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OPEN PEER REVIEW

The Impact of Acute Myeloblastic Leukemia-Derived Small Extracellular Vesicles on the Bone Marrow Mesenchymal Stem Cells: Expression of G1 Cell Cycle Progression Genes

Bentolhoda Kuhestani Dehaghi, Mozhgan Amirpour, Najibe Karami, Mojtaba Shafiei, Leila Noorazar, Ali Keshavarz, Nader Vazifeh Shiran, Mahmoud Dehghani-Ghorbi, Hamide Rahmani Seraji, Mehdi Allahbakhshian Farsani

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About this report

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1 Original submission

1 comment · 17 Aug 2024

Reza Hosseini Reviewer

MAJOR REVISION 17 Aug 2024

Dear Prof. Akbari,
Editor-in-Chief, IJCM

I appreciate your kind invitation to review the manuscript titled " The effect of AML-derived exosomes as a growth factor on the Bone Marrow Mesenchymal Stromal cells: Regulating the G1 Phase of cell cycle".
In this manuscript, the authors have attempted to highlight the effects of AML extracellular vesicles on bone marrow mesenchymal stromal cells. My comments are as follows:

1- The English writing of the manuscript needs to be carefully revised.
2- The AKT genes in the abstract should be revised to AKT1.
3- The conclusion in the abstract is not based on the results obtained. The authors have not examined the PI3K/Akt components at the protein level. Moreover, the expression levels of CCND1, CDK4, and CDK6 can be altered due to the Akt, ERK, MAPK and MTOR pathways. Hence, they can only conclude that AML extracellular vesicles could alter the gene expression associated with the proliferation of BMSCs.
4- Based on the recommendations by MISEV2018, it is suggested that the authors use small extracellular vesicles instead of exosomes throughout the manuscript.
5- Line 48-49: In contrast to what the authors inferred, Ref3 assumes that AML development significantly impairs the hematopoietic progenitors and stem cells.
6- The introduction should be revised completely. It should explain the necessity of the current study based on the existing literature. Furthermore, why the authors have examined CCND1, CDK4, CDK6 and AKT1 should be properly addressed in the introduction.
7- Line 87-90 should be moved and merged with the next section.
8- All the methods sections should be revised to contain the details necessary for reproducibility.
9- The cell culture section in the Methods should be moved earlier in the Methods, before EV isolation.
10- Some references are misused, for example, Ref 20 in the discussion. The authors should ensure that they have used suitable references.
11- In the title, "a growth factor" should be revised to a more sensible phrase, since EVs contain several growth factors. For example, stating that AML EVs could ignite the proliferation of BMSCs might be more sensible.
12- Based on Figure 2D, it seems that there are too many 500nm particles in the purified fraction, in contrast to the DLS results in Figure 2C. How do the authors interpret these discrepancies?
13- Why have the authors chosen 50 microgram/ml for their experiments?
14- Although the authors have claimed that they have done dose-time response experiments, none of the figures support their claim. For example, the MTT and apoptosis assays for 20 and 80 micrograms/ml doses are not presented.
15- Figure 4C is not presented correctly. Indeed, the expression levels of AKT1, Cyclin D, etc. need to be compared with those in the control group. However, the authors have only used a single bar for all the genes they have investigated. It is not acceptable.
16- In Figure 5, the percentage of apoptotic cells should be presented as the percentage of Annexin V+ and Annexin V+PI+ cells. Fold change is not recommended for the presentation of apoptosis results.
17- The legends for all figures are not informative enough and should provide adequate explanations.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Response to Reviewers

Manuscript ID: 152405

Dear Reviewers,

We would like to express our gratitude for your valuable feedback and constructive comments on our manuscript. Your input has been instrumental in enhancing the quality and comprehensibility of our work. In response to your comments, we have made several revisions and addressed each of your concerns:

Reviewer Comments:

Comment 1: The English writing of the manuscript needs to be carefully revised.

Response: Regarding your concern about English writing, we revised the text.

Comment 2: The AKT genes in the abstract should be revised to AKT1.

Response: Thanks to your care and attention, the name of this gene was edited in the entire manuscript.

Comment 3: The conclusion in the abstract is not based on the results obtained. The authors have not examined the PI3K/Akt components in protein level. Moreover, the expression levels of CCND1, CDK4, and CDK6 can be altered due to Akt, ERK, MAPK and MTOR pathway. Hence the can only conclude that AML extracellular vesicles could alter the gene expression associated with the proliferation of BMSCs.

Response: Thank you for your valuable suggestion. The conclusion of the abstract was edited as requested.

Comment 4: Based on the recommendations by MISEV2018, it is suggested that the authors to use small extracellular vesicles instead of exosomes throughout the manuscript

Response: Thank you for pointing this out, we applied your recommendation.

Comment 5: Line 48-49: In contrast to what authors inferred from, Ref3 assumes that AML development significantly impairs the hematopoietic progenitors and stem cells.

Response: Thank you for your attention, unfortunately, there was a mistake in referencing. We reviewed all the references in the article.

According to doi.org/10.1016/j.arcmed.2022.12.008, doi.org/10.1016/j.gendis.2023.01.008, doi.org/10.3390/ijms22041881, and doi.org/10.1038/s41568-021-00363-z articles that referenced in the manuscript, we claim that "Leukemic cells exploit the supportive niche provided by MSCs, which, in turn, undergo significant biological alterations influenced by the leukemic cells".

Comment 6: Introduction should be revised completely. It should contain the necessity of the current study based on the existing literature. Furthermore, why authors have examined the CCND1, CDK4, CDK6 and AKT1 should be properly addressed in the introduction.

Response: We appreciate your emphasis on Introduction. According to your suggestion, we revised the introduction.

Comment 7: Line 87-90 should be moved on and merged with the next section.

Response: we applied your recommendation.

Comment 8: Al the method sections should be revised to contain any details for reproducibility.

Response: According to your request, we added the items that helped the reproducibility of the tests to sections 3.3.1, 3.3.2, 3.3.3, 3.4, and 3.9.

Comment 9: Cell culture section in the methods should be transferred to the earlier parts of the Methods, prior than EV isolation.

Response: we applied your recommendation.

Comment 10: Some references are misused. For example, Ref 20 in the discussion. The authors should ensure that they have used suitable references.

Response: We agree with this and checked and update references according to your suggestion.

Comment 11: In the title, as "a growth factor" should be revised to more sensible phrase, since EVs contain several growth factor. For example AML EVs could ignite the proliferation of BMSCs might be more sensible.

Response: We revised the title to “The Impact of Acute Myeloblastic Leukemia-derived small extracellular vesicles on the Bone Marrow Mesenchymal Stem cells: Expression of G1 Cell Cycle Progression Genes”.

Comment 12: Based on Figure 2D, it seems that there is too many 500nm particles in the purified fraction contrasting the DLS results in Figure 2C. How do the authors interpret these controversies?

Response: Thank you for pointing out the issue with the Figures scale. The scale of the figure was corrected by rechecking to 336nm.

Comment 13: Why do the authors have chosen 50 microgram/ml for their experiments?

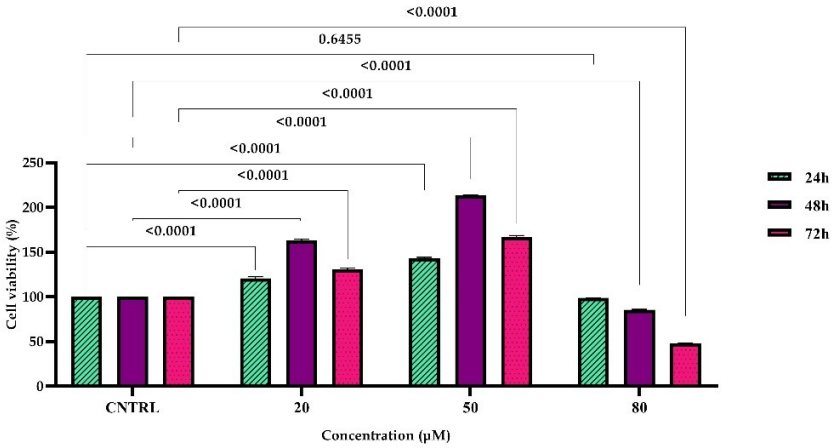
Response: We selected three doses of 20, 50, and 80 over 24, 48, and 72 hours based on our previous study (doi: 10.1007/s11033-024-09674-4.) and other studies. In addition, based on the MTT data, the 50 over 48-hour dose was associated with the highest proliferation rate.

Comment 14: although the authors have claimed that they have done dose-time response experiments, but non of the figures show their claim. Fore example, the MTT and apoptosis assay for 20 and 80 micrograms/ml doses are not presented.

Response: With the increase of time from 24 to 48 and then 72 hours in the doses of 20 and 50 µg /ml, we observed an increase in proliferation compared to the control group, and we saw the highest proliferation in the dose of 50 within 48 hours. While increasing the dose from 50 to 80 in all three times of 24, 48 and 72 hours, apoptosis occurred in the test group compared to the control group. Therefore, we considered a dose of 50 within 48 hours for further investigations.

Overall, since both the MTT results in this study and the results of our previous study (doi: 10.1007/s11033-024-09674-4) showed that the dose of 50 in 48 hours was the optimum dose, so we used the dose of 50 in 48 hours for further investigations.’

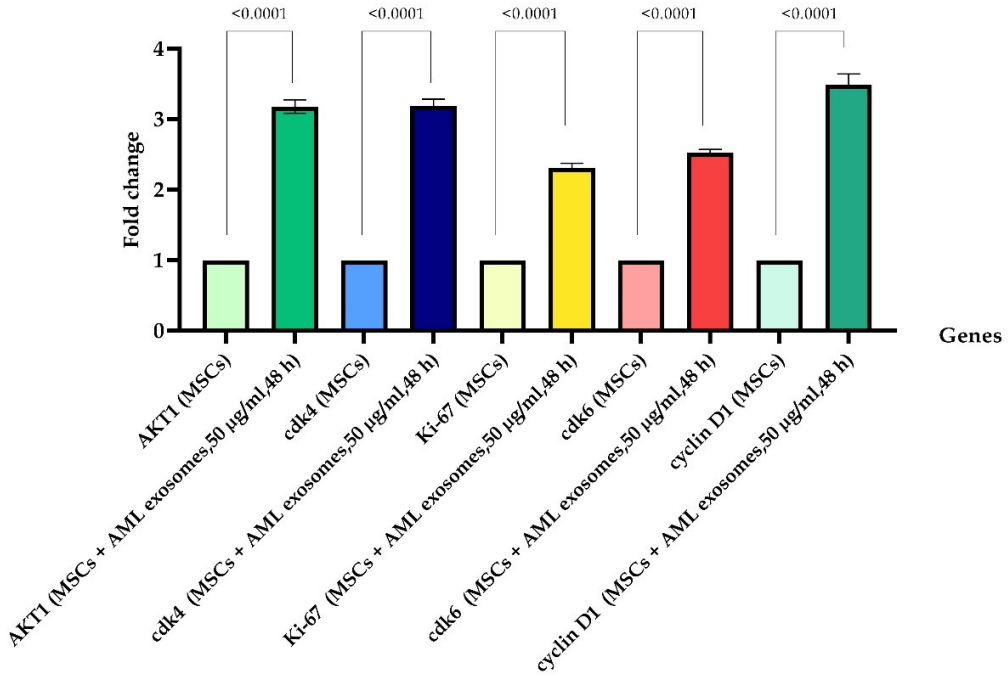
The MTT results are available in the manuscript supplementary



file.

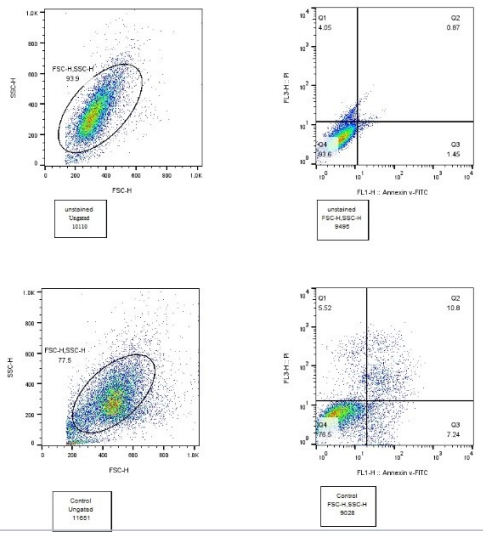
Comment 15: The figure 4C is not presented correctly. Indeed, the expression levels of AKT1, Cyclin D, and etc. need to be compared with expression in the control group. However the authors have only used a single bar for all the genes they have investigated. It is not acceptable.

Response: We calculated the fold change, in order to be able to compare the level of gene expression in the test group with the control group, However, based on your comment, we edited the chart.



Comment 16: In Figure 5, the percentage of the apoptotic cells should be presented as the percentage of Annexin V+ and Annexin V+PI+ cells. Fold change is not recommend for the presentation of apoptosis results.

Response: we calculated the fold change, In order to be able to compare the amount of apoptosis in the test group with the control group. However, we modified the text, charts and legends based on your comment and reported percentage instead of fold change.



In the control group:

- Annexin V +/ PI - : 7.24 %
- Annexin V +/ PI + : 10.8 %

In the test group:

- Annexin V +/ PI - : 5.44 %
- Annexin V +/ PI + : 6.16 %

Comment 17: The legends for all figures are not informative enough and should be properly explained.

Response: We appreciate your feedback on the figure legends. We revised them to provide clearer guidance to readers on the meaning of the images.

Once again, we appreciate your time and effort in reviewing our work, and we believe that these revisions will significantly improve the manuscript's quality. We look forward to sharing the updated manuscript with you for your further evaluation.

2 Revision 1

1 comment · 3 Sep 2024

Reza Hosseini Reviewer

ACCEPT 3 Sep 2024

Dear Editor-in-Chief, IJCM

The manuscript is a good read now and could be accepted for publication.