Round 1

Dear reviewer,

Thank you for reviewing our manuscript and for the constructive suggestions and comments, which greatly helped us to improve the manuscript. We have added some new data and heavily revised the manuscript. Point-by-point responses to your comments are listed below. We hope that your comments have been addressed accurately. The major revised portions of the manuscript are marked with yellow color and the responses are shown in blue text.

Reviewer #1: The authors demonstrated overexpression of the UBE2T in the HCC and the high expression group of HCC showed poor overall survival. And the UBE2T KO induced inhibition of proliferation by cell cycle arrest and apoptosis. In addition, many UBE2T-related genes were found. The topic of this study is of fair interest. The manuscript is well organized and written. There are some concerns, however. 1. It would be appropriate to investigate the lesion of hepatocyte by dividing according to the progression of the lesion in order to study the development like precursor, early and advanced lesions. 2. The author have to investigate the status and effects of p53 because the p53 and UBE2T not only showed relationship in HCC, but p53 controls the apoptosis and cell cycle. 3. It is unnecessary to describe the same things both in the introduction and discussion. 4. The authors should discuss the meaning or effects of UBE2T-related genes. 4. The thought that UBE2T can be a diagnostic candidate for HCC might be not suitable from this study.

Response:

Thank you for this comment. We have revised the discussion section, in which we attempted to avoid describing the same things in both sections. In addition, our gene chips analysis has not identified the dys-regulation of P53. Furthermore, the description “UBE2T is a promising diagnosis factor” has been deleted from the discussion section.

Reviewer #2: This manuscript of JianGuo et al. aims to study the impact of the ubiquitin-conjugating enzyme E2T (UBE2T) in hepatocellular carcinoma (HCC). They confirmed that UBE2T expression was increased in HCCs as compared non-tumor tissue. Using the two HCC cell lines BEL-7404 and SMMC-7721, they showed that UBE2T silencing impaired cell proliferation promoting a G1 to S phase arrest and apoptosis and also decreased tumorigenesis in xenografts. At the molecular level, they performed gene expression profiling by microarray to identify the gene program modified by UBE2T-silencing. My main concern with this study is the use of two cell lines, which have been demonstrated as HeLa-derivative, particularly the SMCC-7721 (Rebouissou, J. Hepatol. 2017). In consequence, the authors had to confirm their study in two non-contaminated HCC cell lines or provide proof of BEL-7404 and SMMC-7721 origins with a panel of short tandem repeats, hepatic gene expression and specific gene mutations. Major points: 1. My main concern is the choice of the two cell lines, BEL-7404 and SMMC-7721 for the study. Firstly, accordingly to the manuscript of Liu et al. in 2017 in BiochemBiophys Res Commun, the expression of UBE2T is not the highest in these cell lines. What is the rationale of the authors for such a choice? Secondly, and more importantly, these cells have been

demonstrated as HeLa-derivative, particularly the SMCC-7721 cells (Rebouissou, J. Hepatol. 2017). In consequence, the authors had to confirm their study in two non-contaminated HCC cell lines or provide proof of BEL-7404 and SMMC-7721 origins with a panel of short tandem repeats, hepatic gene expression and specific gene mutations. 2. Concerning the shRNAs, only one shRNA against UBE2T has been used. Two shRNAs will be more rigorous. Additionally, the shCtrl matches with SLK with 68% homology. What is the effect of theshCtrl on this RNA/protein? 3. Concerning the human analysis, the patients were divided into low or high UBE2T expression. How did the authors divide the patients? What is the threshold value？ This had to be detailed. 4. In figure 3, what is the expression of UBE2T in the xenografts? An analysis of proliferation and apoptosis will be very informative. 5. In figure 6, why did the authors use cells rather than tumors? Only one point for each condition is not sufficient for a microarray analysis. Considering that a number of immune signaling seem to be altered in response to UBE2T silencing, it will be important to perform this analysis on at least two tumors for each condition. Minor points: 1. The expression of UBE2T is increased in HCC but nothing is said about the cause of this upregulation. This had to be explained or hypothesized.

Response:

Thank you for this comment. We used BEL-7404 and SMMC-7721 cells in our study because the UBE2T expression was abundant in these cells according to our Western blot (Figure 2A-2D). In addition, both cells were easily infected by lentivirus. Importantly, about the concerning that these cells have been demonstrated as HeLa-derivative, particularly the SMCC-7721 cells (Rebouissou, J. Hepatol. 2017), we have examined the identity by checking the short tandem repeat (STR) and found that both BEL-7404 and SMMC-7721 were not contaminated by HeLa cells. We have also shown that the shRNA had effective knockdown efficacy in both cells. Furthermore, the UBE2T expression was examined in SMMC-7721 cells which were used for xenografted tumorigenesis.

Round 2

Dear reviewer,

Thank you for reviewing our manuscript and for the constructive suggestions and comments, which greatly helped us to improve the manuscript. We have added some new data and heavily revised the manuscript. Point-by-point responses to your comments are listed below. We hope that your comments have been addressed accurately. The major revised portions of the manuscript are marked with yellow color and the responses are shown in red text.

Reviewer #1: The authors demonstrated overexpression of the UBE2T in the HCC and the high expression group of HCC showed poor overall survival. And the UBE2T KO induced inhibition of proliferation by cell cycle arrest and apoptosis. In addition, many UBE2T-related genes were found. The topic of this study is of fair interest. The manuscript is well organized and written. There are some concerns, however.

1. It would be appropriate to investigate the lesion of hepatocyte by dividing according to

the progression of the lesion in order to study the development like precursor, early and advanced lesions.

Response: We greatly appreciate this important suggestion by the reviewer. According to previous study, the expression of UBE2T is increased in tumors with high pathological grade and vascular invasion in TCGA cohort, thus we don't analyze the development of HCC repeatedly in this manuscript. And we discussed this suggestion in the discussion section (reference 10).

2. The author have to investigate the status and effects of p53 because the p53 and UBE2T not only showed relationship in HCC, but p53 controls the apoptosis and cell cycle.

Response: Previous study revealed that UBE2T facilitated the degradation of p53 protein via enhancing its ubiquitination. And also as we know, p53 controls the apoptosis and cell cycle. But in this study, we found that knockdown of UBE2T doesn't influence the expression level of p53 in HCC cells (data not show). Further, our microarray analysis do not identified the dys-regulation of p53 after UBE2T knockdown.

3. It is unnecessary to describe the same things both in the introduction and discussion.

Response: We revised the main text and attempted to avoid describing the same things both in the introduction and discussion part, which can be followed in the revised version.

4. The authors should discuss the meaning or effects of UBE2T-related genes.

Response: We greatly appreciate this excellent point by the reviewer. In this manuscript, we stated the effects of UBE2T-related genes in the discussion part of the main text.

5. The thought that UBE2T can be a diagnostic candidate for HCC might be not suitable from this study.

Response: We followed the reviewer's suggestion, that we deleted the description "UBE2T is a promising diagnosis factor" from the discussion section.

Reviewer #2: This manuscript of JianGuo et al. aims to study the impact of the ubiquitin-conjugating enzyme E2T (UBE2T) in hepatocellular carcinoma (HCC). They confirmed that UBE2T expression was increased in HCCs as compared non-tumor tissue. Using the two HCC cell lines BEL-7404 and SMMC-7721, they showed that UBE2T silencing impaired cell proliferation promoting a G1 to S phase arrest and apoptosis and also decreased tumorigenesis in xenografts. At the molecular level, they performed gene expression profiling by microarray to identify the gene program modified by UBE2T-silencing. My main concern with this study is the use of two cell lines, which have been demonstrated as HeLa-derivative, particularly the SMCC-7721 (Rebouissou, J. Hepatol. 2017). In consequence, the authors had to confirm their study in two non-contaminated HCC cell lines or provide proof of BEL-7404 and SMMC-7721 origins with a panel of short tandem repeats, hepatic gene expression and specific gene mutations. Major points:

1. My main concern is the choice of the two cell lines, BEL-7404 and SMMC-7721 for the study. Firstly, accordingly to the manuscript of Liu et al. in 2017 in BiochemBiophys Res Commun, the expression of UBE2T is not the highest in these cell lines. What is the rationale of the authors for such a choice? Secondly, and more importantly, these cells have been demonstrated as HeLa-derivative, particularly the SMCC-7721 cells (Rebouissou, J. Hepatol. 2017). In consequence, the authors had to confirm their study in two non-contaminated HCC cell lines or provide proof of BEL-7404 and SMMC-7721 origins with a panel of short tandem repeats, hepatic gene expression and specific gene mutations.

Response: Thank you for your considerable advice. We choose BEL-7404 and SMMC-7721 cells in this study because the UBE2T expression was significantly abundant in these cells according to our western blot result (Figure 2A-2D). In addition, both cells were easily infected by lentivirus for researching. More importantly, about the concerning that these cells have been demonstrated as HeLa-derivative, particularly the SMCC-7721 cells (Rebouissou, J. Hepatol. 2017), we have examined the identity by checking the short tandem repeat (STR) and found that both BEL-7404 and SMMC-7721 were not contaminated by HeLa cells. The STR report could be found in the attachment.

2. Concerning the shRNAs, only one shRNA against UBE2T has been used. Two shRNAs will be more rigorous. Additionally, the shCtrl matches with SLK with 68% homology. What is the effect of the shCtrl on this RNA/protein?

Response: In this study, we designed three shRNA against UBE2T to examine the silencing efficacy in HCC cells that we found that only this shRNA had effective knockdown efficacy in both HCC cells, so that we just choose this shRNA for studying.

And according to our microarray results, the RNA expression level of SLK unchanged, so there is no detectable off-target effect of the shCrtl sequence in this study. Although the shCtrl matches with SLK with 68% homology, publications suggested that a large number of genes with good seed match remain unaffected (A. Birmingham, et al, 3′ UTR seed matches, but not overall identity, are associated with RNAi off-targets, Nat. Methods 3 (2006) 199–204.).

3. Concerning the human analysis, the patients were divided into low or high UBE2T expression. How did the authors divide the patients? What is the threshold value？This had to be detailed.

Response: We thank the reviewer for this insightful comment. We explained how patients divided in more detail in the Methods section.

4. In figure 3, what is the expression of UBE2T in the xenografts? An analysis of proliferation and apoptosis will be very informative.

Response: We examined the UBE2T expression in SMMC-7721 cells xenografts and the results was consistent with the cell line results. In vivo experiment, 8 nude mice are included in each group and observed for 42 days after implantation. Several

xenografts disappeared during this process. Thus, it's difficult to examine the significance difference of the proliferation and apoptosis of UBE2T in xenografts between the two groups.

5. In figure 6, why did the authors use cells rather than tumors? Only one point for each condition is not sufficient for a microarray analysis. Considering that a number of immune signaling seem to be altered in response to UBE2T silencing, it will be important to perform this analysis on at least two tumors for each condition.

Response: As previous studies suggested, in vivo growth of cell lines profoundly alters the transcriptome for most models, whether these changes represent gradual adaptations to growth in a mouse microenvironment or selection-pressure promoting outgrowth of sub-clones remains to be determined (Melinda G Hollingshead, BMC Genomics, 2014). Thus, for the stability of transcripts in HCC and reproducing experimental data, we use HCC cells for a microarray analysis.

Minor points:

1. The expression of UBE2T is increased in HCC but nothing is said about the cause of this upregulation. This had to be explained or hypothesized.

Response: We greatly appreciate this important suggestion by the reviewer. In the reversed version, we stated the potential cause of UBE2T upregulation in HCC in the discussion part.