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Editorial Board of
World Journal of Gastrointestinal Oncology

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Submission of the revised manuscript entitled: "Role of mTORC2 in primary and secondary liver cancer" (manuscript NO: 64949; ID 02543955) to World Journal of Gastrointestinal Oncology

Dear Editorial Board,

please find attached the revised version of our manuscript entitled "*Role of mTORC2 in primary and secondary liver malignancies*" (manuscript NO 64949) to be published in *World Journal of Gastrointestinal Oncology* **(ID 02543955)**

We would like to thank the referee for the fair, thorough and thoughtful review. Based on the comments we now provide the revised manuscript. All changes in the manuscript are marked with yellow colour. We believe that our work has significantly improved by the reviewer's comments. Furthermore, we have addressed the issues raised by the editorial office. A point-by-point revision is provided below.

No portion of the contents of the manuscript or any similar paper have been published in any other primary scientific journal or are currently under review elsewhere.

All authors contributed to the work and agree to the content of the paper.

We appreciate your time in handling and reviewing our manuscript and look forward to your response.

Yours sincerely,

Sven A. Lang, MD

Reviewer 1:

The manuscript by Joechle et al. constitutes a review manuscript on the role of mTORC2 in primary and secondary liver cancer. The authors herein provide a very critical, complete and comprehensive revision on this topic. The manuscript is very well-written and get together all the relevant information in the field. I only have small comments that might increase even more the quality of this great review. - The authors only mention intrahepatic cholangiocarcinoma. What about the other types of CCA? And other types of liver malignancies, such as hepatoblastoma? Mixed HCC-iCCA tumors?

Thank you very much for your excellent review of our manuscript and mentioning this important point. As we focused on intrahepatic malignancies, we primarily did not include distal cholangiocarinoma. However, we have now included a sentence on perihilar cholangiocarcinoma (pCCC) although this is not an intrahepatic tumor per se. Unfortunately, only very few literature is available examining the impact of mTORC2 on tumorigenesis of pCCC. Similarly, there is no literature available focusing on the tumorigenic potential of mTORC2 for combined HCC-iCCC, hepatoblastoma or hepatic angiosarcoma.

Page 9:
Furthermore, the results of another study by Yang *et al.* examining the impact of FXBW7 on EMT and metastasis of iCCC and perihilär CCC (pCCC) might also be interesting although not directly connecting FXBW7 to mTORC2. In this study, silencing of FXBW7 lead to promotion of EMT, stem-like property and metastasis for iCCC and pCCC [1].

Could the authors provide some information regarding the relevance of mTORC2 in pre-tumoral conditions such as fibrosis, NAFLD, viruses, alcohol, PSC, PBC, etc? It would be important to understand if the alterations are already evident in pre-malignant states.

Thank you very much for pointing out this important point. Indeed, mTORC2 is also implicated in pre-tumoral conditions in HCC. However, no data is available regarding the role of mTORC2 in premalignant lesions of iCCC (e.g. primary sclerosing cholangitis, primary biliary cirrhosis, etc.) We have included a statement on the latter on page 10 of the manuscript. Some data on the impact of mTORC2 in premalignant lesions is now included on pages 8/9.

Page 8/9:

While these data show an important role of mTORC2/Rictor in the tumorigenesis and tumor progression of HCC, it also is involved in its pre-tumoral conditions. For example, Reyes-Gordillo et al. could show that in an in vivo two-hit model of alcoholic liver disease the AKT isoforms were activated leading to an increase of mTORC2 and inflammatory, proliferative and fibrogenic genes [69]. In line with these results, blocking of AKT1 and AKT2 lead to a decrease in progression of liver fibrosis. In addition, mTORC2 is involved in the progression of non-alcoholic fatty liver disease (NAFLD) by dysregulation of white adipose tissue. Thereby, de novo lipogenesis, lipolysis, glycolysis and increased glucose uptake by GLUT-4 are the mechanisms by which mTORC2 regulates adiposity and non-alcoholic fatty liver disease [70]. Besides

alcoholic and non-alcoholic liver disease, viral hepatitis is one of the main risk factors for development of HCC. In this context, increased AKT activity was demonstrated for hepatitis B and C. In hepatitis B, activation of AKT by the hepatitis B virus protein HBx leads to a persistent, non-cytopathic virus replication [71]. In hepatitis C, its NS3/4A protease increases AKT activity by enhancing EGF-induced signal transduction [72].

Page 10:
While mTORC2 seems to be also involved in the pre-tumoral conditions of HCC including (non) alcoholic liver disease and viral hepatitis, no data exist focusing on the role of mTORC2 chronic cholangitis, primary or secondary biliary cirrhosis as risk factors for the development of iCC.

This manuscript would greatly benefit from a summary table with the information of mTORC2 (or the mediators of this pathway) regarding levels and correlation with clinics in the several cancers discussed.

Thank you very much, this is a great suggestion to improve our manuscript. We included the following table.

Page 28:

Table 1. Overexpression of mTORC2 and its mediators determine clinical outcome

		mTORC2 mediator	associated with	measured by	reference
primary liver cancer	HCC	p-AKT^{Ser473} overexpression	poor outcome (p<0.02)	IHC	Hu *et al.* [59]
		Rictor overexpression	reduced OS (p=0.0029)	mRNA expression	Xu *et al.* [60]
		Rictor overexpression	reduced RFS (p=0.016)	IHC, mRNA expression	Kaibori *et al.* [61]
	iCCC	p-AKT1 overexpression	improved OS (p=0.0137)	IHC	Lee *et al.* [76]
secondary liver cancer	CRLM	data only available for primary CRC:			
		Rictor expression	increasing tumor stage	mRNA expression	Gulhati *et al.* [49]
		Rictor expression	increasing tumor stage	IHC, mRNA expression	Shuhua *et al.* [81]
		Rictor expression	reduced OS (p=0.0004)	IHC	Wang *et al.* [10]
	breast cancer liver metastases	data only available for invasive ductal breast carcinoma:			
		Rictor expression	lymph node metastasis	IHC	Zhang *et al.* [90]
	melanoma liver metastases	Rictor positivity (primary tumor)	reduced OS (p=0.018)	IHC	Liang *et al.* [100]
		Rictor expression	tumor stage/metastatic disease	mRNA expression	Schmidt *et al.* [101]
	renal cancer liver metastases	no data available			
	gastric cancer liver metastases	data only available for gastric cancer			
		Rictor, p-AKT^{Ser437} expression	tumor stage, reduced RFS and OS	IHC	Bian *et al.* [7]
		Rictor expression	tumor stage, reduced RFS and OS (p=0.012, p=0.014)	IHC	Bian *et al.* [110]
	pancreatic cancer liver metastases	data only available for pancreatic cancer			
		Rictor expression	reduced OS	IHC	Schmidt *et al.* [9]

Abbreviations: CRC, colorectal cancer; CRLM, colorectal liver metastases; HCC, hepatocellular carcinoma; iCCC, intrahepatic cholangiocarcinoma, IHC, immunohistochemistry; OS, overall survival; RFS, recurrence-free survival;

Science editor:

Issues raised: (1) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

With the revised manuscript we also uploaded an approval of the grant to S.A.L and C.H. from the German Research Council (Deutsche Forschungsgemeinschaft, DFG; FOR2127) which supported this study.

(2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

The original figures are now provided using PowerPoint.

(3) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published; and correctly indicating the reference source and copyrights. For example, "Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]". And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable. 6 Recommendation: Conditional acceptance.

The figures are created by ourselves and were not re-used or published anywhere else.

On behalf of all the coauthors I would like to thank the reviewer for the educational and constructive comments that have led to a significant improvement of the manuscript.

Prof. Dr. med. Sven A. Lang

1. Yang, H., et al., *FBXW7 suppresses epithelial-mesenchymal transition, stemness and metastatic potential of cholangiocarcinoma cells.* Oncotarget, 2015. **6**(8): p. 6310-25.