Author's response to reviews

Title: High nuclear expression levels of histone-modifying enzymes LSD1 HDAC2 and SIRT1 in tumor cells correlate with decreased survival and increased relapse in breast cancer patients

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Author's response to reviews: see over
Annie Lyn Bravo
On behalf of The BioMed Central Team
Journal Editorial Office
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Thank you for considering our paper for publication and for your suggestions for improvement. Attached to this document, you find a point-by-point response to the reviewers suggestions. Our manuscript has been revised accordingly; attached you find the revised version. Hereby, on behalf of my co-authors I submit a revised version of the manuscript “High nuclear expression levels of histone-modifying enzymes LSD1, HDAC2 and SIRT1 in tumor cells correlate with decreased survival and increased relapse in breast cancer patients” by R. S. Derr et al. for publication in BMC Cancer.

Thank you for your time and consideration of our revised manuscript.

Sincerely,

Peter J. K. Kuppen, Ph.D.

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Review report 1:

While IHC is a powerful technique for analysis of proteins, the findings presented in this manuscript should be strengthened and verified by another approach such as Western immunoblot.

This is a good suggestion. The strength of our study is the long follow-up time. Unfortunately, due to the long follow-up, good quality frozen material of these patients is not available, which is mandatory to perform reliable Western blot. Therefore we have not performed Western blot or similar antibody validation studies. However, we chose antibodies that have been validated using several molecular techniques by several research groups. We added references for these validation studies in the method section of the manuscript on page 6 lines 28 and 29.
In addition, we added the last follow-up moment in our revised version (lastly March 2013), in line 3 on page 8.

Also, there was no IHC analysis for normal breast epithelial tissues in support of data presented in Fig. 2 and must be included.

We have compared the mean IHC staining percentage for normal and tumor breast tissue in the result section on page 9 as well as in figure 2. In addition, we added pictures of representative normal breast tissue cores stained for each enzyme to figure 1. Accordingly, we changed the text of the legend of figure 1 on page 20 line 6 into normal tissue core (left), a tumor tissue core with expression above median (middle), as well as the text about figure 1 on page 9, line 6 & 7 into "Figure 1 shows representative pictures of normal breast tissue cores immunohistochemically stained individually for each enzyme, as well as representative pictures of breast cancer tissue cores with expression above and below median for each of the enzymes. The brown color is the amount of expression of the enzyme;"

According to Fig. 3, HDAC2 level seems to increase in stage III cancer tissue, perhaps to the same level as that of LSD1, although the median may be different. Authors need to address this issue.

We agree with the reviewer that the range is smaller in stage III and therefore there seems to be a trend towards an increase in HDAC2 levels. However, this trend was not significant in our one-way ANOVA statistical analysis. Therefore we did not address this in detail in our manuscript.

Figs. 4 and 5 are very difficult to read and must be enlarged.

We have adjusted the size of the figures to improve their readability.

Total number of patients has been indicated as 460, but the legend of Fig. 3 indicates 465, when three number are added. These numbers should be corrected.

Thank you for your critical reading of our document. It indeed concerns a typographical error, and the numbers in the legend of figure 3 (page 20) have been corrected (182 instead of 157 and 51 instead of 81).

There are minor typographical errors, for example, in the Fig. 3 legend, line 2, the word "en" it should be "and".

We have corrected typographical errors mentioned throughout the manuscript, highlighted in grey:
  - page 2: conclusions part: ', as well as' changed in 'and correlated with', and added a
  - page 20: legend of figure 3 'en' changed in 'and'
Review report 2:

The only weakness is the lack of a cell system to validate the clinical impression. Functional effects on the growth of cancer cell lines can be generated by treating breast cancer cells with small interfering RNAs (siRNAs) against histone-modifying enzymes and/or inhibitors against the enzymes and investigation of cell viability.

This is a very good suggestion of the reviewer, but it is beyond the scope of our current study, because we aimed to relate the enzymes to a clinical cohort. Previous studies have shown that inhibition of LSD1, HDAC2 or both have an influence on breast cancer cell survival in vitro. We have referred to two publications in our introduction (end of page 4 and beginning of page 5). In our discussion we included a third reference to authors who have performed cell survival experiments. Publications by Huang et al. 2007 (reference number 21), Huang et al. 2012 (reference number 11) and Vasilatos et al. 2013 (reference number 12), discuss experiments with inhibitors of LSD1 or both LSD1 and HDAC2 during cell culture to test the viability of breast cancer cells in presence of these enzymes. In the article of Huang et al. 2012 a microarray study is shown, demonstrating that inhibition of the enzymes LSD1 and HDAC2 led to reexpression of aberrantly silenced genes involved in processes such as cell differentiation and cell proliferation, which are frequently deregulated in cancer. The authors concluded that inhibition of LSD1 and HDAC2 leads to synergistic inhibition of breast cancer cell proliferation as compared to inhibition with a single agent. We referred to this publication from Huang et al. 2012 on page 4 (lines 28 and 29), page 5 (line 1), page 12 (lines 25-30) and page 13 (lines 1 and 2). In our revised manuscript, we added a phrase to highlight the importance of histone demethylases and deacetylases (suggesting an important role for histone demethylases and deacetylases in breast cancer.) on page 5 line 2.