

Peer Review File

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Reviewer A

Comment (i): It suggests that authors should use at least two cell lines in this study.

Reply (i): Using two or more cell lines can make the experiment more rigorous. Our research group used the mouse HCC cell line Hca-P with low lymphatic metastatic potential and the mouse HCC cell line Hca-F with high lymphatic metastatic potential in the preliminary experiment. In the subsequent in-depth study, in addition to Hca-P and Hca-F cells, human liver cancer cells HepG2 and 7721 will also be used for study.

Comment (ii): Authors should identify the role of ATF2 in mediating JNK-Annexin A7 pathway, using ATF2 inhibitor, ATF2 siRNA or ATF2 shRNA...et al.

Reply (ii): In our research group, the JNK-Annexin A7 pathway was studied by ANXA7 shRNA, JNK shRNA and JNK inhibitor (the study has been completed and is being submitted for submission). To further investigate the role of ATF2 in mediating the JNK-A7 signaling pathway, we will use ATF2 inhibitor, ATF2 siRNA or ATF2 shRNA...et al.

Comment (iii): Authors should identify the interaction between ATF2 and LncRNA NONMMUT114121.1 in the JNK-Annexin A7 pathway; or provide the references about the interaction between ATF2 and LncRNA NONMMUT114121.1.

Reply (iii): ATF2 was found to be related to LncRNA NONMMUT114121.1 by RNA-seq, and the expression levels of ATF2 and LncRNA NONMMUT114121.1 were verified by qRT-PCR for down-regulating Annexin A7 and JNK. In the further study, we will study the interaction between ATF2 and LncRNA NONMMUT114121.1 using methods such as ChIRP and CHART.

Comment (iv): It suggests that authors draw a cartoon picture about the findings/results about this study.

Reply (iv): That's an interesting suggestion.

Reviewer B

Comment 1: In lines 22 to 25 in page 3, authors said “In HCC cells, JNK downregulation reduced ANXA7 expression, while ANXA7 downregulation had no effect of JNK expression (our unpublished observations), suggesting that ANXA7 expression is controlled by JNK signaling.” In this study, they did not show how was JNK signaling involved in the induction of ANXA7 expression; authors need to demonstrate it.

Reply 1: Our previous study found that Annexin A7 affecting biological function of mouse hepatocellular carcinoma via JNK-Annexin A7 protein complex in vitro.

Comment 2: Authors showed that the downregulation of ANXA7 and JNK expression reduced hepatocellular carcinoma cell's lymphatic tissue adherence and invasive capacity using only one cell line 'Hca-P cells'. They need to demonstrate it at least in three cell lines.

Reply 2: Using two or more cell lines can make the experiment more rigorous. Our research group used the mouse HCC cell line Hca-P with low lymphatic metastatic potential and the mouse HCC cell line Hca-F with high lymphatic metastatic potential in the preliminary experiment. In the subsequent in-depth study, in addition to Hca-P and Hca-F cells, human liver cancer cells HepG2 and 7721 will also be used for study.

Comment 3: Authors said that the downregulation of ANXA7 and JNK reduced ATF2 mRNA and protein expression.

Reply 3: The mechanisms of downregulation of ANXA7 and JNK reduced ATF2 mRNA and protein expression are complex and diverse. This study found this phenomenon, through consulting the literature, combined with the existing research results put forward reasonable inference. The points raised by peer review are very valuable and will be fully considered in further studies exploring their mechanisms.

Comment 4: Using Immunofluorescence authors showed nuclear localization of ATF2. To confirm their claim, they need to demonstrate ATF2 protein nuclear and cytoplasmic compartmentalization by western blotting after isolating nuclear and cytoplasmic fraction of ATF2.

Reply 4: That's good advice. We used western blotting and immunofluorescence to detect the expression of ATF2 protein. And surprised to find the ATF2 nuclear location.

Comment 5: Authors need to include the list of differentially expressed lncRNAs in the manuscript and demonstrate how the lncRNA NONMMUT114121.1 is related to ATF2. What is the role of lncRNA NONMMUT114121.1 in hepatocellular carcinoma cell's lymphatic tissue adherence and invasiveness? It needs to be addressed experimentally.

Reply 5: We published a paper titled "Long non-coding RNAs co-regulated by Annexin A7 and JNK in hepatocellular carcinoma identified by Whole genome expression Profiling" in BioMed Research International (DOI: 10.1155/2020/5747923). The results of RNA-seq are described in detail in this paper.