

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 34004

Title: Estrogen Receptor (ER) expression in chronic Hepatitis C and Hepatocellular Carcinoma pathogenesis

Reviewer's code: 03259512

Reviewer's country: Bosnia and Herzegovina

Science editor: Ze-Mao Gong

Date sent for review: 2017-03-22

Date reviewed: 2017-03-28

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input checked="" type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Plagiarism	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		<input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Major revision
		BPG Search:	
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

Authors investigated ER expression in liver hepatocytes from normal subjects and patients with Hepatitis C virus-related cirrhosis and Hepatocellular Carcinoma. The expression of ER subtypes, ER α and ER β , were evaluated for the first time in relation to gender. The manuscript is well written and comprehensive. However, the role of ER α and beta isoforms was not described or discussed at all; although the authors detected expression of ER isoform as can be visible on the western blots. Please address the following point: Page 9: The info about Abs is insufficient. "...probed with antibodies specific for human ER α (Fitzgerald Industries International, Acton, MA, United States), human ER β (...)" - authors should specify exactly the region of the receptor that is recognized by the Abs. Are the Abs made in mouse or rabbit? Catalog #? Clone? Etc. Page 10: the positive control IHC should be presented (it can be presented as Supplementary material/figure). The best ER positive control samples are breast cancer tissues. Readers need to see how used (novel) Abs react with positive control

samples and whether the nuclear and cytoplasmic ER can be detected in breast cancer samples using these Abs. Figure 1A. Western blots were overexposed/ bleached. The background level is not visible. The blots look different to those provided in Figure 2 and 3. Why? On Fig2 and 3 the few other ER bands (ER variants) are recognizable, which is normal and happens often with polyclonal Abs. If authors intentionally cut off the upper and lower ER bands, they should replace the figure with larger cuts so it will be possible to see the expression of all ER bands in liver samples. The band size should be also marked. The presence of ERalpha/ beta isoform variants in the liver samples should be described in the results section and in Discussion. Figure 5D – is too small and it is nearly impossible to see the localization of the protein. That makes the figure useless. It has to be enlarged or insertion images with higher magnification should be provided. Discussion section: The role of ERalpha/beta isoforms attracts a lot of attention in the area of various intestinal cancers. Authors should make a statements about this as supporting fact for their investigation and add couple of relevant references including these: ? Xu CY, Guo JL, Jiang ZN, Xie SD, Shen JG, Shen JY, Wang LB. Prognostic role of estrogen receptor alpha and estrogen receptor beta in gastric cancer. *Ann Surg Oncol*. 2010 Sep;17(9):2503-9. doi: 10.1245/s10434-010-1031-2. ? Sukocheva OA, Wee C, Ansar A, Hussey DJ, Watson DI. Effect of estrogen on growth and apoptosis in esophageal adenocarcinoma cells. *Dis Esophagus*. 2013;26(6):628-35. doi: 10.1111/dote.12000. Discussion section page 17: “We also found significant differences in the ER α :ER β expression ratio in males vs females.” Authors should be more specific here and make precisely statement whether the ration is lower or higher in males etc. Page 17: “...there was a significant change in ER α but not ER β expression only in diseased” – again: what change – increase or decrease? – be specific, avoid misleading generalized statements. Discussion section: In majority of the relevant sentences the words “ change”, “ difference” etc should be replaced with “ increase” or “ decrease” according to the observed effects. The study limitations should be mentioned in the Discussion (small group/ sample size etc). Page 20: “We did not evaluate the expression of the variants due to the lack of commercially available reliable antibodies.” It is not entirely true. The sentence should be re-written. The involvement/role of ERalpha/beta isoforms should be presented in more details.