Reviewer’s report

Title: Activation and Function of Receptor Tyrosine Kinases in Human Clear Cell Renal Cell Carcinomas

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Reviewer: Shanshan Wang

Reviewer's report:

Summary
The present study by Qiang Yu et al. identified a set of RTKs that are characteristically phosphorylated in ccRCCs. Besides, using a ccRCC patient derived xenograft models in nude mice, they found out the RTK phosphorylation pattern of the xenograft was different from that of the primary tissue samples and treatment of the xenograft nude mice with corresponding RTK inhibitors can effectively inhibit Erk1/2 signaling pathway as well as the growth of the tumors. The study is logical and straight forward. However, there are some comments below that need to be addressed.

Major comments:

Introduction
1. Line 65, spell the percentage out when start a sentence, also "the kidney cancer" should be "kidney cancer", the sentence should be "Ninety percentage of kidney cancers……". Line 66, sentence "originate from….., and are subdivided into…” is too long, should be "originate from tubular structures of the kidney. They are subdivided……". Line 70, "metastasized cancers" should be "metastasized cancer", also this sentence needs to be re-written to make it easy to understand, e.g. "surgery for localized kidney cancer, targeted therapies and immunotherapies for metastasized cancer". Line 71, again, spell the percentage out when start a sentence.

2. Redundant use of words such as 'of the RTKs' in line 80. Line 82, "the current studies on ..." is really confused, is it should be "the previous study" or "recently published studies from other groups"?

3. Why spend a whole paragraph talk about VHL mutation and its treatment? If its related to RTKs mutation, this needs to be described also in the opening Discussion paragraph to set the narrative of the study, since it's a little bit confused when read through the abstract part.

Methods
1. Line 105, "were" should be "are".
2. Line 126, "was" should be "were".
3. Line 129, "for 2 h" should be "for 2 hours".
4. Line 130, one should not start a sentence with "AND".
5. Line 146, primary antibodies are "anti-phospho-EGFR……".
6. Line 172, "tumor measured" should be "tumor was measured".
7. Line 177, "Mice were euthanized in a CO2 chamber 2 hours after last treatment" should be "Mice were euthanized in a CO2 chamber for 2 hours after the last treatment".

Results
1. Line 193, "There was a lot of extravasated red blood cells" should be "An abundance of extravasated red blood cells were observed in the tumors".
2. Line 220, "To determine if .... were specific, we evaluate..." Should be "To determine whether ...are specific, we evaluated ...".
3. Line 229, when describe phosphorylation pattern in papillary RRC, oncocytoma, etc, should add sample number here also, it's confused because author will need to go back to table 2 to find the correlated information of these sample numbers.
4. Line 239, the "only one" should be more clarified by add the sample number here also lable in the figure9 A and the legend.
5. Line 242, author described that EGFR and HGFR were maintained high level while others decreased, but according to Figure 9A, ERBB2 seems also maintained high level of phosphorylation as EGFR. Quantification graph is needed here to demonstrate authors conclusion.
6. Line 248, "tumorigenicity seemed to correlate with..." may not accurate without any correlation analysis, also it should not appear in result, should move this part to discussion since it is not a solid result from analysis.
7. Line 256, from the poor-quality pictures provided here, hard to tell the result that "PDGFR was absent..."
8. Line 264. "Fig. 9C" appears without any describe of Figure 9B. Is it missing in Line 267, here "Fig. 9D,9E" should be "Fig.9B,9D,9E"? Figure 9B readout is missing here. Line 274, "As shown in figure 9" is not consistent with previous paragraph, it should be "Fig. 9F, 9G, 9H". This paragraph seems lack of organization, which make it hard to read, the whole paragraph should be organized more definitively with describe each result in figure 9 and re-arranged by order.
9. Line 267, when compare these 3 group, lapatinib, crizotinib and combination, should use "In comparison..." instead of "But...".

Discussion
1. Line 286, "These data are consistent with several previous studies on the roles of RTKs in ccRCCs. For example, the activation of VEGF/VEGFR... the expression of HGFR mRNA was upregulated in patients with ccRCCs" needs to be re-written, such as "these data are consistent with several previous studies on the roles of RTKs in ccRCCs that showed VEGF/VEGFR activation and HGFR mRNA upregulation in patients with ccRCCs.
2. Line 290, "correlate" should change to "relate to" or "due to".
3. Line 292, author cited one report but saying "there were reports". Line 295, should add "but not adjacent normal tissues" after the sentence "insulin R was significantly phosphorylated in the ccRCC samples".
4. Line 298, 6 RTKs? Its 9 RTKS through the whole paper.
5. General comment - Discussion seems lack to conceptual organization and should be organized more definitively with concluding sentences after each paragraph. E.g. Line 242. Author just stated that not clear which of these RTKs were activated in the ccRCC cells, and some of them may be expressed in endothelial cells while some of them are expressed in cancer cells, and PDGFR is associated with poor prognosis, so the conclusion "targeting all of these RTKs..." is not accurate, this conclusion needs to be more specific, such as "Thus, targeting these activated RTKs that associated with poor prognosis may be an efficient way to inhibit tumor growth."
Figures & Legends

1. Figure 4. Needs to be re-labeled by each set of pictures. Also WB bands needs to be quantified, graphs are missing here. In the method, quantification by software of Azure system was described.
2. Figure 8. Immunostaining figure is poor quality, needs higher resolution.
3. Figure 9A. Which ccRCC sample is this? should absolutely label the number of this sample because it is the only one grew successfully. that so Quantification graph is needed here to demonstrate authors conclusion as mentioned above.
4. Figure 9 legend, Figure C and D were measure before Figure B, should be rearranged by time order. Figure 9F, what is the number (2,4,5) underneath the group mean? Should be described here. P-akt quantification is missing here, even it's a negative read out. Line 561, Last sentence, "Error bars, means±SEM" is this a full sentence?!!!

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

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