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ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 4403
Title: Bortezomib Effect on E2Fs and Related Genes in Hepatocellular Carcinoma Cell Lines
Reviewer code: 00184525
Science editor: Song, Xiu-Xia
Date sent for review: 2013-06-29 22:40
Date reviewed: 2013-07-14 00:29

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[Y] Grade A: Priority Publishing	Google Search:	[ ] Accept
[Y] 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:	[Y] Minor revision
[ ] Grade E (Poor)		[ ] Existed	[ ] Major revision
		[ ] No records	

COMMENTS TO AUTHORS

Baiz et al aimed to evaluate the effect of the proteasome inhibitor Bortezomib (BZB) on the expression of E2F transcription factors in human hepatocellular carcinoma (HCC) cell lines (HepG2: well differentiated, JHH6: undifferentiated). They concluded that BZB not only affects E2F-1 expression but also that of other E2F members, expanding the existing knowledge about the molecular basis of BZB action as an anti-tumour agent. Furthermore, they examined the effect of E2F8 depletion in HepG2 cell line proliferation. Major Comments 1. Title Page: Title could be more specific as E2Fs control a wide spectrum of genes. In addition, HCC cell lines could be specified as “human”. 2. Introduction: This needs to be expanded by adding data in the following points: a) current epidemiology of HCC (i.e. mEASL–EORTC HCC management guidelines, J Hepatol 2012) b) sorafenib: mention type of inhibitor, how it improves patient survival c) BZB: how does it inhibit proteasome and where does it bind d) specify type of hematological malignancies and of other malignancies that BZB is indicated for. e) current knowledge on the role of all E2F family members in cell cycle and related data in human HCC 3. Material and Methods: mention the characteristics of HCC cell lines and the criteria according which their degree of differentiation was initially defined. Add related reference. 4. Data on positive and negative controls in a separate paragraph. 5. Reasoning of not extending experiments longer should be strengthened Minor Comments 6. Introduction: Add “liver” resection, “radiofrequency” ablation. The selection criteria for HCC cell lines should be mentioned in “Material and Methods” section. Replace “cyclin D dependent kinase” with “cyclin dependent kinases in complex with their cyclin partners” 7. Material and Methods: Reference to origin of cell lines (Caucasian, Asian, other) and if they are affected by HBV or other hepatotropic viruses. Brief description of statistical tests used is missing. 8. Results: HepG2 should be

accompanied by "cell line". Definition of E2F-1 upregulation: protein or mRNA level? Grade of differentiation of HuH7 cell line is missing. Results: Exact p values are missing. Comments on Results should be mentioned in Discussion. 9. Discussion: For homogeneity, is advised to use the same terms throughout the text i.e. "activators and repressors E2Fs" or "anti-proliferative and pro-proliferative E2Fs" 10. Figure legends: Abbreviations need explanation

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

COMMENTS TO AUTHORS

In the paper "Bortezomib effect on E2Fs and related genes in hepatocellular carcinoma cell lines", Baiz D et al. described the changes of gene expression in two hepatocellular carcinoma cell lines (JHH6 and HepG2) induced by treatment of bortezomib. This research group is one of research teams who have reported bortezomib regulates E2F1 expression. Extending from their previous reports, the authors of this study further found that bortezomib treatment induced the changes of E2F2, E2F4, E2F5, E2F6 and E2F8, and such changes differed between undifferentiated JHH6 cells and hepatocyte-like HepG2 cells. To interpret their findings, the authors proposed that the sum of multiple minor anti-proliferative signals may account for the anti-proliferative effects of bortezomib. The findings and discussion of this paper are interesting and potentially important. However, there are several points of this paper to be clarified, as follows. 1. Authors found that bortezomib treatment significantly increased E2F8 expression in JHH6 cells but significantly decreased it in HepG2 cells. E2F8 has been regarded as an anti-proliferation transcription factor. So the finding of bortezomib-elevated E2F8 could be used for explaining the cell suppression effects of bortezomib in JHH6 cells, but such finding put E2F8 as a contradicting role in HepG2 cells. The authors subsequently knocked down E2F8 gene levels and did not detect the change in cell number (Figure 3), concluding that decreased E2F8 expression had nothing to do with cell proliferation. To strengthen their statements, I urge the author repeat this experiments in JHH6 cells and discuss the results to the revised Figure 3. 2. The findings that bortezomib treatment induced differential expression of E2F family members between JHH6 and HepG2 cells are interesting. Considering the different phenotypes of these two cell lines, I am wondering whether their differentially responsive E2Fs expressions are related to epithelial-mesenchymal transition (EMT) of hepatocellular carcinoma.

Since the authors have already had microarray data in hand, I suggest them to analyze the bortezomib induced changes in EMT-related genes. A minor comment to this paper: English editing by a native English-speaking colleague could much improve the language quality of this paper.

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COMMENTS TO AUTHORS

The article Bortezomib Effect on E2Fs and Related Genes in Hepatocellular Carcinoma Cell Lines is an original research paper focusing on the proteasome inhibitor bortezomib (BZB) effects on E2Fs transcription factors and related genes in Hepatocellular carcinoma. The study is well structured, the subject is actual and interesting, providing a rationale for performing the research. The article has a recent bibliography and the manuscript is correctly written. The authors used advanced techniques and clearly highlighted the conclusions that are justified by the results found in the study. We are suggesting a few minor revisions such as spelling check and removal from legends text of reference to materials and methods section. For future papers, in order to confirm the data obtained and to avoid differences between human tumors and standardized tumor cell lines, I would suggest that the authors consider an in vivo study on bortezomib antitumor actions, using possibly derived patients cell lines.