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PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 72372

Title: Extracellular vesicles' miR-224-5p from hypoxia preconditioned mesenchymal stem cells alleviates myocardial injury by targeting TXNIP-Mediated HIF1α Pathway at the early stage of myocardial infarction

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05935626
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: Indonesia

Author's Country/Territory: China

Manuscript submission date: 2021-10-13

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-10-17 04:52

Reviewer performed review: 2021-10-17 06:57

Review time: 2 Hours

Scientific quality	[Y] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection

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Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

I would like to congratulate the authors for this study. I have some comments about the manuscript: Introduction: please include previous studies with correlation to this study. Material and method: There were total of 80 mice used, 20 mice per group. please clarify briefly your sample size determination. About the collection of neonatal mouse cardiomyocytes, please clarify how many mice used and the detailed criteria / specification. Result: where is figure 1A and 4D mentioned in the text? Please arrange the figures accordingly in the text. Discussion: please give the reasoning of this study compared to other study and provide the limitation of this study.

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Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 04244067
Position: Peer Reviewer
Academic degree: MD
Professional title: Doctor
Reviewer's Country/Territory: Japan
Author's Country/Territory: China

Manuscript submission date: 2021-10-13

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-10-14 00:34

Reviewer performed review: 2021-10-19 02:29

Review time: 5 Days and 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection

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Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Although many attempts have been made to use EVs derived from ADSCs to alleviate cardiac damage such as MI and to promote repair, the present study is unique in that it prepares ADSCs that have been exposed to hypoxia beforehand and shows that EVs released from these cells have a more pronounced cardioprotective effect against myocardial infarction. This effect was observed in normoxic ADSCs, however, since this effect is stronger than that of EVs generated from normoxic ADSCs, these differences led to the extraction of important miRNA factors. To further elucidate the molecular mechanism, they analyzed the regulation of target proteins by miRNAs and the nuclear export of HIF1a in cultured strain cells and primary cultured cells. Although the molecular mechanism of miR224-TXNIP-HI1a has already been clarified before such as in pancreatic cancer cells, the present study was able to verify the existence of a similar mechanism using cultured myocardium. On the other hand, in order to prove that the cardioprotection of ADSC-derived EVs against MI is due to the above molecular mechanisms, it may be necessary to verify the molecular dynamics in the myocardium after MI. 1. For the ADSC-cas9-miR224 used in the article, the method of cell establishment and data confirming KO should be presented. 2. The amount of TXNIP and HI1a in the nucleus and cytoplasm, as well as their ubiquitination, should be verified in mouse hearts after MI.

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PEER-REVIEW REPORT

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Manuscript NO: 72372

Title: Extracellular vesicles' miR-224-5p from hypoxia preconditioned mesenchymal stem cells alleviates myocardial injury by targeting TXNIP-Mediated HIF1α Pathway at the early stage of myocardial infarction

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00462474
Position: Editorial Board
Academic degree: PhD
Professional title: Full Professor
Reviewer's Country/Territory: Italy
Author's Country/Territory: China

Manuscript submission date: 2021-10-13
Reviewer chosen by: AI Technique
Reviewer accepted review: 2021-10-18 09:43
Reviewer performed review: 2021-10-25 13:53
Review time: 7 Days and 4 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection

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Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Mao et al. aim to analyze the cardioprotective mechanism of extracellular vehicles (EVs) generated by hypoxia-preconditioned mesenchymal stromal/stem cells (MSCs) in an in vivo myocardial injury (MI) model and in an in vitro hypoxia model. They hypothesize that hypoxia-preconditioned extracellular vehicles (HP-EVs) could be more effective against myocardial injury-associated cardiomyocyte death than EVs derived from normoxic MSCs (NC-EVs). They analyze the morphological and molecular changes associated with MI-induced apoptosis in cardiomyocytes and hypotize a role for HP-EVs-associated miRNAs in cardiomyocyte survival. Although the paper is of interest several issues have to be addressed before considering it suitable for publication.

Major issues 1. The paper needs to be revised by a native English speaker. 2. The Introduction paragraph lacks a proper definition of MSCs. Authors have to underlie their characteristics and function (see recent literature PMID: 34398443; PMID: 30001217).

3. It is well known that oxidative stress in the heart is increased in response to ischemia/reperfusion and heart failure. Indeed, in pathological situations, ROS accumulate due to excessive production or insufficient degradation, leading to oxidative stress (PMID: 28861421). ROS are among the most harmful DNA-damaging agents. A major product of oxidative damage to DNA is 8-oxo-2′-deoxyguanosine. In addition to apoptosis assessment, authors must address this issue in their experimental plan by analyzing at least this DNA damage marker (see for experimental setting PMID: 19804370; 20697355). 4. Author state that adipose-derived mesenchymal stem cells (ADSCs) were isolated from adipose tissue from C57BL/6 mouse adipose tissue as

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previously described without providing the reference. In addition, authors have to indicate at which culture passage they performed the experiments. To obtain the required number of cells, MSCs need to be cultured for several passages. Although in vitro expansion is a necessary procedure to guarantee the required number of MSCs, it is also considered to pose important issues. It has been demonstrated that in vitro growth of MSCs can give rise to replicative senescence. Have the authors addressed this issue? (see PMID: 32223893). 5. Figure 1A is not mentioned in the text.

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PEER-REVIEW REPORT

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Manuscript NO: 72372

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Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05817439
Position: Peer Reviewer
Academic degree: PhD
Professional title: Senior Postdoctoral Fellow
Reviewer's Country/Territory: Brazil
Author's Country/Territory: China

Manuscript submission date: 2021-10-13

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-10-14 17:16

Reviewer performed review: 2021-10-29 06:24

Review time: 14 Days and 13 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection

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Re-review	[ ] Yes [Y] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

My observations during the review of the manuscript are as follows: 1- Isolation and culture of mouse ADSCs In the phrase "ADSCs were isolated from adipose tissue from C57BL/6 mouse adipose tissue as previously described (?)– authors should add the reference here. 2- Methods Section: Isolation and characterization of EVs Authors should add the total number of cells used to extract EVs 3- Please review this paragraph: On groups 3 and 4, EVs were administered at a dose of 1μg/1g of mice body weight via injecting into the border zone of infarcted heart at three sites.(???? lack of a phrase) post MI surgery immediately. Authors should clarify this phrase. 4- Results Section: In Figure 3 B , authors should add a better description of x and y axis of each graph to clarify which one corresponds to CCK8 assay. In conclusion, I suggest minor corrections.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 72372

Title: Extracellular vesicles from hypoxia-preconditioned mesenchymal stem cells alleviates myocardial injury by targeting thioredoxin-interacting protein-mediated hypoxia-inducible factor-1α pathway

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 04244067
Position: Peer Reviewer
Academic degree: PhD
Professional title: Professor
Reviewer's Country/Territory: Japan
Author's Country/Territory: China

Manuscript submission date: 2021-10-13

Reviewer chosen by: Jia-Ru Fan

Reviewer accepted review: 2021-12-25 11:19

Reviewer performed review: 2021-12-27 00:25

Review time: 1 Day and 13 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The revised manuscript is considered to have answered this reviewer's request. Thank you for all the effort you put into this.