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ESPS Peer-review Report
Name of Journal: World Journal of Biological Chemistry
ESPS Manuscript NO: 9964
Title: Hepatic Hepcidin Expression is not Regulated by Fas-mediated Apoptosis
Reviewer code: 00255764
Science editor: Ling-Ling Wen
Date sent for review: 2014-03-06 22:21
Date reviewed: 2014-03-25 19:25

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	[ ] Existed	[ ] High priority for publication
[Y] Grade C (Good)	[ ] Grade C: a great deal of language polishing	[ ] No records	[ ]Rejection
[ ] Grade D (Fair)	[ ] Grade D: rejected	BPG Search:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

This work is based on investigating how Fas signalling may regulate the expression of the important iron exporter Hepcidin-1 (Hep-1). Hypothesis: Their work follows up on studies identifying p53 (Weizer-Stern et al) and Fas (Li et al) as regulators of Hep-1 expression. The authors attempt to reproduce the findings made by Li et al, which failed to provide evidence linking Fas-mediated apoptosis to the regulation of Hep-1 expression. The authors appear to pose a hypothesis that the process of apoptosis itself, as defined by them as effector caspase activity, regulates Hep-1 expression. It was not clear to me what they really meant by apoptosis and whether they were trying to address the role for Fas-mediated complex I versus complex II formation, or non-apoptotic initiator caspase activity for example. It was never clearly stated. Conclusions: Through their work they conclude that "hepcidin gene expression... does not correlate with induction of caspase activation, and thus is not regulated by apoptosis." The above conclusion is somewhat odd. Fas-mediated formation of the pro-apoptotic Complex II is responsible for activating caspases and mediating the destruction of the cell's DNA and transcriptional/translational potential. The fact that the cell ceases to exist clearly provides a very potent means of regulating gene expression. Hence I am uncomfortable with their statement that "hepcidin gene expression… is not regulated by apoptosis." The authors must be more specific and clearly state that they cannot correlate changes in caspase activity with changes in hepcidin gene expression. In contrast to the end point of apoptosis (cell deletion), work in Drosophila clearly demonstrates that initiator caspase activity is required to drive transcriptional events in an apoptotically committed, but not yet 'deleted', cell. However, due to lack of reference to relevant background literature it was not clear to me that the authors were addressing this specific

role for initiator caspase function. Experiments to block the late stages of cell death (effector caspase inhibition) would allow the cells to be kept in an 'undead-like' state, allowing harvesting of material for gene expression analysis. Layout: The justification for this work only really became apparent once I read the discussion. I would recommend a thorough re-write of the Introduction, incorporating some background currently in the discussion section. This would help justify why this work was done and clearly state how it differs from the work published by Li et al. Specific points: Figure 1B. Positive control would be desirable. Could the authors include either a HAMP cDNA plasmid or treat the cells with known induces of HAMP expression – IL-6 or BMP6? Figure 4. The conclusion drawn from the P-STAT3 blots are not convincing. Levels in P-STAT3 vary between the NaCl-treated animals @1 and 6hrs, with the levels at 1hr somewhat comparable to those see in the JO2-treatment 6hr samples. Blot not labelled as "Phospho-p65" Figure 5. Negative controls for the CHIP assays are essential. Positive control would be preferable.

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

COMMENTS TO AUTHORS

Summary In this study, the authors designed to investigate the effect of apoptosis on the regulation of hepcidin expression in the liver, the authors built up in vitro and in vivo models of Fas-mediated apoptosis to examine their hypothesis. According to the author's present results indicated that despite the activation of Stat3 signaling, the Fas-mediated extrinsic apoptotic pathway is not involved in the regulation of human or mouse hepcidin gene expression in the liver of C57BL/6 mice. Comments 1. Over all, the study is designed well. The conclusion is supported by the results. 2. The results provide a new and clear illustration that Fas-mediated extrinsic apoptotic pathway did not play a significant role in the regulation of human (HAMP) or mouse (hepcidin-1) hepcidin gene expression in the liver.

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[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

In this paper, Lu et al. aimed to determine the regulation of human hepcidin (HAMP) and mouse hepcidin (hepcidin-1 and hepcidin-2) gene expression in the liver by apoptosis using in vivo and in vitro experimental models. The role of hepcidin in liver fibrosis, via apoptosis, has emerged in recent years. Given the goal of achieving an explanation about the role of hepcidin is a growing concern, the analysis is justified and the aim of the study is clinically relevant. It is a well-designed study and the conclusions are consistent with the results. The fact of that the Fas-mediated extrinsic apoptotic pathway is not involved in the regulation of hepcidin gene expression in the liver is a novel conclusion which could improve the management of liver fibrosis.