ANSWERING REVIEWERS

April 28, 2022

Dear Prof Ma and Reviewers,

Please find enclosed the edited manuscript in Word format **(No:74064).**

Title: **Effects of Targeted-edited Oncogenic IGF-1R with Specific-sgRNA on Biological Behaviors of HepG2 Cells**

Author: **Min Yao, Yin Cai, Zhijun Wu, Ping Zhou, Wenli Sai, De-Feng Wang, Li Wang and Deng-Fu Yao**

Name of Journal: **World Journal of Clinical Cases**

Reviewer 1 comments:

Thanks to Reviewer 1 for your very kindly comments.

Thank you for submission of the article. The topic is very interesting and the result are very encouraging. However, major revisions are needed before acceptance. Please see my revisions attached.

Thank you. English expressions have been checked and edited.

Reviewer 2 comments:

Thanks to you for your very kindly comments.

1) General Comments In this manuscript, the authors explored the expressions of insulin-like growth factor-1 receptor (IGF-1R) and P-glyco-protein (P-gp) in hepatocellular carcinoma (HCC), surrounding liver tissues, and sera to show the important biological roles in hepatocar- cinogenesis, HCC progression, and therapeutic managements. After editing the target sequence using Crispr/Cas9 system, cell proliferation, apoptosis, cell cycle arrest, migration, and invasion were quantitatively evaluated in a hepatoma cell line. Furthermore, the synergistic effects on cell growth of the IGF-1R editing and anti-cancer agents were investigated. Although the strategies were straightforward, data presentation is insufficient. Novel evidence is scarce. There is no direct evidence suggesting the conclusion that IGF-1R gene is a potential modulator to reverse multidrug resistance (MDR) in HCC cells. The followings are several concerns that the authors may wish to consider:

2) Specific comments Major concerns:

1. Because the crucial roles of IGF-1R in hepatocarcinogenesis, HCC progression, and therapeutic managements have been reported as the authors mentioned, all the results presented in this manuscript are similar with the

evidence that have been reported in the literature except for the synergistic effects on cell growth of the IGF-1R editing and anti-cancer agents. Although the authors expected that the synergistic growth inhibitory effects are achieved by reversing MDR character of HCC specifically through the function of P-gp, there is no direct evidence suggesting a molecular link neither between IGF-1R and MDR nor between IGF-1R and P-gp. Without the direct evidence suggesting two molecules, it is difficult to draw the conclusion.

In previous studies, although the relationship between hepatic IGF-1R expression at mRNA or protein and malignant transformation of hepatocytes has been explored. In the meantime, it is interesting to find that hepatic P-gp expression is closely positive correlated with IGF-1R expression. However, whether hepatic IGF-1R signal could regulate liver P-gp expression and both interaction needs to be further studied.

2. In comparisons among three or more groups, I believe the authors would perform statistics using ANOVA first and follow post hoc tests to see the probabilities in a specific combination. Unfortunately, however, there are no explanation for post hoc test. Only one probability is presented and is unclear if it is for ANOVA or for one of specific combinations. Furthermore, the chi-square values and probabilities are not consistent with my calculation. For example, "Differentiation Group" of IGF-1R in Table 2 shows chi-square value of 4.699 and probability of 0.030, which are calculated as 7.131 and 0.0076, respectively, using GraphPad Prism 8 software. In addition, TNM stage and other factors such as tumor size and number are confounding each other. They should not be analyzed together. In summary, statistical methods and results should be checked again and presented more precisely.

Thank you . These data have been rechecked.

			χ^2	P		χ^2	P
Well	21	17			16		
Middle	49	47	4.2014	0.0404	43	1.4843	0.2231
Poor	23	23	4.8190	0.0281	23	6.1783	0.0219

Minor concerns:

1. Many typos, poor English expressions, careless mistakes of referencing, and so on. Carefully rewrite and edit English.

Thank you. English expressions have been checked and edited.

2. In "Editing IGF-IR with cell proliferation inhibition" paragraph of Result section, the relative ratio of IGF-1R to β-actin expression of Western bolting in the control group was reported as 31.22 ± 0.13. Is it correct?

Thank you, sorry to say, it's wrong. It was corrected. The relative ratios of IGF-1R to β -actin were 1.32 ± 0.13 in the control group, 1.14 ± 1.23 in the

sgRNA-neg group, 1.01 ± 0.94 in the sgRNA1 group, 0.43 ± 0.79 in the sgRNA2 group, and 0.99±0.82 in the sgRNA3 group, respectively.

3. In "Effects of edited IGF-IR on the biological features of HepG2 cells" paragraph of Result section, the actual numbers of apoptotic cells should be described.
Added. Apoptotic rates of HepG2 cells in the sgRNA2 group (56.25 %) were significantly higher ($P < 0.01$, Fig.3A Left) than those in the sgRNA-neg group (5.98 %) or control group (5.66 %).

4. There is not description for Figure 3C at all.
In "Synergistic effect of sgRNA with anti-cancer drugs" paragraph of Result section, the corresponding table should not be Table 3. It should be Table 4.
Thank you. It is mistake, Fig. 3C has been marked, and Table 4 has been corrected.

Reviewer 3 comments:

Thanks to you for your very kindly comments.
In this manuscipt, the authors report the "Effects of Targeted-edited Oncogenic IGF-1R with Specific-sgRNA on Biological Behaviors of HepG2 Cells". This is a very interesting study which represents a useful contrbution to the medical literature It deserves to be published after minor English language editing
Thank you. English expressions have been checked and edited.

We also appreciate the reviewers' careful and thoughtful suggestions, since the comments are all valuable and helpful for improving our paper. We have studied comments and made some modifications according to the reviewers' comments or suggestions
Thank you again for publishing our manuscript in the **WJCO**.

Sincerely yours,

Dengfu Yao, M.D. & Ph.D., Professor,

Dengfu Yao