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Reviewer 2

Submitted manuscript presents study investigating prognostic significance of RUVBL1 and HIF1A expression levels in clear cell renal cell carcinoma (ccRCC) patients. The authors used in silico analysis TCGA datasets and immunohistochemistry (IHC) analysis of formalin-fixed paraffin-embedded specimens. Their findings suggest that simultaneous evaluation of RUVBL1 and HIF1A expression levels in cancerous tissues can be considered as useful prognostic biomarker in patients with ccRCC. Submitted study provides novel data, combining well-known prognostic/predictive biomarker HIF1A with one of its cofactor, RUVBL1. The results are clearly presented and the findings support conclusions. However, there are some remarks that must be addressed in the revised version of the article before it could be considered for publication.

1. Specify the type of positive controls used for IHC. Did the authors carry out any negative controls?

Response: Quality control was performed using positive controls as recommended by the manufacturer and in accordance with the literature. For RUVBL1 protein, fallopian tube tissue was utilized as a positive control, while stomach tissue was used for HIF-1α protein. Additionally, the authors conducted several negative control slides by substituting the primary antibody with PBS.

2. It is unclear which cells were analyzed for their RUVBL1 and HIF1A immunoreactivity, especially in sections of non-cancerous, normal renal tissue.

Response: Thank you for bringing this issue to our attention. In our study focusing on the expression of RUVBL1 and HIF1A proteins in non-tumor tissue sections, we assessed immunoreactivity specifically in tubular epithelial cells. This was done in accordance with the adopted and described methodology: „This evaluation was conducted using the modified Remmele-Stegner Index (IRS) scale. The final score, which falls within the range of 0 to 12, is calculated by multiplying two factors: the percentage of cells or areas displaying positive staining (ranging from 0 to 4) and the intensity of staining (ranging from 0 to 3).”

3. Why kidney medulla is shown in Figure 3? Which cells of the unchanged renal tissue were investigated for RUVBL1 and HIF1A immunoexpression? Since there is a consensus on the origin of ccRCC cells, immunoreactivity in shall be evaluated.

Response: Thank you for your feedback regarding Figure 3. As previously mentioned, the evaluations were indeed conducted in tubular epithelial cells. In order to provide clearer results, we have made revisions to Figure 3.

4. Figure 3 Legend must be carefully revised since much more comprehensive description of IHC results and localization of immunoreactive cells is necessary. Authors shall add letters to images and references in the main text body and/or figure legend.

Response: We have carefully revised Figure 3 to provide a more comprehensive description of IHC results.

Figure 3. Representative microphotographs illustrating immunohistochemical expression of RUVBL1 (A,C,E) and HIF-1α (B,D,F) in ccRCC. RUVBL1 staining in adjacent normal tissues (A); absence of RUVBL1 staining (C); presence of RUVBL1 staining in ccRCC (E); HIF-1α staining in adjacent normal tissues (B); absence of HIF-1α staining (D); presence of HIF-1α staining in ccRCC (F); The original magnification is 20×.

5. The presented tables with Cox regression suggest that the multivariate analyses were carried out separately. When building a model for multivariate Cox regression both RUVBL1 and HIF1A shall be included in a model (in order to support or invalidate them as independent prognostic factors) Did authors carry out such an analysis?

Response: Thank you for your comment. The results of the analysis have been included in our manuscript.

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6. The discussion shall be much more concise and focused on authors' findings; e.g. lines 249-273 shall be transferred (after shortening) to the introduction.

Response: Thank you for your appreciated review. Following your suggestion, we have moved the specified section to the introduction after shortening it.

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