Dec., 3, 2012

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: WJG-417-final.doc).

Title: Establishment of an orthotopic transplantation tumor model of hepatocellular carcinomas in mice

Author: Zhao Gui-Jun, Xu Li-Xia, Chu Eagle SH, Zhang Ning, Shen Jia-Yun, Alatangaole, Li Xiao-Xing

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 417

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

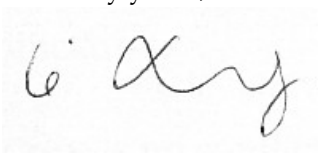
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer. All the revisions have been highlighted by underline. Please find the point by point responds following this letter.

3 References and typesetting were corrected

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

Sincerely yours,



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Comments to the authors

Reviewer 1

In general, the topic is attractive but the organization is poor, hard to read. The authors should rewrite many parts of the manuscript and make it easily understandable. A great deal of English polishing is required.

Title:

It's better to make the model clearer, such as adding "in mice" at the end of the present title.

Thank you for the suggestion. We have revised the title to "Establishment of an orthotopic transplantation tumor model of hepatocellular carcinomas in mice".

Abstract:

Aim:

Not clear. "To improve the outcome of orthotopic transplantation, …" Readers may think it a model of organ transplantation, but the content is not. What kind of tumor transplantation?

Thank you for the suggestion. We have revised the aim part accordingly. "To improve the outcome of orthotopic transplantation in mouse model, we…".

Methods:

Hard to understand. According to the description, hepatoma cells were injected subcutaneously to the nude mice. That's subcutaneous tumor model. Then here came the establishment of an orthotopic liver transplantation model. What's the connection? Where is the procedure of establishing an orthotopic transplantation of HCC in the liver of nude mice?

We have revised the method part abstract accordingly. "MHCC-97L hepatocellular carcinoma (HCC) cells with stably expressing luciferase gene were injected into the subcutaneous region of nude mice. After one week, the ectopic tumors were harvested and transplanted into the left lobe of liver of nude mice."

Results:

There are results of comparison between several groups but the authors did not mention it in Methods.

We have added the information accordingly. "The mice were divided into 3 groups: I) AGS-, II) AGS+/PAX5-, and III) AGS+/PAX5+."

Conclusion:

no comment

Thanks.

Key words:

hepatocellulcar carcinoma (HCC) is the standard words, but the authors used many "hepatocarcinoma" or "liver cancer" in the manuscripts. Better to fit the key words.

All the "hepatocarcinoma" and "liver cancer" have been replaced with "hepatocellulcar carcinoma" or "HCC".

Introduction:

I believe that the goal of this study is to investigate the value of using absorbable gelatin sponge in liver tumor orthotopic transplantation, compared with routine procedure. If so, the authors should mention it much clearer.

We appreciate this suggestion. We have revised the text according to this comment.

Materials and Methods:

Cell line:

No comment

Thanks.

Animal model:

The authors firstly said that" Six-week-old..mice weighting 16-18g were used in this study." But in the next paragraph, tumor cells were injected into flank of four-week-old mice weighting 12-15g. Which description is real? The last half parts of the two paragraphs were highly similar, better to rewrite.

This part has been rewrited accordingly. The 4-week-old mice were used for the tumor cell subcutaneous injection, and the 6-week-old mice were used to perform the surgery and establish the orthotopic transplantation tumor model.

"MHCC97L cells (1 × 107 cells in 0.1 mL PBS) transfected with PAX5 or pcDNA3.1 were subcutaneously injected into the dorsal left flank of 4-week-old male Balb/c nude mice (1/group); the body weights of all mice were between 12-15g. Six-week-old athymic male Balb/c nude mice weighing between 16-18 g were used for the xenograft tumor transplantation."

Establishment of an orthotopic transplantation liver tumor model in nude mice:

Confused content and hard to understand. This is one of the key points of the manuscript. Please rewrite and make it clear.

Thank you very much for the important suggestion. This part has been revised accordingly.

No comments for the rest parts of this section.

Thanks.

Results:

1. Only demonstrate results in this section. Don't use some words such as "To evaluate…""…could be…".

We have revised the text according to this comment.

2. 40% of mice in control group died one day after the surgery. The mortality rate is really high. Didn't the authors use any methods to stop bleeding after removal of left liver lobe of mice? If not, why?

We did not remove the left lobe of the liver. We just pulled out the left lobes for the surgery, and put it back after our operation. We also noticed the post-operative mortality was high in group I. This may due to the routine surgery using incision suture in liver takes a long time. Another

reason was probably due to the fast post-operative recovery and frequent activities caused by pain.

Discussion:

1.Is there any reference talks about tumor cell leakage rate during injection of tumor cells into the liver of mice? If so, please ref it.

It would be difficult to find such reference. However, based on our experience, the intra-operative leakage rate is about 50% and the post-operative rate is hard to estimate, especially in the cases which only part of the cells were leaked.

2. The use of gelatin sponge in this study seems to stop bleeding after transplantation. Compared with routine way, what are the other advantages? Moreover, why not use it when inject tumor cells in the liver, which may help prevent occurrence of leakage? Have the authors tried such a methods?

The use of gelatin sponge could prevent the implanted solid tumor from dropping out. However, in cells injection model, as the cells were suspended in medium, if the leakage happened, the sponge would do no help but only aggravate the leakage as it hygroscopic. Meanwhile, the bleeding is minimal in injection model, no particular homeostasis strategy is needed.

3. The whole section should be reorganized after rewriting the former two sections.

We reorganized the Discussion and add the section about the former questions. However, since we do not have actual statistics for the first question, we feel it's better not to show our estimated data in the paper. The second question was carefully discussed.

Reviewer 2

Good for publication

Thank you so much for your kind comment.