

Peer Review File

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Replies to Reviewer #A:

Comments:

1. Page 3, line 67. The sentence of “the role of POLA2 in HCC was unexplored so far” should be modified. There are at least two papers exhibiting same findings. The authors should add these two articles, and strengthen the difference and novelty of this study in the text.

Answer: Thank you for your professional comment. We have modified the sentence to “Recently, POLA2 was confirmed to be involved in HCC progression. Nevertheless, the mechanism of POLA2 involved in HCC has not been explored”, and cite the two papers related to POLA2 in HCC as shown in line 67-68.

2. Upstream mechanism regarding the binding of E2F1 and E2F4 is important findings by ChIP-seq public datasets. I think that this is one of the novel findings in the present study. However, these findings were not confirmed by experiments using cell line. If possible, the authors should examine whether or not knockdown of E2Fs suppress POLA2 expression in HCC cells. Alternatively, are there any evidences of ChIP-seq data that E2Fs does not bind the POLA2 promoter in POLA2-negative HCC cell line? Moreover, what is the consensus binding motif of E2Fs and does POLA2 promoter contain it? If the authors do not have these evidence, their claim (POLA2 is regulated by E2Fs) should be weaken in the text.

Answer: Thank you for your professional comment. We confirmed this conclusion in the E2F1-knockdown and E2F4-knockdown HCC cell line HepG2 by western blotting. Western blotting results showed that knockdown of E2F1 but not E2F4 could suppress POLA2 expression in HCC cell, which was added in the Figure5K-Q.

Replies to Reviewer #B:

1. I found the paper entitled “Overexpression of POLA2 in hepatocellular carcinoma is involved in immune infiltration and predicts a poor prognosis. Long Liu et al., 2023. Cancer Cell International.” which showed similar result.

“The Biological Function of POLA2 in Hepatocellular Carcinoma. Yang et al., 2023. Comb Chem High Throughput Screen” which showed the function of POLA2 in HCC. “Upregulation of E2F transcription factor 3 is associated with poor prognosis in hepatocellular carcinoma. Zeng et al., 2014. Oncol Rep” which showed a E2F function on the HCC. So, authors need to update the reference with related papers.

Answer: Thank you for underlining this deficiency. We have added these papers in line 67 and line 253.

2. At Fig. 1, authors need to provide how to quantify the western blot results.

Answer: Thank you for your constructive comment. We add the method in the line 104-105.

3. At line 140, authors need to provide more evidence that show the relationship between POLA2 expression and malignancy, because it is not clear only high expression of POLA2 is corelated with malignancy.

Answer: Thank you for underlining this deficiency. We detected the expression of POLA2 in HCC lines (HepG2, Huh7) and the immortal normal liver cell line (MIHA) by using western blotting, we found POLA2 protein level was increased in HCC lines compared to normal liver cell line, as shown in Figure1.P.

4. At line 197, authors provide some evidence that E2F1 and 4 should regulate the expression of POLA2, but there is no direct evidence, so authors need not to say “directly”.

Answer: Thank you for underlining this deficiency. We had deleted “directly”, and we found that knockdown E2F1 could suppress POLA2 expression in HCC cell, as shown in Figure5K-Q.

Replies to Reviewer #C:

In this study, Teng and Liu seek the roles of POLA2 in HCC. There are previous studies, Liu et al. 2023 Cancer Cell International and Yang et al. 2023 Comb Chem High Throughput Screen. Both studies used TCGA-LIHC data and showed that high POLA2 expression is associated with poor prognosis. Therefore, this study lacks novelty. The authors used the same data set and showed same results. In addition, the authors say like “POLA2 is upregulated by transcription factors E2F1...”, but data provided in this manuscript do not support this claim. Data shown in Figure 5 are merely computational results and Figure 5H-J show the correlation only. The authors can say something like “POLA2 expression positively correlates E2F1 expression”, but cannot conclude that POLA2 is upregulated by E2F1. Same issues for other claims, such as immune cell infiltration. This study is based on only database search and computation, not experimental evidence. Overall, this study lacks novelty and experimental evidence to support the authors’ conclusion.

Answer: Thank you for your professional comment. We confirmed this conclusion “POLA2 is upregulated by transcription factors E2F1” in the E2F1-knockdown and E2F4-knockdown HCC cell line HepG2 by western blotting. Western blotting results showed that knockdown of E2F1 but not E2F4 could suppress POLA2 expression in HCC cells, which was added in the Figure5K-Q.