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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 14947
Title: Autophagy in anti-apoptotic effect of augmenter of liver regeneration in HepG2 cells
Reviewer's code: 01568246
Reviewer's country: Norway
Science editor: Ya-Juan Ma
Date sent for review: 2014-11-03 08:21
Date reviewed: 2014-11-17 17:54

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	PubMed Search:	[Y] Accept
[Y] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[Y] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The aim of the present study was to determine whether "augmenter of liver recognition" (ALR) could reduce apoptosis by enhancing autophagy. HepG2 cells were used for this purpose. It has earlier been shown that ALR may control the apoptotic process of regenerating liver following partial hepatectomy in rats (ref 17). The authors conclude, based on the presented data, that ALR protects cells against apoptosis partly through increasing autophagic activity in HepG2 cells. ALR does not prevent apoptosis if autophagy at the same time is prevented by means of the autophagy inhibitor 3MA (3methyl adenine). The methods used seem to be adequate to determine whether ARL control apoptosis through its ability to stimulate autophagy, and the results are obtained by carefully conducted experiments. Neverthesless, the authors should deal with the following points: (1) The molecular mechanisms whereby ALR increase autophagy and reduce apoptosis are still unknown. The authors should discuss/suggest possible explanations. (2) When using accumulation of LC3-II to measure autophagy it is necessary to distinguish whether autophagosome accumulation is due to autophagy induction or rather a block in downstream steps, for instance reduced fusion of

autophagosomes/amphisomes with lysosomes. One possibility to measure the real autophagic activity would be to inhibit degradation in lysosomes (autolysosomes). This could be done by means of protease inhibitors such as leupeptin (used by Seglen and coworkers, Autophagy 3:3 181-206 2007). The actual increase in autophagic activity would be the difference in accumulation with or without the inhibitor. This problem is discussed in one of the papers referred to in the present manuscript (ref 12). (3) A main problem with the paper is the language. In several places in the article it is difficult and even not possible to understand what the authors mean. Some examples: In Abstract (Background): (a) The sentence starting with : "Multiple evidences.." is not clear and should be rewritten. It should give real information about the processes. What are the mechanistic overlap and the interaction between the apoptotic machinery and autophagy proteins? Maybe this information could be given in the Introduction. (b) The sentence starting with "HepG2 cells were treated by.." should be rewritten. The cells were not treated by inhibition of autophagy, and "to observe" should be deleted and the rest of the sentence should read: Apoptosis were observed by fluorescence microscopy and flow cytometry". (3) "The counts of apoptotic cells were much more…" should be replaced with "The counts (number) of apoptotic cells were much higher…" In Introduction: (1) The first sentence in Introduction is not clear: What can stimulate DNA synthesis? HSS or partial hepatectomy? The sentence should be rewritten! (2) The second paragraph (section) in the Introduction is very muddled and should be abbreviated and give a clearer overview of what is known about the relation between autophagy and apoptosis. In Results: (1) In second paragraph: "As well as we know" change to: "As far as we know". (2) In "ALR increased autophagic activity in HepG2 cells": "After starving for 24 hours…green puncta in HepG2 cells treated with ALR were more than that in control" change to "….green puncta in HepG2 cells treated with ALR were more numerous than in control cells". Later in same paragraph: "the number of autophagosomes…..was more than that in control", change to "was higher than that in control". (3) In "3MA suppressed autophagic activity in HepG2 cells". The first sentence is difficult to understand. What is meant with "to inhibit autophagic formation for the importance of Beclin 1/class-III PI3 kinase complex"? Rewriting is needed.

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ESPS PEER-REVIEW REPORT

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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
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[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
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[ ] Grade E: Poor		[Y] No	[ ] Major revision
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		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The present manuscript aimed to determine the effect of ALR on autophagy by transfecting ALR plasmid to HepG2 cells. The paper is well written and very interesting, while there are some minor concerns, please respond some queries accurately. 1. Why only one cell line, HepG2 was used? One would wonder what the effects in other hepatoma cancer cells. 2. Discussion includes a careful description of the experimental data but lacks the discussion of the global meaning of the results. The comparison of the findings with other scientific reports, as well as a final sentence on the future perspectives of the research are absolutely required. Conclusions needs to be extended with a more general statement. 3. English needs to be edited in the whole manuscript and checked for typos.

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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
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		[Y] No	

COMMENTS TO AUTHORS

Honbo et al. investigated the role of ALR in autophagy and cell death, using human HepG2cell line. Changes in autophagy-related proteins (Atg), cell death, and autophagy were analyzed in the presence or absence of ALR. The authors attempted to elucidate the mechanisms of ALR-induced anti-apoptosis. However, there are numerous major concerns that authors need to address. 1. This is a poorly written paper with numerous grammatical and spelling errors. 2. Authors' conclusion is quite contradictory to the data presented, and could be misleading. 3. Autophagy is highly dynamic and the changes in static levels of LC3 do not reflect this important feature of autophagy. Although authors mentioned the autophagic flux on page 5, its validity and interpretation are questionable. 4. All assays were performed in combination with both ALR and starvation. ALR alone should be assessed. 5. The concentration of 3-MA used in this study is microM, which is not enough to block autophagy. 6. The interpretation of Fig. 4 is not appropriate since fluorescence imaging of Annexin-V and PI does not support apoptosis.

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ESPS PEER-REVIEW REPORT

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		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

In this manuscript, the authors studied whether augmenter of liver regeneration (ALR) played a role in induction of autophagy in HepG2 (hepatocarcinoma cell line) and evaluated its anti-apoptotic effect in the presence of serum deprivation. They found that ALR increased the autophagy induced by serum deprivation and that inhibition of autophagy by 3-MA increased the counts of apoptotic cells in HepG2 cells treated with both ALR and 3MA compared to those treated with ALR only. They conclude that the anti–apoptotic effect of ALR may be related to autophagy. The manusript is very confusing and the englush needs a strong revision. The authors did not clearly introduced their working hypothesis and several conrols are missing in the experimental plan. Western blot of p62, the read-out of autophagy should be shown. Experiments with genetic interference of autophagy should be performed. An inhibitor of apoptosi should be used to better evaluate the role of ALR-induced autophagy. Specific points: 1) ALR should be better explained in the Introduction. In the Introduction section, the mechanisms of autophagy and apoptosis are confusing and their overlap is not clear neither is the role of ARL. 2) In the Abstract, Background section, where is the

verb for the sentence: "Multiple evidences suggested that mechanistic overlap and interaction between the apoptosis machinery and autophagy proteins"? 3) In the Introduction section, which is the reference number in the sentence: "Polimeno et al. demonstrated that ALR can…."? 4) Which is the reference for those two plasmids: GFP-LC3B and ALR. 5) In the Material and methods' section the authors claimed that: "For quantification of autophagic cells, GFP-LC3 puncta were determined from triplicates by counting a total of more than 30 cells". The number of counted cells is quite small, the authors should increase such number. The same is for electron microscopy evaluation. 6) In the figure legend, Fig 1, the authors claim that "the number of typical autophagosome…", however, they did not show any graphs. What is such number? 7) In Fig 2, it is not clarified which sample is transfected with control and with LC3 plasmid, making the comprehension of the figure very difficult. For instance, what are the four lanes of the western blot in Fig 2A? 8) To claim that: "All results indicated that ALR increased autophagic flux in HepG2 cells…", in pag 6, the authors should perform also western blot of the p62 protein, as read-out of the autophagic flux. 9) In Fig 3, again, what are the samples in the western blot? The figure legend is very confusing. 10) In the Discussion section the authors claim: "Based upon these premises, we verified the hypothesis of an involvement of autophagy in the anti-apoptosis effect of ALR on hepatocytes", however, they used a hepatocarcinoma cell line, instead of hepatocyrs. Please explain this discrepancy.

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ESPS PEER-REVIEW REPORT

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[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	PubMed Search:	[ ] Accept
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		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

This is a good paper on the role of augmenter of liver regeneration in regulation of autophagy and apoptosis. However, there are some problems with data interpretation, which may require additional studies. 1. You made a conclusion that ALR activates authophagy based on induction of LC3-II. However, LC3 in autophagosome indicates only upstream level of autophagy induction, which may be also related to impairment of lysosomal function. To show that there are no changes in lysosomal degradation, you need at least to measure p62, which is a substrate for lysosome. If you get an accumulation of p62 after transfection of ALR plasmid, it will mean that ALR suppresses lysosomal degradation and it will indicate the reduction in autophagy even LC3 levels go up. 2. LC3 induction by itself cannot indicate autophagic flux. 3. You need to provide the data on efficiency of plasmid transfection. 4. There are lot of misspelings in the text.