Prof. Tong Cao DDS PhD, September 7, 2018

Editor-in-Chief,

World Journal of Stem Cells

Department of Oral Sciences, National University of Singapore,

Singapore 119083, Singapore

Dear Prof. Cao,

MINIREVIEW in Basic Studies “Protein factors based on liver cirrhosis with adipose-derived mesenchymal stem cells transplantation”

We would like to thank you for the opportunity to revise the paper that we submitted. We greatly appreciate the high-level comments of the four reviewers who improved the quality of our paper.

- Reviewer 1 suggested that we summarize this paper in a manner that is easy for the reader to understand. We added a sentence to the Abstract based on the advice of the reviewer.
- Reviewer 2 pointed out that the title does not represent the essence of this paper; we therefore revised the title. In addition, Reviewer 2 noted that the pathological definitions and names of liver diseases should be clearly explained. We added these explanations. Furthermore, Reviewer 2 instructed us to describe the background for selecting each of the protein components described in this paper in detail. We added this explanation.
- Reviewer 3 indicated their acceptance of the paper.
- Reviewer 4 instructed us to give our opinions as to what we think about the use of FBS (a component derived from bovine serum). We noted that we have not used FBS for clinical cell cultures. Reviewer 4 also instructed us to improve the clarity of our descriptions as there were many points of ambiguity. We added extra sentences to the text to resolve this matter.

We hope that our manuscript is now suitable for publication in the *World Journal of Stem Cells*.

Respectfully yours,

Hirofumi Noguchi, MD, PhD. (Editorial Board Member of WJSC)

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Response letter

Manuscript NO: 41507
Title: Protein factors in adipose-derived mesenchymal stem cell transplantation for liver cirrhosis"
Authors: Saifun Nahar, Yoshiki Nakashima, Chika Miyagi-Shiohira, Takao Kinjo, Zensei Toyoda, Naoya Kobayashi, Issei Saitoh, Masami Watanabe, Hirofumi Noguchi and Jiro Fujita.

RESPONSE TO REVIEWER #1:
CONCLUSION: Major revision
We appreciate the reviewers' comments. The reviewer's comments are important for communicating the contents of the paper to the reader in an easy-to-understand manner. Changes to the text are shown in red, and deleted text is shown in red strikethrough.

Comment : *Previous studies demonstrated that adipose-derived mesenchymal stem cells (ADSCs) are a treatment cell source for patients with chronic liver injury. The review described the various cytokines and chemokines produced by ADSCs promote the healing of liver disease.*

Response: We think that the reviewer's comments are important for correctly conveying the contents of this paper to the reader. Based on the reviewer's suggestion, we added the following text to the Abstract:

Previous studies demonstrated that ADSCs are a treatment cell source for patients with chronic liver injury. This review describes the various cytokines and chemokines produced by ADSCs that promote the healing of liver disease. (Line 102).

RESPONSE TO REVIEWER #2:

CONCLUSION: Major revision

We appreciate the reviewer's comments, which have helped to greatly enhance the quality of this paper. The modifications made in response to the comments are shown in red text.

Comment 1: *The title as Main content may be revised to indicate the context of the contents.*

Response: In accordance with the reviewer's suggestion, we changed the title to read as follows: ~~Protein factors in adipose-derived mesenchymal stem cell transplantation for liver cirrhosis~~ Cytokines in adipose-derived mesenchymal stem cells promote the healing of liver disease (Line 9).

Comment 2: *The symptoms of acute liver failure may be described more in detail in the first paragraph.*

Response: In accordance with the reviewer's comment, we added an explanation about acute liver failure in the main text:

Liver failure is a defined as a group of diseases associated with the development of symptoms such as jaundice, ascites, hepatic encephalopathy, bleeding tendency, or the like, due to a decrease in the number of hepatocytes or a decrease in their function. Acute liver failure is defined by the presence of necrosis and inflammation in normal liver tissue, with the period until the onset of symptoms of hepatic insufficiency being within 8 weeks. Cases in which the onset of symptoms is 8–24 weeks or >24 weeks are classified as delayed liver failure (late onset hepatic failure; LOHF) and chronic liver failure, respectively. The American Association for the Study of Liver Diseases (AASLD) published a position paper on acute liver failure in 2005 to unify the terms and disease concepts [45]. (Line 207).

Comment 3: *The reason for selecting growth factors, inhibition of inflammation of hepatic stellate cells and angiogenic factors as important factors for improvement of chronic liver failure symptoms may be added briefly around line 200 in page 7.*

Response: In accordance with the reviewer's suggestion, we added the reasons for focusing on "Growth factors", "Inflammation inhibitor of hepatic stellate cells", and "Angiogenic factors" to the Main text:

When the disordered repair process is delayed or inhibited after liver damage from drugs, trauma, inflammation, or other insults, liver regeneration is insufficient and hepatic failure develops. In hepatic tissue repair, in addition to growth factors that promote hepatocyte proliferation, angiogenic factors that promote hepatic microvascular remodeling are important. In addition, the extracellular matrix in the liver is mainly produced in hepatic stellate cells. The ability of hepatic stellate cells to produce extracellular matrix is low in the normal liver. However, in the fibrous liver, it is known that hepatic stellate cells are activated to differentiate into myofibroblasts and their ability to produce extracellular matrix markedly increases. Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), hepatocyte growth factor (HGF), and other factors secreted from hepatic stellate cells (HSCs), hepatic sinusoidal endothelial cells (HECs) and Kupffer cells are thought to have the greatest influence on hepatocyte proliferation [47]. Angiogenesis is a physiological phenomenon in which a new blood vessel branch is branched from an existing blood vessel to construct a vascular network. The various factors involved in angiogenesis include fibroblast growth factor (FGF), vascular endothelial cell growth factor (VEGF), angiopoietin, and platelet derived growth factor (PDGF). (Line 228).

Comment 4: *The abbreviations for POSTN, SAP, SEM7A and PTK7 etc. should be described at the first time described.*

Response: We defined the abbreviations on first use according to the reviewer's instruction, as follows:

The proteins associated with a growth function (GO analysis), identified by the presence of ADSC-CM, were Periostin (POSTN), P component (SAP), semaphorin 7A (SEM7A), and Inactive tyrosine-protein kinase 7 (PTK7) [40, 41]. Periostin, which is encoded by the POSTIN gene, has been reported to be an extracellular factor that promotes hepatosteatosis [59, 60]. Nevertheless, much remains unknown about proteins with the ability to promote the cellular proliferation of hepatocytes. For example, SAP, a protein that is expressed in hepatocytes and secreted into serum, is known to be involved in processes associated with immune regulation, such as the action of opsonins [61], but whether SAP is involved in the cellular proliferation of hepatocytes is unknown. Further, SEM7A is known to contribute to TGF-β-mediated hepatic fibrosis [62], but whether it promotes hepatocyte cell proliferation is unknown. (Line 268).

Comment 5: *In Conclusion, the description about CXCL5 as a component to promote hepatocyte proliferation in front of (3) ADSC-secreted VEGF, HGF, EGF, MMP2, POSTN and MFGM are,,, is quite confusing since CXCL5 is classified as (2) inhibitors of inflammation of hepatic stellate cells in the main text. The conclusion may be revised to be clearer.*

Response: In accordance with the reviewer's comment, the conclusion was rearranged as follows:

The factors necessary for improvement of the symptoms of chronic liver failure (i.e. liver cirrhosis) are: (1) growth factors, (2) inhibitors of hepatic stellate cell inflammation, and (3) angiogenic factors. (1) It is certain that ADSC-secreted HGF and VEGF are among the factors that promote the proliferation of hepatocytes. In addition, CXCL5 was identified as a component that promotes hepatocyte proliferation. (2) MIF, which was one of the ADSC-secreted proteins that we identified that suppressed liver fibrosis. ~~In addition, CXCL5 was identified as a component that promotes hepatocyte proliferation.~~ Finally, (3) ASDC-secreted VEGF, HGF, EGF, MMP2, POSTN and MFGM are factors that promote hepatic angiogenesis. (Line 430).

Comment 6: *This manuscript can be accepted without figures 3 and 4, if it is a minireview as shown in the file. The materials and methods and more detailed description are needed, if it is basic study research article.*

Response: Based on the opinions that the other reviewers expressed in relation to Figures 3 and 4, we moved Figures 3 and 4 to the Supplemental section.

RESPONSE TO REVIEWER #3:
CONCLUSION: Accept (General priority)
We appreciate the reviewer's evaluation of our paper.

Comment : *My comments included in the attached manuscript.*
Response: We appreciate this acceptance from the reviewers.

RESPONSE TO REVIEWER #4:

CONCLUSION: Minor revision

We appreciate the high-level comments made by the reviewers, who brought a broad perspective to the argument of the paper. The reviewer's comments were very important for strengthening the clinical evidence of our research. Changes to the manuscript are shown in red text.

Comment : *The manuscript has the merit of offering an expert review regarding a not yet well explored topic, the mechanism of action of stem cell, specifically adipose derived stem cells in treating liver cirrhosis. The text is well written and organized easy to follow and presents relevant information. Below are point by point comments*

Comment 1: *Abstract informs with accuracy about the content of the manuscript , however in my opinion the core tip is only a brief repetition of it, maybe this point could be reformulated to be shorter , concise and inviting for the reader.*

Response: In accordance with the reviewer's instructions, we rewrote the Abstract using shorter sentences.

Adipose-derived mesenchymal stem cells (ADSCs) are a treatment cell source for patients with chronic liver injury. ADSCs are characterized by being harvested from the patient's own subcutaneous adipose tissue, a high cell yield (i.e., reduced immune rejection response), accumulation at a disease nidus, suppression of excessive immune response, production of various growth factors and cytokines, angiogenic effects, anti-apoptotic effects, and control of immune cells via cell-cell interaction. We previously showed that conditioned medium of ADSCs promoted hepatocyte proliferation and improved the liver function in a mouse model of acute liver failure. Furthermore, as found by many other groups, the administration of ADSCs improved liver tissue fibrosis in a mouse model of liver cirrhosis. A comprehensive protein expression analysis by LC-MS/MS showed that the various cytokines and chemokines produced by ADSCs promote the healing of liver disease. In this review, we examine the ability of expressed protein components of ADSCs to promote healing in cell therapy for liver disease. Previous studies demonstrated that ADSCs are a treatment cell source for patients with chronic liver injury. This review describes the various cytokines and chemokines produced by ADSCs that promote the healing of liver disease. (Line 90).

Comment 2: *The chapter "main content could be maybe named introduction or background . It is just a minor formal element but can affect the general impression on the work presented. In the introductory part , R 166-168 presents ADSCs but the phrase has, in my opinion a little bit of problem. ADSCs are indeed obtained usually by liposuction but this accounts for their easiness in procurement (as being obtainable in large quantities through a minimally invasive procedure). ADSCs claimed lack of immunogenicity is a story that has been challenged. MSCs (ADSCs included as a form of MSCs) might be only immune evasive not immune privileged therefore any form of allo-MSC therapy should be regarded with caution and tested from this perspective (Ankrum, 2014, Berglund, 2017), Furthermore, ADSCs based therapies ARE NOT a mainstream procedure in any medical field (unfortunately in my opinion) therefore one should acknowledge only several clinics worldwide practice this form of science based therapy.*

Response: We apologize that our initial explanation in the Main text caused misunderstanding. We rewrote the passage pointed out by the reviewer, as follows:

~~Cell therapy using adipose-derived mesenchymal stem cells (ADSCs) [35, 36] has the advantage of requiring only the minimally invasive collection of fat from subcutaneous fat in the patient's abdomen, and can therefore be performed without immunosuppression [37-39].~~ Since adipose-derived mesenchymal stem cells (ADSCs) [35, 36] are obtained from the patient's abdomen by liposuction, cell procurement is relatively easy (large numbers of cells

can be obtained by minimally invasive treatment). ADSCs can avoid immune rejection if they are autografted [37-39]; however, similarly to other cell sources, they are subject to immune rejection if allogeneic or xeno cell transplantation is performed. Mesenchymal stem cells are used for medical treatment worldwide. Since ADSCs are not a mainstream therapeutic cell, we have been performing clinical studies of treatments using ADSCs. (Line 170).

Comment 3: *The problem of using animal based supplements (such as FBS) in the culture media for expansion of clinical grade stem cell population is under debate. What is the author opinion in this respect? Does this influence the immunogenicity or any other aspect of cultivated cell biology? What about batch reproducibility when trying to scale up cell manufacturing (cell growth and even surface markers have been shown to be affected by FBS batch variability) . CM from FBS treated cells definitely contain a large amount of xenogenic proteins, how this could be accommodated with the requirements of a clinical grade therapy?*

Response: In accordance with the instructions of the reviewer, we described the opinion on FBS, which is an animal-derived product used to prepare the medium, in the Main text, as follows:

However, using animal-derived components in the process of culturing cells for treatment is associated with a risk of transmitting pathogenic infections derived from animals (e.g., bovine spongiform encephalopathy [BSE] or swine fever). Furthermore, when animal-derived ingredients are used the risks and quality may vary in individual lots. Thus, the use of chemically defined media with recombinant protein is recommended for large-scale culture conditions, such as the manufacturing of therapeutic cells for industrial use. In this background, we use the medium containing FBS as the control medium for the cultivation of research cells, while chemically-defined media is the first choice for culturing therapeutic cells. In addition, we do not deny the option of using a supplement that uses infectious and highly safe virus-tested human serum as a raw material for culturing therapeutic cells. (Line 183).

Comment 4: *R 177 "Terms defined in GO are called GO terms tries to explain a notion using the same terminology, maybe this could be reformulated. GO is rather a classification than a description of biological phenomena which associates genes with their so far known (reported by the existent literature) biological role structured based on given criteria.*

Response: We apologize that the explanation about the concept of GO was inadequate. The reviewer's comments were used to improve the explanation about GO. The revised passages reads as follows:

Terms defined in GO are called GO terms (GO is a classification of biological phenomena that associates genes with their known [reported in the existing literature] biological role structured based on given criteria), which are divided into three categories: biological processes, cellular components, and molecular functions. (Line 194).

Comment 5: *Maybe the subtitle growth factors could be complemented with " growth factors improving liver chrrhosis symptoms" or similar for enhance clarity Have the authors used ADSCs cell population as cell therapy in their in vivo studies, ADSC-CM or both, if it is the case when and how, maybe this should be summarized in a phrase or a table. It seems that the therapeutic effect on cirrhosis symptoms is based on ADSC released growth factors , on anti-inflammatory effect over the stellate cells, anti fibrotic and angiogenic effect of ADSCs released proteins. Do the authors consider this could be summarized in a phrase/table, for improving clarity?*

Response: In accordance with the reviewer's comment, we rewrote the section title as follows:

~~**Growth factors**~~
Growth factors improving the symptoms of liver cirrhosis (Line 244)

Furthermore, according to the comments of the reviewer, I added the following summary sentence to the Conclusion:
It seems that the therapeutic effect on the symptoms of cirrhosis is based on ADSC-secreted growth factors, anti-inflammatory effects on stellate cells, and the anti-fibrotic and angiogenic effects of ADSC-secreted proteins. (Line 438)

Comment 6: *The manuscript has two tables presenting a crude relationship between two kind of potentially active elements but a summary with all ADSCs expressed proteins that are presumable active in liver cirrhosis eventually indication on which basis is made this presumption (reference) should be of a help. They are only summarized in the conclusion, a little bit to late for the reader to follow. Instead maybe the conclusion should orient what could be the practical importance and relevance of deschpering these factors as potential therapeutic ones.*

Response: : Based on the reviewer's suggestion and comments from other reviewers, we added the following text to the Abstract:
Previous studies demonstrated that ADSCs are a treatment cell source for patients with chronic liver injury. This review describes the various cytokines and chemokines produced by ADSCs that promote the healing of liver disease. (Line 102).

Comment 7: *Can stem cells used as a therapy be selected based on the expression of these factors?*

Response: Based on the reviewer's suggestion, we added the following sentences at the end of Main text:
Recently, we reported that ADSC strongly expresses MFGM and the result that human MFGM protected dopamine neurons in rats of Parkinson's disease model. At the present stage, there is no report on a therapeutic method for disease-specific selection of therapeutic cells with reference to the list of protein components expressed by therapeutic stem cells. (Line 377).

Comment 8: *Is ADSC CM enough to act as therapeutic agent and if yes what could be the formulation of a proposed therapeutic intervention ?*

Response: I appreciate positive comments in relation to the clinical application. We added the following passage on cell therapy using ADSCs to the text:
Regarding cell therapy using ADSCs, we believe that excellent effects on immune response control by cell adhesion can be expected based on reports in the literature. For treatment with ADSC-CM, we used a method to concentrate ADSC-CM 20 times using a 10k filter. ADSC-CM is a liquid and has the advantage of being able to pass through both a 0.22-μm sterilizing filter and a 0.10-μm virus removal filter. We think that lowering the hurdles to cell therapy by taking advantage of the simple adjustment of the solution will contribute to a wide range of medical needs. (Line 385).

Comment 9: *Regarding ADSCs released factors, maybe it would be good to advance an opinion is the symptoms improvement based on real liver parenchyma regeneration or is it a transient decrease in inflammation and functional improvement based on a transitory supply of growth factors?*

Response: Based on the reviewer's suggestion, we added the following passage to the text:
A study of one-shot stem cell therapy reported the effects of bone marrow-derived mesenchymal stem cell treatment in 53 cirrhosis patients [99]. Although study period was sufficient to observe short-term symptomatic improvement over a period of days to weeks, it has been reported that there is no improvement in symptoms over the longer term (more than a few months). Although this study did not involve the use of ADSCs, it showed that stem cell treatment improves the symptoms of cirrhosis over the short term. However, it shows that one-shot stem cell therapy does not reset the pathological state of cirrhosis through natural healing

power leading to recovery. In this paper, we reported that ADSC has three angiogenesis-inducing effects, which promoted the proliferation of hepatocytes, which suppressed the fibrosis of the liver tissue. We believe that medical stakeholders and patients will be more likely to challenge clinical studies of ADSCs in the treatment of cirrhosis. However, it is unlikely that ADSCs play a direct role in controlling all of the pathological conditions of cirrhosis in the body. Thus, we are of the opinion that cell therapy using ADSCs and treatment using ADSC-CM will be useful as supplementary treatments. (Line 397).

(prior to the revision)

Name of Journal: World Journal of Stem Cells

Manuscript Type: Minireview

(< 12 words)

Title: ~~Protein factors in adipose-derived mesenchymal stem cell transplantation for liver cirrhosis~~ Cytokines in adipose-derived mesenchymal stem cells promote the healing of liver disease

Nahar S et al. Protein factors in cirrhosis treatment

Author list

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Author contributions: study design, SN, YN, CS, HN; study conduct, SN, YN, CS, HN; data collection, SN, YN, CS, TK, ZT, HN; data analysis, SN, YN, CS, TK, ZT, HN; data interpretation, SN, YN, CS, TK, ZT, HN; provision of materials, TM, NK, IS, MW, JF; drafting of the manuscript, SN, YN, HN; revision of the content of the manuscript, SN, YN, HN; approval of the final version of the manuscript, SN, YN, CS, TK, ZT, NK, IS, MW, JF, HN. SN takes responsibility for the integrity of all of the data analyses.

Supported by: This work was supported by the Research Laboratory Center, Faculty of Medicine, and the Institute for Animal Experiments, Faculty of Medicine, University of the Ryukyus. This work was supported in part by the Japan Society for the Promotion of Science (JSPS; KAKENHI Grant Number JP16H05404, JP16K10435, JP18K08545), Japan Agency for Medical Research and Development, The Naito Foundation, and Okinawa Science and Technology Promotion Center (OSTC).

Institutional review board statement:

Not applicable (this paper does not report clinical studies that required institutional review).

Institutional animal care and use committee statement:

All experimental protocols were performed according to the guidelines for the care and use of laboratory animals set by Research Laboratory Center, Faculty of Medicine, and the Institute of Animal Experiments, Faculty of Medicine, University of the Ryukyus (Okinawa, Japan). The experimental protocol was approved by the Committee on Animal Experiments of University of the Ryukyus (permit number: A2017101).

Conflict-of-interest statement:

According to the definitions of the World Journal of Stem Cells, the authors of this manuscript have no conflicts of interest to disclose.

Data sharing statement:

Biostatistics Review Certificate: not applicable (no significant difference test was carried out in this paper).

ARRIVE guidelines statement:

ARRIVE Guidelines have been adopted.

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Abstract (192 < 200 words)

Adipose-derived mesenchymal stem cells (ADSCs) are a treatment cell source for patients with chronic liver injury. ADSCs are characterized by being harvested from the patient's own subcutaneous adipose tissue, a high cell yield (i.e., reduced immune rejection response), accumulation at a disease nidus, suppression of excessive immune response, production of various growth factors and cytokines, angiogenic effects, anti-apoptotic effects, and control of immune cells via cell-cell interaction. We previously showed that conditioned medium of ADSCs promoted hepatocyte proliferation and improved the liver function in a mouse model of acute liver failure. Furthermore, as found by many other groups, the administration of ADSCs improved liver tissue fibrosis in a mouse model of liver cirrhosis. A comprehensive protein expression analysis by LC-MS/MS showed that the various cytokines and chemokines produced by ADSCs promote the healing of liver disease. In this review, we examine the ability of expressed protein components of ADSCs to promote healing in cell therapy for liver disease. Previous studies demonstrated that ADSCs are a treatment cell source for patients with chronic liver injury. This review describes the various cytokines and chemokines produced by ADSCs that promote the healing of liver disease.

Keywords (5-10): adipose-derived mesenchymal stem cells (ADSCs); cell transplantation therapy; cytokine; hepatocytes; liquid chromatography with tandem mass spectrometry (LC-MS/MS); liver cirrhosis

Core tip (96 < 100 words)

We previously showed that conditioned medium of ADSCs promoted hepatocyte proliferation and improved the liver function in a mouse model of acute liver failure. Furthermore, as reported by many other groups, the administration of ADSCs improved liver tissue fibrosis in a mouse model of liver cirrhosis. A comprehensive protein expression analysis by LC-MS/MS showed that the various cytokines and chemokines produced by ADSCs have the ability to promote the healing of liver disease. In this review, we examine the ability of the expressed protein components of ADSCs to promote healing in cell therapy for liver disease.

Saifun Nahar[1], Yoshiki Nakashima[2*], Chika Miyagi-Shiohira[2], Takao Kinjo[3], Zensei Toyoda [3], Naoya Kobayashi[4], Issei Saitoh[5], Masami Watanabe[6], Hirofumi Noguchi[2**], and Jiro Fujita[1]

Protein factors in adipose-derived mesenchymal stem cell transplantation for liver cirrhosis

Main text

Main content

We and others have conducted clinical studies in patients with symptoms of autoimmune liver disease [1], hepatitis C [2, 3], bacterial infection [4], acute liver failure liver [5], nonalcoholic fatty liver disease (NAFLD) [6] and cirrhosis. We have also conducted translational research that bridges basic research using hematopoietic cells [7-14], hepatic stellate cells (HSCs) [15-18], embryonic stem (ES) cell-derived hepatocytes [19-22], bioartificial livers [23-26], animals [27-33] and clinical research in humans.

Thanks to their expected therapeutic efficacy, mesenchymal stem cells (MSCs) [34] are currently under clinical evaluation as a cell source for cell therapy in trials of regenerative medicine for a broad spectrum of diseases. ~~Cell therapy using adipose-derived mesenchymal stem cells (ADSCs) [35, 36] has the advantage of requiring only the minimally invasive collection of fat from subcutaneous fat in the patient's abdomen, and can therefore be performed without immunosuppression [37-39].~~ Since adipose-derived mesenchymal stem cells (ADSCs) [35, 36] are obtained from the patient's abdomen by liposuction, cell procurement is relatively easy (large numbers of cells can be obtained by minimally invasive treatment). ADSCs can avoid immune rejection if they are autografted [37-39]; however, similarly to other cell sources, they are subject to immune rejection if allogeneic or xeno cell transplantation is performed. Mesenchymal stem cells are used for medical treatment worldwide. Since ADSCs are not a mainstream therapeutic cell, we have been performing clinical studies of treatments using ADSCs. For this reason, cell therapy using ADSCs is now performed in many public and private hospitals worldwide. In this review, we examine the effects of ADSCs in cell therapy for liver diseases, focusing on the proteins secreted by ADSCs. We previously reported that the proteins expressed by human ADSCs cultured using Dulbecco's Modified Eagle's medium (10% fetal bovine serum (FBS)) and clinical-grade chemically-defined medium (CDM) showed 98% similarity, demonstrating that the proteins expressed by ADSCs cultured in media for research and clinical use largely coincide [40, 41]. However, using animal-derived components in the process of culturing cells for treatment is associated with a risk of transmitting pathogenic infections derived from animals (e.g., bovine spongiform encephalopathy [BSE] or swine fever). Furthermore, when animal-derived ingredients are used the risks and quality may vary in individual lots. Thus, the use of chemically defined media with recombinant protein is recommended for large-scale culture conditions, such as the manufacturing of therapeutic cells for industrial use. In this background, we use the medium containing FBS as the control medium for the cultivation of research cells, while chemically-defined media is the first choice for culturing therapeutic cells.

In addition, we do not deny the option of using a supplement that uses infectious and highly safe virus-tested human serum as a raw material for culturing therapeutic cells.

Gene ontology (GO) facilitates [42, 43] the development of a common vocabulary to describe biological concepts. Terms defined in GO are called GO terms (GO is a classification of biological phenomena that associates genes with their known [reported in the existing literature] biological role structured based on given criteria), which are divided into three categories: biological processes, cellular components, and molecular functions. The Gene Ontology Consortium (http://www.geneontology.org/) is a database of functional information that aims to describe biological phenomena in standardized terms. In recent years, liquid chromatography with tandem mass spectrometry (LC-MS/MS) has been used to perform a GO classification of comprehensive expression data using protein analysis software programs. Both a comprehensive expression analysis of proteins using LC-MS/MS and a protein GO analysis were performed according to methods that we reported previously [40, 41, 44].

In normal liver tissue, blood flows from the portal vein through the central veins, each of which supplies sufficient blood to the hepatocytes of a single hepatic lobule (Figure 1). The blood supply to hepatocytes remains sufficient in carbon tetrachloride (CCL4: 0.5 ml/kg)-administered acute liver failure model in mice [40, 41]. Liver failure is a defined as a group of diseases associated with the development of symptoms such as jaundice, ascites, hepatic encephalopathy, bleeding tendency, or the like, due to a decrease in the number of hepatocytes or a decrease in their function. Acute liver failure is defined by the presence of necrosis and inflammation in normal liver tissue, with the period until the onset of symptoms of hepatic insufficiency being within 8 weeks. Cases in which the onset of symptoms is 8–24 weeks or >24 weeks are classified as delayed liver failure (late onset hepatic failure; LOHF) and chronic liver failure, respectively. The American Association for the Study of Liver Diseases (AASLD) published a position paper on acute liver failure in 2005 to unify the terms and disease concepts [45].

In contrast to this acute model, the preparation of the CCL4 (2.0 ml/kg)-administered cirrhosis model mouse requires more than 6 weeks. The liver tissue in this mouse model of liver cirrhosis shows a marked increase in the fiber component of the fibrous septa. This results in the separation of some lobules by fiber components, and the creation of pseudo-lobules. At the same time, the blood supply to the lobules decreases and liver cell necrosis occurs (Figure 2). Chronic liver injury also results in the differentiation of astrocytes into myofibroblastoid cells, in turn causing the pathogenesis of fibrotic liver injury [46]. Given this background, the factor most necessary for the improvement of acute liver failure symptoms is hepatocyte

growth factor. In contrast, the most important factors for the improvement of the symptoms of chronic liver failure (*i.e.*, liver cirrhosis) are: (1) growth factors, (2) inhibition of the inflammation of hepatic stellate cells, and (3) angiogenic factors.

When the disordered repair process is delayed or inhibited after liver damage from drugs, trauma, inflammation, or other insults, liver regeneration is insufficient and hepatic failure develops. In hepatic tissue repair, in addition to growth factors that promote hepatocyte proliferation, angiogenic factors that promote hepatic microvascular remodeling are important. In addition, the extracellular matrix in the liver is mainly produced in hepatic stellate cells. The ability of hepatic stellate cells to produce extracellular matrix is low in the normal liver. However, in the fibrous liver, it is known that hepatic stellate cells are activated to differentiate into myofibroblasts and their ability to produce extracellular matrix markedly increases. Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), hepatocyte growth factor (HGF), and other factors secreted from hepatic stellate cells (HSCs), hepatic sinusoidal endothelial cells (HECs) and Kupffer cells are thought to have the greatest influence on hepatocyte proliferation [47]. Angiogenesis is a physiological phenomenon in which a new blood vessel branch is branched from an existing blood vessel to construct a vascular network. The various factors involved in angiogenesis include fibroblast growth factor (FGF), vascular endothelial cell growth factor (VEGF), angiopoietin, and platelet derived growth factor (PDGF).

~~**Growth factors**~~

Growth factors improving the symptoms of liver cirrhosis

Hepatocyte growth factor (HGF) acts as a hepatocyte growth factor and metabolic regulator and promotes hepatocyte proliferation [48]. The hepatic development of the liver is the origin of the gut tube, which is formed by the accumulation of hematopoietic cells [49]. Thus, the idea that HGF expressed by hematopoietic cells promotes the regeneration of hepatocytes is plausible. Liver tissue regeneration using HGF may accordingly be considered a treatment method that reproduces the original development of the liver. HGF is expressed by both HSCs and ADSCs.

Our group previously investigated the clinical application of organ preservation solution [50-56]. We found that the expression level of HGF mRNA did not decrease in ADSCs, even when they were stored in preservation solution for 16 hours after separation from adipose tissue. In addition, we found no difference between the expression levels of HGF using glucose-free University of Wisconsin (UW) and glucose-containing (5.6 mmol/L) Hank's Balanced Salt Solution (HBSS). This result shows that the expression of HGF by ADSCs does

not decrease after separation from adipose tissue. Moreover, the expression is not affected by the glucose concentration. In addition, the expression of vascular endothelial growth factor (VEGF) showed a similar tendency. In short, ADSCs constantly express HGF and VEGF both *in vivo* and *in vitro* [57]. A recent theory suggests that the biliary tree functions as a source of liver and pancreatic stem cells and progenitor cells. VEGF is secreted by the biliary tree as a response to stress [58]. From these developmental perspectives, HGF and VEGF secreted by ADSCs appear to have a marked promoting effect on hepatocyte proliferation.

Our experiments showed that the administration of ADSC conditioned medium (CM) from a single vein rapidly promotes the cellular proliferation of mouse hepatocytes (Supplemental Figure 1). The proteins associated with a growth function (GO analysis), identified by the presence of ADSC-CM, were Periostin (POSTN), P component (SAP), semaphorin 7A (SEM7A), and Inactive tyrosine-protein kinase 7 (PTK7) [40, 41]. Periostin, which is encoded by the POSTIN gene, has been reported to be an extracellular factor that promotes hepatosteatosis [59, 60]. Nevertheless, much remains unknown about proteins with the ability to promote the cellular proliferation of hepatocytes. For example, SAP, a protein that is expressed in hepatocytes and secreted into serum, is known to be involved in processes associated with immune regulation, such as the action of opsonins [61], but whether SAP is involved in the cellular proliferation of hepatocytes is unknown. Further, SEM7A is known to contribute to TGF-β-mediated hepatic fibrosis [62], but whether it promotes hepatocyte cell proliferation is unknown. Future studies should therefore investigate whether the growth-associated proteins that are newly identified by GO analyses promote the cellular proliferation of hepatocytes in CCL4-induced liver impairment. What is certain is that HGF and VEGF secreted by ADSCs are among the key factors promoting the proliferation of hepatocytes.

Inflammation inhibitor of hepatic stellate cells

The JNK signaling pathway is involved in the activation of HSCs [63, 64]. JNK1 plays a major role in the upregulation of the α-SMA expression in HSCs under the stress conditions induced by TGF-β during liver fibrosis [65]. We previously reported the clinical application of organ preservation solution with a JNK inhibitory peptide (11R-JNKI) [66-68] and 8R-sJNKI(-9) [69]. The design of these cell-permeable inhibitory peptides is not only significant for *in vivo* studies, but also for future attempts to design inhibitors of liver fibrosis for the clinical treatment of liver cirrhosis. In addition, we previously reported that Arg-Gly-Asp (RGD) peptide [70] and Rho-kinase (ROCK) inhibitor [71] suppresses liver fibrosis. Our experiments show that the administration of ADSCs (1×10^6 cells) from a total of three veins at a twice

weekly interval rapidly improves the fibrosis of excessive mesenchyme around mouse hepatocytes (Supplemental Figure 2). When ADSCs are administered via the mouse tail vein, they cause pulmonary embolism, which has a high probability of causing the death of the mouse. Our group developed a method to safely administer ADSCs using heparin [72]. The proteins associated with the immune system process (GO analysis) identified by the presence of ADSC-CM were FINC, CO1A2, CO1A1, CATB, TSP1, CFAH, GAS6, LEG1, PTX3, C1S, SEM7A, CLUS, G3P, PXDN, SRCRL, CD248, SPON2, ENPP2, CD109, CFAB, CATL1, MFAP5, MIF, CXCL5, ADA M9, and CATK (Table 1) [40, 41]. Among these ADSC-secreted proteins, we found no studies reporting a relationship in the field of liver cirrhosis and hepatic stellate cells for FINC, CO1A2, CATB, CFAH, LEG1, C1S, SEM7A, CLUS, G3P, PXDN, SRCRL, SPON2, ENPP2, CD109, CFAB, CATL1, MFAP5, ADAM9, or CATK. It is expected that these proteins will be investigated in future studies.

Type I collagen and fibronectin are also reported to be components of hepatic fibrosis [73, 74]. It is therefore unlikely that CO1A1 and CO1A2, which are secreted by ADSCs, suppress the excess activity of HSCs. Thrombospondin-1, a matricellular glycoprotein that is secreted by many cell types, modulates a variety of cellular functions by binding to extracellular proteins and/or cell surface receptors. Thrombospondin-1 might contribute to liver fibrosis not only as an activator of TGF-β, but also as a modulator of angiogenesis [75]. In the normal liver, growth arrest-specific gene 6 (Gas6) is mainly expressed in Kupffer cells. The expression of Gas6 increases in activated HSCs and macrophages after acute CCL4 administration [76]. Given that Gas6 and Axl are reported to be necessary for HSC activation [77], Gas6 secreted by ADSCs seems to have no effect in inhibiting the activity of HSCs. Pentraxin 3 (PTX3) is expressed and released by hematopoietic cells and stromal cells and is an essential component of innate immunity. Interleukin-1 (IL-1) induces the production of PTX3 by Kupffer cells, endothelial cells and biliary duct epithelial cells. PTX3 is reported to be a biomarker of liver fibrosis in response to hepatic injury [78]. These reports indicate that PTX3 secreted by ADSCs is unlikely to suppress the activation of HSCs. CD248 (endosialin) is a stromal cell marker expressed on fibroblasts and pericytes. During liver injury, myofibroblasts are the main source of fibrotic matrix. Liver fibrosis was reported to be suppressed in CD248 knockout mice [79], suggesting that it is unlikely that CD248 secreted by ADSCs inhibits hepatic fibrosis. Macrophage migration inhibitory factor (MIF) is a pleiotropic inflammatory cytokine that has been implicated in various inflammatory diseases. MIF knockout mice were reported to have strongly increased fibrosis in a mouse model of chronic liver injury model. This phenomenon was accompanied by no change in the infiltration of intrahepatic immune cells. MIF has an

anti-fibrotic effect on the liver via the MIF receptor (CD74). In addition, recombinant MIF protein has a similar anti-fibrotic effect [80]. These results indicate that MIF secreted by ADSCs is a major component in the suppression of liver fibrosis. CXCL5 is best known for its function as a neutrophil chemotactic factor and activator, with a molecular structure similar to that of IL-8 4, 5. CXCL5 is released from monocytes, neutrophils, epithelial cells, fibroblasts and smooth muscle during inflammation. Interestingly, CXCL5 has a proliferative effect on rat hepatocytes. The use of a neutralizing antibody of CXCL5 slowed the liver regeneration rate after partial hepatectomy [81]. The plasma CXCL5 levels are low in patients with chronic liver disease, and CXCL5 may be involved in the pathogenesis of chronic liver disease [82]. These results strongly indicate the possibility that CXCL5 secreted by ADSCs also promotes hepatocyte proliferation. These findings indicated that MIF is one of the components that suppress liver fibrosis among the ADSC-secreted proteins that we identified. In addition, CXCL5 was identified as a component that promotes hepatocyte proliferation. Of course, the function of these proteins has been previously reported. Table 1 shows 26 different proteins classified as immunomodulatory (GO analysis). The 18 proteins indicated by N/A have not been reported in the liver field, and further research into them is anticipated. Note that proteins that were not classified as immunomodulatory (by a GO analysis) may also have effects on the liver.

Angiogenic factors

EGF, VEGF and HGF, which are expressed by ADSCs, have a strong angiogenic effect on liver tissue [83-85]. Table 2 lists eight types of proteins (PAI1, FSTL1, POSTN, MMP2, TSP1, TIMP1, FBLN3 and MFGM) affecting angiogenesis from among 101 types of proteins secreted by ADSCs. Plasminogen activator inhibitor 1 (PAI1) is a member of a family of proteins that inhibit plasminogen activators [86]. Although the binding of VEGF to vitronectin induces strong angiogenic signaling, this is inhibited by competitive binding to PAI1 [87]. PAI1 secreted from ADSCs is therefore thought to inhibit angiogenesis by VEGF in liver tissue. Follistatin-related protein 1 (FSTL1) is a secretory glycoprotein belonging to the follistatin and SPARC family. FSTL1 was reported to be highly expressed in fibrotic human liver tissue and activated HSCs [88]. FSTL1 has the effect of promoting the activity of HSCs. It is therefore unlikely that FSTL1 secreted by ADSCs affects angiogenesis. Periostin (POSTN), an extracellular matrix (ECM) molecule of the fasciclin family, has roles in vascular cell differentiation and migration [89]. We therefore hypothesize that POSTN secreted by ADSCs may promote angiogenesis of the liver. Matrix metalloproteinases (MMPs) are a family of over 24 zinc-dependent endopeptidases

capable of degrading virtually any component of the ECM [90]. MMP2 plays an important role in the preservation of liver vascular homeostasis via its participation in the TGF-β activation process [91]. MMP2 secreted by ADSCs—and many other cell types—is therefore considered to be one of the main factors. TSP1 was reported to be increased in HSCs isolated from the liver in a mouse model of CCL4-induced cirrhosis. In liver samples of patients with alcohol cirrhosis and non-alcoholic steatohepatitis-related cirrhosis, TSP1 levels were reported to be increased [92]. It is thought that TSP1 expressed in the liver has the effect of promoting liver fibrosis. If so, it would be unlikely that TSP1 secreted by ADSCs promotes liver angiogenesis. Metalloproteinase inhibitor 1 (TIMP1) is a widely expressed inhibitor of matrix metalloproteinases (MMPs). Given that the inhibition of TIMP1 promotes angiogenesis by increasing cell motility during fibrovascular invasion [93], TIMP1 secreted by ADSCs may inhibit liver angiogenesis. Fibulins (FBLNs), a versatile family of extracellular matrix proteins, comprise a small family of widely expressed extracellular matrix (ECM) proteins [94]. FBLN3 has been reported to be an angiogenesis antagonist that regulates cell morphology, growth, adhesion and motility [95]. It is therefore unlikely that FBLN3 secreted by ADSCs promotes liver angiogenesis. Lactadherin (MFGM) interacts with alphavbeta3 and alphaV-beta5 integrins and alters both VEGF-dependent Akt phosphorylation and neovascularization [96]. MFGM was reported to promote angiogenesis via enhanced PDGF-PDGFRβ signaling mediated by cross-talk of the integrin growth factor receptor [97]. Thus, MFGM secreted by ADSCs is considered to be one of the main components promoting liver angiogenesis. We recently reported that ADSCs strongly express MFGM and that human MFGM protected dopamine neurons in a rat model of Parkinson's disease model [98]. At the present stage, there are no reports on a therapeutic method for the disease-specific selection of therapeutic cells that reference to the list of protein components expressed by therapeutic stem cells.

Regarding cell therapy using ADSCs, we believe that excellent effects on immune response control by cell adhesion can be expected based on reports in the literature. For treatment with ADSC-CM, we used a method to concentrate ADSC-CM 20 times using a 10k filter. ADSC-CM is a liquid and has the advantage of being able to pass through both a 0.22-μm sterilizing filter and a 0.10-μm virus removal filter. We think that lowering the hurdles to cell therapy by taking advantage of the simple adjustment of the solution will contribute to a wide range of medical needs.

These results suggest that VEGF, HGF, EGF, MMP2, POSTN, and MFGM secreted by ADSCs promote hepatic angiogenesis. Among the 101 proteins expressed by ADSCs, we identified three proteins that promote angiogenesis from among eight proteins reported to be

involved in angiogenesis. The further development of research into the 93 other proteins is expected.

A study of one-shot stem cell therapy reported the effects of bone marrow-derived mesenchymal stem cell treatment in 53 cirrhosis patients [99]. Although study period was sufficient to observe short-term symptomatic improvement over a period of days to weeks, it has been reported that there is no improvement in symptoms over the longer term (more than a few months). Although this study did not involve the use of ADSCs, it showed that stem cell treatment improves the symptoms of cirrhosis over the short term. However, it shows that one-shot stem cell therapy does not reset the pathological state of cirrhosis through natural healing power leading to recovery. In this paper, we reported that ADSC has three angiogenesis-inducing effects, which promoted the proliferation of hepatocytes, which suppressed the fibrosis of the liver tissue. We believe that medical stakeholders and patients will be more likely to challenge clinical studies of ADSCs in the treatment of cirrhosis. However, it is unlikely that ADSCs play a direct role in controlling all of the pathological conditions of cirrhosis in the body. Thus, we are of the opinion that cell therapy using ADSCs and treatment using ADSC-CM will be useful as supplementary treatments.

Conclusion

The factors necessary for improvement of the symptoms of chronic liver failure (i.e. liver cirrhosis) are: (1) growth factors, (2) inhibitors of hepatic stellate cell inflammation, and (3) angiogenic factors. (1) It is certain that ADSC-secreted HGF and VEGF are among the factors that promote the proliferation of hepatocytes. In addition, CXCL5 was identified as a component that promotes hepatocyte proliferation. (2) MIF, which was one of the ADSC-secreted proteins that we identified that suppressed liver fibrosis. ~~In addition, CXCL5 was identified as a component that promotes hepatocyte proliferation.~~ Finally, (3) ASDC-secreted VEGF, HGF, EGF, MMP2, POSTN and MFGM are factors that promote hepatic angiogenesis. It seems that the therapeutic effect on the symptoms of cirrhosis is based on ADSC-secreted growth factors, anti-inflammatory effects on stellate cells, and the anti-fibrotic and angiogenic effects of ADSC-secreted proteins.

Acknowledgements

We thank Naomi Kakazu (University of the Ryukyus) for clerical assistance and Maki Higa, Yuki Kawahira and Saori Adaniya (University of the Ryukyus) for providing technical support.

Figure legends

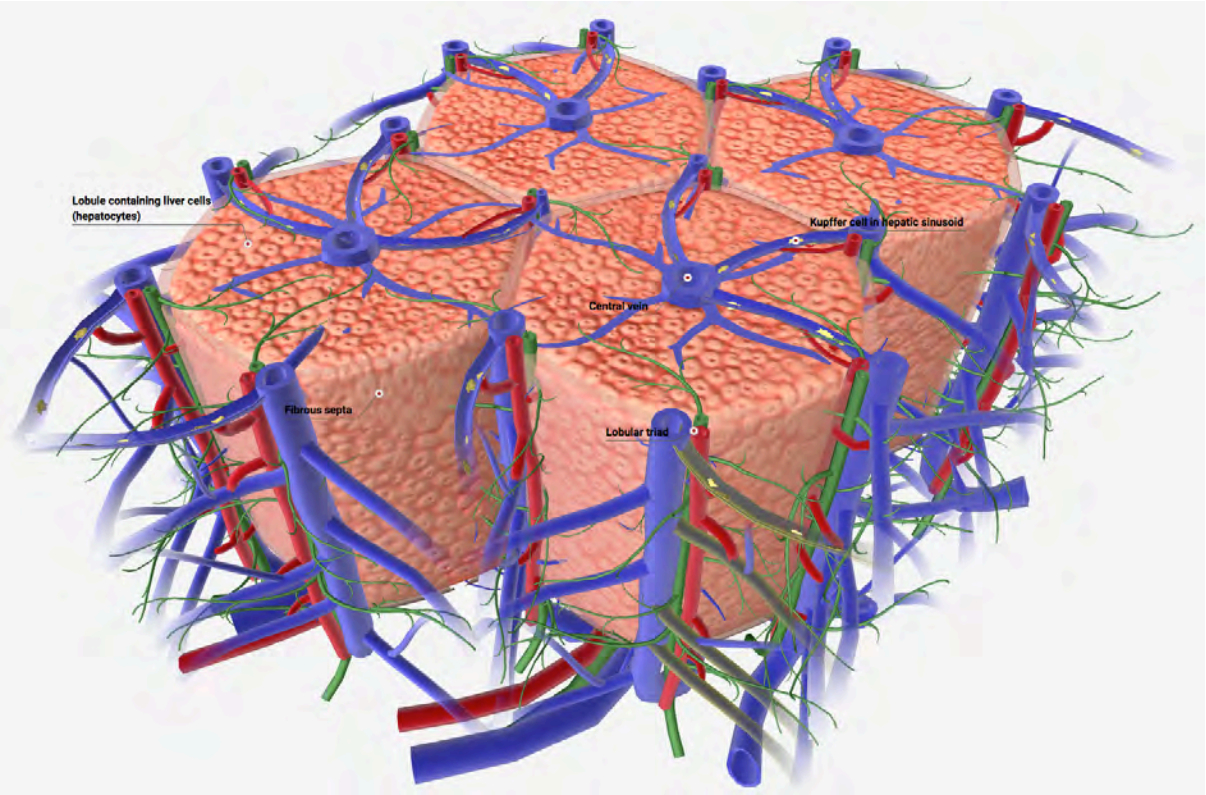


Figure 1. Schematic diagram of a normal liver tissue model. The liver is a digestive organ that filters and detoxifies blood from the digestive tract. It also produces proteins, such as albumin, and synthesizes cholesterol and bile. The functional portion of the liver tissue is organized into hexagonal columns called liver lobules. Each liver lobule contains hundreds of individual liver cells (hepatocytes) and a large central vein. Lobular portal triads, which contain branches from the hepatic portal vein, hepatic artery and bile duct, are located at the points of the hexagonal lobule. Blood from the branches of the hepatic artery joins the blood of the hepatic vein branches, forming hepatic sinusoids. Hepatic sinusoids are lined with specialized cells called Kupffer cells, which help collect debris and detoxify the blood. All hepatic sinusoids in the liver lobule drain into the central vein. Adjacent lobules are separated by a thin fibrous septa. Images were obtained from BIODIGITAL HUMAN 3.0 (https://human.biodigital.com/index.html) (BioDigital, Broadway, NY, USA).

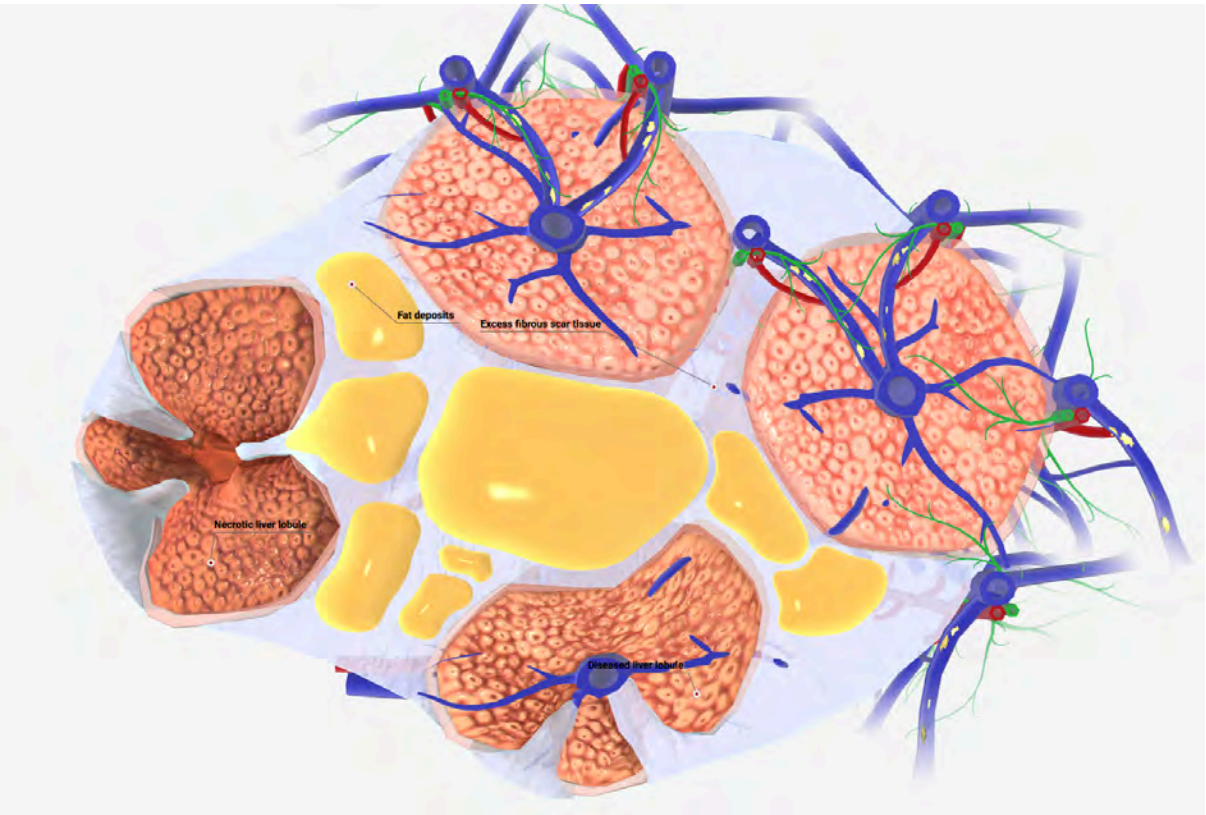
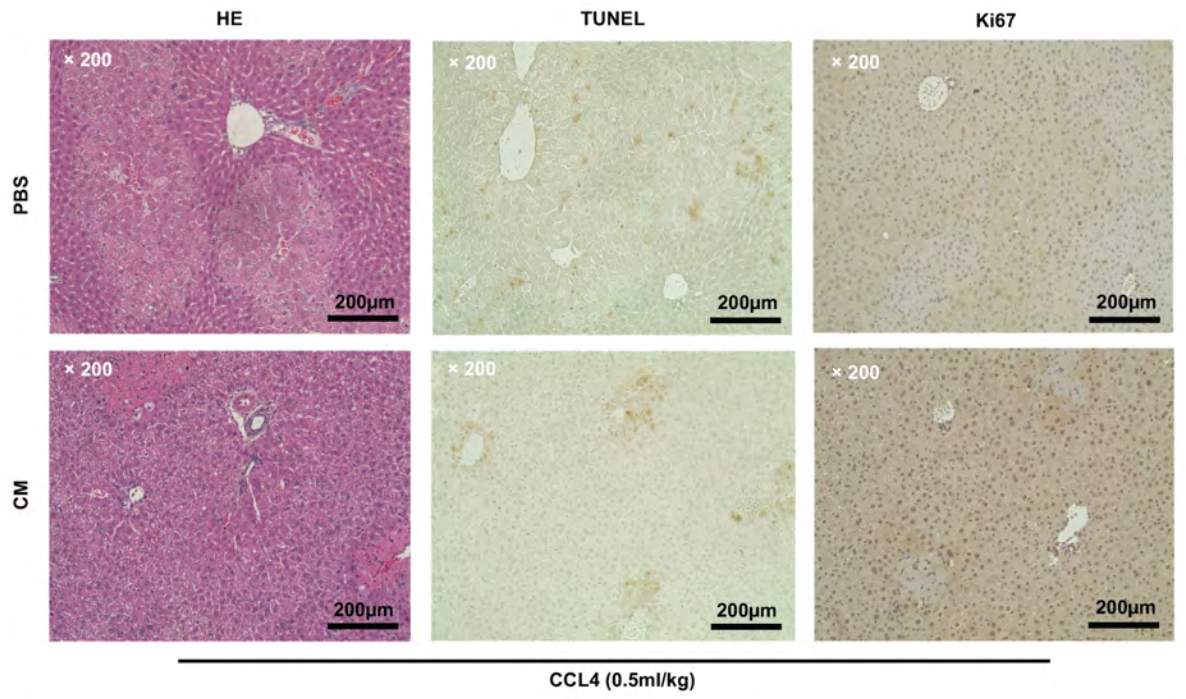
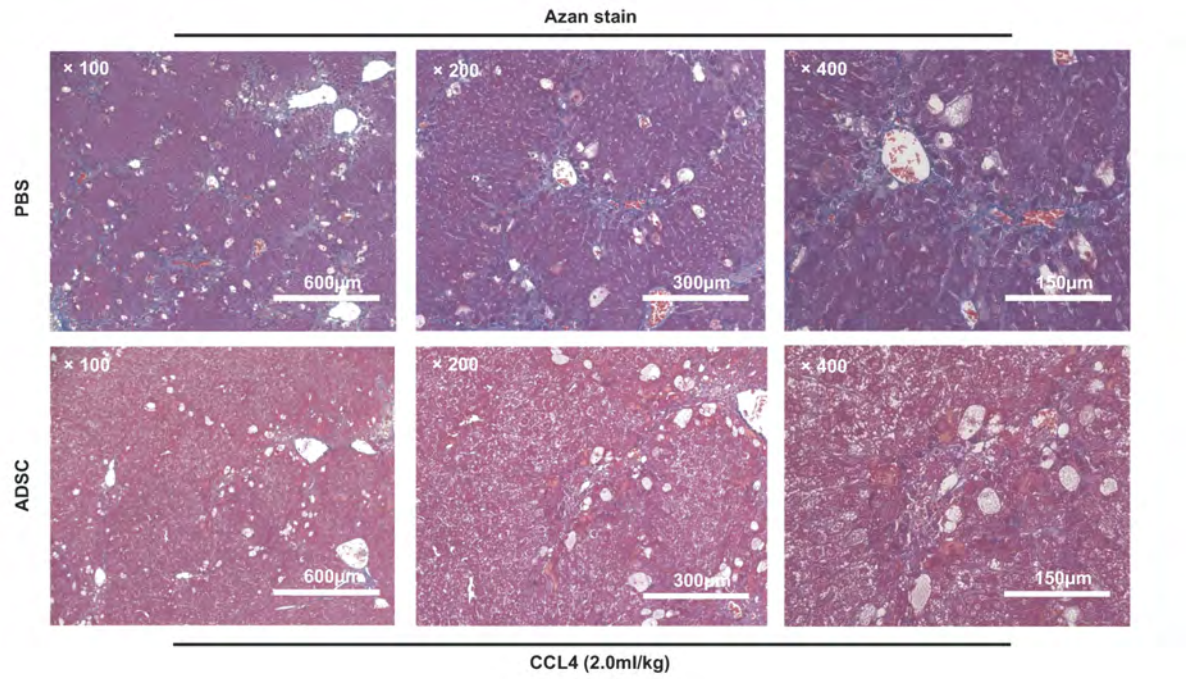


Figure 2. Schematic diagram of a liver cirrhosis tissue model. The functional portion of the liver tissue is organized into hexagonal columns called liver lobules. Each liver lobule contains hundreds of individual liver cells (hepatocytes). In healthy liver tissue, adjacent lobules are separated by a thin fibrous septa. However, liver cirrhosis involves thickening of the fibrous septa that separate lobules, and the deposition of fat. As a result, the blood flow in the lobules is disturbed and hepatocyte necrosis occurs. Also, the fibrous septa that separate the lobules transform the lobules and produce pseudolobules. Although it has a wide variety of causes, liver cirrhosis is most commonly caused by chronic alcohol abuse, chronic hepatitis, and nonalcoholic fatty liver disease. Images were obtained from BIODIGITAL HUMAN 3.0 (https://human.biodigital.com/index.html) (BioDigital, Broadway, NY, USA).



Supplemental Figure 1. Culture supernatant concentrate significantly improved the symptoms of acute liver failure caused by the administration of CCL4. Micrographic images of H&E staining (A, left panel), TUNEL assay (A, middle panels) and tissue immunostaining of Ki67 (A, right panel) of liver specimens. Microscopic images of liver specimens 20 hours after the administration of PBS (upper panels) and CM (lower panels) via the mouse tail vein. Scale bar = 200 μm. Fragmented DNA generated in the process of apoptosis can be detected by the TUNEL (TdT-mediated UTP nick end labeling) method. Ki67 protein present in the nucleus of cells in G1, S, G2 and M cycles (cell growth phase) was detected using immunostaining to identify cells in the growth phase in liver tissue. It was also used to count the number of positively stained cells in images of TUNNEL-stained sections (× 400). The numbers of positively stained cells in the PBS and CM groups were 14.00 ± 4.54 and 8.25 ± 5.57, respectively (n = 4; P = 0.19). The numbers of cells with positively stained nuclei on images of Ki67-stained sections (× 400) were also counted. The numbers of cells with positively stained nuclei in the PBS and CM groups were 9.25 ± 7.61 and 116.25 ± 3.06, respectively (n = 4; ** P < 0.01).



Supplemental Figure 2. ADSC improved symptoms of tissue fibrosis in cirrhosis caused by the administration of CCL4. Micrographic image of Azan staining of liver specimens. Azan staining is a fibrous connective tissue staining method that differentiates collagen fibers and muscle fibers. The fibrous connective tissue in the tissue section was stained blue. Microscopic images of the liver specimens 20 hours after the administration of PBS (upper panels) and ADSC (lower panels) via the mouse tail vein. In the ADSC administration group, fibrosis and pseudolobule formation were ameliorated. Microscopic images (×100 - 400) of the same tissue section. Scale bar = 150 - 600 μm.

Table 1. Report on the relationship between immunomodulatory protein secreted by ADSC and liver cirrhosis

UniProt/SWISS-PROT ID	Description		Reference
FINC	Fibronectin	Fibronectin expression is critical for liver fibrogenesis in vivo and in vitro.	[74]
CO1A2	Collagen alpha-2(I) chain	N/A	
CO1A1	Collagen alpha-1(I) chain	Type I collagen has also been reported to be one of the components of hepatic fibrosis.	[73]
CATB	Cathepsin B	N/A	
TSP1	Thrombospondin-1	TSP1 might contribute to liver fibrosis not only as an activator of TGF-β, but also as an modulator of angiogenesis.	[75]
CFAH	Complement factor H	N/A	
GAS6	Growth arrest-specific protein 6	Gas6/Axl pathway is activated in chronic liver disease and its targeting reduces fibrosis via hepatic stellate cell inactivation.	[77]
LEG1	Galectin-1	N/A	
PTX3	Pentraxin-related protein PTX3	The pentraxins PTX3 and SAP in innate immunity, regulation of inflammation and tissue remodelling.	[78]
C1S	Complement C1s subcomponent	N/A	
SEM7A	Semaphorin-7A	N/A	
CLUS	Clusterin	N/A	
G3P	Glyceraldehyde-3-phosphate dehydrogenase	N/A	
PXDN	Peroxidasin homolog	N/A	
SRCRL	Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D	N/A	
CD248	Endosialin	CD248 reduces susceptibility to liver fibrosis via an effect on PDGF signalling.	[79]
SPON2	Spondin-2	N/A	
ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	N/A	
CD109	CD109 antigen	N/A	
CFAB	Complement factor B	N/A	
CATL1	Cathepsin L1	N/A	
MFAP5	Microfibrillar-associated protein 5	N/A	
MIF	Macrophage migration inhibitory factor	MIF exerts antifibrotic effects in experimental liver fibrosis via CD74.	[80]
CXCL5	C-X-C motif chemokine 5	Plasma CXCL5 levels in patients with liver cirrhosis were lower than in healthy controls.	[81, 82]
ADAM9	Disintegrin and metalloproteinase domain-containing protein 9	N/A	
CATK	Cathepsin K	N/A	

Table 1. Relationship between immunomodulatory protein secreted by ADSC and liver cirrhosis.

Table 2. Report on the relationship between angiogenesis protein secreted by ADSC and liver cirrhosis

UniProt/SWISS-PROT ID	Description		Reference
PAI1	Plasminogen activator inhibitor 1	PAI-1 regulates angiogenesis via effects on extracellular matrix proteolysis and cell adhesion.	[87]
FSTL1	Follistatin-related protein 1	Knockdown of Fstl1 attenuates hepatic stellate cell activation through the TGF-β1/Smad3 signaling pathway.	[88]
POSTN	Periostin	POSTN, a ligand of αvβ3/5 integrins, as an effector protein in SULF2-induced angiogenesis.	[89]
MMP2	72 kDa type IV collagenase	MMP2 has an important role in the preservation of liver vascular homeostasis.	[91]
TSP1	Thrombospondin-1	TSP1 was reported to be increased in HSCs isolated from the liver of CCl4-induced cirrhosis model mice.	[92]
TIMP1	Metalloproteinase inhibitor 1	Inhibition of TIMP1 was reported to promote angiogenesis by increasing cell motility during fibrovascular invasion.	[93]
FBLN3	EGF-containing fibulin-like extracellular matrix protein 1	FBLN3 has been reported as an angiogenesis antagonist regulating cell morphology, growth, adhesion and motility.	[95]
MFGM	Lactadherin	MFGM promote angiogenesis via enhanced PDGF-PDGFRβ signaling mediated by cross-talk of the integrin growth factor receptor	[97]

Table 2. Relationship between angiogenesis protein secreted by ADSC and liver cirrhosis.

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