that AFMSCs participate in the suppression of liver local inflammatory responses and may be also down regulating systemic immuno-inflammatory responses; this study section still needs further researches *in vivo*. Transfused AFMSCs not only differentiates into hepatocyte-like cells with expression ALB in the liver, but also repair damage hepatocytes and accelerates hepatocyte, and suppresses lesion in hepatocytes in FHF model during post-cells perfusion period. These AFMSCs graft subsequently differentiates into hepatocyte-like cells and is instrumental in increasing the survival rate with FHF.

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Abbreviations: AFMSCs, Amniotic fluid derived mesenchymal stem cells; **FHF,** fulminant hepatic failure; **GFP,** green fluorescent protein; **BM-MSCs,** bone marrow-derived mesenchymal stem cells; **BAL,**

bioartificial liver; **ESCs,** embryonic stem cells; **iPSCs,** induced pluripotent stem cells; **ALT,** alanine aminotransferase; **AST,** aspartate aminotransferase; **ALB,** albumin; **Lv,** lentiviral vectors.

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