Author's response to reviews

Title: Caveolin 1 protein expression in renal cell carcinoma predicts survival

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Author's response to reviews: see over
Dear Prof. Patel,

Thank you for your letter concerning our manuscript entitled:

“Caveolin 1 protein expression in renal cell carcinoma predicts survival”

We have carefully studied all of the reviewers’ comments as well as the editorial request and revised the manuscript accordingly.

Attached please find the revised manuscript as well as a list providing a detailed explanation of all revisions.

We hope that the manuscript is now suitable for publication in “BMC Urology”.

Yours sincerely,

Markus A. Kuczyk
Major Compulsory Revisions

The expression of CAV1 has already been studied in a number of different tumor types including ccRCC. According to the literature different CAV1 antibodies, IHC protocols and criteria have been used for analyzing CAV1 expression without validating the antibodies. This obviously lead to contradictory data.

1. In light of the large ccRCC cohort used here, the results of this study would gain more importance if the authors are able to validate the CAV1 binding specificity of the used antibody (i.e. Western blot analysis of some CAV1 negative and positive tumors (negative, weak, strong + reference). Replacing the primary antibody by non-immune serum is widely used in pathology labs but this not sufficient to justify it as negative control.

Answer: The rabbit polyclonal anti-caveolin-1 was obtained from Becton Dickinson Biosciences, Franklin Lakes, NJ, USA. This antibody recognises both the a and b isoforms of caveolin-1 as assessed by Western blotting. Negative controls run in parallel comprised sections where the primary antibody had been omitted. Caveolin-1 staining of peripheral endothelial cells and non-neoplastic tissue adjacent to the tumour were used as the positive controls. In negative controls, the primary antibody was omitted.

2. As the cores of tissue microarray elements are very small, the use of a score addressing the percentage of tumor cells is rather arbitrary. There is no data describing how many ccRCC were negative, weak, moderate and strong positive (cytoplasm and membranous). It would be interesting to see the associations between CAV1 expression (single groups and combined (i.e.0+1 and 2+3 or neg/pos) and clinical parameters by applying a 3 or 4-tiered staining score.

Answer: A 3-tiered staining score is shown in the supplementary material. However, the authors believe, that its inclusion in the article would not lead to significantly more information for the average reader. However, if favored by the editor, we are absolutely willing to include it in the manuscript.

3. There is a significant correlation between membrane and cytoplasmic positive ccRCC. It is surprising that only cytoplasmic CAV1 expression is significantly correlated with pathological parameters and survival?

Answer: CAV1 protein expression in the tumor cell cytoplasm and cell membrane correlated significantly, but only moderately (r=0.52 !). This is most probably the explanation for the fact, that cytoplasmic CAV1 expression was significantly associated with survival whereas there was only a trend for membranous CAV1 expression.

Minor Essential Revisions
4. The previous CAV-1 mRNA expression study was not cited.  
Answer: It was included (Reference 29). Thank you for this remark.
Reviewer's report:
The authors' manuscript deals with the caveolin 1 expression in renal cancer. Results collected from nearly 300 tissue specimen indicated a negative correlation between cytoplasmic caveolin 1 expression and patient prognosis. The article provides interesting and novel information and is well structured. I have just few minor concerns which should be taken care of.

Minor Essential Revisions:
1. Abstract, methods: The term IQR should be written out in full when mentioned the first time.  
   Answer: DONE

2. It should also be explained that both cytoplasmic and membranous caveolin was analyzed.  
   Answer: DONE

3. Abstract, results: The formulation “higher than average” and “higher and lower than average” is difficult to understand. How is “average” defined?  
   Answer: Higher than average is defined as higher than the median staining intensity score, which was 3 (compare results section). This has been clarified in the abstract and material and method section.

4. Introduction: CAV1 should be written out in full the first time (introduction, third para) and replaced by CAV1 thereafter (introduction, last para).  
   Answer: DONE

5. Results, first chapter, second para: The difference between “a high CAV1 expression” (first line) and “a higher than average CAV1 expression” (third line) should be explained. Secondly, how is “average” defined?  
   Answer: see answer to 3.

6. Table 1+2: CAV1 staining is given as “cytoplasm < median” and “cytoplasm ≥ median”. Does it mean that “cytoplasm < median” includes scoring values 0,1,2,3, whereas “cytoplasm > median” is related to scoring values 4+5?  
   Answer: The reviewer is right, we did not explain the difference between the pure staining score and staining intensity properly. The slightly modified pure staining score, as previously described by Tamskar 2003, includes degrees 0-5 (i.e. maxium staining per tumor specimen). This score was multiplied by the percentage of the stained area in our specimens. The result was the staining intensity score, on which our study was based. Here the median intensity score of all specimens was 3 (compare Results-Section). Thus the staining intensity “>median” was equivalent to a staining intensity score of 4 or more. We clarified the different definitions in the manuscript.

7. Discussion, third para: “Previously, we were able to show....” should be
underlined by a reference.

**Answer:** DONE, Reference 29

All changes in the text and tables are indicated in red ink.