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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 13860
Title: Gene expression profiling of MYC-driven tumor signatures in porcine liver stem cells by transcriptome sequencing
Reviewer code: 00069371
Science editor: Ya-Juan Ma
Date sent for review: 2014-09-04 17:12
Date reviewed: 2014-09-29 16:18

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

In this study, the authors identify the overall genes expression in a liver stem cell after induction by c-MYC plasmid transfection in generating hepatic tumors Major comments: The METHODS: conduction and explanation were sound and direct to the results. The RESULTS: are interesting but the presentations were run around (- not well organized), and mixed up with what supposed to be in the discussion part, especially, on the differential gene expression. Those descriptive results have confused the readers a lot. 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. The summary for the groups of under-expressed/ absence genes (ANGPT1, FIGF, RSPO2, SELENBP1, CDO1, DKK2 – in the abstract) and C22orf39, CDO1, DKK2, ENPEP, GPX6, SRPX2- in the text abstract) were listed differently, why? Which of them were the targets or critical in term of functional significant? The genes not regulated by MYC should be put last. The DISCUSSION: The present discussion was too far expanded beyond what they have shown in the results. Several genes did not even show in the data results, eg MDR1, etc. Please, check to be more convincing. How possibly the under-expressed and the absence genes played a role in this tumorigenesis should be also discussed. Which gene should be selected as the best ? CONCLUSION: "MYC-driven genes may serve as a promising candidates for the development of HCC therapeutics that would not have deleterious effects on other cell types in the liver" – How, please example the ideas, since the

expression seems to be under epigenetic control? Minor comments: Language: page 16, paragraph 3, line 2 – It is was…. page 16, paragraph 3, line 3….to suppress to…. Figure 2 – the proliferation is very minor response, only a few dividing cells, this could be compare with the PICM-19 cell implantation

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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
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Title: Gene expression profiling of MYC-driven tumor signatures in porcine liver stem cells by transcriptome sequencing
Reviewer code: 00032726
Science editor: Ya-Juan Ma
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Date reviewed: 2014-10-21 20:52

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[Y] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[Y] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

This manuscript aims to identify the genes induced and regulated by the MYC protein in generating tumors from liver stem cells. The authors used an immortal porcine liver stem cell line PICM-19, to identify MYC-driven differential gene expression, transcriptome sequencing was carried out by RNA sequencing and genes identified by this method were validated using real-time PCR. And finally find that MYC-driven genes may serve as promising candidates for the development of HCC therapeutics that would not have deleterious effects on other cell types in the liver. There are some comments need to be proposed. 1) The abbreviation first appears need to be explained use its full name. 2) The groups of under-expressed/absence genes that listed in the text and the abstract are different. Please check it. And I think you need to clarify the function of the important genes what you list in this manuscript. 3) I think the authors need to do some mechanistic experiments to draw the conclusions.

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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
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Science editor: Ya-Juan Ma
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[ ] Grade D: Fair	[ ] Grade D: Rejected	BPG Search:	[ ] Rejection
[ ] Grade E: Poor		[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

This study provides the information about gene expression profiling of MYC-driven tumor signatures in liver stem cells. Further, there are some confusing questions and suggestions in this study. 1.The control groups need to be provided 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. 3.Why the genes in table 1 and 2 do not present in table 3 and 4? 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.

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ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology
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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] Existing	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] No records	
[Y] Grade D: Fair		BPG Search:	[ ] Rejection
[ ] Grade E: Poor	[ ] Grade D: Rejected	[ ] Existing	[ ] Minor revision
		[ ] No records	[Y] Major revision

COMMENTS TO AUTHORS

The manuscript by Aravalli et al, "Gene expression profiling of MYC-driven tumor signatures in porcine liver stem cells by transcriptome sequencing" seeks to link the development of the cancer stem cell (CSC) state in HCC to MYC driven processes. Using transcriptomic analysis, six genes are implicated as key suppressors to the CSC state in MYC driven tumor progression. The methodology with respect to RNAseq appears to be sound and identification of genes with are upregulated or downregulated in the population of PICM-19-CSCs appears to be sound, however the manuscript appears to lacking other key mechanistic experiments such that key conclusions are difficult to make with respect to the six gene set, MYC, and CSC development. Major Criticisms 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. While it may be the case that MYC drives the development of HCC, proof is not provided that this population has a CSC phenotype. Indeed as many as one million cells are required to form tumors (HCCs) in NOD-SCID mice. Limiting dilution analysis would need to be performed to demonstrate that a small population of these cells is capable of recapitulating HCC either in vivo or in sphere assays in vitro. CSC markers may indeed allow identification of a subset of PICM-19-MYC cells which may have this capacity including increased expression of stemness related genes. 2) A very strong implication is made regarding the silencing of a six gene set in PICM-19-MYC cells being

perhaps suppressors of HCC tumor progression. In addition, implication is provided regarding the numerous genes which are strongly induced in PICM-19-MYC cells (Table 3). However no specific gain of function or inhibitory studies (e.g. with shRNA) are provided to help functionally assess whether there is any mechanistic link at all. Therefore most of the data is observational with respect to the transcriptomic signature. Additionally, no clinical correlative studies are performed to either assess linkage of the gene expression changes to clinically relevant prognostic outcomes in HCC patients or with respect to correlations of gene expression in HCC patients to known gene products consistent with the CSC state.