Author's response to reviews

Title: Identification of a novel microRNA signature associated with intrahepatic cholangiocarcinoma (ICC) patient prognosis

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Author's response to reviews: see over
Rebuttal Letter

Editors Comments:
In the corrected version of the manuscript, the authors do not sufficiently address the necessary changes that had been recommended by the reviewers.

In particular,

Comment 1: Referring to the comment 2 of the LG review, the description of treatment/the lack of treatment description as well as the question of cancer-related death needs to be supplemented in the patients and methods chapter and the issue of AFP vs. survival should supplement the discussion, where the authors could refer to "data not shown"

Response: The description of treatment was added to the Patients paragraph (line 149-153: “After hepatectomy, the patients were not given any other therapies except the regular liver protection treatment. If patients had hepatitis B virus (HBV) infection, serum alanine aminotransferase (ALT) elevation (>40 U/L) and serum positive for hepatitis B surface antigen (HBsAg), hepatitis B extracellular antigen (HBeAg) and HBV DNA, they would undergo antiviral therapy.”).

And the description of cancer-related death was added to the Follow-up paragraph (line 173-176: “The overall survival (OS) was computed from the date of hepatectomy to the date of death, and disease-free survival (DFS) was computed from the date of hepatectomy to the first relapse, distant metastasis, or death. During this follow-up period, all the deaths were cancer-related.”)

The discussion of AFP vs. survival was presented in Discussion section (line 387-394: “With univariate Cox regression analysis, AFP concentration was found to be associated with overall survival. Kaplan-Meier curve analysis also showed that patients with high-level AFP had much poorer survival than
those with low-level AFP (P < 0.05, data not shown). However, no report has shown that high-level AFP is correlated with overall survival of HCC patients. Moreover, we found that all of the four patients with higher AFP concentration died within 18 months after surgery. Therefore, we speculate that this significant correlation might be caused by the statistical bias because of the very limited sample size of patient with high-level AFP.

Comment 2: referring to the L. K-H review

Comment ①: the main issues raised in the authors' response should supplement in the methods/results/discussion, and it should be clearly listed which miRs of those reported by others to be associated with prognosis were included to assess the predictive power.

Response: The ROC curve analysis and result were described in the result “Identification of 3-miRNA signature associated with survival in ICC” (line 267-276: “To find the best predictor for survival, we performed receiver operating characteristic (ROC) analysis on single miRNAs, as well as different combinations of the three miRNAs. In decreasing order of performance, the results showed that the predictive performance of the 3-miRNA signature is the best (area under the curve (AUC): 0.747, p = 0.002), followed by single miR-675-5p (AUC: 0.686, p = 0.021), the combination of miR-675-5p and miR-652-3p (AUC: 0.686, p = 0.021), the combination of miR-675-5p and miR-338-3p (AUC: 0.686, p = 0.021), single miR-652-3p (AUC: 0.622, p = 0.130), single miR-338-3p (AUC: 0.622, p = 0.130), and the combination of miR-652-3p and miR-338-3p (AUC: 0.587, p = 0.281).”).

After searching PubMed, we found that the downregulated miR-338-3p in colorectal carcinoma and overexpressed miR-675 in pancreatic cancer were found to be associated with survival of patients. We described this in the Discussion chapter (line 375-382: “Of the three miRNAs, miR-675 has been reported to be over expressed and correlates with survival of pancreatic cancer patients [29], and miR-338-3p was down-regulated and associated with
prognosis of colorectal carcinoma [30], which supports a similar expression pattern for these miRNAs in other cancers. However, miR-338 has been reported to be over-expressed and linked to a poor outcome in gastric cancer [31], which is inconsistent with the pattern we observed for miR-338 in ICC. This discrepancy might be caused by the different kinds of cancers in the two studies (ICC vs. gastric cancer).”

**Comment ②**: it has to be made clear (with the results shown or at least described) and discussed that neither a single miR or a combination of any of the two out of the 3-miR prognostic signature had a prognostic value.

**Response**: Now we described and showed the predictive performance of each single miRNA or different combinations of the 3 miRNAs in “Identification of 3-miRNA signature associated with survival in ICC” of the Results chapter (line 267-276: “To find the best predictor for survival, we performed receiver operating characteristic (ROC) analysis on single miRNAs, as well as different combinations of the three miRNAs. In decreasing order of performance, the results showed that the predictive performance of the 3-miRNA signature is the best (area under the curve (AUC): 0.747, p = 0.002), followed by single miR-675-5p (AUC: 0.686, p = 0.021), the combination of miR-675-5p and miR-652-3p (AUC: 0.686, p = 0.021), the combination of miR-675-5p and miR-338-3p (AUC: 0.686, p = 0.021), single miR-652-3p (AUC: 0.622, p = 0.130), single miR-338-3p (AUC: 0.622, p = 0.130), and the combination of miR-652-3p and miR-338-3p (AUC: 0.587, p = 0.281).”).

**Comments ③ and ④**: please supplement these issues in the discussion

**Response**: We discussed these issues in Discussion chapter (line 382-386 for comments ③: “Considering that the three miRNAs (miR-675-5p, miR-652-3p and miR-338-3p) are highly dysregulated in ICC and other cancers, these miRNAs may play an important role in ICC carcinogenesis. Therefore, we are conducting further studies on the biological function and the
regulation of miR-675-5p and miR-652-3p expression in ICC cells.”, and line 332-339 for comment ④: ‘Comparing the 3-mRNA signature with the 30-miRNA signature, we found that miR-675-5p and miR-338-3p were shared between the two signatures, while miR-652-3p was not included in the 30-miRNA signature. The reason for this phenomenon was that miRNAs with more than 2-fold change were selected for establishing the signature for distinguishing ICC from NIBD, while those with more than 1.5-fold change were chosen for constructing the prognostic signature. Consequently, miR-652-3p (1.52-fold change) was not presented in the 30-miRNA signature.”).

Comment ⑥: since very little is known in ICC, please discuss the results in the context of the selected, main results in other cancers.

Response: We had fully discussed the related issues with 3 new references in Discussion chapter (line 375-382: “Of the three miRNAs, miR-675 has been reported to be over expressed and correlates with survival of pancreatic cancer patients [29], and miR-338-3p was down-regulated and associated with prognosis of colorectal carcinoma [30], which supports a similar expression pattern for these miRNAs in other cancers. However, miR-338 has been reported to be over-expressed and linked to a poor outcome in gastric cancer [31], which is inconsistent with the pattern we observed for miR-338 in ICC. This discrepancy might be caused by the different kinds of cancers in the two studies (ICC vs. gastric cancer).”

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