Author's response to reviews

Title: Expression pattern of matrix metalloproteinases in human gynecological cancer cell lines

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Author's response to reviews:

Dear Dr Cordes,

Please find enclosed the revised version of our manuscript “Expression pattern of matrix metalloproteinases in human gynecological cancer cell lines”; MS: 2071669303359599, which my colleagues and I would like to resubmit for publication in BMC Cancer. In our revised version of the manuscript we followed the referees' suggestions as answered point-by-point below.

Reviewer: Rafael Fridman

Discretionary Revisions:

1. Although not a pre-condition for acceptance by this reviewer, a zymogram will be more informative for the gelatinases because it clearly detects the latent and active forms of these MMPs and it is by far more sensitive that western blots.
   >We performed the analysis of the gelatinolytic activity of MMP-2 and MMP-9 secreted in serum-free cell culture supernatants by zymography. The results of gelatin zymography are added in the manuscript in Figure 3.<

2. How were the antibodies evaluated in terms of specificity?
   >We only used antibodies, which were described to have no cross-reactivity with other MMP family members. Since all antibodies were well characterized and obtained from commercial sources, we had no doubt on their specificity. Please see Table 2 describing the details of the antibodies in question.<

3. Any information or plans to measure about TIMP levels in these cells? At least a comment in this regard will be useful, as at the end the activity of MMPs is regulated by the levels of TIMPs.
   >Regulation of the expression of MMPs by TIMPs is indeed a very important aspect. However, in this study we focused specifically on the expression pattern
of MMPs in gynecological cancer cell lines. Our aim was to provide basic information on MMP expression necessary before starting functional analysis. Our data can help researchers to choose model system for further analysis on MMP regulation in cancer. A comment in this regard was involved in the manuscript, page 12.<

4. A table describing the cell lines used will be very useful.

> The cell lines used in our study are described in Table 1 adding the most prominent publications describing the use of them in research. However, all of them are well established commercially available cell lines and detailed information are subsumed on the data sheets available via the commercial cell lines service homepage (http://www.cell-lines-service.de) where we purchased the stocks.

Major Compulsory Revisions:
1. The paper needs serious English editing.

> English editing was done.<

Reviewer: Gregg B. Fields

This is a very interesting, ambitious, and thorough study, examining all human MMPs on the mRNA level and implicated MMPs on the protein level. Overall, the results indicate no real pattern for MMP expression and either cancer type or metastatic potential. If anything, this study points out problems with expression profiling for correlation of MMPs with cancer type or stage. This is not necessary bad, but simply warns agains overinterpretation of expression data. The authors should probably discuss this point.

> Indeed, our data indicate that there is no real pattern of MMP expression related to cancer type or metastasis. Our previous analysis of breast cancer and glioblastoma showed also that even the same stage of the cancer has diverse expression pattern of MMPs. Comment in this regard was involved in the manuscript, page 16, see references (Stojic et al., 2007; Köhrmann et al., 2009) cited in this respect.<

Also, is there any correlation between specific MMPs and the lack of protein production? In other words, are some MMP mRNAs less stable (more susceptible to degradation) than others?

> There is evidence about the alteration of the mRNA stability of some MMPs, like e.g. MMP-9 in cancer. Modulation of the MMP-9 mRNA stability might be important during malignant conversion and metastasis, when tumor cells need to induce or maintain MMP-9 levels in response to changing environmental cues. Comment in this regard was involved in the manuscript, page 12, see references (Iyer et al., 2005; Morini et al., 2005) cited in this respect.<

Finally, why are there so many forms of MMP-28 protein (Table 3 and Figure 2)?

> To date there are very few publications on the most recently described
MMP-28. There are several splicing variants of MMP-28 transcript differentially expressed in human tissues (Lohi et al., 2001) and barely any information about its protein size. Using the antibody for MMP-28 we detected bands of approximately 62, 58, 50, 48 and 46 kDa (there are two human MMP-28 sequences, isoform 1 and isoform 2, which encode proteins of 520 and 393 amino acids with predicted respective masses of 58.9 and 44.5 kDa). However, we did not have enough data to discriminate inactive and active forms of this protein. We have added this aspect into the discussion section, page 14.<

We hope that we revised the manuscript satisfactorily and are looking forward to hear whether it is now acceptable for publication in BMC Cancer.

Thank you very much for your time in processing this manuscript.

Sincerely yours,

Jelena Anacker