Reviewer’s report

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

**Version:** 0 **Date:** 26 Apr 2019

**Reviewer:** Peter Schraml

**Reviewer's report:**

Zhang and colleagues investigated the phosphorylation patterns of receptor tyrosine kinases in human renal cell carcinoma (RCC) tissue samples, cell lines and patient-derived xenograft models in nude mice using phospho-RTK arrays. They found 9 RTKs in clear cell RCC whose phosphorylation patterns were similar among each other, but different to those seen in adjacent normal tissue, cell lines and other kidney tumor types. They suggest that synergistical inhibition of RTKs by combinatorial use of RTK inhibitors may be a novel strategy to treat ccRCC.

This is an interesting approach, the paper is well written, there are, however, several concerns which should be clarified by the authors.

1. A-498 and ACHN are considered papillary RCC cell lines (see Brodaczewska et al. and refs herein, Mol Cancer 2016, 15:83). This should be corrected throughout the manuscript accordingly.

2. The images shown in Fig. 1A/B are of bad quality. The size of "adjacent" normal tissue is uncommon.

3. Despite the known heterogeneous phenotype and genotype of ccRCC it is surprising that the RTK phosphorylation patterns are similar among ccRCCs. This suggests that some RTKs may be preferentially bound. 2-3 cell lines or tumor tissue samples other than RCC should be included as additional controls to show activity of other RTKs (f.e. EphA, ALK).

   It's a bit hard to follow the sequence of figures and paragraphs.
   Fig 9A should be combined with Fig 7. The paragraph "PDGFRb was expressed…” should be placed to the end of the results section and the Figure numbers should be corrected accordingly.

5. The authors claim that PDGFRb is present in glomeruli, interstitium, peritubular and stroma cells but not in ccRCC cells. In contrast, in Figure 3 PDGFRb is much higher than in the adjacent tissue suggesting the tumors might be strongly contaminated with non-tumorous material? This should be explained.

6. A supplementary schematic illustration of the RTK array showing the localization of the RTKs would help the reader to compare and interpret the RTK phosphorylation patterns.
Minor:
What is meant by the VHL gene and its partners?
VHL loss of function prevents HIFa from proteasome degradation, which leads to HIFa stabilization rather than overproduction.

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.

No

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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