June 6th, 2019

Dear Editor and Reviewers:

We are very grateful that you reviewed and provided constructive suggestions for our manuscript titled "*LncRNA HULC promotes exosomes secretion from hepatocellular carcinoma cells by sponging miR-372-3p, which targets Rab11a*". Based on your helpful suggestions, we have made corresponding modifications, which are highlighted in the revised manuscript. We hope these changes will be acceptable. Our responses are detailed below.

Thank you for your consideration.

Sincerely yours,

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Reviewer #1：

The authors investigate the role on the lncRNA HULC in a translational Setting of hepatocellular carcinoma. They Show that HULC promotes exosome secretion and that the mir-372-ep Targets Rab11a and thus interferes with cell Proliferation and survival. The study is well designed, results are clearly presented. The study also adressed a current Topic and is therefore of high interest and relevance.

Reply: We thank Reviewer #1 for the insightful and constructive comments. We have now revised our manuscript according to these comments.

Minor comments:

1. Please explain "ceRNA" in the introduction part.

Reply: Thank you for the constructive comments. We have explained "ceRNA" in the Introduction section of the revised manuscript. CeRNA could regulate mRNA through competitive miRNA sharing[1].

2. Please avoid the term "gender" (in Tab 1/2) when you refer to biologic sex.

Reply: Thank you for the insightful comments. We have replaced "gender" with "sex" in Table 1 of the revised manuscript.

3. What was the etiology of the investigated HCC cases? Are there differences between e.g. HBV, HCV or NASH triggered HCC? Did patients also have fibrosis/cirrhosis? Please add this Information to the tables.

Reply: Thank you for your valuable comments. In China, 80.50% patients had HBV infection, while only 3.84% had HCV infection. 80.12% patients with HCC had liver cirrhosis (with 8.85% severe cirrhosis, 28.33% moderate cirrhosis, and 42.95% slight cirrhosis)[2]. All our patients had hepatitis B cancer with cirrhosis. This explanation has been added in the "Sample collection" subsection of the MATERIALS AND METHODS in the revised manuscript. In the future, we will collect more clinical cases for analysis.

Reviewer #2：

The authors present a study in which every experiment went through what a standard study would supposedly have completed, from basic measurements of a lncRNA "HULC" in exosomes, in vitro transfection assays, to dual-Luciferase reporter assays. The results from this study are new in hepatocellular carcinoma but lack of substantial progression and genuine novelty for the field of cancer-exosome-non-coding RNA associations.

Reply: We thank Reviewer #2 for the positive comments and valuable detailed suggestions. We have now revised our manuscript according to these comments.

Major points

1. It's well known that the storage conditions can affect the physical and functional properties of exosomes [PMID: 25536933; Lőrincz, et al. J Extracell Vesicles. 2014 Dec 22;3:25465]. Usually the exosome isolation should be completed within 7-28 days to ensure the quality of exosomes. This study collected the samples from serum and liver tissues over a time period of 8 months. It would be good to know how the authors processed these samples when isolating the exosomes. Were these samples processed altogether after all samples collected in the end of patient enrollment, or was processed one by one at each time of a patient enrolled? If it's the latter, how did authors deal with the batch effect? In addition, the authors should show the shape and size for exosomes isolated at early, middle, and late time of sample collection period to validate the integrity of exosomes analyzed throughout the study.

Reply: Thanks for the constructive comments. We collected the patients' blood first and separated serum within 30 minutes. The serum were stored at - 80°C before use. These samples

were processed altogether after all samples collected in the end of patient enrollment.

Since our experiment required RNA stability, exosomes were the suitable carrier. Previous studies had shown that RNA in exosomes does not degrade during the long-term preservation and even after storage of plasma samples at room temperature for over 42 h or − 80°C for 12 years[3] . In addition, Our process was the same as the following articles (Table 1).

Table 1, Articles in the same way

samples	Preservation time	articles
serum	15months	Pan L.et al [4]
serum	15months	Wu T .et al[5]
serum	12months	Kitagawa T .et al[6]

The OD260/280 values of total RNA in serum exosomes in our test were 1.8-2.0 before and after storage. The RNA of our experiment is stable.

We apologize that this information was not conveyed clearly in the original manuscript. This explanation has been added in the “Sample collection” subsection of the MATERIALS AND METHODS in the revised manuscript. The extraction of exosomes from cell lines ensures that the PCR experiments are performed within 3 days. Due to the long span of 8 months, we did not acquire electron microscopy photographs of exosomes in the early, middle, and late stages. This is a goal of our subsequent research.

2. The authors started from measuring the levels of HULC in serum and liver tissues, and then focused experiments on miR-372-3p and Rablla. This seemed reasonable by following the literature to find study focuses. Since “Rab11a” was the result from the target prediction analysis, it would be good to show how the authors locked “Rab11a” in the focus. A table showing the prediction scores with a kind of ranking method could fulfil this.

Reply: Thanks for the insightful comments. We have added a table (table 2) showing the prediction scores in supplementary material of revised manuscript.

Table 2, Predicted consequential pairing of miRNA and Rab11a

miRNA	Position in the UTR	seed match	context++ score	context++ score percentile	weighted context++ score	conserved branch length	Pct
miR-372-3p	1346-1353	8mer	-0.42	99	-0.02	3.96	0.64

Minor points

1. On the Title, authors should change ", which targets" to "that targets".

Reply: Thank you for the helpful comments. We have modified the title according to your and the editor's suggestions.

2. In the "Core tips", the "HULC/miR – 372-3p/Rab11a axis" should be HULC/miR-372-3p/Rab11a axis (remove the space around hyphen sign – and change hyphen to dash).

Reply: Thanks for the insightful comments. We removed the space around hyphen sign and change hyphen to a dash in the revised manuscript.

3. In paragraph of "Transfection" in the Methods section, "miRNA-373-3p" should be "miR-372-3p" (change 373 to 372, miRNA to miR).

Reply: Thank you for the useful comments. We changed 373 to 372, and miRNA to miR in the revised manuscript.

4. In Figure 2C, move the "r=0.633,P<0.05" underneath the plots.

Reply: Thank you for the useful comments. We moved "r=0.633,P<0.05" underneath the plots in the revised manuscript.

5. In Figure 1, the images A and B can be separated with more space and this will allow a better alignment between the images and the labels. The labels C-F can be aligned better with corresponding images as well.

Reply: Thanks for the valuable comments. We modified the Figure1 in the revised manuscript.

6. The font size is too small to be legible in all Figures, and it's even smaller in Figures 3-5. A common standard is that the minimal font size should be above 2 mm. If it's in some difficulties to make the font larger, the authors should re-consider by arranging some charts/plots into supplementary.

Reply: Thank you for the insightful comments. We modified Figures 3-5 according to your and the editor's suggestions.

7. In addition, the height of Figures 4-5 might exceed the limit of common journal formats, usually it's 247 mm.

Reply: Thanks for the friendly comments. We modified the Figures 4-5 according to your and editor's suggestions.

8. In Figure 4A, the markers on the lines are not distinguishable between the two groups. It would be better that the authors change the markers to the solid-empty dot pair from the round-triangle pair.

Reply: Thanks for the valuable comments. We have modified the Figure 4A in the revised manuscript.

9. Tables 1 and 2 can be combined to show the results efficiently. The sole difference between the two tables is the statistics values and there are plenty of space left blank in the table.

Reply: Thanks for the constructive comments. We have combine the two table in the revised manuscript.

Reviewer #3：

First of all I want to congratilate the authos for their research My annotations: This is a nice paper with a very nice proposal to increase the armamentarium of biological markers of HCC, not only for diagnosis but for staging and prognosis also I think there is need more clinical data with their use, to reinforce the importance of including HULC in the protocols for HCC study in the pretreatment scenario I don´t think also that this paper is the kind of articles for World Journal Of Gastroenterology.

Reply: We sincerely thank Reviewer #3 for the careful review and valuable suggestions. Indeed, more clinical data are needed in further studies. The effect of HULC in the protocols for HCC study in the staging and prognosis will be investigated in further studies.

We demonstrated that HULC enhances the secretion of exosomes by sponging miR-372-3p, which in turn targets Rab11a. Our findings provide novel insights into the mechanism of action of HUL*C* in HCC. Similar effects on non-coding RNA articles in WJG have also been published, for example "*Construction of an oesophageal cancer-specific ceRNA network based on miRNA, lncRNA, and mRNA expression data*"[7] and "*Identification and prediction of novel non-coding and coding RNA-associated competing endogenous RNA networks in colorectal cancer*"[8].

Once again, thank for your very helpful suggestions and careful review.

Reviewer #4：

This is a detailed, complex and well written manuscript that studies the mechanism by which long chain non-coding RNA regulate gene expression in hepatocellular cancer. This is a novel area of research and one of these long chain RNAs is HULC which appears to have a role in HCC progression and metastases. The manuscript investigates how up regulation of HULC might influence these actions in HCC. The study is well carried out, detailed and well written.

The subject matter is complex and tricky to understand but the manuscript is broken down into bite size pieces.

Reply: We thank Reviewer #4 for the positive comments and valuable detailed suggestions. We have now revised our manuscript according to these comments.

My only criticism is that it is a bit too long with too many figures and could be paired down to make comprehension & reading a little easier. The English is good and the manuscript deserves publication.

Reply: Thanks for the insightful comments. We modified the Figures according to your and editor's suggestions in revised manuscript.

References:

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