Author's response to reviews

Title: GOLPH2 expression in renal cell cancer

Authors:

Florian R Fritzsche (florian.fritzsche@usz.ch)
Mark-Oliver Riener (mark-oliver.riener@usz.ch)
Manfred Dietel (manfred.dietel@charite.de)
Holger Moch (holger.moch@usz.ch)
Klaus Jung (klaus.jung@charite.de)
Glen Kristiansen (glen.kristiansen@usz.ch)

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Author's response to reviews: see over
Submission of manuscript

Dear Dr. Bucceri,

Thank you very much for reviewing our manuscript “GOLPH2 expression in renal cell cancer”. We found the comments of the reviewers very helpful to improve the manuscript. Please find attached our answers to these comments itemised point by point.

Reviewer 1:
The authors should clearly define how the immunostain results were graded, i.e., what was considered to be negative, weak, moderate and strong staining.

Answer:
For the evaluation of the GOLPH2 staining we used a four-tier grading system and applied a 10% threshold for any positivity and for each individual intensity grade. Tumours were considered negative, if no specific GOLPH2 immunostaining was detectable in the tumour cells or if there was a GOLPH2 staining in less than 10% of the tumour cells. Determination of staining intensity is, like any evaluation of immunostainings in routine surgical pathology, a subjective measure. In our study, the stainings were evaluated by two experienced urogenital pathologists to create more objectivity. To achieve an even greater uniformity of the evaluation, the first step was to construct a panel with four illustrative examples pictures, of which a hardcopy lay next to the microscope. For each staining intensity category (weak, moderate, strong),
the 10% threshold was also valid. For instance, in a case in which the tumour cells displayed mainly weak GOLPH2 expression but more than 10% of the tumour cells showed a moderate GOLPH2 expression, the tumour was considered “moderately positive” for GOLPH2. The description in the M&M section was accordingly improved.

Reviewer 1
Have the authors also looked at other renal tumours, such as oncocytoma or pelvic urothelial carcinoma? Have they looked at GOLPH2 expression in sarcomatoid and rhabdoid RCC?

Answer
In the current study we focussed on the analysis of renal cell carcinomas and did not include non-malignant renal tumours (oncocytoma) or urothelial tumours. Accordingly, the tissue microarray used in this study did represent renal cell carcinomas with representative fractions of the three most common histologic subtypes. Especially the analysis of urothelial carcinomas is of interest for future studies of GOLPH2. We included this point in the discussion.

Reviewer 1
The outcome data are bit confusing. Cases with higher GOLPH2 expression had slightly worse (although not statistically significant) survival. Yet chromophobe and papillary RCCs, which have better survival than clear cell RCC, had higher GOLPH2 expression. The authors may want to try to explain this perplexing result.

Answer
We absolutely agree with the reviewer that these results are somewhat perplexing. On the other hand, as stated in the manuscript and by the reviewer, the log rank test remained insignificant wherefore care should be taken when interpreting such trends (p=0.162). GOLPH2 was consistently found in normal renal tissue and in most of the papillary and chromophobe RCC. Meanwhile less than one third of the clear cell RCC subtype were considered highly positive for GOLPH2. One possible explanation could be that GOLPH2 is normally down-regulated in clear cell RCC and that the re-up-regulation could be seen as a correlate of molecular de-differentiation. However, there were no correlations between the GOLPH2 expression and the Fuhrman grade.
(Table 1). We also analyzed this for the group of clear cell RCC. In support of this hypothesis the trend for shortened patient survival for patients with GOLPH2 positive tumours was accentuated in the subgroup of clear cell RCC \((p=0.057)\). We included this point in the result and the discussion section.

Reviewer 2
The authors report that 108 patients were enclosed in this study and they report most data relative to the entire series. However, they also clearly report that in four of the 108 cases nor tumour tissue could be evaluated and, thus, all of the relevant data only refer to 104 patients. It would be much better to only report these 104 patients overall in the study.

Answer
We agree with the reviewer and recalculated all data with the 104 patients. We still kept the original 108 patients in the TMA construction part of the M&M section.

Reviewer 2
In the Discussion section the authors appear to be concerned mainly for the possible prognostic value in non-clear cell RCC, but as they note the number of cases is too limited to draw any conclusion. Thus I would avoid any comparisons with the results obtained in HCC and prostate cancer.

Answer
The Discussion section was thoroughly revised and an extensive discussion of HCC and prostate cancer has been avoided.

Reviewer 2
I would also eliminate in the Discussion section the speculations regarding the way in which GOLPH2 acts and interacts and the significance of possible splicing variants as well as various glycosilation sites of GOLPH2.
Answer
The respective points were omitted from the Discussion section as requested.

Reviewer 2
Manuscript should be checked throughout for English.

Answer
The whole manuscript was thoroughly revised for English style and orthography.

Concerning the level of interest we can understand the reviewers’ ratings for apparently the results are mainly negative and no novel diagnostic or prognostic marker of RCC can reliably be deduced from our findings. Still, since GOLPH2 has very recently been described as a promising biomarker in prostate and hepatocellular cancer and serum analyses of this marker are underway, research on tissue levels of GOLPH2 in other tumours is important. Therefore this work represents a logical and scientifically important step in the thorough characterisation of GOLPH2 in human neoplasias.

We hope that the revised manuscript will be considered acceptable for publication in BMC cancer. Please do not hesitate to contact us for further information.

Best regards

Florian Fritzsche  Glen Kristiansen