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Human umbilical cord mesenchymal stem cells reduce platelet α-granule release in rats via the AKT/MEK/ERK pathway during acute exposure to high-altitude hypoxia

Response Letter to Reviewers' Comments

Comments from the Editor:
Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are provided below:

Response: We thank all the editors and reviewers for their valuable comments and suggestions.We have carefully revised the manuscript to enhance its clarity and facilitate the understanding of the readers. Our point-to-point responses are presented in the following. We hope that the revision.would satisfactorily address the comments and concerns of the editors and reviewers.

Reviewer#1:
The article titled "hUC-MSCs reduce platelet α-granule release in rats via the AKT/MEK/ERK pathway during acute exposure to high-altitude hypoxia" is good work but I have few queries/comments that need to be resolved before final recommendation of the article.

Response: Thanks for the positive comments.

1.Title & Abstract: 1.1. The title is clear but could be more concise while retaining key elements. 1.2. The abstract is well-structured but should briefly highlight the potential clinical applications of the findings. 1.3. Some minor grammatical issues need correction, e.g., "as also as the expression of" should be "as well as the expression of."

Response: Thanks for the comments. 1.1. We have partially revised the title to make it clearer, and now the title is: Human umbilical cord mesenchymal stem cells reduce platelet α-granule release via the AKT/MEK/ERK pathway during acute exposure of rats to high-altitude hypoxia. 1.2. We have highlighted the potential clinical applications of our findings in the abstract: The results indicate that hUC-MSCs may represent a promising and effective approach for the prevention and treatment of acute high-altitude-associated thrombosis, providing an experimental foundation for the development of clinical applications. 1.3. Grammatical issues have been modified.

2. Introduction: 2.1. The introduction provides a strong rationale but could emphasize

the novelty of the study more. 2.2. The discussion on high-altitude thrombosis is good but could reference more recent studies. 2.3. Some claims, such as "Recent studies have demonstrated promising therapeutic effects," need specific citations.

Response: 2.1. we have emphasized the novelty of the study more: This study presents the first systematic elucidation of the regulatory mechanisms by which hUC-MSCs modulate α-granule release from platelets, using a rat model of acute high-altitude hypoxia. 2.2. We have added recent references to the discussion section. 2.3. "Recent studies have demonstrated promising therapeutic effects" has been followed by the latest references and retouched in the language.[1-3]

3. Methods: 3.1. The methodology is detailed, but the selection criteria for animals should be clearer. 3.2. A separate subheading for statistical methods would improve readability. 3.3. Justification for using CD62p as an activation marker in flow cytometry should be included. 3.4. The preparation of hUC-MSCs needs more details on culture conditions and passage numbers. 3.5. ELISA and Western blot methods should specify the number of independent experiments performed.

Response: 3.1. We have added animal selection criteria to the manuscript: Rats were chosen as experimental animal models in this study as rats are more suitable for pathophysiological studies than mice, and platelet gene and protein expression in rats closely resembles that of humans [28]. 3.2 We have listed separate subheadings for statistical methods. 3.3. The rationale for using CD62p as an activation marker in flow cytometry has been added: The levels of CD62p, a key membrane glycoprotein stored in platelet α-granules and considered a classic marker of platelet activation [29], were determined using flow cytometry. 3.4. We have re-described the preparation process of hUC-MSCs as well as the details of culture conditions and passage times. 3.5. We have added the number of independent experiments performed to the ELISA and Western blot methods: Eight independent replicates were used for all measurements. The experiment was repeated three times independently for each protein.

4. Results: 4.1. The results are well-presented, but some sections contain redundant information. 4.2. Figure legends should explain comparisons more clearly. 4.3. The discussion on platelet activation should be better linked to clinical relevance. 4.4. Individual variability in response to hypoxia should be acknowledged.

Response: 4.1. We conducted a thorough review of the results and identified that there was indeed overlapping information present in both the descriptions and notes. Consequently, redundant content was removed to enhance the conciseness and clarity of the results. 4.2. Figure legends have been explained more clearly. 4.3. We have better linked the discussion of platelet activation to clinical relevance and have added the latest relevant references: In addition, recent studies have demonstrated that activated platelets play a pivotal role in various physiological and pathological processes, including inflammation, innate immunity, growth and development, angiogenesis,

wound healing, and cancer metastasis [46, 47]. 4.4. A description of individual differences in response to hypoxia has been added to the first paragraph of the discussion: It is important to highlight that there are substantial individual variations in physiological responses to hypoxic stress. These differences may stem from the interplay of genetic differences, baseline health conditions, and other pathophysiological regulatory factors, particularly in terms of the extent of platelet activation and the threshold of hematopoietic responses.

5. Discussion: 5.1 The discussion effectively explains results but could relate findings more to prior studies. 5.2 Limitations should be expanded, especially regarding sample size and generalizability. 5.3 Alternative pathways to AKT/MEK/ERK activation should be briefly discussed. 5.4 A final paragraph summarizing the translational relevance of findings would strengthen the discussion.

Response: 5.1. We have linked our results to more previous studies in the discussion section to make the results more convincing. 5.2. We have added descriptions of limitations, particularly in terms of sample size and generality: Further studies with larger sample sizes should be undertaken to verify the potential of using human umbilical cord mesenchymal stem cells for treating thrombotic diseases, and their applications in other populations, environments, or conditions. 5.3. Alternative pathways to AKT/MEK/ERK activation should be briefly discussed: However, activation of the AKT and MEK/ERK pathways is complex, and associated with several non-classical pathways involving GPCRs, integrins, oxidative stress, and cytokines. These mechanisms play important roles in tumorigenesis, immune responses, and metabolic diseases, and targeted cross-regulation of multiple pathways is recognized as a research hotspot in the development of strategies for disease treatment.

6. Language & Style: 6.1.The manuscript contains minor grammatical errors and awkward phrasing. Examples include: • "as also as the expression of" → "as well as the expression of." • "was found to reverse these changes" → "was found to mitigate these changes." 6.2.Passive voice is frequently used; active voice may enhance readability in some sections.

Response: 6.1. We have addressed the grammatical errors and improved the wording in the manuscript as outlined in the comments. 6.2. Part of the manuscript has been changed from passive voice to active voice to improve the readability of the manuscript.

7. Conclusion: 7.1. The conclusion restates findings well but should highlight future directions, such as human trials.

Response: 7.1. Human trials in future directions have been highlighted in the conclusions: In terms of clinical applications, the current findings can be used as a reference for the construction of standardized clinical transformation protocols. The efficacy of these could be evauated in thrombotic diseases, degenerative

osteoarthropathy, graft-versus-host disease, and the treatment of diabetic foot ulcers using multi-center randomized controlled trials, and biomarker-based efficacy prediction models could be established at the same time. Finally, we believe that there is an urgent need to establish a long-term follow-up database of the epigenetic stability, tumorigenic risk, and immunogenic evolution of hUC-MSCs to provide evidence-based references for clinical applications.

Reviewer#2:
Acute exposure to high-altitude hypoxic environments is associated with an increased risk of thrombosis; however, current preventive measures remain inadequate. Recent studies have demonstrated promising therapeutic effects of human umbilical cord mesenchymal stem cell (hUC-MSC) transplantation on the prevention and treatment of various clinical conditions, including thrombotic diseases. Platelets are crucial for thrombus formation, with their α-granules serving as key determinants of platelet function. However, little is known of the influence of hUC-MSCs on platelet α-granules. This study investigated the influence of hUC-MSCs on platelet α-granules in rats during acute exposure to high-altitude hypoxia. Acute exposure to high-altitude hypoxia increased the platelet count, altered platelet morphology, and increased α-granule density and release. These effects were reversed by hUC-MSC treatment, mediated by the AKT/MEK/ERK pathway. The findings demonstrate that acute exposure to high-altitude hypoxia increases the platelet count, alters platelet morphology, increases α-granule density, and promotes the release of α-granule contents in rats. Treatment with hUC-MSCs can reverse these changes, possibly through the AKT/MEK/ERK signaling pathway. These results provide novel insights into the prevention of acute high-altitude-associated thrombosis using hUC-MSCs. Nevertheless, given the complexity of thrombosis formation involving multiple factors and mechanisms, further investigation is needed to verify the role of hUC-MSCs in preventing acute high-altitude-associated thrombosis. These findings provide new insight into the application of hUC-MSCs for the prevention and treatment of thrombosis. The content of this study is detailed and innovative. The content is logical and clear. The language expression standard in the text. The reference introduction is reasonable and appropriate. This article can be accepted after minor revisions. There is no need to review the manuscript again.

Response: Thanks for the positive comments.

1. In lines 281 to 286, the labeling order of Figure B and C is reversed.

Response: 1. We have adjusted the order of figures B and C in lines 281 to 286.

2. The A，B，C in Figure 4 should be enlarged to ensure sufficient clarity.

Response: 2. We have enlarged A, B, and C in Figure 4 to ensure sufficient clarity.

3. Please upload the ethics approval form again.

Response: 3. We have re-uploaded the ethics approval form.

Added references:

1 **Zhu Z,** Zhang Q, Liu L, Xu J. Human Umbilical Cord Mesenchymal Stem Cells' Cultivation and Treatment of Liver Diseases. *Curr Stem Cell Res Ther* 2023; **18**: 286-298 [PMID: 35747963 DOI: 10.2174/1574888X17666220623111406]

2 **Gomes A,** Coelho P, Soares R, Costa R. Human umbilical cord mesenchymal stem cells in type 2 diabetes mellitus: the emerging therapeutic approach. *Cell Tissue Res* 2021; **385**: 497-518 [PMID: 34050823 DOI: 10.1007/s00441-021-03461-4]

3 **Pan Z,** Chen Q, Ding H, Li H. MicroRNA-342-3p loaded by human umbilical cord mesenchymal stem cells-derived exosomes attenuates deep vein thrombosis by downregulating EDNRA. *J Thromb Thrombolysis* 2022; **54**: 411-419 [PMID: 36006542 DOI: 10.1007/s11239-022-02694-6]

4 **Singh LC.** High Altitude Dermatology. *Indian J Dermatol* 2017; **62**: 59-65 [PMID: 28216727 DOI: 10.4103/0019-5154.198050]

5 **Jenne CN,** Urrutia R, Kubes P. Platelets: bridging hemostasis, inflammation, and immunity. *Int J Lab Hematol* 2013; **35**: 254-261 [PMID: 23590652 DOI: 10.1111/ijlh.12084]

6 **Zhou L**, Zhang Z, Tian Y, Li Z, Liu Z, Zhu S. The critical role of platelet in cancer progression and metastasis. *Eur J Med Res* 2023; **28**: 385 [PMID: 37770941 DOI: 10.1186/s40001-023-01342-w]

7 **Scopelliti F,** Cattani C, Dimartino V, Mirisola C, Cavani A. Platelet Derivatives and the Immunomodulation of Wound Healing. *Int J Mol Sci* 2022; **23** [PMID: 35955503 DOI: 10.3390/ijms23158370]