ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 5208

Title: Differential gene expression of chemokines in KRAS and BRAF mutated colorectal cell lines: Role of cytokines

Reviewer code: 00503455

Science editor: Wen, Ling-Ling

Date sent for review: 2013-08-23 18:22

Date reviewed: 2013-09-02 14:01

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
[ ] Grade A (Excellent)	[Y] Grade A: Priority Publishing	Google Search:	[Y] 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	[ ] No records	
[ ] Grade D (Fair)	language polishing	BPG Search:	[ ]Rejection
[ ] Grade E (Poor)	[ ] Grade D: rejected	[ ] Existed	[ ] Minor revision
		[ ] No records	[ ] Major revision

COMMENTS TO AUTHORS

None

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 5208
Title: Differential gene expression of chemokines in KRAS and BRAF mutated colorectal cell lines: Role of cytokines
Reviewer code: 02458759
Science editor: Wen, Ling-Ling
Date sent for review: 2013-08-23 18:22
Date reviewed: 2013-09-28 15:55

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

COMMENTS TO AUTHORS

The abstract of this study gives a clear delineation of the research background, objectives, materials and methods, results and conclusions and results provide sufficient experimental evidence and data to draw firm scientific conclusions. Systematic theoretical analyses and valuable conclusions are provided in the discussion.

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 5208
Title: Differential gene expression of chemokines in KRAS and BRAF mutated colorectal cell lines: Role of cytokines
Reviewer code: 02569061
Science editor: Wen, Ling-Ling
Date sent for review: 2013-08-23 18:22
Date reviewed: 2013-09-29 02:06

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

COMMENTS TO AUTHORS

The authors of the manuscript entitled "Differential gene expression of chemokines in KRAS and BRAF mutated colorectal cell lines: Role of cytokines" tried to build an interesting proposal. Although a current practice in science, I do not want to jeopardize the efforts from these authors with irrelevant criticism; instead I want to help. Unfortunately, I do not think that this work is ready for publication, because: - Aims are not unclear, and do not bring novelty to the matter; o Authors should draw a line for what do they want to show; ? The best way would be they draw schemes for themselves before doing experiments; ? It would help clarify what they want to show; o Unclear aims = unclear experimental design, results, and discussion. - Source of cell lines: o Normally the source for these cell lines is ATCC. Thus, authors should prove the authenticity of the cell lines used herein. o As much as I tried to understand, please, from where these mutated cell lines are coming? Unfortunately, mutated cell lines (Colo205, HT29, and DLD-1) just appear in the results and reader cannot find their source. - Most of this research was just based on gene expression o Although I am totally pro of gene expression analyses, I do not feel that just this technique will prove the author's goal. ?   Authors can argue that also did WB and siRNA; but, have those techniques solved the problem of lack of structure? For example: Figures 4 and 5… is Fig. 5 adding something? No, it is a repetition of Fig. 4, but with WB. - "The results of this study may be helpful to build a rationale for the understanding of microenvironment remodelling and tumor-microenvironment interactions in view of the different mutations." o Sorry, but how have you come to this conclusion? Based on which evidences? ? Starting the results sections, authors describe levels of gene encoding cytokines, and they afterwards stimulate these cells with same cytokines   ? This, by itself, blows out the idea of

microenvironment that authors want to describe. There, they indeed are talking about an autocrine-loop for cytokines that tumor cells have o If they had at least tried co-cultures, or in vivo experiments, they could then talk about microenvironment, but, unfortunately, not here. - English o The last of my observations, the most annoying point but less important now, is their English writing style.　? Authors should carefully think having an English native speaker correcting their manuscript before submission. - Suggestions o Think about: ? Keep the track ? You have too many open questions; be focus in one, but go to its deepest point ? Proliferation ? Signaling pathways (from receptors to transcription factors) ? Co-culture experiments ? In vivo experiments

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 5208
Title: Differential gene expression of chemokines in KRAS and BRAF mutated colorectal cell lines: Role of cytokines
Reviewer code: 00054915
Science editor: Wen, Ling-Ling
Date sent for review: 2013-08-23 18:22
Date reviewed: 2013-09-30 22:41

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

COMMENTS TO AUTHORS

General comments: o Overall it looks like a good idea for a manuscript and the experiments performed seem worthwhile. I would classify it in Grade C, however it was quite difficult to understand what the goal was they wanted to achieve. o The language can be considered a grade B. o I would accept the manuscript, after some minor revisions. Specific comments: ? The title: o I had the feeling that the effect of mutation status was the main focus of the manuscript. So I would make that obvious in the title, except of talking about the 'role of cytokines'. ? Abstract o Good abstract o Colorectal cancer is written without a hyphen (first sentence) ? Introduction o Following sentence is repeated in the introduction: "These chemokines attract immune cells which act on the tumor cell and its microenvironment, thereby multiplying the inflammatory effects and subsequent tumor initiation and promotion." o The attractions of immune cells to the microenvironment seems to be a bad thing in this manuscript. Isn't it possible that the presence of immune cells in the microenvironment might be a good prognostic feature (e.g. MSI T-lymphocytes infiltrates). This might be a good thing to discuss too. ? Materials and methods o RNA isolation and RT-PCR: is it sufficient to look at relative expression levels while only using one   housekeeping gene? o RNA interference: "20nM siRNA (6µl) and 10nM lipofectamine (12µl) which was considered to be a combination which resulted in an acceptable KRAS knock down after 48h and 72h incubation time for the following experiments". This sentence seems a bit off. And maybe define wat 'acceptable' is.   o Small detail: when mentioning "(Table. 1 & 2)" make sure it is mentioned exactly the same way next time. In the 'materials and methods' (Table. 1&2) is also seen. ? Results o The results are nicely structured. Also the figures look good.   o It might have been nice to also check the effect of a BRAF-inhibition.   Just to complete the

whole picture. And to see whether differences could be observed between KRAS and BRAF mutated cell line. o The activation of the NF-κB pathway is analyzed. However, no mentioning of this pathway happens in the introduction, and it is thus not really clear why you analyzed this. ? Discussion o "The aim of the study was to understand the influence of these CRC mutationS in view of the regulation and induction of inflammatory cytokines…". The plural way to talk about mutations (KRAS and BRAF), makes you disappointed when the effect of the inhibition of BRAF has not been explored. I would hardly recommend to do this to, to complete the picture. o Same remark as in the results. The NF-κB pathway results are mentioned, and a little explanation as to why you analyzed this is mentioned. But the overall intention is not really clear.

ESPS Peer-review Report
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 5208
Title: Differential gene expression of chemokines in KRAS and BRAF mutated colorectal cell lines: Role of cytokines
Reviewer code: 02456377
Science editor: Wen, Ling-Ling
Date sent for review: 2013-08-23 18:22
Date reviewed: 2013-10-07 22:38

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

COMMENTS TO AUTHORS

This article conducted a vitro study to explore the association among KRAS, BRAF mutation and CRC regulation. The conclusion was that basal chemokine gene expression(CXCL1, CXCL8, CXCL10) for pro-angiogenic chemokines was higher in mutated as compared to wild type cell-lines. Major concerns: 1. Novelty: In CRC, the mutation of KRAS, BRAF have been found long before, but the role of KRAS or BRAF mutational status in tumor immune regulation has never been reported. The affection of KRAS or BRAF in Chemo-attractant cytokines expression may help to rationalize the choice of molecular targets for suitable therapeutic investigation in clinical studies. 2. Data: In page11, the results in paragraph 1 and 2 both demonstrated the basal changes in mRNA expression of acute phase cytokines in IECs. I am confused about the differences.Then in paragraph 3, the basal mRNA expression of CXCL8 in Colorectal Cell lines were missing. As the mutation of KRAS, BRAF both have been reported to play a important role in CRC immune regulation in this study. I wonder why the author chose KRAS knockdown not BRAF in the exploration and an animal experiment would be better in confirming the effects of KRAS, BRAF in CRC immune regulation. By the way, the references are too old. 3. Language: The grammar, spelling and conciseness of the language needs major improvements. Conclusion: major revise