Author's response to reviews

Title: IQGAP1 and IQGAP2 are Reciprocally Altered in Hepatocellular Carcinoma

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Author's response to reviews: see over
Dr Melissa Norton  
Editor-in-Chief – BMC Gastroenterology  

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Dear Dr Norton,  

Thank you for indicating that our manuscript entitled “IQGAP1 and IQGAP2 are Reciprocally Altered in Hepatocellular Carcinoma” may be acceptable for publication after we address the referees’ comments. Enclosed is a revised version. A detailed response to each item raised by each reviewer is outlined below.  

Thank you for reconsidering this manuscript. We hope that it is now suitable for publication in BMC Gastroenterology.  

Sincerely,  

Valentina A. Schmidt, Ph.D.
Reviewer 1 – Jun-ichi Okano

Thank you for reviewing and commenting on our manuscript. We appreciate your recognition that this work is of importance in this field. We have made several changes in response to your review. These are summarized below:

“They examined the expression levels of IQGAP1 and IQGAP2 in normal liver, adenoma, cirrhosis, and HCC. They need to see them in dysplastic nodules, which can be often difficult to differentiate from HCC morphologically. The results may give an answer if IQGAP1 and IQGAP2 can be useful markers in discriminating HCC and dysplastic nodules.”

We appreciate the reviewers valid comment and acknowledge that it is correct. Nevertheless, the possible utility of IQGAP staining in the differentiation of HCC from dysplastic nodules was not within the remit of the current investigation. As we state in the background section of our manuscript “In the present study, our aim was to examine IQGAP1 and IQGAP2 expression in human HCC, their sensitivity and specificity as biomarkers of this type of tumor, and the methylation profile of the Iqgap2 promoter”. We feel that these aims have been successfully achieved. The reviewers provocative question will form the basis of future investigations.

“In table 2, although they say that no significant correlation was noted between expression of IQGAP1 or IQGAP2 and any clinical or pathological features, there is no such information in table 1. Expression levels of IQGAP1 and IQGAP2 need to be demonstrated by every parameter, even when there is no significant correlation between them.”

We acknowledge the reviewers point and have modified the manuscript accordingly. Our conclusions were based exclusively on pathological information. As such, we have removed the following sentences from the manuscript: “No significant correlation was observed between IQGAP protein expression and clinicopathological factors” from the abstract and “No significant correlation was noted between expression of IQGAP1 or IQGAP2 and any clinical or pathological features (Table 1)” from the results section.

“They say in discussion that increased IQGAP1 and/or decreased IQGAP2 expression may be a characteristic of a more invasive and metastatic HCC phenotype. This appears to contradict the results of figure 3 showing no correlation between expression levels of IQGAP1 and/or IQGAP2 and tumor stages. How would they compromise with this point?”

Thank you for bringing this to our attention. We agree with the reviewers point. We have therefore added a sentence on pages 13-14 of the revised manuscript that reads “Nevertheless, as we did not observe a difference in IQGAP1 positivity or IQGAP2 negativity between different HCC grades, it is also possible that these observations are a consequence of cell line immortalization. Future studies are necessary to reconcile these discrepant data”.

“As they described in the background session, IQGAP1 binds a variety of signaling molecules including Cdc42 and Rac1, ERK and MEK kinases, beta-catenin, E-cadherin, APC, and mTOR. It would be curious to see if these molecules are activated in HCC cells and tissues with high expression levels of
IQGAP1. If they could find some of these molecules are simultaneously activated with IQGAP1, this could further clarify the pathogenesis of HCC and could be useful in increasing the accuracy of the diagnosis of HCC.

We agree that these studies will clarify the precise mechanism by which IQGAPs contribute to the pathogenesis of HCC. As such, the putative role of each of the IQGAP1 binding partners you describe (and more) is currently under investigation in our laboratory. Our initial data support a function for some of these proteins in the onset and progression of HCC. For a variety of reasons, however, we do not feel that these results should be included in the current manuscript. For example, 1) we believe that these data would detract from our current results describing the actual expression of IQGAP proteins in liver cancer. 2) We are currently investigating the possible role of a large number of IQGAP1 binding partners. This is a complex time consuming process and requires a large amount of tissue. 3) Nothing is known about which proteins (if any) bind IQGAP2. Based on our current findings, the stochiometry between IQGAP1 and IQGAP2 expression appears to be critical to the pathogenesis of HCC. We therefore hypothesize that both IQGAP1 and IQGAP2 binding partners will be important in this process. Given the current state of scientific understanding in the field, and as you highlighted that any changes in response to this comment were discretionary, we respectfully suggest that these experiments will be published in future articles.
Reviewer 2 – Shinji Osada

Thank you for reviewing and commenting on our manuscript. We appreciate your recognition that this work is of outstanding merit and interest in this field. We also note that you have suggested no compulsory or discretionary revisions. Therefore, we have made no changes in response to your review.
Reviewer 3 – Yinkun Liu

Thank you for reviewing and commenting on our manuscript. We have made several changes in response to your review. These are summarized below:

“Although the western blot and IHC validation was done, according to the description, a sufficient number of individual independent specimens need to be quantified by analysis. Moreover, expression of IQGAP1 and IQGAP2 on same single sample should be presented, rather than only expression rates.”

The reviewers point is difficult to interpret as written. As we stated in the original manuscript, two independent pathologists quantified all samples individually. A semi-quantitative (positive or negative) method was used. The majority of surgical pathologists agree that IHC positivity or negativity varies between institutions and/or staining batches. Furthermore, cytoplasmic staining is nearly impossible (even to a expert pathologist) to quantify accurately and varies largely depending on the area of tissue studied. Accurate quantification is therefore challenging and of no scientific or clinical relevance. The accepted quantification system, and the one we have utilized, is a semi-quantitative method. This system forms the basis of a large number of high-impact publications in high-impact journals.

“There will be serious remodeling of liver at the cirrhosis stage. Is the cirrhosis compensated? What clinical criteria were used to match the groups? There is no information about such clinical criteria; details have to be provided to exclude the possibility of bias.”

The reviewers comment is erroneous as this was not a clinical study. In the study groups section of the materials and methods (pages 5-6), we clearly state “Hematoxylin and eosin stained sections were reviewed independently by two pathologists for confirmation of the diagnoses and grading of the tumors (according to International Union against Cancer (UICC) guidelines”. These diagnoses were established on published criteria. With regards to immunostaining, both pathologists were blinded to the antibodies used for IHC. The authors feel that these measures further exclude any possibility of bias. We have added the word “blindly” to the immunostaining interpretation section of the materials and methods. The first sentence now reads “IQGAP1 and IQGAP2 staining was blindly evaluated by two independent pathologists with a high degree of interobserver agreement (>90%)”.

“The reciprocal IQGAP expression pattern may be a characteristic of a more invasive and metastatic HCC phenotype. More evidence should be given by the functional assays on cell adhesion, motility, invasion as well as the colony formation in soft agar. In another point of view, how does this alternation of IQGAPs to be regulated during HCC progression.”

As we highlighted in response to reviewer 1, functional assays are currently underway in our laboratory. Our initial data support our hypothesis regarding the reciprocal expression of IQGAPs in HCC. Nevertheless, as we stated in our original submission, this is a diagnostic liver pathology manuscript – functional assays and mechanistic insight will form the basis of our next publication. In response to the second part of your comment, the putative role of each of the IQGAP1 binding partners in the pathogenesis of HCC is currently under investigation. Our initial data support a function for some of
these proteins in the onset and progression of HCC. For a variety of reasons, however, we do not feel that these results should be included in the current manuscript. For example, 1) we believe that these data would detract from our current results describing the actual expression of IQGAP proteins in liver cancer. 2) We are currently investigating the possible role of a large number of IQGAP1 binding partners. This is a complex time consuming process and requires a large amount of tissue. 3) Nothing is known about which proteins (if any) bind IQGAP2. Based on our current findings, the stoichiometry between IQGAP1 and IQGAP2 expression appears to be critical to the pathogenesis of HCC. We therefore hypothesize that both IQGAP1 and IQGAP2 binding partners will be important in this process. For these reasons, and given the current state of scientific understanding in this field, we respectfully suggest that these experiments will be published in future articles.

"In the study, a high degree of sensitivity and specificity for IQGAP1 positivity and IQGAP2 negativity in HCC should be supported by more strong evidences, not only positive expression rates. Does it only depend on the ability of metastasis?"

The reviewers criticism is impossible to interpret. Positive expression by immunohistochemistry is the sole accepted criteria in diagnostic sensitivity/specificity studies.

"Based on pyrosequencing, authors concluded methylation of the lqgap2 promoter is not the principle mechanism by which IQGAP2 is downregulated in HCC. What be contributed to the paper objective by the result?"

Silencing of tumor suppressor genes by promoter methylation is the most widely studied epigenetic mechanism in tumorigenesis [1-3]. In addition, hypermethylation of the lqgap2 promoter has previously been reported to account for downregulation of IQGAP2 in gastric cancer [4]. We hypothesized that loss of IQGAP2 expression in HCC may be due to hypermethylation of the lqgap2 promoter. Furthermore, we used a novel pyrosequencing technique to test this theory. Although we did not detect lqgap2 hypermethylation, we feel that this information has relevance to both the IQGAP and HCC fields. In addition, the publication of this complex method will allow other investigators to easily utilize this technique.

"Needs some language corrections before being published"

All authors on this manuscript are either native or fluent English speakers. The English is grammatically correct and does not require revision.
References