Author's response to reviews

Title: Monocarboxylate transporter 4 (MCT4) and CD147 overexpression is associated with poor prognosis in prostate cancer

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Author's response to reviews:

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Dear Editor,

We are grateful for the opportunity to resubmit our Manuscript entitled “Monocarboxylate transporter 4 (MCT4) and CD147 overexpression is associated with poor prognosis in prostate cancer”, Ms. 106329936516644. We have considered the reviewer’s comments and have made the suggested changes. We believe this will improve the manuscript.

Our detailed response to the reviewers’ comments is attached below.

We look forward to hearing from you again soon with your decision.

Yours sincerely,

Fátima Baltazar

Assistant Professor, PhD
Life and Health Sciences Research Institute (ICVS), School of Health Sciences,
In reply to Reviewer Sven Perner:

Major points:

1. The main conclusion of this manuscript stating that MCT1/4 and CD147 are associated with features of unfavourable clinical course, is not novel, and has already been shown by Hao et al. The only new finding is the overexpression of MCT2 in prostate cancers, which revealed, besides younger age at diagnosis, no association with clinicopathological data.

We agree that associations of MCTs and CD147 with unfavorable prognosis not novel; however, there is only one study (Hao et al.) showing this association for MCT4 and MCT1. Thus, we believe that knowledge is not based on a single study and the present study adds important information to the literature. In addition, our results showed for the first time the overexpression of MCT2 in prostate tumour compared to non neoplastic tissue, and although not associated with clinicopathological data, MCT2 seems to be important for the maintenance of the tumour. Also, alpha-methylacyl-CoA racemase, an enzyme currently used in prostate cancer diagnosis, since is overexpressed and recognized as a marker in prostate cancer, is not associated with prognosis data. Thus, if this is the case for MCT2, it is not surprising that MCT2 has no prognostic value.

2. The immunohistochemical evaluation of the TMA sections pertaining to cytoplasmic or plasma membrane staining seem to be very subjective. The manuscript would profit by including pictorial representations of scoring and staining intensity parameters for easier comprehension and clear definition. Also, the authors fail to state the reason for a combined scoring method. Was there a significant difference observed in the staining of a given area or a gradation in staining intensity?

To clarify immunoreaction evaluation, we included pictures (new Figure 1) in the Results section showing examples of positive cases (score # 4) with weak, moderate and strong staining intensity for each protein. We were the first to describe the scoring system used in the present manuscript (refs. 6, 7, 9, 10). We believe that a combined scoring system reflecting intensity plus extension has higher biological significance. In this scoring system, to classify a case as positive, if extension score is 1 (# 5% of stained cells), intensity has to be 3 (strong), likewise, for intensity score 1 (weak), extension score has to be 3 (# 50%). As stated by the reviewer, IHC evaluation is somewhat subjective and, in this way, we believe that evaluating 2 parameters instead of 1, decreases subjectivity and would have higher biological significance. A sentence on this issue was included in the Discussion section.
3. The authors should explain why the positive cases were categorized into two groups (ISG and HSG). Also, this detail should be included in the methods and not the results section.

We categorized the positive cases in two groups to further clarify the significance of the immunoexpression of MCTs and CD147 in prostate carcinoma. Also, these are new markers (MCTs) for which we do not have definitive information on the most adequate score to use. There are cases considered positive but this positivity was different among the cases and we thought that would be more interesting for the study to explore these results having into account these differences. As suggested by the reviewer, details on the category score definition were included in the Material and Methods section and further referred in the Results section.

4. Referring to their introduction, it would be interesting to study the correlation of gp70 with MCT2 on neoplastic and non-neoplastic tissue. The gp70 antibody could be tested by IHC and then correlated to the patients' clinicopathologic characteristics.

As suggested by the reviewer, we performed IHC reactions for gp70. We added this information in the Material and Methods section. However, we found that gp70 was expressed only in very few cases of prostate cancer and was negative in all normal, adjacent non-neoplastic tissues and PIN lesions. This might indicate an alternative regulation of MCT2 in prostate cancer that is not totally dependent on gp70, as it is described for MCT isoforms 1 and 4 (Slomiany et al, 2009).

The Results and Discussion sections were modified accordingly (in red).

5. Contrary to the report by Hao et al., Gomes and collaborators found MCT1 to be expressed less intensively in neoplastic tissue as compared to benign tissues, suggesting a physiologically role of this protein. Still, the expression in tumours is reported to be associated with less favourable features. How do you explain this? The authors should expand their contrary finding in the discussion.

The reviewers comment is pertinent, however the role of MCT1 is prostate tissue is not clear. Similarly to MCT4 and CD147, MCT1 function in prostate tumours might be related to the glycolytic and invasive phenotype of tumours, through facilitation of lactate transport, as discussed in the Discussion section. We replaced Table 1 by a figure (Figure 1), which we believe that helps to visualize the differences in expression of the markers analyzed.

6. As suggested by the authors’ discussion, insight into the MCTs and CD147 expression in normal prostate tissue and PIN lesions would provide a better understanding of protein function. This should be included in their study. Normal prostatic tissue is easily accessible through cystoprostatectomy material. Also,
access to PIN lesions should not be a significant problem for the collaborating pathologists.

As suggested by the reviewer, we have incorporated in this study some cases of normal prostatic tissue obtained from cystoprostatectomy material and also PIN lesions. The Material and Methods, Results and Discussion sections were modified accordingly.

7. Figure 1 shows images with very faint IHC staining and counter staining. The image quality should be improved significantly. Former Figure 1 (now Figure 3) was updated with the new results and the quality of the pictures was improved.

Minor points:

1. The authors very often use the term ‘correlation’ for MCTs and CD147. This word should be substituted with ‘association’ since a pure statistical association does not proof a correlation between two events. As suggested, the word “correlation” was replaced by “association”.

2. Table 4 is not homogeneously formatted. The significant p-values should be made bold, thus making the significant results more eye-catching. These “errors” were corrected.

On the whole, this study has no biological explanation for the differences in protein expression and has been based on an a-priori formulated hypothesis. Therefore, this study has a marginal impact on the understanding of prostate cancer. In the conclusion, the authors state- ‘Importantly, since MCT4 and CD147 were associated with markers of poor prognosis, assessment of the expression of these molecules may help in the prediction of prognosis in prostate cancer’- This statement is too strong for a study where a small panel of antibodies were assessed on a cohort in order to expect a significant association.

As suggested, the sentence “Importantly, since MCT4 and CD147 were associated with markers of poor prognosis, assessment of the expression of these molecules may help in the prediction of prognosis in prostate cancer” was replaced by “MCT4 and CD147 should be further explored as markers of poor prognosis”. (in red)

In reply to Reviewer Roman Nawroth:

5. The authors discuss their results in context to previous findings by other groups and draw appropriate conclusions from their own and others data. However, there are some minor essential revisions to be addressed.

The authors might have to demonstrate the value of the proteins examined for
prognosis in prostate cancer more thoroughly. Although the results clearly demonstrate a correlation of CD147 and MCT4 expression and clinical marker of poor prognosis in prostate cancer, the additional value of these biomarkers to the standard clinical parameter in use is not obvious. Although discussed properly, it would be of interest if differences within the published data and the present dataset found for MCT1 staining indeed result from the different antibodies used. This might be of particular importance because future work on these proteins as biomarker in cancer should follow a certain standard for reproducible and comparable results. Thus, I consider a comparison of different antibodies used in different studies in a limited amount of prostate cancer specimen as a valuable addition to the article.

In the past, we have tried different antibodies for MCT1, however the one used in the present manuscript as well as in previous publications, either our own (ref. 6,7 and 9) or from other authors (ref.30 and Végran et al, 2011**), proved to be very specific. Very recently, Végran et al., silencing MCT1 with siRNA, confirmed this specificity. Thus, we do not believe that the use of the antibody from Santa Cruz (H-70) would add any value to our study. We agree that this is an important issue, however, comparison of different antibodies would be a different study which was not the aim of the present study.


** Végran F, Boidot R, Michiels C, Sonveaux P, Feron O. Lactate Influx through the Endothelial Cell Monocarboxylate Transporter MCT1 Supports an NF-{kappa}B/IL-8 Pathway that Drives Tumor Angiogenesis. Cancer Res. 2011 Apr 1;71(7):2550-60