Response of Authors to Reviewer's Comments

Manuscript NO: 50941

Title: Micro-RNA signature in patients with hepatocellular carcinoma associated with type 2 diabetes.

Reviewer ID: 00004011

Thank you very much for your valuable comments.

Honorable Reviewer #2

Thank you very much for your valuable comments. Kindly refer to the following table with our responses to each of your questions.

Best Regards
Authors

Reviewer's comments	Authors' response
1. The authors stated that they recruited a group of 200 diabetic patients without liver disease. Did they exclude NAFLD in these patients? The majority of diabetic patients have NAFLD, did the authors mean that those diabetic patients are without cirrhosis? Please clarify	You are absolutely correct. The majority of diabetic patients have some form of NAFLD. However, only a minority (about 12%) will develop steatohepatitis, steatocirrhosis and/or HCC. Therefore, we chose this group to be either NAFLD-free or have simple steatosis without any laboratory evidence of inflammation or fibrosis. We added a phrase to clarify these selection criteria in the "Patients/samples inclusion criteria" section of the manuscript: "200 diabetic patients without liver disease (persistently normal liver profile parameters for at least 6 months and no cirrhosis by ultrasound examination)"
2. The patients were recruited from 2 research centers, were they stratified from the 2 centers?	The patients were initially stratified in the 2 centers according to their clinical, imaging and laboratory profile, but the stratification was revised by the authors to ensure the uniformity of criteria of stratification.
3. The authors did not mention the number of patients who underwent	Actually most of the patients either refused biopsy or were not fit for biopsy procedure (due to low platelet count or

liver biopsy and what were the biopsy findings.	high INR). So, we didn't consider the histopathology results of the few cases who agreed to do biopsy to be useful for our study. As regards HCC cases, it is now – of course – almost obsolete to perform biopsy for them due to the risk of tumor seeding along the biopsy track.
4. How did the authors identify F4 patients? (not all patients had a biopsy) did they use non-invasive markers or elastography?	They were evaluated by ultrasound-based transient elastography. We added a phrase to clarify this in the "Patients/samples inclusion criteria" section of the manuscript: "Only patients with F4-fibrosis stage, i.e. cirrhosis (by ultrasound-based transient elastography, with a cut-off value of 12.2 kPa for diagnosing F4-fibrosis stage) were enrolled for homogeneity of study population."
5. Why patients with HCV and HBV excluded from the study? It would be helpful to add a group with viral related HCC to assess a possible effect on miRNAs	This is due the known carcinogenic effect of HCV and HBV itself, possibly because viral replication impairs cellular DNA damage responses, thereby promoting instability of the infected host cell genome. We added a phrase to clarify this in the "Patients/samples inclusion criteria" section of the manuscript: "Active hepatitis C virus (HCV) and hepatitis B virus (HBV) infections were excluded by screening for serum HCV antibodies and HBs antigen, respectively and confirmation by real time PCR, whenever necessary (to exclude the potential carcinogenic effect of HBV and HCV)."
6. Was NAFLD excluded in the group of 225 healthy controls?	Yes, it was excluded by abdominal ultrasound screening and normal liver panel tests. We added a phrase to clarify this in the "Patients/samples inclusion criteria" section of the manuscript: "225 healthy control subjects (normal ultrasound picture of the liver and normal liver profile parameters) were enrolled."