Peer Review File

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Reviewer Comments

The authors present a study in which they explored the expression of SMARCC1 in ccRCC as well as the correlation between SMARCC1 expression and the clinical parameters and overall survival of the patients with ccRCC. The authors used tissue microarrays (TMAs) and real-time PCR analysis. They reveal that “SMARCC1 expression in ccRCC tissues was significantly lower than that in paired para-tumor tissues; A significant positive correlation between SMARCC1 expression and pathological grade and correlation of high SMARCC1 expression with a good prognosis in ccRCC"

There are a couple of questions related to the methodology;

Materials and Methods
Comment 1: It is not clear which classification system of renal cell tumors was used? A complete cohort of “clear cell renal cell carcinomas and the clear cell renal carcinoma group with other pathological types” should be included in the Table 1.
Reply 1: Thank you for the insightful comments. The World Health Organization / International Society of Urological Pathology (WHO/ISUP) classification system of renal cell tumors was used in this study. We acknowledged that the cohort of “clear cell renal cell carcinomas and the clear cell renal carcinoma group with other pathological types” should be included in the Table 1.
Changes in the text: We added “All the patients were diagnosed with ccRCC based on the World Health Organization / International Society of Urological Pathology (WHO/ISUP) classification system of renal cell tumors.” in the text (see Page 3, line 80). We included a cohort of “clear cell renal cell carcinomas and the clear cell renal carcinoma group with other pathological types” in the Table 1 as advised (see Page 13, line 391).

Comment 2: It should be stated which grading system was used for ccRCC? Was the same system applied for “clear cell renal carcinoma group with other pathological types”? Was the comparation done high (III-IV) vs. low (I-II) or I vs, II, III, IV?
Reply 2: Thank you for the insightful comments. The World Health Organization / International Society of Urological Pathology (WHO/ISUP) grading system was used

in this study. The same WHO/ISUP grading system applied for “clear cell renal carcinoma group with other pathological types”. The comparation was done between high (III-IV) and low (I-II), not I vs. II-IV.
Changes in the text: We stated the specific grading system in the sentence “All the patients with ccRCC were graded using the WHO/ISUP grading system and divided into two groups by tumor pathology” (see Page 3, line 84). We added the sentence “In addition, all the patients were divided into several sub-groups by gender (male or female), pathological grade (low pathological grade: grades I and II, high pathological grade: grades III and IV), and tumor size (≤5 cm or >5 cm)” in the text (see Page 4, line 108).

Comment 3: Which TNM edition has been used for these cases?
Reply 3: Thank you for the insightful comments. The American Joint Committee on Cancer (AJCC) 7th Edition Cancer Staging System has been used for these cases.
Changes in the text: We added the sentence “The American Joint Committee on Cancer (AJCC) 7th Edition Cancer Staging System was used for all included cases in this study” in the text (see Page 4, line 110).

Comment 4: It is not clear from the methods how TMA was constructed. Importantly, the methodology does not detail how many cores was taken from tumor and normal renal parenchyma and whether the tissue cores were selected from most high-grade areas of tumor or not. It should be explained how the IHC scoring was performed according to the cores?
Reply 4: Thank you for the insightful comments. The tissue microarray was made by using a core needle to punch a core column from the wax block with a fixed diameter of 1.5 mm. The tissue cores were selected from most high-grade areas of tumor, and 128 cores were taken from tumor and normal renal parenchyma. After the array block was completed, 4 μm slices were cut out. The IHC scoring method was detailed in the second paragraph of “Immunohistochemistry” (see Page 5, line 136).
Changes in the text: We added a “tissue microarray” paragraph in the text to detail the used methods in this study (see Page 4, line 116).

Comment 5: It was not clear how the tissue was prepared for RT-PCR analysis and how many samples was used.
Reply 5: Thank you for the insightful comments. A total of 30 tissue samples were prepared for the RT-PCR analysis according to the manufacturer's instructions.
Changes in the text: We added the sentence “A total of 30 tissue samples (15 cancer tissues and 15 adjacent tissues) were prepared for the cDNA microarray (MecDNA-HKidE030CS01) according to the manufacturer's instructions” in the text (see Page 5,

line 149).

Results

Comment 6: In which structures of normal renal parenchyma IHC expression of SMARCC1 was observed?

Reply 6: Thank you for the insightful comments. In the cortex of normal renal parenchyma IHC expression of SMARCC1 was observed in our study.

Changes in the text: We detailed the specific structure "in the cortex" of normal renal parenchyma IHC expression of SMARCC1 in the text (see Page 6, line 179).

Comment 7: "The results revealed a significant positive correlation between SMARCC1 expression and pathological grade". It should be precise whether the higher expression of SMARCC1 was correlated with high grade or not?

Reply 7: Thank you for the insightful comments. The higher expression of SMARCC1 was correlated with high pathological grade in our study.

Changes in the text: We modified the expression in the related text as advised (see Page 6, line 184).

Comment 8: It is unnecessary to highlight the statistic method elsewhere in the manuscript. Furthermore, sub-grouping of the patients according to gender, pathological grade, and tumor size should be detailed in the section material and methods.

Reply 8: We acknowledged that the statistic method highlighted in the "Results" section should be deleted and the sub-grouping of the patients should be detailed.

Changes in the text: We deleted the statistic method highlighted in the "Results" section, and detailed the sub-grouping of the patients in the "Methods" section (see Page 4, line 108).

Comment 9: Was the RT-PCR analysis performed only on tumor vs. normal renal parenchyma? Are there any results of mSMARCC1 RT-PCR expression in various grades of ccRCC?

Reply 9: Thank you for the insightful comments. The RT-PCR analysis was performed only on tumor vs. normal renal parenchyma. We didn't analyze mSMARCC1 RT-PCR expression in various grades of ccRCC due to limited cases.

Changes in the text: No changes.

Discussion

Comment 10: It is unnecessary to repeat the sentences from the introduction

Reply 10: We acknowledged that some sentences in the "Discussion" section should

be modified.
Changes in the text: We deleted the redundant sentences in the “Discussion” section and adjusted the related reference literatures (see Page 7, line 209).

Comment 11. The summarized data relates entirely to clear cell renal cell carcinoma and it would be interesting perhaps to see at least some of this data across other subtypes of RCC (e.g. renal oncocytoma and chromophobe renal cell carcinomas, as an example of benign and malignant entity).
Reply 11: We acknowledged that it would be better to have more data across other subtypes of RCC. Unfortunately, we didn’t analyze data relates to other types of RCC in this study due to their limited cases. It might be addressed in our following studies.
Changes in the text: No Changes.