Rebuttal Letter (Point by Point response):

We are grateful for the valuable comments and suggestions from the editor and the reviewers for this manuscript. The rigorous review has helped us immensely improving our manuscript. The reviewers' comments have been closely followed and revisions have been made accordingly. Please see below our point by point response.

Reviewer #1

1. Figure 6 and the description of the Figure 6 in text may be revised to represent summary of the research and introduce the diabetic microenvironment more clearly.

Response: Thank you for your suggestion, we elaborated the description of figure 6 in the discussion part where it was positioned.

Reviewer #2

1. According to the citation of the article, it is known that mesenchymal stem cells used to treat diabetes mainly include bone marrow mesenchymal stem cells, Wharton's jelly mesenchymal stem cells, umbilical cord mesenchymal stem cells and amniotic mesenchymal stem cells and induced pluripotent stem cells. Choosing adipose-derived stem cells as the research object to realize the investigation of high diabetes or hyperglycemia on the cellular and molecular characteristics of MSCS needs to be clear.

Response: We thank the reviewer for paying careful attention to these details. MSCs of different sources have relatively similar regenerative and therapeutic potential for many chronic and acute diseases. Many studies have already used Adipose tissue -derived MSCs (AD-MSCs) for treating diabetes and the results showed that AD-MSCs can induce the regeneration of Beta cells in pancreas, improve insulin sensitivity, and reduce the risk of long term complications, but these beneficial effects were hindered by their short -lived engraftment in DM. So our choice to select AD-MSCs was based on these studies. Below are some studies that have used AD-MSCs for DM.

1.Wang L, Zhang L, Liang X, Zou J, Liu N, Liu T, Wang G, Ding X, Liu Y, Zhang B, Liang R, Wang S. Adipose Tissue-Derived Stem Cells from Type 2 Diabetics Reveal Conservative Alterations in

Multidimensional Characteristics. Int J Stem Cells. 2020 Jul 30;13(2):268-278. doi: 10.15283/ijsc20028. PMID: 32587133; PMCID: PMC7378902.

2. Yu, S., Cheng, Y., Zhang, L. et al. Treatment with adipose tissue-derived mesenchymal stem cells exerts anti-diabetic effects, improves long-term complications, and attenuates inflammation in type 2 diabetic rats. Stem Cell Res Ther 10, 333 (2019). https://doi.org/10.1186/s13287-019-1474-8

3. Agnieszka Mikłosz, Adrian Chabowski, Adipose-derived Mesenchymal Stem Cells Therapy as a new Treatment Option for Diabetes Mellitus, The Journal of Clinical Endocrinology & Metabolism, Volume 108, Issue 8, August 2023, Pages 1889–1897, https://doi.org/10.1210/clinem/dgad142

2. As far as I know, the study of the biological behavior changes of adipose-derived stem cells under high glucose conditions, there have been studies (high glucose-induced reactive oxygen species generation promotion stemness in human adipose-derived stem cells https:/ /PubMed.ncbi.nlm.nih.gov/26780864/) , so the author uses the phrase "Little is known about the impact of diabetic microenvironment, granular hyperglycemia" is inaccurate, and this part has not been discussed in the article.

Response: We thank the reviewer for reviewing this article. The introduction part regarding the impact of hyperglycemia on MSCs was modified according to your note. Furthermore, the article provided in your comment (https:/ /PubMed.ncbi.nlm.nih.gov/26780864/) discussed that despite lower proliferative activity and higher senescence in a diabetic environment (high glucose), AD-MSCs also exhibited enhanced stemness and neurogenic transdifferentiation potential via a ROS-mediated mechanism if they were placed in induction medium that contains antioxidants and spec fic growth factors. So the impact of high glucose on MSCs still controversial and nees thorough investigation.

3. LDH is used to detect cytotoxicity, but there is only one normal glucose culture in the control vs. high sugar culture conditions of different days?

Response: We thank the reviewer for reviewing the article. We have revised the LDH cytotoxicity assay and provided the required low glucose controls time points.

4. According to the official instructions (https://www.abcam.com/products/assay-kits/tmre-mitochondrial-membrane-potential-assay-kit-ab113852.html), TMRE fluorescence intensity is only qualitative test, and I personally think that other experiments are needed to verify the results again.

Response: We thank the reviewer for his comment. The TMRE assay used can be reported by detecting the fluorescent intensity and by taking fluorescent images at TEXAS RED filter and this have been clearly mentioned on ABCAM website as they stated that the Detection method: is Fluorescent and the Platforms for detection are Microplate reader, Fluor. microscope, Flow cyt. We followed exactly what have been reported in various studies that have used TMRE for mitochondrial membrane potential measurement and what have been provided in the company protocol.

5. The balance of NAD+/NADH pool is very important to the balance of mitochondrial membrane potential, but whether there are other factors, such as high glucose leading to high osmotic pressure environment, has not been elaborated in this research.

Response: We thank the reviewer for his suggestion. It's out of study focus since we are not studying the impact of high glucose on mitochondrial membrane potential only, but on different mitochondrial parameters. So we measured NAD+/NADH pool since it's an important parameter for Mitochondrial dynamics besides doing molecular analysis for various regulators. We will focus on investigating the impact of high glucose on osmotic pressure environment in our future studies.

6. "It has been reported that mTOR is important to preserve mitochondrial dynamics and generate the required mitochondrial potential to produce ATP. PI3K is required to remove the inhibitory effect of tuberous sclerosis tumor suppressor (TSC1) which binds mTOR and in activations it." This key part lacks citation. Moreover, it is different from

the mTOR signal path (https://www.genome.jp/pathway/map04150) related to the KEGG website

Response: We thank the reviewer for his comment. Relevant references have been added in the revised manuscript to this part.

Reviewer #3

General/Miscellaneous:

1. This article has too little experimental validation, and the pathway selection builds on the literature. It is recommended that the authors perform RNA sequencing of the treated cells and combine it with bioinformatics analysis for more in-depth mechanistic exploration.

Response: Thank you for your valuable comment. Unfortunately, we don't have currently an access to perform RNA sequencing, but your comment is very important, so we conducted deep analysis using western blot for mitochondrial complexes to better understand the impact of high glucose on these important functional proteins. The results showed that high glucose downregulated complex I, IV, and V, while complex II and III didn't change. These new data have been provided in figure 6 in the revised manuscript and were added to the result section as well.

2. Throughout the manuscript, there are instances of repeated information (e.g., the properties of MSCs, the characteristics of DM). Streamlining these will enhance readability.

Response: we thank the reviewer for his comment. We tried to avoid repetition but sometimes we used similar terms since the alternative terms can look unscientific.

3. There are occasional grammatical errors. Consider a thorough proofreading or editing pass.

Response: we thank the reviewer for his comment. The grammatical typos have been followed and corrected in the revised manuscript by an expert.

4. It would be beneficial to have a figure showing the impact of high glucose on MSCs in a schematic diagram, detailing all the changes observed in the study.

Response: we appreciate your suggestion. A graphical summary was provided in figure 7 of the revised manuscript and its description was added in the discussion part of the revised manuscript.

5. Ensure all figures mentioned in the text (e.g., Figure 1a, Figure 2 a &b) are provided for review. Confirm the consistency in the use of abbreviations throughout the manuscript (e.g., "hMSCs" vs. "hAD-MSCs").

Response: we thank the reviewer for his valuable comment. We inserted the figures in the manuscript provided initially, and also we will be uploading a PowerPoint file having all figures. We checked these abbreviations for consistency in the revised manuscript.

Abstract:

1. The phrasing "reputable type of stem cells that has enchanted regenerative abilities" seems colloquial and lacks scientific precision. Suggest revising for clarity and accuracy.

Response: We thank the reviewer for paying careful attention to these important details. We replaced this sentence with more scientific one in the revised manuscript as the following. “Mesenchymal stem cells (MSCs) are a type of stem cells that possess relevant regenerative abilities”

2. Provide specific data in the results section of the abstract to provide quantifiable evidence of the findings.

Response: We thank the reviewer for pointing this out. Specific data were added to the results section of the abstract in the revised manuscript.

Introduction

1. The introduction provides a comprehensive overview of the current knowledge on the topic. However, it would benefit from being more concise and directly relevant to the study's objectives.

Response: Thank you for your comment. Some sentences in the introduction were deleted to make it more concise.

2. **In the last sentence of the introduction, specify what "these changes" refer to, for clarity.** References [2,3] discuss the positive outcomes of MSCs but then state there were short-lived therapeutic results. Please clarify this discrepancy.

Response: We thank you for your comment. The last senteces of the introduction have been clarified in the revised manuscript.

Materials and Methods

1. For the glucose conditions (low and high), it would be beneficial to provide a rationale for the specific concentrations chosen. → *Is there any reference for those?*

Response: We thank you for your comment. The low glucose medium is the ideal and control medium for growing MSCs which contains 1 g of glucose per 1 L (around 5.6 mmol/L). Growing and maintaining MSCs in low glucose have been proven by many studies. Exposing MSCs to high glucose medium that has around 25 mmol/L glucose have been selected based on previous studies. Below are some references to support the selection of these glucose concentrations and they were added in the revised manuscript..

References:

1. Al-Qarakhli, A.M.A., Yusop, N., Waddington, R.J. et al. Effects of high glucose conditions on the expansion and differentiation capabilities of mesenchymal stromal cells derived from rat endosteal niche. BMC Mol and Cell Biol 20, 51 (2019). https://doi.org/10.1186/s12860-019-0235-y

2. Chang TC, Hsu MF, Wu KK. High glucose induces bone marrow-derived mesenchymal stem cell senescence by upregulating autophagy. PLoS One. 2015 May 11;10(5):e0126537. doi: 10.1371/journal.pone.0126537. PMID: 25961745; PMCID: PMC4427318.

2. Clarify the source and characteristics of the hAD-MSCs, such as their passage number at the beginning of the experiment.

Response: Thank you for this comment. We used MSCs of passage 5 and 6 for performing our experiments since they are chronologically close and this has been clearly mentioned in the revised manuscript.

3. For the cytotoxicity assays, provide more details on the LDH Assay methodology, including the number of cells seeded and any controls used.

Response: Thank you for this valuable comment. We revised the LDH results by adding more controls based on your comment and other reviewer's comments and this has been added to figure 1 of the revised manuscript. The number of cells seeded were $5X10^4$ per well and was added in the result section of the revised manuscript.

4. The procedure for the apoptosis assay should be elaborated upon. It's essential to understand the exact steps taken to ensure reproducibility.

Response: Thank you for this important comment. The apoptosis procedure was elaborated in the revised manuscript.

Results

1. In section 3.1, it would be beneficial to provide actual percentages or quantitative data regarding cell viability and cytotoxicity, instead of qualitative terms like "remarkably low" or "significantly greater."

Response: Thank you for this important comment. The p values were added in the results to represent the qualitative terms used and were added in the revised manuscript.

2. In the mitochondrial dynamics section (3.2), provide specific values for the changes in TMRE fluorescence intensity and NAD+/NADH ratios.

Response: Thank you for this important comment. The p values were added in the results to represent the qualitative terms used and were added in the revised manuscript.

3. For the western blot results (3.4), include the actual fold-changes or percentages to understand the magnitude of the observed effects. Additionally, consider presenting representative blots for clarity.

Response: Thank you for this important comment. We used the relative expression percentages after normalizing the values of each protein to B actin and these percentages were demonstrated in the graph bars of each protein in figure 5 and the new figure 6.

4. The results section is missing statistical information. State the statistical significance for each data point and consider adding p-values to the figures

Response: Thank you for your suggestion. The p values were added in the results as well as in the figure legends of the figures.

Discussion:

1. The discussion provides a broad overview of the findings and their implications. However, it would benefit from a more focused discussion, directly related to the study's results.

Response: This is very important to be considered. The discussion was modified to be more focused and related to the study's results.

2. The paragraph discussing the discrepancies in mTOR regulation in different cell types is quite lengthy. Consider streamlining it or presenting it in a more structured manner to enhance readability.

Response: Thank you for your suggestion. This part in the discussion has been modified and modified to be more focused and easy to be followed

3. The sentence "The findings of our study deserve consideration in broader context..." seems slightly biased. Reframe to a more neutral tone.

Response: This is very important to be considered. This sentence has been reframed and revised in the discussion.

Conclusion

1. The conclusion adequately summarizes the main findings. However, it largely mirrors the abstract. Consider adding future implications or research directions.

Response: we thank you for your suggestion. We added future directions in the conclusion part of the revised manuscript.

Revision reviewer

I think the author's answer based on the question is scientific. At present I agree to the publication of this article。

Response: Thanks for your comments.