November 4, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13860-revised.doc).

Title: Gene expression profiling of MYC-driven tumor signatures in porcine liver stem cells by transcriptome sequencing

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Name of Journal: *World Journal of Gastroenterology*

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We thank the reviewers for their valuable comments, which helped greatly in enhancing the quality of this manuscript. As per their suggestions, the following changes have been made in this revision:

Reviewer 1

1. *The results are interesting but the presentations were run around. The summary for the groups of under-expressed/ absence genes were listed differently …. Each of the major different genes that was significant in term of driven tumor and their known, well accepted functions should be raised separately and clarified.*

We have discussed major genes in this revised manuscript. These are highlighted in yellow. As suggested by this reviewer, a clarification has been given on these genes and their known functions. We thank the reviewer for pointing out the differences in the gene list and the text. These errors were corrected in this revised manuscript.

2. *The present discussion was too far expanded beyond what they have shown in the results.*

Since we were investigating genes that are differentially expressed in PICM-19 as well as PICM-CSCs at a global scale, we felt that there is a need to discuss their role/known functions at length so that we could underscore their importance in tumorigenesis. Many of these genes were earlier shown to be involved in various cellular signaling pathways. Therefore, the discussion was a bit elaborate to highlight various cellular mechanisms that are potentially induced by the MYC overexpression.

3. *How possibly the under-expressed and the absence genes played a role in this tumorigenesis should be also discussed.*

As suggested by this reviewer, roles played by under-expressed and silenced genes has been clarified in this revised manuscript.

Reviewer 2

1. *The abbreviation first appears need to be explained use its full name.*

As suggested by the reviewer, we have carefully reviewed the text and included the full name of each gene when it first appeared prior to using its abbreviation.

2. *The groups of under-expressed/absence genes that listed in the text and the abstract are different.*

We thank the reviewer for pointing out this error. The gene list has been corrected in this revision.

3. *I think the authors need to do some mechanistic experiments to draw the conclusions*.

We agree with the reviewer that mechanistic studies are needed. As mentioned in the manuscript title and in the text, this study was solely aimed at obtaining gene expression profile of MYC-induced genes so that we get an idea on the potential mechanisms of tumorigenesis to carry out follow-up studies. Currently, these mechanistic follow-up studies are underway, and will be the subject of a separate manuscript in the near future.

Reviewer 3

1. The control groups need to be provided in fig.1A and fig.2.

As reviewer suggested, we have provided the control groups in Fig. 1A and Fig. 2.

2. Due to the gene expression normalized process should be done before compared with interesting genes in PICM-19 and PICM-19 CSC, the significance of table1 and 2 is the same with table 3 and 4. Therefore, the table 1 and 2 are not necessary.

We have indeed normalized the gene expression of PICM-19 and PICM-19-CSC separately prior to comparing them. We disagree with the reviewer that the significance of tables 1 and table 2 are the same as tables 3 and 4, respectively. Tables 1 and 2 refer to the differential gene expression in PICM-19 stem cells themselves and Tables 3 and 4 refer to those that are either downregulated or upregulated by MYC overexpression. Moreover, Table 1 is important because those genes were completely silenced by MYC in stem cells.

3. Why the genes in table 1 and 2 do not present in table 3 and 4?

In Tables 2 and 4, we listed only the top 25 genes that are differentially expressed. Therefore, some of the genes from Tables 1 and 2 are missing in Tables 3 and 4. However, we have given the entire list of differentially expressed genes in supplementary Tables 1 and 2 that show the expression of genes that are missing in Tables 1 and 2.

4. .The p-value and standard error should be provided in the real-time PCR data about PICM-19 and PICM-19-CSCs genes expression

As suggested by the reviewer, we have now included the p-value and standard error in the figure legend for Fig. 4.

Reviewer 4

1. While the base cell line, PICM-19, has been characterized to be representative of porcine normal hepatic stem cells, experiments showing that MYC drives development of a CSC state is lacking.

PICM-19 is the only porcine liver stem cell type that can be propagated in culture for an extended period of time. That was the primary reason we chose it for our studies. In this manuscript, we did show the cancer phenotype of PICM-19-CSCs that expressed MYC gene by performing *in vivo* animal experiments as shown in Fig. 2. Thus PICM-19 cells, which are primary stem cells, were indeed converted into cancer stem cells by MYC overexpression.

2. A very strong implication is made regarding the silencing of a six gene set in PICM-19-MYC cells … however, no specific gain of function or inhibitory studies are provided.

Each of these six genes was involved in a distinct signaling pathway. Therefore, extensive and elaborate mechanistic studies are needed to deduce their role in CSCs. We will perform follow-up studies on these genes,

and will compare PICM-19-CSCs with CSC from human HCC specimens and cell lines. Results from these studies will be the subject of another manuscript in the near future. This study is an important first step to gain an understanding on potential genes that play critical roles in MYC-induced tumorigenesis.

All the changes in the text were **highlighted in yellow**, and all available PMID and DOI numbers were included for references, together with the names of all authors.

On behalf of my co-authors and me, I thank you again for the opportunity to publish our manuscript in the *World Journal of Gastroenterology*.

Yours sincerely,

Raj Aravalli