Author's response to reviews

Title: Increased MiR-221 Expression in Hepatocellular Carcinoma Tissues and Its Role in Enhancing Cell Growth and Inhibiting Apoptosis in vitro

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Author's response to reviews: see over
Dear Editor:

Please find enclosed a manuscript entitled: *Increased MiR-221 Expression in Hepatocellular Carcinoma Tissues and Its Role in Enhancing Cell Growth and Inhibiting Apoptosis in vitro*. Authors: Minhua Rong, Gang Chen and Yiwu Dang. Our article was previously submitted to *Molecular Cancer* and it was suggested to submit to your journal.

MiR-221 is over-expressed in human hepatocellular carcinoma (HCC), but its clinical significance and function in HCC remains uncertain. The aim of the study was to investigate the relationship between miR-221 overexpression and clinicopathological parameters in HCC formalin-fixed paraffin-embedded (FFPE) tissues, and the effect of miR-221 inhibitor and mimic on different HCC cell lines in vitro. MiR-221 expression was detected using real time RT-qPCR in FFPE HCC and the adjacent noncancerous liver tissues. The relationship between miR-221 level and clinicopathological features was also analyzed. Furthermore, miR-221 inhibitor and mimic were transfected into HCC cell lines HepB3, HepG2 and SNU449. The effects of miR-221 on cell growth, cell cycle, caspase activity and apoptosis were also investigated by spectrophotometry, fluorimetry, fluorescence microscopy and flow cytometry, respectively. The results showed that the relative expression of miR-221 in clinical TNM stages III and IV was significantly higher than that in the stages I and II. The miR-221 level was also upregulated in the metastatic group compared to the nonmetastatic group. MiR-221 over-expression was related to the status of tumor capsular infiltration in HCC clinical samples. Functionally, cell growth was inhibited, cell cycle was arrested in G1/S-phase and apoptosis was increased by miR-221 inhibitor in vitro. Likewise, miR-221 mimic accelerated the cell growth. In conclusion, expression of miR-221 in FFPE tissues could provide predictive significance for prognosis of HCC patients. Moreover, miR-221 inhibitor could be useful to suppress proliferation and induce apoptosis in HCC cells. Thus miR-221 might be a critical targeted therapy strategy for HCC.

We declare that all the authors meet the criteria of your journal and all authors and acknowledged contributors have read and approved the manuscript. We declare no conflicts of interest (both financial and personal) and the content of the manuscript is original, and it has not been previously published or accepted for publication.

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Thank you very much considering our manuscript for publication in your journal.

Yours sincerely,

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