Author's response to reviews

Title: A modified method for isolation of bladder cancer stem cells from a MB49 murine cell line

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Author's response to reviews: see over
Dear Editors:

Thank you very much for your comments and suggestions.

As suggested, we have revised the manuscript according to the comments and suggestions point by point. The paper has been revised significantly throughout the text.

I am looking forward to hearing from you soon.

With kindest regards,

Yours Sincerely

Wanlong Tan, PhD. MD

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REPLIES TO THE COMMENTS

Referee 1:

1-“In Fig3A, the authors purposely did not collect the same 'events' on the FACS, it is clear that the MCSCs events collected are significantly smaller than that of MB49 cells. They should collect and show the same event number.”

Reply: Following the suggestion, we have collect the same "events" on the FACS in Fig3A.

2-“In Fig3 and Fig4, it is accustomed to put the control group in the left and experimental group in the right. It is quite used to mark * as the distinctive sign instead of □. These images should be modified”

Reply: Following the suggestion, we have modified the Figure 3 and Figure 4.

3-“It will be great if the authors can perform limiting dilution experiment in Fig 4E to show that at a certain cell dose MB49 doesn’t grow while MCSC grow.”

Reply: The revised manuscript has been performed the limiting dilution experiment in animal experiment. The cells were injected with gradient concentration subcutaneously into the nude mice, at a number from $1 \times 10^2$ to $1 \times 10^4$ in MCSCs and from $1 \times 10^4$ to $1 \times 10^6$ in MB49 cells (Page 11 line208-210).
The result showed that immune deficient nude mice injected with $1 \times 10^6$ in MB49 cells or $1 \times 10^3$ in MCSCs formed xenografts, those injected with $1 \times 10^5$ in MB49 cells or $1 \times 10^2$ in MCSCs did not (Figure 4E) (Page 14 line266-268).

Referee 2:

1-“it is unclear what is "new" about their method compared to numerous papers published in the field, please elaborate. The manuscript should include a comparison to available approaches in order to provide evidence for the advantage that the proposed new method provides.”

Reply: In the revised version of the manuscript, we have changed the title of manuscript to “A modified method for isolation of bladder cancer stem cells from a MB49 murine cell line”. Meanwhile, the revised manuscript include a comparison to provide evidence for the advantage as following:

As we known, the combination of the limited dilution method and SFM method has not been used to isolate the CSCs, which could improve the purity of cell sorting (Page 5 line76-78);

To facilitate the transition of MCSCs to vaccine applications, advances in expanding MCSCs had become an absolute necessity. There were three methods that have been used to isolate CSCs from tumors: specific cell surface markers, SFM, and side population cells. These methods were limited due to the lack of purity of CSCs or the purity was not enough for CSCs (Page
The limited dilution method in our study was different, which allowed MCSCs sphere formation to originate from a single cell before improving the purity of CSCs (Page 14 line279-280).

2-“English need to be improved under the suggestions of native specialist.”

Reply: The revised manuscript has been edited and proofread by the ivy-editing company. The added or changed parts or sentences in revised manuscript have been underlined in original manuscript with the red font.

Referee 3:

1-“In the introduction, the authors mentioned CSCs were isolated in a number of diseases. Since their paper focuses on bladder cancer, they should cite other primary references on human and mouse bladder CSCs. A good summary of the individual sources is available recently at Nature Reviews Urology 9, 583-594 (October 2012).”

Reply: Following the suggestion, we have provided with reference 4 (Ho PL, Kurtova A, Chan KS: Normal and neoplastic urothelial stem cells: getting to the root of the problem. Nat Rev Urol 2012, 9(10):583-594) in the introduction section.
2-“The method section is written with some important information missing, e.g. none of the dosages for chemotherapy drugs are mentioned”

Reply: In the revised version of the manuscript, the dosages for chemotherapy drugs have been supplemented in Table 4 (Page 21 line 419).

3-“In the results section, none of the Figure 1 panels were described in the manuscript.”

Reply: In the revised version of the manuscript, the Figure 1 panels were described in the results section (Page 12 line 223-227).

4-“Please send the manuscript to English editors, the grammar and the typing need to be corrected in some parts.”

Reply: The revised manuscript has been edited and proofread by the ivy-editing company. The added or changed parts or sentences in revised manuscript have been underlined in original manuscript with the red font.

Referee 4:

1-“I would recommend the authors to change the title of the manuscript because the method employed for isolation of stem cells have been
previously used for various other cancers. Use of defined media to propagate stem cells using B27, FGF, EGF etc have also been extensively used. Therefore, this method is not new but a modified version to isolate stem cells from an established cancer cell line. Therefore, even thought the protocol followed is novel to MB49 cells, it is not new. Highlighting the findings from MCSC might be more appropriate.”

Reply: In the revised version of the manuscript, we have changed the title of manuscript to “A modified method for isolation of bladder cancer stem cells from a MB49 murine cell line”.

2-“Please mention that MB49 is a murine cell line.”

Reply: In the revised version of the manuscript, we have added “murine” in the title and the method part (Page 6 line 98).

3-“Figure 2 needs scale bars and magnification details.”

Reply: Following the suggestion, we have modified the scale bars in Figure 2.

4-“Please provide with references, especially in the methods section.”

Reply: Following the suggestion, we have provided with references (Number 12, 13, 14) in the methods section.
Editorial Request:

1-“Please can you include more context in the background section of the Abstract, in addition to the study objectives. Also, please can you provide more detail in the Conclusions section of the Abstract to provide a brief summary of your findings and the potential implications.”

Reply: Following the suggestion, we have include more context in the background section and the conclusion section of the abstract. The added sentences in revised manuscript have been underlined with the red font.

2-“Please could you also highlight with colored text all changes made when revising the manuscript to make it easier for the Editors to give you a prompt final decision on your manuscript.”

Reply: All changes in revised manuscript have been highlighted with the red font.