

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 11137

Title: Weichang'an Inhibits Colorectal Tumor Growth and Hepatic Metastasis in Transplant Mouse Model

Reviewer code: 00070916

Science editor: Ya-Juan Ma

Date sent for review: 2014-05-11 12:48

Date reviewed: 2014-05-22 17:16

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> Existing	<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"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor	<input type="checkbox"/> Grade D: Rejected	<input type="checkbox"/> Existing	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Tao et al. analyzed the effect of WCA, a traditional Chinese medicine on colorectal carcinoma in a clinically interesting orthotopic model. Their major finding was a reduction in tumor weight and size as well as the number of metastases when WCA was combined with 5-FU. They also speculate that a decreased expression of b-Catenin and MMP-7 may be involved in the anti-tumorigenic properties of WCA. The design, technical performance and some of the conclusions made in this study are comprehensible. The authors address a clinically very relevant topic. The manuscript is overall written in a very good English. However, there are several major and minor points, the authors must address. Major points: 1. The orthotopic implantation procedure was described – but since for this case, the technical procedure of the purse-string suture is not yet described in the literature, the authors should provide a more detailed description including some pictures. This may be of interest to many people working in the field. 2. The phenomenon of lymphocyte infiltration in the nude mouse system is rather unexpected and we did not see this in comparable studies. What is the cell type? NK cells? B cells? Others? 3. Honestly, claiming 100% tumor take with only 1/12 mice dying after such an invasive surgical procedure is hard to believe. Please provide reasons/data how you trained your mouse-surgeons to reach such a fantastic standard. Moreover, any disadvantages of the model used (as for example the use of a very old cell line of the compared to CIN rare molecular phenotype MSI; thus being most likely not an ideal model for 5-FU based therapies) are not at all

addressed in the discussion. Finally, the fact that more mice died in the WCA groups is also not at all discussed. 4. The claim: "Our findings suggest that WCA additively enhances the efficacy of 5-FU to inhibit colon cancer growth and metastasis" in the discussion is not supported by the data delivered, isn't it? 5. The differences between the mRNA and W-Blot results versus immunohistochemistry for MMP7 and b-Catenin are neither plausible nor discussed. 6. What is the effect of WCA on colorectal cells in vitro? A simple proliferation/cytotox test using at least HCT116 must be provided or – if possible – cited from the literature. Minor points: 1. There are several smaller mistakes in style and form as for example Catenin versus catenin. Another example is the use of 0 in the 4th line of the discussion – "close to zero" would be superior. If finally accepted, the manuscript should thoroughly be revised concerning these things. 2. Since the WCA alone did not significantly reduce hepatic metastasis, the title of the manuscript has to be changed! 3. The treatment started 7 days after orthotopic tumor implantation – this is very early. At least, the authors should comment on that in the discussion. Would a later treatment still have effects on tumor development? 4. Classically, the nuclear translocation is analyzed by immunohistochemistry. The authors should ideally provide such data or at the very least comment on why they did use W-Blot instead. 5. 2 digits after the decimal point should always be used. 6. In the heading of Table 2 the authors write "HCT116 cell injection". Is this just a mistake – or is this a hint towards another technical procedure used in this study than the orthotopic implantation described?

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Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 11137

Title: Weichang'an Inhibits Colorectal Tumor Growth and Hepatic Metastasis in Transplant Mouse Model

Reviewer code: 00503512

Science editor: Ya-Juan Ma

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

COMMENTS TO AUTHORS

Tao et al. investigate the therapeutic efficacy of WCA in a mouse model of colorectal cancer. They find that WCA, alone or in combination with 5-FU has some effect on tumor growth and metastatic rate. The paper is well written and presented, and the findings might be interesting. Please consider the following critical points: 1) Abstract: it is too descriptive, please add p values and other objective measures to back up your statements 2) Tab 1 and 2 are hard to read, since the titles of the columns are misplaced. Please correct 3) Please indicate the statistical test employed after each p value, in the text and/or in the legends. 4) The most critical point is to show that WCA has an effect on colorectal cancer growth/metastasis, especially when added to 5FU. This point is not well investigated. The authors should directly compare 5FU and 5FU plus WCA in terms of growth rate, metastatic potential. Is there any significant advantage of adding WCA to classical chemotherapy?

ESPS PEER REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 11137

Title: Weichang'an Inhibits Colorectal Tumor Growth and Hepatic Metastasis in Transplant Mouse Model

Reviewer code: 02533276

Science editor: Ya-Juan Ma

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

COMMENTS TO AUTHORS

In this manuscript, Tao et al analyzed the therapeutic effect of WCA on colorectal cancer in an animal model. According to their results, WCA enhances the efficacy of 5-FU to inhibit colon cancer cells growth and hepatic metastases. They also describe the effect of WCA on the expression of the proteins beta-catenin and MMP-7, both of them involved in metastasis development in colorectal cancer. This is an interesting work addressing a very relevant topic in clinical gastroenterology. Authors used properly different techniques, qPCR, IHC and immunoblotting, in order to confirm their results about the effect of WCA on the expression of beta-catenin and MMP-7. The manuscript is written in a good English and only some minor mistakes must be revised. However, I have some major and minor points that authors should address: Major points Methods: The description of Real time PCR is not clear. Moreover, authors must include the amplification efficiency values of each primer pair used for beta-catenin, MMP-7 and GAPDH amplification in order to confirm that those values are right. In addition, some authors have described that GAPDH is not the best reference gene for real time PCR in colorectal tissues. Authors must address this question. Results: Authors determine WCA concentration measuring hesperidin concentration. Is hesperidin the compound responsible for the therapeutic effect of WCA? In literature, there are reports describing hesperidin effect on proliferation in induced colon carcinogenesis in mice (Inflamm Res 2013, 62:425-40) as well as on apoptosis in human colon cancer cells (Phytomedicine 2008, 15:147-51). Authors must discuss



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this topic. Figure 4: Authors must indicate, as they do in figure 5, the statistical significance of their IHC results Discussion: Authors must discuss the discrepant results about MMP-7 expression found by immunoblotting, IHC and real time PCR. Minor points: Introduction: The paragraph “The principal element in WCA are....., previous clinical studies have indicated..... will benefit from WCA treatment “ is not comprehensible. In my opinion, it must be split into 2 sentences. Material and Methods: For the statistical analysis considering the small number of mice included in each group, I would user non-parametric tests instead of the ANOVA test. Results: Quality of figures must be improved. Figure 4: Authors must indicate, as they do in figure 5, the statistical significance of their IHC results