effects that significantly contribute to tissue repair in injured livers [2]. MSCs can be isolated from various adult connective tissues, including bone marrow and adipose tissues, the placenta, amniotic fluid, and umbilical cord blood [3, 4]. MSCs initially attracted research interest due to their ability to differentiate into cells of the mesodermal lineage. However, in recent years, greater attention has been devoted to exploring their capacity to secrete cytokines and growth factors [2, 5–7]. To date, numerous animal studies have demonstrated that MSCs are therapeutically beneficial for the treatment of liver diseases.

Several animal models for acute liver disease have been proposed, and these models have provided a great deal of insight with respect to evaluating the therapeutic efficacy of MSCs for these diseases. The most widely used model of acute liver disease is the carbon tetrachloride (CCl_4) treatment model [8–13]. In this model, hepatitis is induced by reactive metabolic trichloromethyl radicals ($\cdot CCl_3$) and peroxytrichloromethyl radicals ($\cdot OOCCl_3$), which are mainly metabolized from CCl_4 by cytochrome P450 2E1 (CYP2E1) [14]. Because CYP2E1 is preferentially localized in the pericentral zone of the liver acinus, the main sites of liver injury in the CCl_4-induced model are these pericentral regions. Similarly, acetaminophen (AAP) can also be used to generate an acute hepatitis model in rodents [15]. An overdose of AAP results in the generation of *N*-acetyl-*p*-benzoquinoneimine by CYP2E1 [16] and thereby produces hepatocyte necrosis. In contrast, concanavalin A (ConA) causes acute hepatitis through an excessive auto-immune reaction induced by the overproduction of various cytokines, such as tumor necrosis factor-α and interferon-γ [17]. It has been reported that the immunosuppressive effects of MSCs can improve ConA-induced acute hepatitis [18, 19]. The co-administration of lipopolysaccharide, a component of gram-negative cell walls, and D-galactosamine, another hepatotoxin, has also been used for the induction of acute hepatitis in mice [20].

Using the CCl_4-induced hepatitis model, we have demonstrated that human adipose tissue-derived MSCs (hADSCs) significantly contribute to tissue repair in acute hepatitis. Our research group has previously reported that hADSC-derived hepatocyte-like cells (hADSC-Heps) could be generated from hADSCs [9, 11] stimulated with growth factors that induce the differentiation of embryonic stem (ES) cells into hepatocyte-like cells [21]. Importantly, we confirmed that transplanted hADSC-Heps ameliorated liver injury in the CCl_4-induced mouse hepatitis model [9, 11]. Interestingly, however, we observed that in this model, undifferentiated hADSCs produced greater therapeutic effects than hADSC-Heps [10]. This finding has provided support for the notion that the therapeutic effects of hADSCs are mainly produced by the paracrine factors secreted by these cells rather than MSC

functions related to the repopulation of the liver mass. In this chapter, we describe a method to evaluate the therapeutic efficacy of the systemic administration of hADSCs in the CCl_4-induced acute hepatitis mouse model [10].

2 Materials

2.1 Animals

Six-week-old female BALB/c nude mice (CLEA Japan Inc., Tokyo, Japan) were used in this study (*see* **Note 1**).

2.2 Isolation and Culturing of hADSCs

1. 0.15 % type I collagenase in Dulbecco's phosphate-buffered saline without calcium and magnesium (PBS(−)) (*see* **Note 2**).
2. Sterilized surgical scissors.
3. Water bath equipped with a heating circulator.
4. Dulbecco's modified Eagle's medium (DMEM; high glucose, Invitrogen).
5. Fetal bovine serum (FBS).
6. 160 mM NH_4Cl.
7. 40 μm cell strainer (BD).
8. Hemocytometer.
9. MesenPRO RS™ Medium (Invitrogen).
10. Antibiotic-Antimycotic (Invitrogen).
11. GlutaMAX (Invitrogen).
12. CellBIND™ Surface 100 mm dish (Corning).

2.3 Routine Culturing of hADSCs

1. MesenPRO RS™ Medium (Invitrogen).
2. Antibiotic-Antimycotic (Invitrogen).
3. GlutaMAX (Invitrogen).
4. CellBIND™ Surface 100 mm dish (Corning).
5. Accutase.
6. PBS(−).

2.4 Systemic Administration of hADSCs in the CCl_4-Induced Mouse Model of Acute Liver Disease

1. Carbon tetrachloride (CCl_4).
2. Olive oil.
3. 26-G needle.
4. 1 mL syringe.
5. 27-G needle.
6. Mouse holder for intravenous injections.
7. 40 μm cell strainer.

2.5 Sampling of Serum and Liver Tissue

1. Isoflurane.
2. 24-G needle.
3. 1.5 mL tube.
4. PBS(−) containing 10 % formalin.

2.6 Histological Analyses of Mouse Liver Sections After Cell Transplantation

1. Hematoxylin.
2. Eosin.
3. Anti-human leukocyte antigen (HLA) class I antibody (clone W6/32; Sigma, 1:250).
4. Alexa Fluor 594 (Invitrogen).

3 Methods

3.1 Isolation and Culturing of hADSCs

This portion of the methods section is based on a protocol that was previously published by our laboratory [22].

1. Use surgical scissors to mince adipose tissue into pieces that are less than 3 mm in size. Collect these tissue pieces into a tube, add an equal volume of PBS(−), and mix vigorously at room temperature.
2. Let the mixture stand at room temperature until it separates into two phases.
3. Collect the upper phase, which contains stem cells, adipocytes, blood, and PBS(−), into a new tube, and wash this phase three times with fresh PBS(−). Discard the lower phase.
4. Add an equal volume of PBS(−) containing 0.15 % type I collagenase (thus achieving a final collagenase concentration of 0.075 %), and shake the resulting mixture for 30 min in a 37 °C water bath.
5. Add an equal volume of DMEM containing 10 % FBS, shake the resulting mixture well, and allow this mixture to incubate for 10 min. The mixture will separate into two phases during this incubation.
6. Discard the upper phase. Centrifuge the lower phase at 280 × *g* for 5 min at room temperature.
7. Resuspend the cellular pellet in 5 mL of 160 mM NH_4Cl over the course of 3 min. Filter the resulting mixture through a 40 μm cell strainer into a new tube containing 5 mL DMEM with 10 % FBS.
8. Centrifuge at 280 × *g* for 5 min at room temperature.
9. Dissolve the cell pellet in MesenPRO RS™ complete medium (*see* **Note 3**), and seed the cells onto CellBIND™ Surface 100 mm dishes at 1.0–5.0 × 10^4 cells/cm^2 (*see* **Note 4**).

3.2 Routine Culturing of hADSCs

When cells reach 70–90 % confluence, passage them as follows.

1. Wash the cells twice in PBS(−).
2. Add 1 mL accutase to each 100 mm dish. Incubate each dish for 5 min at 37 °C.
3. Tap the dish, use 5 mL/dish of MesenPRO RS™ complete medium to collect cells into a tube, and centrifuge cells at 220 × *g* for 5 min at room temperature.
4. Resuspend the cells in MesenPRO RS™ complete medium, count the cell number, and seed the cells into new CellBIND™ dishes at a concentration of 5×10^3 cells/cm^2.

3.3 Intravenous Administration of hADSCs in the CCl_4-Induced Mouse Model of Acute Liver Disease

1. To ensure the acquisition of sufficient numbers of cells, plate hADSCs 2–5 days prior to the intravenous injection of these cells into mice.
2. Prepare diluted CCl_4 solution by mixing one volume CCl_4 with nine volumes olive oil.
3. Intraperitoneally inject mice with 100 μL diluted CCl_4 solution/20 g body weight (10 μL CCl_4/20 g body weight).
4. To establish a sham operation, intraperitoneally inject mice with 100 μL olive oil/20 g body weight.
5. Twenty-four hours after CCl_4 injection, perform the intravenous injection of hADSCs as follows.
6. Wash the cells twice in PBS(−).
7. Add 1 mL accutase per 100 mm dish, and incubate dishes at 37 °C for 5 min.
8. After tapping the dishes, use 5 mL/dish of MesenPRO RS™ complete medium to collect cells into a tube.
9. Remove cell aggregates by filtering the cell suspension through a 40 μm cell strainer into a new tube.
10. Centrifuge the cells at 220 × *g* for 5 min at room temperature.
11. Resuspend these cells in 0.5–1 mL PBS(−), and count the number of cells obtained.
12. Use PBS(−) to dilute the cell suspension to 7.5×10^6 cells/mL, and store the suspension on ice until it is injected into mice (*see* **Note 5**).
13. Load the hADSC suspension into a 1 mL syringe and equip this syringe with a 27-G needle (*see* **Note 6**).
14. Slowly inject 200 μL ADSC suspension/mouse (1.5×10^6 cells/mouse) into the tail veins of the mice (*see* **Notes 7** and **8**).

3.4 Blood and Tissue Sampling

1. Use isoflurane to anesthetize mice 24 h after the injection of hADSCs.
2. Open the chest of each mouse with surgical scissors to expose the heart.

3. Insert a syringe with a 24-G needle into the left ventricle. Slowly collect blood into the syringe (*see* **Note 9**).
4. After collecting this blood sample, extract the liver of the mouse. Wash the liver once in PBS(−), and fix the extracted liver by soaking it in 10 % formalin.
5. Collect blood into a 1.5 mL tube and incubate this tube at room temperature for 30 min.
6. Incubate the tube at 4 °C for 1 h.
7. Centrifuge the tube at 2,200 × *g* for 20 min at 4 °C.
8. Transfer the supernatant into a new tube.
9. Centrifuge this tube at 2,200 × *g* for 5 min at 4 °C.
10. Carefully collect the supernatant (serum) and transfer it into a new tube (*see* **Note 10**).
11. Use serum samples for blood tests, or store these samples at −20 °C until use (*see* **Note 11**).

3.5 Histological Analyses of Liver Tissue

1. Fix the collected liver tissue in PBS(−) containing 10 % formalin, and prepare a paraffin block. Use a general sectioning procedure to obtain 3–5 μm sections.
2. Utilize a generally accepted procedure to perform hematoxylin and eosin (HE) staining (*see* **Note 12**).
3. To detect human hADSCs in the livers of immunodeficient mice, perform immunofluorescent staining of mouse liver tissue using an anti-HLA-1 antibody (*see* **Note 13**).

4 Notes

1. Animals are maintained in an isolator unit at a constant temperature of 20 °C and subjected to a 12 h light–dark cycle. Mice receive a standard sterilized diet and water ad libitum. All experiments were performed in accordance with national laws and institutional regulations.
2. Dissolve 0.015 g collagenase in 10 mL PBS(−) by layering the powder on the surface of the liquid to avoid clumping. After the powder has completely dissolved, sterilize the solution by filtration through a 0.22 μm filter.
3. Prepare MesenPRO RS™ complete medium by supplementing 500 mL of basal medium with 10 mL growth supplement, 5 mL Antibiotic-Antimycotic, and 5 mL GlutaMAX.
4. The cells exhibit a spindle-shaped morphology that is characteristic of MSCs (Fig. 1a) and express CD105 (endoglin) (Fig. 1b). CD105, which is a component of the receptor complex of transforming growth factor (TGF)-β, is involved in various cellular

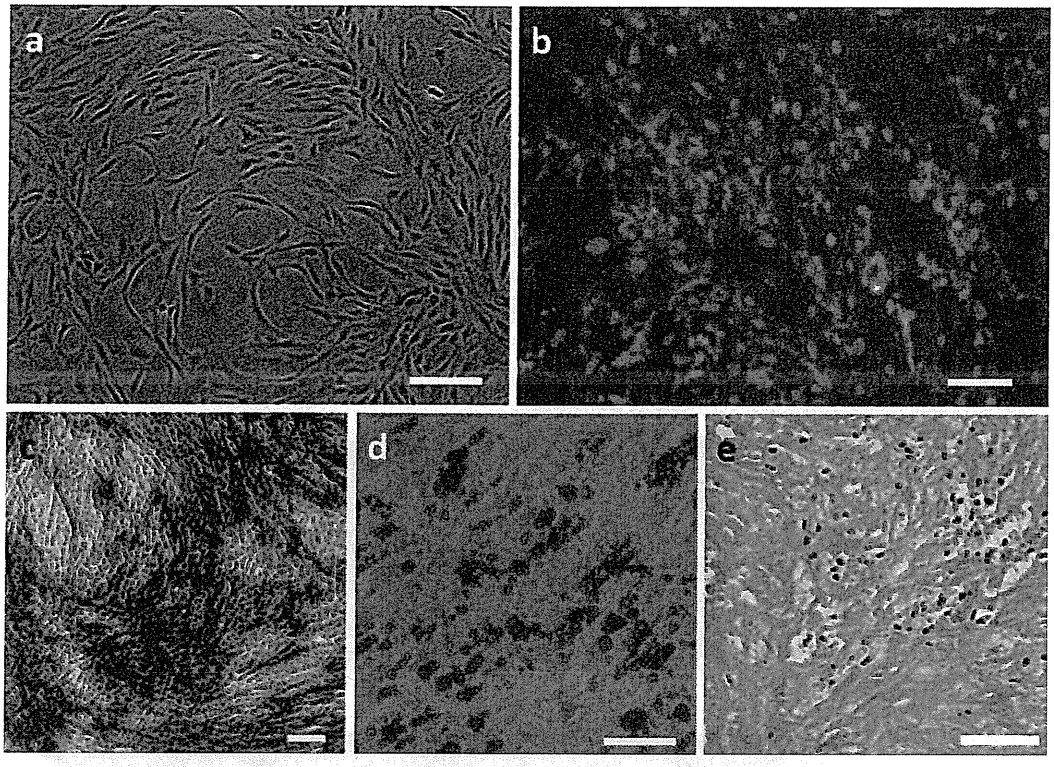

Fig. 1 Characterization of isolated hADSCs. (**a**) Phase contrast images of isolated hADSCs indicate the spindle-shaped morphology of these cells, which is a characteristic feature of MSCs. Scale bar: 200 μm. (**b**) hADSCs are positive for CD105 (*green*), an important molecule for maintaining MSC characteristics [9]. Nuclei are counterstained with Hoechst 33342 (*blue*). Scale bar: 100 μm. (**c**) Alkaline phosphatase staining reveals the osteogenic differentiation of hADSCs. Scale bar: 100 μm. (**d**) Oil red O staining reveals the adipogenic differentiation of hADSCs. Scale bar: 100 μm. (**e**) Alcian blue staining reveals the chondrogenic differentiation of hADSCs. Scale bar: 50 μm

events, including proliferation, differentiation and migration. The cultured cells are also multipotent. In particular, these cells can differentiate into adipocytes, osteoblasts, and chondrocytes (Fig. 1c–e). In accordance with the manufacturer's instructions the following commercial kits are used for the differentiation of hADSCs into three mesodermal lineages: hMSC Mesenchymal Stem Cell Adipogenic Differentiation Medium (Lonza), hMSC Mesenchymal Stem Cell Chondrocyte Differentiation Medium (Lonza), and hMSC Mesenchymal Stem Cell Osteogenic Differentiation Medium (Lonza). Surface marker characterization by flow cytometry indicates that these cells are positive for CD105, CD73, CD90, and CD44 but negative for CD45, CD31, and CD34 [23].

5. hADSCs are likely to aggregate at room temperature. This aggregation may cause pulmonary embolisms in mice injected with these cells.

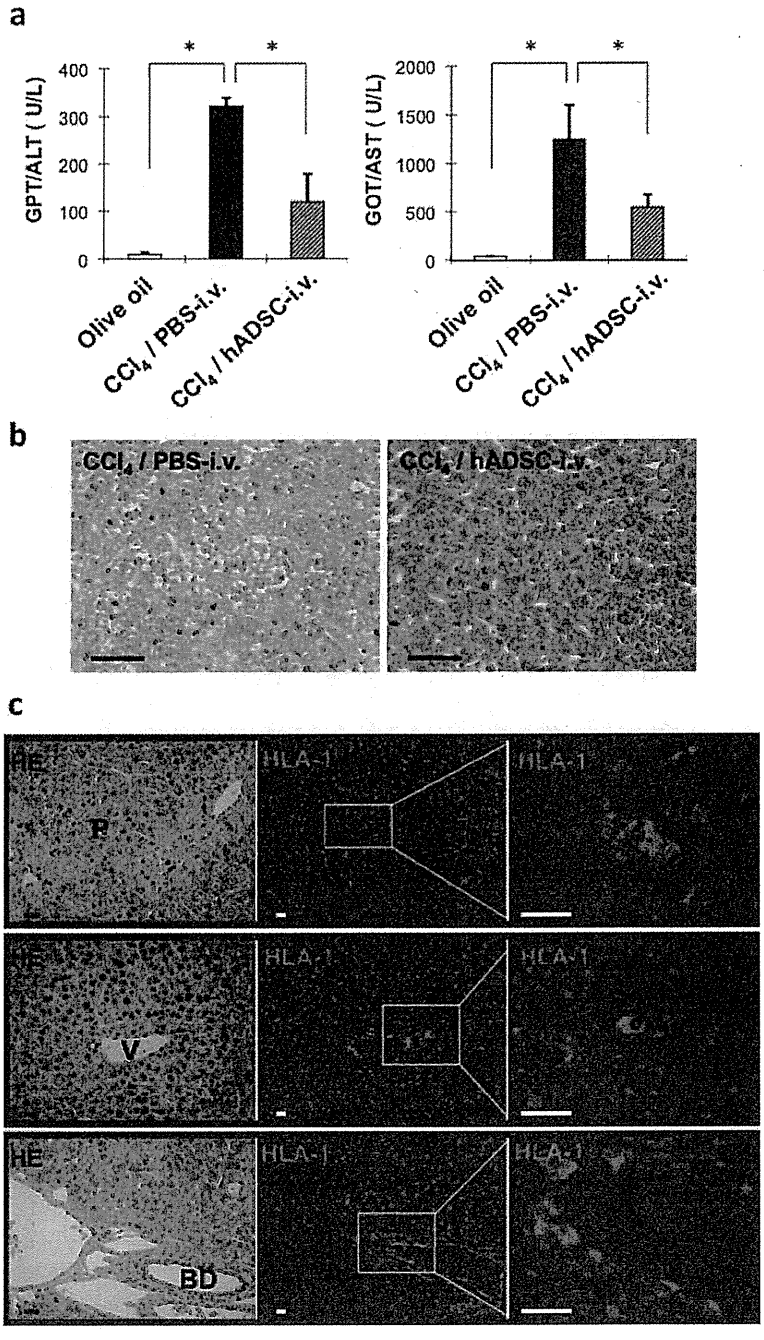

Fig. 2 Therapeutic efficacy of systemically transplanted hADSCs in the CCl4-induced acute hepatitis mouse model. (**a**) Biochemical analysis of mouse blood serum samples for the liver injury markers GPT/ALT (*left*) and GOT/AST (*right*). Immunodeficient mice received an intraperitoneal injection of either olive oil (a control for the CCl_4 injection) or 10 μL CCl_4/20 g body weight. At 24 h after this injection, the CCl_4-injected mice received an intravenous injection of either PBS(–) (a control for hADSC administration) or 1.5×10^6 hADSCs/mouse. Data are expressed as means ± S.D. and subjected to analysis using the Bonferroni

6. When loading cells into a 1 mL syringe, thoroughly mix the cell suspension by pipetting because the cells will tend to fall to the bottom wall of the tube. Do not attach a needle to the syringe prior to loading the cells because the use of a syringe with an attached needle may damage the cells.
7. In our experience, a tail vein injection of more than 2×10^6 cells/mouse is associated with an increased risk of pulmonary embolism.
8. If more than 5 min are required to complete an injection, reloading of the cell suspension is recommended to avoid precipitation of cells.
9. Rapid drawing of the syringe may cause hemolysis.
10. Leave a small portion of the supernatant in the tube to ensure that the serum samples are not contaminated by the pellet.
11. In our laboratory, we use the DRI-CHEM system (Fuji) to measure blood markers of liver injury, such as serum levels of GPT/ALT, GOT/AST, ammonia, uric acid, and blood urea nitrogen. We have observed significant improvement in liver injury markers, particularly with respect to GPT/ALT and GOT/AST levels (Fig. 2a).
12. This staining reveals that hADSC administration produces significant morphological changes in hepatocytes in non-necrotic regions (Fig. 2b). Relative to injured livers from control mice, injured livers from mice that received hADSCs exhibit lower levels of vacuolar degeneration caused by the dilatation of mitochondria and the rough endoplasmic reticulum.
13. We detected hADSCs within the injured mouse liver 24 h after these cells were injected into the mice. HLA-1 positive cells were found in various areas of the examined mouse livers, including the parenchyma, vessels, and bile ducts (Fig. 2c).

Fig. 2 (continued) correction; $n=3$. (*$p<0.05$). (**b**) Histological analysis of CCl_4-injured liver sections. This figure presents HE-stained images of mouse livers 24 h after an intravenous injection of either PBS(–) (*left*) or 1.5×10^6 hADSCs/mouse (*right*). These mice received an intraperitoneal injection of CCl_4 24 h prior to the administration of hADSCs. Scale bars: 100 μm. (**c**) Immunohistochemical analyses for human leukocyte antigen 1 (HLA-1)-positive cells in mouse liver sections after the administration of hADSCs. HLA-1-positive cells are present in different areas of the liver, including the parenchyma (P), vessels (V), and bile duct (BD). The left side of this figure presents the results of HE staining for these areas of the examined liver sections. Scale bars: 500 μm. This figure is reproduced from ref. 10

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Review Article

Adipose Tissue-derived Mesenchymal Stem Cells in Regenerative Medicine Treatment for Liver Cirrhosis – Focused on Efficacy and Safety in Preclinical and Clinical Studies

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- Adipose
- Mesenchymal stem cell
- Liver cirrhosis
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Abstract

Stem cell therapy, including mesenchymal stem cell (MSC) therapy, is a promising therapeutic option for treating several diseases. Adipose tissue-derived mesenchymal stem cells (AT-MSCs) have been identified as a candidate for stem cell therapy. Sources of MSCs include bone marrow, umbilical cord, amniotic fluid, and adipose tissue. Adipose tissue can be easily harvested using procedures that are minimally invasive compared with those used to obtain the other sources, and it is suitable for regenerative medicine treatments.

End-stage cirrhosis and chronic liver failure are life-threatening liver diseases. Liver transplantation is an effective therapy for end-stage liver disease, but most patients are unable to undergo liver transplantation because of the limited supply of donors, the complex surgical procedure, rejection, pre-existing disease recurrence, and high costs.

AT-MSCs are a promising candidate for regenerative medicine to treat liver cirrhosis. Over the past decade, the literature on non-clinical studies and clinical trials for liver diseases has been accumulating, and we can speculate on the efficacy and safety of MSC therapy. The mechanisms of the curative effects of AT-MSCs have been clarified insufficiently. However, a large number of reports indicate that the hepatoprotective effect of AT-MSCs is related to a paracrine effect of soluble mediators rather than the differentiation potency of the cells. In this review, we summarize the efficacy and safety of AT-MSC use and the current preclinical studies and clinical trials of AT-MSCs.

ABBREVIATIONS

AT-MSCs: Adipose-Tissue derived Mesenchymal Stem Cells; **BM-MSCs:** Bone Marrow-derived Mesenchymal Stem Cells; **CCl_4:** Carbon Tetrachloride; **ECM:** Extracellular Matrix; **HGF:** Hepatocyte Growth Factor; **HLA:** Human Leukocyte Antigen; **HSCs:** Hepatic Stellate Cells; **IL:** Interleukin; **MELD:** Model for End-Stage Liver Disease; **MMP:** Matrix Metalloproteinase; **MSCs:** Mesenchymal Stem Cells; **NASH:** Non-Alcoholic Steatohepatitis; **PDGF-β:** Platelet-Derived Growth Factor-β;

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TGF-β: Transforming Growth Factor-β; **TIMP:** Tissue Inhibitor of Matrix Metalloproteinase; **UC-MSCs:** Umbilical Cord-derived Mesenchymal Stem Cells;

INTRODUCTION

End-stage cirrhosis and chronic liver failure are life-threatening liver diseases. The most effective therapy for patients with advanced cirrhosis is liver transplantation. However, most patients are unable to undergo liver transplantation because of the limited availability of donors, the complex surgical procedure, rejection, pre-existing liver disease recurrence, and high costs [1,2].

Stem cell therapies, including those using mesenchymal stem cells (MSCs), are promising for the treatment of end-stage liver disease [3]. The tissue origins of MSCs include bone marrow [4], umbilical cord [5], amniotic fluid [6,7], and adipose tissue [8,9].

Adipose tissue can be easily harvested through a less invasive procedure than used to obtain MSCs from other sources, and it is a promising source of MSCs to be used as a regenerative medicine treatment for various diseases, including hepatic failure [10-12]. There is accumulating evidence for hepato-curative effects of adipose tissue-derived mesenchymal stem cells (AT-MSCs). In this review, we summarize the efficacy and safety of AT-MSC use and the clinical trials of AT-MSCs.

AT-MSCs

MSCs are a promising candidate for regenerative medicine. According to recommendation, International Society for Cell Therapy, the criteria to define human MSCs are that they must adhere to plastic in standard culture conditions and that the cells must express the markers CD105, CD90 and CD73 but not the markers CD45, CD34, CD14, CD11b, CD79a, CD19 and human leukocyte antigen (HLA) -DR. Moreover, the cells must have osteogenic, adipogenic and chondrogenic differentiation potential under standard in vitro differentiation conditions [13]. Bone marrow was the first organ reported to be a source of MSCs, but the isolation procedure for bone marrow is the most invasive procedure of all of the MSC sources.

In particular, it is thought that AT-MSCs are ideal for developing regenerative medicine. The advantages of using adipose tissue as a source of MSCs include its abundance and easy access for harvesting [10,14]. Furthermore, a comparative analysis of MSCs from bone marrow, umbilical cord and adipose tissue has been reported [15]. According to this report, adipose tissue contains MSCs at the highest frequency, and there are no morphological or immune phenotypic differences among bone marrow, umbilical cord and adipose tissue as sources.

Many reports indicate that AT-MSCs have more therapeutic effects than other sources of MSCs. MSCs have immunomodulatory effects on various immune cells such as T-cells [16], B-cells [17], natural killer cells [18] and dendritic cells [19]. These properties of MSCs make it possible to control the autoimmune diseases and graft-versus-host-disease [20]. Many studies indicate that AT-MSCs have more pronounced immunomodulatory effects compared with other MSCs sources such as bone marrow and umbilical cord [21,22]. Furthermore, other pre-clinical studies demonstrated that treatment of AT-MSCs are more effective on hindlimb ischemia [23], wound healing [24] and spinal cord injury [25] than bone-marrow derived MSCs (BM-MSCs).

Pathogenesis of liver cirrhosis

Liver cirrhosis is characterized by extensive fibrosis caused by chronic hepatic injury. The major causes of liver fibrosis are infection with hepatitis B virus, infection with hepatitis C virus, alcoholic steatohepatitis and nonalcoholic steatohepatitis (NASH). Liver fibrosis is the excessive accumulation of extracellular matrix (ECM) in the space of Disse following both the increased synthesis and decreased degeneration of ECM [26,27].

Hepatic stellate cells (HSCs) are the key source of ECM synthesis in the damaged liver. Quiescent HSCs, which synthesize a small amount of ECM, are activated by soluble mediators and differentiate into myofibroblasts, which are the main source of ECM. It is well known that augmented tissue inhibitor of matrix metalloproteinase (TIMP1) expression derived from hepatic myofibroblasts plays a pivotal role in tipping the balance between the production and the degradation of ECM components [28]. TIMP1 promotes the accumulation of ECM in damaged liver through the inhibition of matrix metalloproteinases (MMPs), a family of enzymes that degrade ECM components.

HSCs are activated by soluble mediators, such as transforming growth factor-β (TGF-β) [29,30] and platelet-derived growth factor-β (PDGF-β) [31]. TGF-β is considered to play a pivotal role in the progression of liver fibrosis through the augmentation of ECM synthesis by HSCs. In addition, TGF-β suppresses ECM degeneration through not only the blockade of MMP expression but also the facilitation of TIMP1 expression [29]. In contrast, it is thought that PDGF-β is the most potent mitogen for HSCs. PDGF-β is upregulated in the fibrotic liver, and PDGF-β inhibition attenuates liver fibrosis in vivo [31] (Figure 1).

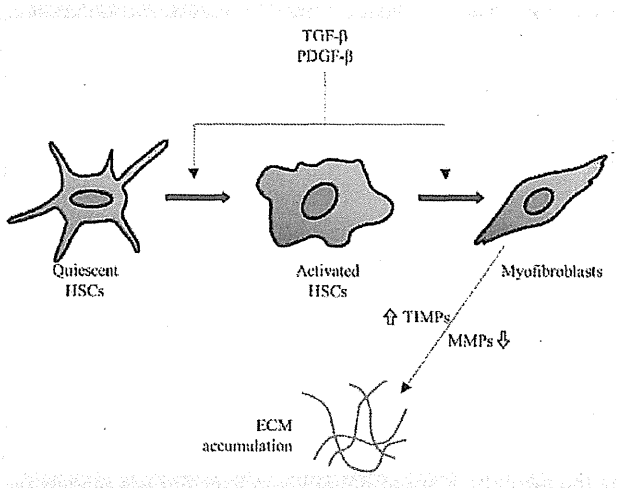

Figure 1 Quiescent hepatic stellate cells (HSCs) are activated by soluble mediators such as transforming growth factor-β (TGF-β) and platelet-derived growth factor-β (PDGF-β). The activated HSCs are further activated by soluble mediators and differentiate into myofibroblasts. These myofibroblasts up-regulate the expression of tissue inhibitor of matrix metalloproteinases (TIMPs) in damaged liver. TIMPs promote the accumulation of extra celler matrix (ECM) in damaged liver through the inhibition of ECM degradation by matrix metalloproteinases (MMPs) activity [17].

Therapeutic potential of AT-MSCs

Many pre-clinical studies have demonstrated that AT-MSCs have a hepato-curative effect in animal models of acute and chronic liver diseases [32-43]. AT-MSCs attenuate impaired liver function and tissue damage in rodent models of acute hepatitis induced by carbon tetrachloride (CCl_4) [32], concanavalin A [33,34], acetaminophen [35], ischemia reperfusion [36,37] and combination of retrorsin and allylalcohol [38]. Furthermore, AT-MSCs ameliorate the liver dysfunction and the histological changes that occur with the fibrogenesis induced by CCl_4 [39,40], thioacetamide [41] and NASH [42] in mice. It has also been reported that AT-MSCs have therapeutic efficacy in the acute-on-chronic liver failure rabbit model [43].

The mechanisms of the protective effects of AT-MSCs on hepatic injury are not fully understood, but they can be ascribed to several possible mechanisms. However, there is still debate about these potential mechanisms. MSCs have a homing capacity to injured organs [44,45]. MSCs home to the endothelial cells through interactions with integrins and vascular cell adhesion molecule-1 [46]. Additionally, MSCs display rolling and adhesion behavior on endothelial cells, where CXC-chemokine receptors-4 and its ligand stromal-derived factor-1 play a crucial role in this behavior [47]. MSCs then migrate across the endothelium and invade the injured organ. Tracking AT-MSCs with an in vivo imaging system has revealed that AT-MSCs accumulate in damaged livers in mice [14].

Many studies have demonstrated that MSCs secrete various molecules, such as cytokines, chemokines and growth factors [48]. AT-MSCs secrete many soluble factors, such as interleukin (IL) -1RA, IL-6, IL-8, hepatocyte growth factor (HGF), nerve growth factor, monocyte chemoattractant protein-1, granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. AT-MSCs secrete these factors more abundantly than either BM-MSCs or normal human dermal fibroblasts [22]. In particular, it is thought that HGF has hepato-protective effects through the inhibition of HSC activation [49]. HGF has preventive and therapeutic effects on liver cirrhosis in rats through growth inhibition and apoptosis induction in myofibroblasts that are activated in cirrhosis. HGF also has a suppressive effect on collagen I and IV synthesis in HSCs [50]. Furthermore, HGF enhances MMP expression and activity [51].

In the case of BM-MSCs, TIMP1 and MMP expression is affected by BM-MSC administration in liver fibrosis models. According to these studies, the application of BM-MSCs suppresses the upregulated expression of TIMP1 in mice with liver fibrosis [52,53]. In contrast, MMP expression is promoted by the administration of BM-MSCs in liver fibrosis models [53-55]. AT-MSCs are also expected to regulate TIMP1 and MMP expression in cirrhosis.

Taken together, it is suggested that AT-MSCs home to damaged livers, where they secrete various molecules, such as HGF. These molecules suppress the activation of myofibroblasts in fibrotic livers, resulting in the degeneration of ECM, which most likely occurs through the suppression of TIMP1 expression and the promotion of MMP expression.

Safety issues of AT-MSCs

Preclinical toxicity and tumorigenicity tests of AT-MSCs conducted under Good Laboratory Practice conditions have been reported [56]. Toxicity symptoms were found not to occur for 13 weeks in mice, even at the highest dose of AT-MSCs (2.5×10^8 cells/kg) administered via the tail vein. Similarly, with a subcutaneous injection at the same dose, no evidence of tumorigenicity was found for 26 weeks using the toxicity test in immunodeficient mice. For large animals, a 6-week toxicity study using an intravenous administration route for $2x10^6$ and $1x10^7$ cells/kg umbilical cord-derived MSCs (UC-MSCs) in cynomolgus monkeys has been reported, and this report suggested that the transplantation of UC-MSCs does not affect the general health of cynomolgus monkeys [57]. Moreover, the intravenous infusion of AT-MSCs in cats has no complications during or after administration [58].

In contrast, several reports have indicated that transplanted cells may be entrapped in the lungs during their first pass through systemic organs. The intravenous injection of neural progenitor stem cells results in death immediately after administration to mice [59]. Another study has reported that blood microcirculation is interrupted in mice when AT-MSCs are injected into the aorta owing to the large cell diameter [60]. The tissue factor has a critical role in promoting MSC-mediated coagulation in mice, and its expression likely leads to thromboembolism. An anti-coagulant agent has also been suggested to be useful for avoiding embolism [61]. Recently, another group has indicated that cell size and infusion velocity are critical factors for developing safe protocols for intracarotid stem cell transplantation in rats [62].

AT-MSCs have advantageous characteristics that allow allogeneic transplantation without immune rejection. AT-MSCs are immunoprivileged because they have intermediate expression levels of HLA-I and undetectable expression levels of HLA-II and because they do not express co-stimulatory molecules, such as CD80, CD86 and CD40 [63,64].

In summary, most of the pre-clinical toxicity reports indicate no side effects resulting from the administration of MSCs, including AT-MSCs. However, there is a risk of embolism induced by the intravenous injection of MSCs in accordance with the studies using rodents. Hence, we should be careful to avoid embolism when clinical trials are conducted.

Clinical trials

In this review, we summarized some of the clinical trials in which MSCs have been used to treat liver cirrhosis. In the clinical trial database (ClinicalTrials.Gov), 47 protocols using MSCs to treat patients with liver cirrhosis are ongoing in the clinical setting (Table 1). Most of these trials are using BM-MSCs or UC-MSCs.

Clinical trials of BM-MSC administration to treat patients with liver failure have been reported. Kharaziha P et al. conducted clinical trials using BM-MSC administration to treat several patients with end-stage liver disease, including hepatitis B and hepatitis C induced disease and alcoholic and cryptogenic cirrhosis [65]. The augmented Model for End-Stage Liver Disease (MELD) score and prothrombin activity in these patients were

Table 1: A summary of the clinical trials of MSCs in cirrhosis.

Source of MSCs	Indication	Phase	Enrolment	Clinical Trials. Gov Identifier	References
adipose	Cirrhosis	-	4	NCT01062750	
bone marrow	Alcoholic cirrhosis	2	11	NCT01741090	[67]
	Chronic hepatitis B-induced liver failure	1/2	Treatment: 53 Control: 105	NCT00956891	[66]
	Cirrhosis(Hepatitis B, Hepatitis C, alcoholic, cryptogenic)	1/2	8	NCT00420134	[65]
umbilical cord	Primary Biliary Cirrhosis	1/2	7	NCT01662973	[71]
	Cirrhosis(Chronic Hepatitis B)	1/2	Treatment: 30 Control: 15	NCT01220492	[69]
	Acute-on-chronic liver failure	1/2	Treatment: 24 Control: 19	NCT01218464	[70]

Abbreviations: MSCs: Mesenchymal Stem Cells

decreased by treatment with BM-MSCs. Peng L et al. and Jang YO et al. reported a curative effect when BM-MSCs were used to treat chronic hepatitis B-induced liver failure and alcoholic cirrhosis [66,67]. Terai et al. reported that bone marrow cell infusions caused a significant amelioration of serum levels of albumin and total protein, and they reported that the Child-Pugh scores in these patients were not adversely affected [68].

Several clinical trials using UC-MSCs to treat cirrhotic patients have been performed. Zhang Z et al. administered UC-MSCs to chronic hepatitis B patients with decompensated liver cirrhosis and ascites [69], and they reported a reduction in ascites volume as well as an improvement in liver functions and MELD scores resulting from treatment with UC-MSCs. Other studies have shown that UC-MSCs improve the survival rate, MELD score and various liver functions in acute-on-chronic liver failure patients [70].

A clinical trial using adipose tissue-derived stromal cells to treat four patients with eligible liver cirrhosis has recently started. This is the first clinical trial using AT-MSCs in cirrhotic patients. In this trial, patients will receive autologous adipose tissue stromal cells through intrahepatic arterial administration, and the endpoint for this trial is safety (ClinicalTrials.Gov NCT01062750).

CONCLUSION

Stem cell therapies, including those using MSCs, are promising alternatives to liver transplantation. AT-MSCs are especially promising because adipose tissue is an abundant and easily accessible source in the body. There is accumulating evidence that AT-MSCs have curative effects on acute and chronic liver failure in animal models. In addition, many toxicological studies have confirmed the safety of AT-MSCs.

Whereas clinical studies of BM-MSC and UC-MSC treatments for patients with serious liver disease have been conducted, clinical trials using AT-MSCs have not yet been conducted, although clinical trials using AT-MSCs are anticipated. According to the clinical trials using BM-MSCs and UC-MSCs, these cells are effective for treating patients with severe liver disease and do not have obvious side effects. AT-MSCs may also have therapeutic potential for liver cirrhosis.

In conclusion, AT-MSCs are a promising regenerative medicine candidate for treating liver cirrhosis. Researchers are exploring the clinical potential of AT-MSCs while considering the safety issues.

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