Author's response to reviews

Title: Histological evaluation of AMPK signalling in primary breast cancer

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Version: 3 Date: 2 June 2009

Author's response to reviews: see over
Dear Andrea;
Thank you very much for considering our paper for publication. We can confirm that ethics approval has been given to this study, this is now commented upon in Materials and methods – patients’ section, paragraph 2. We also appreciate the constructive comments made by the reviewers and have addressed the individual suggestions below:

Reviewer's report 1
Title: Histological evaluation of AMPK signalling in primary breast cancer
Version: 2 Date: 3 April 2009
Reviewer: Carsten Denkert
Reviewer's report:
Generally this is a well written manuscript on an interesting topic. In particular, the approach using two separate cohorts makes the data more reliable. However, the data is not presented well in the tables.

Major compulsory revisions:
Table 1: It would be important to know what are the statistical differences between both cohorts, the authors should perform statistical tests.

Response:
This has been added in to table 1 and commented upon in Discussion, paragraph 2.

Table 2: This table should include all relevant clinical parameters, including HR status, Her2 status.

Response:
Table 2 now highlights the 3 parameters (tumour size, nodal status and tumour grade) used in clinical practice for prognostication (e.g. Nottingham Prognostic Index). Table 3 contains the biological markers (ER, HER2 …etc.)

The nodal status is not significant in cohort 2, however, this result is not mentioned in the text, where only the results from table 3 are included. This issue should be discussed.

Response:
Now commented upon in Discussion, paragraph 2

Table 3: This table is not adequate, as only the p-Values are reported. To get an overview on the results, we need numbers of cases in the various groups, as well. The authors should extend this table to show all relevant data.
Response:
As suggested, table 3 has now been extended to show all relevant data

Furthermore, they should be box blots for selected markers.

Response:
Box and Whisker plots are used in the analysis of continuous data for the display of means and medians, confidence intervals, data ranges and outliers. However, the biomarker data used in the research presented in this paper are ordinal, and therefore Box and Whisker plots are not appropriate.

Table 3: Disease free survival should not be tested by Fisher’s test, a Kaplan-Meier approach should be used.

Response:
Kaplan-Meier analysis could not be performed for disease-free survival in both cohorts, we could perform Fisher’s Exact Tests using the disease free status, and this is what has been reported in the table. We have also briefly signified this in Table 3. Kaplan-Meier analysis was used for overall survival using the Log Rank test (also see Table 3).

The authors should add a new table with the univariate survival data for all relevant clinical and pathological variables including the biomarkers.

Response:
Now in table 3.

Figure 1: The authors should include a higher magnification. The Her2 staining looks like a 2+ staining on the image, the image should be replaced and on slide controls for Her2 should be included as inserts.

Response:
This figure has been modified as suggested.

The authors should formally discuss the limitations of their study.

Response:
Now discussed in Discussion, paragraph 2
Reviewer's report

Title: Histological evaluation of AMPK signalling in primary breast cancer
Version: 2 Date: 1 May 2009
Reviewer: Zhijun Luo

Reviewer's report:
The major findings in this manuscript are that phosphorylation of AMPK at the thr 172 site, an indicator of AMPK activation, and phosphorylation of ACC, an immediate downstream target of AMPK, are decreased in breast cancer specimens. The decreased phosphorylation correlates to pathological grade and metastatic states. This is the first report showing changes of AMPK in clinical samples, which provides important information.

Response:
We are grateful for this positive review.

However, the date with enough number on scoring of matched normal breast epithelial cells should be provided. Without this, it is hard to judge if low detection rate of signal is due to technical issue or is truly reflective of differences in cancer tissues.

Response:
Normal breast tissue cores were constructed alongside the cancerous cores for every patient. In all of these normal cores, where there were identifiable ductal epithelial cells, phospho-AMPK was expressed strongly without exception. This is commented upon in “TMA construction” section, paragraph 1, and “Results section”, paragraph 3.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

Many thanks,
Sirwan