ANSWERING REVIEWERS

March 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9203-Edited.doc).

Title: Reversible immortalization of human hepatocytes mediated by retroviral transfer and site-specific recombination

Author: Fan-ying Meng, Li Liu, Feng-hui Yang, Chun-you Li, Jun Liu, Ping Zhou

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9203

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer #1 : This study aims to establish reversibly immortalized human hepatocytes for use in cell therapies, such as bio-artificial liver therapy. Reversible immortalization of primary human hepatocytes was successfully achieved using methods involving retrovirus-mediated transfer of the SV40 T antigen and subsequent excision by site specific recombination. Although the authors successfully achieved their aims, the paper lacks novelty as the methods used in this paper and the generation of reversibly immortalized human hepatocytes from primary cells have been reported previously. Language polishing and formatting corrections are required.

Answer: Thanks a lot for your valuable opinion. As you said that we herein successfully established reversible immortalization of human hepatocytes which may offer a good and safe hepatocytes source for bio-artificial liver in the near future. In fact, we have made great efforts to establish liver cell lines for cell therapies. We first successfully established porcine reversibly immortalized cell lines in 2009. However, in using xenogenic porcine cells, there is concern that species-specific pathogens can be transmitted in recipients. Then, we expanded the novel strategy of reversible immortalization of human hepatocytes, using the method we used before. Though the hepatocytes can be transduced with retroviral vectors, the efficiency of transduction was significantly low. You see, it was rather hard work to collect suitable tissues for human cells isolation. It took us about 4 years to succeed in achieving the aim.

Though other alternative sources of reversibly immortalized human hepatocytes have been explored, we herein established a totally new human hepatocyte cell line. The population of reverted cells exhibited the characteristics of differentiated hepatocytes. Furthermore, our ultimate aim is to find a good and safe hepatocytes source for a BAL system. In fact, we now focusing on decellularized liver matrix, which could be reseeded with the reversibly immortalized human hepatocytes we established. We believe that it was meaningful to establishing an alternative sources of reversibly immortalized human hepatocytes.

We are very sorry for our incorrect writing. We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We hope that the correction will meet with approval.

Special thanks to you for your good comments. Your valuable opinion is greatly appreciated.

Reviewer #2

The author made great efforts in establishing a human liver immortalized cell line. However, lots of studies regarding on establishing the immortalization of human hepatocytes have published.

Reviewer #3

This paper is a good presentation of the authors' work and achieves good linearity, but no innovative contribution was found in the methods.

Answer: Both Reviewer #2 and Reviewer #3 considered our paper lacks novelty. We answered Reviewer #1 the similar question. Though other alternative sources of reversibly immortalized human hepatocytes have been explored, we herein established a totally new human hepatocyte cell line. The population of reverted cells exhibited the characteristics of differentiated hepatocytes. Furthermore, our ultimate aim is to find a good and safe hepatocytes source for a BAL system. In fact, we now focusing on decellularized liver matrix, which could be reseeded with the reversibly immortalized human hepatocytes we established. We believe that it was meaningful to establishing an alternative sources of reversibly immortalized human hepatocytes. We hope that the explaination will meet with approval.

Once again, thank you very much for your comments and suggestions.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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