Peer Review File

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Reviewer Comments:

Comment 1: English should be reviewed throughout. There are many weird phrasings (eg "after learn TCGA datasets"? What does it mean? "To learn the expression of SKA3", etc)

Reply 1: We appreciate the reviewer's suggestions. With the help of AME Editing Service, we have modified our text to ensure the accuracy of the language. ("after learn TCGA datasets" means "after examining TCGA data sets". "To learn the expression of SKA3" means "To examine the expression of SKA3")

Comment 2: What is the meaning of the sentence page 11, line 235: "Logistic regression analysis showed…" Please re-phrase.

Reply 2: The review's suggestion is very valuable. The meaning of the sentence is that we found that SKA3 level was one of the categorical dependent variables (according to the median value of expression) related to pathological features using the logistic regression analysis. We have re-phrased the sentence in our text (see Page 14, line 271 - 273).

Comment 3: The authors keep naming SKA3, Spindle Mitochondrial associated protein complex while it is the Spindle and Kinetochore Associated complex. Why? I

do not see the role played by mitochondrion in this story.

Reply 3: We are grateful for the careful review of our manuscript. We have modified the name of SKA3 in our whole text. In the future work, we will be more careful and rigorous.

Comment 4: Introduction: The lesser proliferative capacities associated with SKA1 should not be mentioned there but in results section.

Reply 4: As you suggested, we have deleted the description of the lesser proliferative capacities associated with SKA1 in the introduction part (see Page 6, line 98 - 99).

Comment 5: Material and methods: Authors seemed to have used a single Si/ShRNA. Why? Iit is acknowledge that 3 should be tested and the best one kept for further experiments.

Reply 5: We are very appreciated for this practical suggestion. In our previous study, we had selected three sequences of siRNA. But because my negligence, we did not show the result, now we have put the result on the text (see Page 9, line 163 - 166 and *Figure 2A*). After screening the siRNA, we used the screened sequence to make the virus. The virus effectively downregulated the expression of SKA3 in HCC cells, so we used a single shRNA for animal experiments.

Comment 6: Results: What is the fold of SKA1-3 overexpression in HCC (mean or median). What is the proportion of HCC displaying such an overexpression?

Reply 6: The review has identified an important issue. By analyzing the TCGA data sets, we found that the log2FC of SKA1 was 0.68, the log2FC of SKA2 was 2.21, and the log2FC of SKA3 was 17.55 (see Page 13, line 245 - 247). The SKA3 expression level in HCC tumour tissues was significantly higher than in adjacent tissue. Because the expression of SKA1, SKA2, and SKA3 in HCC detected is non-mutational, the proportion of HCC displaying such an overexpression cannot be simply calculated. Kaplan-Meier analysis revealed that high levels of SKA1 and SKA3 (but not SKA2) were associated with the poor overall survival of patients with HCC. By conducting a pretest study, we found that SKA3 had a greater ability to promote the proliferation and metastasis of HCC cells than SKA1. Thus, we chose SKA3 as our target gene for the further study.

Comment 7: There is no interpretation of figure 1D in the text. Please correct this.

Reply 7: We are very grateful for your careful review. We have modified our text as advised (see Page 13, line 262 - 266).

Comment 8: Stem cell properties: The authors indicated that correlation with CD44 or OCT4 (POU5F1) were sufficient clues to suggest a stemness property of SKA3 because they observed this correlation in clinical samples. The same is true for NOTCH and CBF1 or even EPCAM mentioned in discussion. But what were the evolution of these genes in cell line with SKA3 inhibition or overexpression? Did the cells behaved as clinical samples?

Reply 8: Thank you very much for your carefully reading and important suggestion. To study the relationship between SKA3 and HCC stemness, we analyzed the correlations among SKA3 and various stemness markers in TCGA at the GEPIA website. The results showed that SKA3 had positive correlations with CD44 and Oct4 in HCC, so SKA3 might play a role in regulating HCC stemness. We added some cell experiments and found that inhibiting SKA3 expression suppressed the expression of Oct4 and CD44 in MHCC-97h and SNU-398, while overexpressing SKA3 increased the expression of Oct4 and CD44(see Page 16, line 325 – 328; *Figure 4D* and *Supplementary Figure 4*). The results showed that the cells behaved as clinical samples. Our previous study demonstrated that in HCC cells, SKA3 could affect the expression of Notch1 (see Page 18, line 354 - 356). RBPJ is a transcription factor that can activate human Notch1(1 - 2). SKA3 was positively correlated with RBPJ, so RBPJ might be the intermediate molecule between SKA3 and Notch1. This provides a direction for the mechanism study, and we will do further research in the future.

Comment 9: Discussion, page16, line342: Reference for Abad not Maria.

Reply 9: We are grateful for the careful review of our manuscript. We have modified our text as advised (see Page 19, line 386). Thank you very much.

Comment 10: Please use the same names in text and figures OCT4-POU5F1 and CBF1-RBPJ.

Reply 10: We do apologize for the careless writing. We have modified our figure and text to make them consistent as advised. We have unified to use Oct4 and RBPJ in the

text and figures (see *Figure 4A*, Page 16, line 314 – 315, and Page 20 - 21, line 413 - 417).

References

1. Xu T, Park S-S, Giaimo BD, et al. RBPJ/CBF1 interacts with L3MBTL3/MBT1 to promote repression of Notch signaling via histone demethylase KDM1A/LSD1. The EMBO journal 2017;36:3232-49.

2. Lu FM, Lux SE. Constitutively active human Notch1 binds to the transcription factor CBF1 and stimulates transcription through a promoter containing a CBF1-responsive element. Proc Natl Acad Sci U S A 1996;93:5663-7.