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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 16198
Title: Nemo-like kinase is upregulated in human colorectal cancer and plays an important role in proliferation and migration of colorectal cancer cells
Reviewer's code: 00503404
Reviewer's country: Hungary
Science editor: Ya-Juan Ma
Date sent for review: 2015-01-03 17:15
Date reviewed: 2015-01-16 06:24

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

COMMENTS TO AUTHORS

This is an interesting paper assessing the role of nemo-like kinase in colorectal cancer cells. The methods and set of experiments is up-to- date and well designed and the conclusions are supported by the findings and a large clinical dataset. Comments; 1. Authors should re-do all analysis separately for colon and rectal cancers since prognosis and therapy of these cancers are different. 2. If possible right-sided cancers should be also analysed separately for the same purpose.

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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 16198
Title: Nemo-like kinase is upregulated in human colorectal cancer and plays an important role in proliferation and migration of colorectal cancer cells
Reviewer's code: 00070916
Reviewer's country: Germany
Science editor: Ya-Juan Ma
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Date reviewed: 2015-01-25 19:24

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

COMMENTS TO AUTHORS

The manuscript by Zhang et al., deals with the gene/protein NLK and its role in CRC. The authors analyse a large series of clinical cases using TMA and correlate the expression findings with clinical parameters. As an add-on, some functional in vitro studies are conducted which shall confirm the hypothesis basing on the biomarker analysis: NLK is a relevant molecule predicting CRC aggressiveness and prognosis. The language is acceptable and with the exceptions named in the following, the methods are suited to address the questions raised. The results are not always as convinsing as wished by the authors. Thus, there are a number of major concerns which have to be ruled out by the authors: 1. Please indicate the number of disagreements (between X.H.G. and J.H.) for the IHC staining procedure. 2. Contrary to what is stated by the authors, there is no Figure showing the data of qRT-PCR of CRC and paired normal mucosa. 3. If you find that NLK expression is significantly higher in rectal than in colon cancer, then you must consequently perform the complete statistical analyses (shown in Table 1 and 2) separately for rectal and colon cancer - either in addition or instead of the "CRC" analysis. 4. My biggest problem lies in the in vitro data - they are

overall not at all convincing and even the authors state in their discussion part that there are contrary effects described for DLD-1 as those found by the authors for HT29. First of all, please analyse more cell lines concerning then level of NLK expression; at least 5 cell lines of each molecular subgroup of CRC may give a better picture. Then, all functional analyses have to be done at least for one additional cell line. Additionally, I missed an explanation why MMP-2 was analysed as a functional target of NLK. Finally, the data of the influence of NLK on the cell cycle - do you really consider a difference of maybe 4% between G1 and S phase as relevant? First, I doubt that these experiments have been properly performed. With all respect, but I've never seen such tiny SDs for real biological replicates of cell cycle analyses! And then, even if you may find it statistically sound - please have a unprejudiced look on Figure 6b - would this be convincing to you when reading it in a manuscript of a competitor? 5. What about the clinical cases and their attribution towards the different molecular subclasses of CRC? These data should be generated and correlated with the NLK expression in order to get a clear picture. Here, I would agree that it may be too much work for "just one" manuscript. But, if you continue this work, consider this additional analyses. 6. I miss Lynch or HNPCC clinical cases to complete the picture. 7. Please discuss the results of PMID: 25371216 and 24972723 which describe similar findings. Minor points: 1. Did you really use 95% O2 in the incubators? 2. Introduce "CRC" as an abbreviation. 3. HEK293T is not a CRC cell line. 4. Which lentivirus kit was used? 5. It is not of interest to the reader, why you did not use 742 cases but 712 cases. It may be of interest, why you did not replace the "missing" 30 ones.

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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
ESPS manuscript NO: 16198
Title: Nemo-like kinase is upregulated in human colorectal cancer and plays an important role in proliferation and migration of colorectal cancer cells
Reviewer's code: 00058348
Reviewer's country: United States
Science editor: Ya-Juan Ma
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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[Y] Grade A: Excellent	[ ] Grade A: Priority publishing	PubMed Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[Y] High priority for publication
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[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

NLK is an important regulator of several signal transduction pathways including Wnt and Notch signaling pathways, both of which play critical roles in tumorigenesis. Deregulation of NLK is closely associated with progression of several cancers. In this study, the authors found that NLK is overexpressed in colorectal cancer, and proposed that upregulation of NLK is an independent prognostic factor for colorectal cancer. Findings from this study are important for both cancer biology and translational cancer research. Specific comments: 1. The title may be modified as "Up-regulation of Nemo-like kinase is an independent prognostic factor in colorectal cancer". 2. Abstract: The transition from the background to the purpose of the study should be smoother. Write the aim, not the procedure, of the study, e.g. "The aim of the present study was to…..", or "The present study was performed to ….." 3. What is the rationale that triggered you to assess NLK expression as a potential biomarker to predict colorectal cancer? This part needs extensive words, sentences, and paragraphs reorganization. 4. Introduction: The background and rational reasons to perform this study are well introduced, but the transition from the background to the purpose of the

study should be smoother. The transition sentence "Towards this end, we focused on nemo-like kinase (NLK),…." is unusual. The authors should first described the current knowledge of NLK in tumorigenesis, and then raise a question or hypothesis to assess the excessive NLK expression as a potential biomarker to predict colorectal cancer. 5. Results: The authors stated that "Furthermore, overexpression of NLK protein in the tumor tissues was further verified using western blot, and overexpression of NLK mRNA was verified using qRT-PCR with 10 cases of colorectal cancer and paired normal mucosae (Fig. 1). The level of NLK mRNA in the tumor tissues was significantly higher than that in the normal tissues ($p < 0.05$).", but I did not find these data, please provided the Western blots and qRT-PCR data. 6. Overall, the manuscript is fairly written and organized. However, there are a few grammatical and language expression problems throughout the manuscript.