Author's response to reviews

Title: Increased tartrate-resistant acid phosphatase (Trap) expression in several malign tumors, patients sera and cell lines, an investigational study.

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Author's response to reviews: see over
Dear Sir or Madam:

Thank you very much for your comments on our manuscript “Increased tartrate-resistant acid phosphatases….”. We have revised the manuscript in light of the reviewers’ comments as shown below.

**Answers to reviewer No. 1 (Wolfgang Janni)**

- **Following the reviewers’ suggestions**
  
  The manuscript has now been subjected to language editing by a native speaker. Changes like i.e. such as malign to malignant, progress to progression have now been made.

- **(Material and Methods)**
  
  The reviewer has a very good point mentioning that there is a lack of a methods section in the Abstract, which has now been added. We added such a methodology section to the abstract.

- **(General design)**
  
  In order to follow the recommendations of reviewer 2 we have tried to improve the manuscript by making it clearer and more concise. We have deleted the results regarding the pertaining to TRAP expression in placenta and serum TRAP serum levels in pregnant women in order to focus on the major subject of the paper.
(General design) Furthermore we have made substantially improved our tables clearer and more concise by abolishing unnecessary information and made them clearer and concise. The tables now clearly show reflect our main statements.

(Methods) The reviewer is right that some of the information given in the Methods section are belongs in the Results. In our improved version We have now removed the tables with the results from the Methods section and put them into the Results section. No statistical analysis or quantification was performed due to the descriptive nature of the manuscript.

(RT-PCR: cut off): RT-PCR was considered positive when a clear band of the expected size was visible on an ethidium bromide-stained gel. This is now mentioned in the Material and Methods section.

(Immunohistochemistry: cut off): For evaluation, the cells and tissues were compared with control sections incubated with an IgG control antibody. These evaluation criteria are now given at the end of the Immunohistochemistry paragraph in the Material and Methods section.

(Results) We describe a relatively new finding and therefore performed various different assays, evaluating several cell types, in order to be sure that cancer cells express TRAP. To some extent the manuscript is therefore descriptive.

(Discussion) The reviewer was right in pointing out that malignant melanoma results are not an integral part of the study. The central aim of the study was to
prove that cancer cells express TRAP. The question was whether only breast cancer cells express TRAP or whether TRAP expression is a common feature of malignant transformed cells. Therefore, we evaluated various different cancer entities including malignant melanoma. In order to make the results on malignant melanoma an integral part of the study we have now included RT-PCR results for TRAP mRNA detection in malignant melanoma cells. We also evaluated cultured malignant melanoma cells for TRAP expression using immunocytochemistry and have included these results in our improved version of the manuscript.

(Discussion) The reviewer asked why healthy people like pregnant women express TRAP. It is a well known fact that at some stage of gestation the placenta expresses almost every protein that has been linked to malignant transformation of cells at some stage of gestation. Placental tissue is used as a positive control in many fields of basic cancer research dealing with cancer. The point is that we aimed to exemplify this finding by describing the fact that TRAP expression is not restricted to cells of the monocytic lineage like osteoclasts and dendritic cells, but is also found in placental tissue. Therefore, TRAP expression is not confined to a particular type of cell or lineage. Such a more widespread expression of the protein makes expression of TRAP in tumors seem possible. However, since we have now omitted the information on TRAP expression in the placenta according to the reviewer's suggestion, this information is not given in the text.

Answers: Response to the reviewer No. 2 (Alison Hayman)
1. **We agree with the reviewer** is right about the extraordinary high TRAP values determined by ELISA. **We did not appropriately describe the methodology of determining the concentrations was not correctly described** in the previous version of our manuscript. Now we described the method of dilution and calculation of the TRAP concentration in the ELISA paragraph of our Material and Methods section.

2. Page 11, last sentence: **We thank the reviewer** for their valuable comments. Following the suggestion we added RT-PCR results. We now show for the mRNA detection of TRAP mRNA in stimulated dendritic cells, which we detected by RT-PCR. (Figure 4).

3. **Page 9 paragraph 3:** We have omitted the section on gestational tissues as well as the results dealing with serum TRAP serum concentrations in pregnant women. Almost any protein that seems to play a role for in malignant transformation is expressed in the placenta during some point of gestation. This is not surprising, because the placenta shows invasive growth at least until week 20 of gestation. Furthermore, this adds to the point that TRAP expression is not restricted to osteoclasts but can be detected in placental tissue as well as a variety of different cancer cells.

4. **It is a justified comment of the reviewer** if you deal with an enzyme whether The question as to whether concentration or activity was determined is of course an important one. We clarified this issue in the Material and methods section and made it clear that we did not measure TRAP activity but concentration of TRAP protein. In addition, we exchanged the expression TRAP “level” by
with TRAP “concentration” wherever serum concentrations were mentioned.

5. Table 1: Units/ml have now been added in the column.

6. We thank the reviewer for her more than valuable comments and have made the following changes. We have subjected the manuscript to language editing by a native speaker and corrected the points mentioned by the reviewer.
   a) The word “malign” has been replaced by “malignant” throughout the text.
   b) “Bone affection” has been replaced by “bone disease” or “bone involvement”
   c) The word “cultivated” is replaced by “cultured”.
   d) The reviewer asked for the correction of minor grammatical errors. We asked for the help of a native speaker to correct these.

7. We have mentioned in the discussion that although we detected a distinctive marked TRAP protein expression in cancer cells, we cannot comment on TRAP activity, because we did not attempt to measure the enzyme’s activity in the various tissues and cells we investigated.

Yours sincerely

Arnd Honig