TRANSLATIONAL ANDROLOGY AND UROLOGY

Peer Review File

Review Comments
Reviewer A

The authors present a study of the expression of mRNA PRAS40 expression level in clear cell renal cell carcinoma (ccRCC) and its correlation to clinicopathological factors and survival in ccRCC patients using using the data from TCGA-KIRC cohort. In order to asses diagnostic value and functional characteristics of mRNA PRAS40 in ccRCC, authors performed ROC curve and gene set enrichment analysis (GSEA).

The authors suggested that this is suggest that "PRAS40 was a promising diagnostic and prognostic biomarker for ccRCC". The authors found that "higher mRNA expression of PRAS40 was correlated with histological grade, clinical stage, T classification, distant metastasis, while higher mRNA expression of PRAS40 was correlated with poor overall survival".

There are a couple of minor comments and suggestions;

Introduction:

The authors should be more precise with the abbreviations when first time mention in the text (e.g. AKT). It should consider the abbreviations "mRNA PRAS40 expression level" and be consistent with using it.

Results

High PRAS40 expression in ccRCC patients
Comment 1: This sentence is not completely clear: "Furthermore, the levels of mRNA expression of PRAS40 were difference in groups based on age..."

Comment 2: Although clearly presented in the tables, the correlation between mRNA PRA40 expression level and grade and stage (low or high) should be precised in the text. The same should be done in all manuscript.

Discussion

The authors stated that "Sample size of G1 and G4 is small, which might result in some deviations in the results. "
As clinical behavior of tumor stage T1 is similar to T2, and T3 to T4, it would be perhaps

interesting to analyze the PRAS40 mRNA expression in T1/T2 versus T3/T4 stage group. Likewise, tumor grades should be grouped as low grade (I/II) and high grade (III/IV). The results should be compared.

Response to reviewer's comments:

Comment 1: The authors should be more precise with the abbreviations when first time mention in the text (e.g. AKT).

Reply 1: Thank you for your suggestion. we have added more precise with the abbreviations when first mentions in the text.

Changes in the text: PRAS40: Proline-rich Akt substrate of 40 kDa; AKT1S1: AKT serine/threonine kinase 1 substrate 1; PRL11: Ribosomal Protein L11; mTORC1: The mechanistic Target of Rapamycin Complex 1; PI3K: Phosphatidylinositol 3-kinase; AUC: Area Under the Curve (see Page1-3).

Comment 2: It should consider the abbreviations "mRNA PRAS40 expression level" and be consistent with using it.

Reply 2: Thank you for your suggestion. we have modified our text as advised

Changes in the text: We have modified "mRNA PRAS40 expression level" as "PRAS40 mRNA expression" in all manuscript.

Comment 3: This sentence is not completely clear: "Furthermore, the levels of mRNA expression of PRAS40 were difference in groups based on age..."

Reply 3: Thank you for your professional language editing. we have modified our text as advised.

Changes in the text: Furthermore, different PRAS40 mRNA expression were observed in groups based on age, gender, T classification, clinical stage, distant metastasis and histological grade. (see Page 4)

Comment 4: Although clearly presented in the tables, the correlation between mRNA PRA40 expression level and grade and stage (low or high) should be precised in the text. The same should be done in all manuscript.

Reply 4: Thank you for your suggestion. We have modified the manuscript as advised.

Changes in the text: The PRAS40 mRNA expression of patients with T3/T4 classification were higher than that of patients with T1/T2 classification (P=6.139e-04). Patients who were in high histological stage had higher PRAS40 mRNA expression than patients who were in low histological stage (P=8.262e-06). Patients with a positive distant metastasis had higher PRAS40 mRNA expression than patients with a negative status. High grade groups (G3/G4) had higher PRAS40 mRNA expression than low grade groups (G1/G2) (P=1.86e-06) (see Page 4).

Comment 5: The authors stated that "Sample size of G1 and G4 is small, which might result in some deviations in the results."

As clinical behavior of tumor stage T1 is similar to T2, and T3 to T4, it would be perhaps interesting to analyze the PRAS40 mRNA expression in T1/T2 versus T3/T4 stage group. Likewise, tumor grades should be grouped as low grade (I/II) and high grade (III/IV). The results should be compared.

Reply 5: It is a good suggestion. The analysis of T classification and grade have modified as advised.

Changes in the text: PRAS40 mRNA expression was analyzed in T1/T2 versus T3/T4 and G1/G2 versus G3/G4. In the revised manuscript, we have replaced the figure (see Figure 2 C and E)