Reviewer #1:

I read the Editorial “Mechanism of Mesenchymal Stem Cells in Liver Regeneration: Insights and Future Directions.” The mesenchymal stem cells come to liver after a potential liver injury and differentiate into hepatocyte-like cells (HLCs). They will acquire the function of hepatocytes like glucose, lipid metabolism, excretion of lipids and synthesis of different proteins. They will also acquire the ability to excrete blood urea and shall be involved in detoxification process. However, even after multiple positive functions acquired by hepatocyte-like cells, practically, this is not sufficient and may not result into clinically meaningful outcomes. I have few comments.

1. Authors have not quoted the article “Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease” in references for which they are writing Editorial.

Response: Thank you very much for your valuable feedback. Regarding the issue of not citing the article "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease", we have added this article to the references.

2. The study for Mesenchymal stem cells has not been provided and we cannot assess the editorial without accompanying article. We do not know what actually was done in that study and how mesenchymal stem cells were assessed and evaluated. And how the mesenchymal stem cells were used as a therapeutic strategy in non-alcoholic fatty liver disease patients.

Response: Thank you for your reminder. I apologize for the confusion caused by our neglection. The original article "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease" is a review, we have added this article to the references. We understand the reviewers' concern about the details of the mesenchymal stem cells research. We have provided more background information regarding the application of mesenchymal stem cells in non-alcoholic fatty liver disease in the editorial (see Lines 69-76).

3. However, Authors have written a review on mesenchymal stem cells. Definitely, editorial is only written for studies where we find the study to have good research protocol, analysis and its outcomes have future implications. In Editorial, author should try to discuss the shortcomings of the study and strength of the study. And, finally with the help of review of existing literature should be giving an expert opinion from their side.

Response: Thank you very much for your valuable feedback. We understand the differences between a review and an editorial. According to the reviewer's suggestion, we have supplemented the discussion on

the advantages and disadvantages of this study and provided some opinions (see Lines 92-96,137-143 and 149-155).

4. The therapeutic potential of mesenchymal stem cells was not discussed. Whether MSCs have been evaluated in other liver diseases.

Response: Thank you for posing such insightful and in-depth professional questions. As you mentioned, stem cells have been confirmed by numerous studies to possess many functions, such as immune regulation, differentiation into hepatocytes, and repair of damaged tissues. In addition to NAFLD, the therapeutic effect of mesenchymal stem cells is relatively ideal for the treatment of liver diseases such as liver cirrhosis, liver failure, auto-immune hepatitis, and hepatocellular carcinoma. (see Lines 77-88).

5. What parameters were taken to assess liver regeneration potential of MSCs.

Response: Thank you for your question. Parameters used to assess the liver regeneration potential of MSCs may include:

1). Liver function tests: Such as measuring levels of liver enzymes (e.g., alanine aminotransferase, aspartate aminotransferase), bilirubin, albumin and glucolipid metabolism, detoxification functions to evaluate the overall function of the liver [1].

2). Histological analysis: Using techniques such as ultrasound, computed tomography, or magnetic resonance imaging to monitor changes in liver volume, structure, blood flow and fibrosis, which can provide indirect evidence of liver regeneration [2].

3). Expression of genes related to liver regeneration: Analyzing the expression of genes involved in liver regeneration pathways, such as HGF (hepatocyte growth factor), TGF-β (transforming growth factor-beta), and VEGF (vascular endothelial growth factor) [3].

4). Detection of inflammatory cytokines: Monitoring the levels of inflammatory cytokines, such as TNF-α (tumor necrosis factor-alpha), IL-6 (interleukin-6), and IL-1β, to assess the anti-inflammatory effect of MSCs on the liver [4].

5). Analysis of oxidative stress: Measuring markers of oxidative stress, such as reactive oxygen species and antioxidant enzymes, to determine the extent of oxidative damage and the ability of MSCs to mitigate it [6].

These additional parameters can provide more comprehensive information about the liver regeneration potential of MSCs and help researchers better understand the mechanisms of MSC-mediated liver regeneration. However, the specific parameters used may vary depending on the study design and the focus of the research.

Literature cited

[1] Zhao H, Shang Q, Pan Z, Bai Y, Li Z, Zhang H, Zhang Q, Guo C, Zhang L, Wang Q. Exosomes From

Adipose-Derived Stem Cells Attenuate Adipose Inflammation and Obesity Through Polarizing M2 Macrophages and Beiging in White Adipose Tissue. Diabetes. 2018;67(2):235-247.
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[3] Farouk S, Sabet S, Abu Zahra FA, El-Ghor AA. Bone marrow derived-mesenchymal stem cells downregulate IL17A dependent IL6/STAT3 signaling pathway in CCl4-induced rat liver fibrosis. PLoS One. 2018;13(10):e0206130.
[4] Ohara M, Ohnishi S, Hosono H, Yamamoto K, Yuyama K, Nakamura H, Fu Q, Maehara O, Suda G, Sakamoto N. Extracellular Vesicles from Amnion-Derived Mesenchymal Stem Cells Ameliorate Hepatic Inflammation and Fibrosis in Rats. Stem Cells Int. 2018;2018:3212643.
[5] Yao J, Zheng J, Cai J, Zeng K, Zhou C, Zhang J, Li S, Li H, Chen L, He L, Chen H, Fu H, Zhang Q, Chen G, Yang Y, Zhang Y. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells alleviate rat hepatic ischemia-reperfusion injury by suppressing oxidative stress and neutrophil inflammatory response. FASEB J. 2019;33(2):1695-1710.

6. Authors writing an editorial may comment upon methodology of the article. Whether they find it suitable or they feel few ares for improvement. However, I myself the reviewer can not comment as original study has not been shared.

Response: Thank you for your reminder. I apologize for the confusion caused by our neglection. The original article "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease" is a review, we have added this article to the references.

7. The authors of Editorial extensively discussed the mechanism of liver regeneration by mesenchymal stem cells. We need how there regenerative potential can be utilized for disease treatment. They should have discussed how we are utilizing role of mesenchymal stem cells. Its de novo or already present in patient's. Which cytokines, chemokine or growth factor have been used to differentiate these specialized cells.

Response: Thank you for your question. In liver diseases, MSCs can be administered exogenously, meaning they are introduced into the patient from an external source. This approach is often used in experimental and clinical studies to explore the therapeutic potential of MSCs in treating liver diseases. In a study, MSC derived from embryonic stem cells were transplanted into a rat model of congenital hyperbilirubinemia. The MSC cells survived in the liver for up to 2 months and could differentiate into functional hepatocytes, promoting liver regeneration [1]. Another study found that MSC could promote liver regeneration by

maintaining the homeostasis of liver function in the acute phase and reducing reactive oxygen species (ROS) damage caused by ischemia-reperfusion within 2 weeks after transplantation, and by differentiating into functional hepatocytes within 2 to 4 weeks after transplantation to prolong survival and prevent acute liver failure [2].

Literature cited

[1] Spitzhorn LS, Kordes C, Megges M, Sawitza I, Götze S, Reichert D, Schulze-Matz P, Graffmann N, Bohndorf M, Wruck W, Köhler JP, Herebian D, Mayatepek E, Oreffo ROC, Häussinger D, Adjaye J. Transplanted Human Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Support Liver Regeneration in Gunn Rats. Stem Cells Dev. 2018;27(24):1702-1714.

[2] Lin NC, Wu HH, Ho JH, Liu CS, Lee OK. Mesenchymal stem cells prolong survival and prevent lethal complications in a porcine model of fulminant liver failure. Xenotransplantation. 2019;26(6):e12542.

To differentiate MSCs into specialized cells for therapeutic purposes, various cytokines, chemokines, and growth factors can be used. For example, hepatocyte growth factor (HGF) [1], fibroblast growth factor (FGF) [2], and transforming growth factor-β (TGF-β) [3] have been shown to play important roles in directing the differentiation of MSCs towards hepatocyte-like cells. Additionally, interleukin-6 (IL-6), IL-10, and other immune-modulatory cytokines can influence the immunomodulatory properties of MSCs, which is crucial for their therapeutic effects in liver diseases associated with inflammation [4-6].

Literature cited

[1] Wu L, Tian X, Zuo H, Zheng W, Li X, Yuan M, Tian X, Song H. miR-124-3p delivered by exosomes from heme oxygenase-1 modified bone marrow mesenchymal stem cells inhibits ferroptosis to attenuate ischemia-reperfusion injury in steatotic grafts. J Nanobiotechnology. 2022;20(1):196.

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[3] Xuan J, Feng W, An ZT, Yang J, Xu HB, Li J, Zhao ZF, Wen W. Anti-TGFβ-1 receptor inhibitor mediates the efficacy of the human umbilical cord mesenchymal stem cells against liver fibrosis through TGFβ-1/Smad pathway. Mol Cell Biochem. 2017;429(1-2):113-122.

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[5] Zhang Y, Li Y, Wang Q, Zheng D, Feng X, Zhao W, Cai L, Zhang Q, Xu H, Fu H. Attenuation of hepatic ischemia-reperfusion injury by adipose stem cell-derived exosome treatment via ERK1/2 and GSK-3β signaling pathways. Int J Mol Med. 2022;49(2):13.

[6] Piao C, Zhang Q, Xu J, Wang Y, Liu T, Ma H, Liu G, Wang H. Optimal intervention time of ADSCs for

hepatic ischemia-reperfusion combined with partial resection injury in rats. Life Sci. 2021;285:119986.

8. How the improvement in non-alcoholic fatty liver disease was documented. Whether this was biochemical improvement, radiological improvement or monitoring of non-invasive markers assessment.

Response: Thank you for your question. The improvement in NAFLD can be documented through various methods. Biochemical improvements can be measured by monitoring changes in liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [1], as well as lipid profiles including triglycerides, cholesterol, and low-density lipoprotein (LDL). Additionally, markers of insulin resistance, such as fasting blood glucose and insulin levels, can also be assessed to evaluate the metabolic improvements in NAFLD. Moreover, radiological improvements can be detected using imaging techniques such as ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI) [2]. These methods can help visualize changes in liver fat content, fibrosis, and the structure of the liver. In addition, non-invasive markers assessment can include the use of biomarkers such as cytokeratin-18 fragments, adiponectin, and fetuin-A, which can provide information about liver inflammation, fibrosis, and metabolic dysfunction [3]. In summary, a combination of these methods is often used to comprehensively document the improvement in NAFLD, providing a more accurate assessment of the disease progression and the effectiveness of treatment.

Literature cited

[1] Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, Subbarayan S, Webb A, Hecht J, Cusi K. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. J Clin Endocrinol Metab. 2015;100(6):2231-8.

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[3] Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. Cell Metab. 2019;30(3):607.

9. And finally, we do not know whether the original study "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease" involved human subjects or animals.

Response: Thank you for your reminder. I apologize for the confusion caused by our neglection. The

original article "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease" is a review, we have added this article to the references. Animal and cellular studies on MSCs for NAFLD were summarised in the review, however, clinical studies were not well explored.

10. Whether liver biopsy was done in "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease." at baseline and follow up. How much follow up duration was planned in that study for response of mesenchymal stem cells in patients with no alcoholic fatty liver disease.

Response: Thank you for your reminder. I apologize for the confusion caused by our neglection. The original article "Current perspectives on mesenchymal stem cells as a potential therapeutic strategy for non-alcoholic fatty liver disease" is a review, we have added this article to the references. The review aims to elucidate the potential roles of MSCs in alleviating the progression of NAFLD by alteration of underlying molecular pathways. The study did not examine how NAFLD patients responded to MSCs, how long follow-up was set, or whether liver biopsies were performed at baseline and follow-up.