Author's response to reviews

Title: Tight correlation between expression of the Forkhead transcription factor FOXM1 and HER2 in human breast cancer

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Author's response to reviews: see over
Tight correlation between expression of the Forkhead transcription factor FOXM1 and HER2 in human breast cancer

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Responses to reviewers' comments:

Dear reviewers,

we would like to thank you for your valuable comments which helped us to further improve the manuscript. Please find enclosed our response letter. The revised parts of the text have been marked (underlining) in the manuscript text.

Reviewer 1: Brigitte Schlegelberger

Major Compulsory Revisions

Comment 1:

The authors mentioned in their abstract in the methods section: “Using semiquantitative realtime PCR and immunohistochemistry (IHC) we systemically analysed FOXM1 expression in human invasive breast carcinoma (n=229) and normal breast tissues (n=58).” These figures seem to be true for the IHC analyses but regarding the real-time PCR the results reveal that only 25 primary human breast cancers and 12 samples of normal mammary tissue have been investigated. The data should be corrected and explained.
Response 1:

In the “Abstract” we assigned the sample number to the corresponding method as suggested by the reviewer as follows: “Using immunohistochemistry (IHC) we systematically analysed FOXM1 expression in human invasive breast carcinomas (n=204) and normal breast tissues (n=46) on a tissue microarray. Additionally, using semiquantitative realtime PCR, a collection of paraffin-embedded normal (n=12) and cancerous (n=25) breast tissue specimens as well as benign (n=3) and malignant mammary cell lines (n=8) were investigated for FOXM1 expression”.

Comment 2:

The breast cancer tissue collected for the tissue microarrays has been collected from women diagnosed between 1994 and 2002. Did the women in the FOXM1 positive and negative groups receive comparable treatment? This information should be added.

Response 2:

Unfortunately, we do not have the clinical data available considering the therapy of the patients. Thus, we cannot exclude a different therapeutic approach between the FOXM1 positive and negative groups. We have added this information to the text in the discussion: “These data have to be confirmed in a prospective multi-centre trial including treatment information on FOXM1 positive and negative patients before FOXM1 overexpression in breast cancer can be considered as an established prognostic marker potentially useful for stratification of patient treatment”
Comment 3:
Taking into consideration the retrospective nature of the study, it has to be mentioned that the data have to be confirmed in a prospective multi-centre trial before FOXM1 overexpression is an established prognostic marker and can be used for stratification of patients.

Response 3:
With respect to the retrospective nature of our study we replaced the sentence “Larger confirmation studies will be done to evaluate the prognostic relevance of FOXM1 in breast cancer” on page 12 (“Discussion”) by the sentence “These data have to be confirmed in a prospective multi-centre trial including treatment information on FOXM1 positive and negative patients before FOXM1 overexpression in breast cancer can be considered as an established prognostic marker potentially useful for stratification of patient treatment “

Minor Essential Revisions

Comment 1:
Although real-time PCR experiments for FOXM1 were performed in triplicate and the expression levels were measured in relation to the expression of the housekeeping gene GAPDH, some details should be mentioned about the quality of the RNA. In particular, regarding the RNA isolated from paraffin-embedded tissue, since only 37 samples were analysed by real time PCR. What is the reason for this? What was the drop-out rate?

Response 1:
The collection of paraffin-embedded breast cancer and normal breast tissues was recently described by our group (Zafrakas M et al.; Int J Cancer 2006;118:1453-9). We have added this information in the methods part (page 5). In this collection of RNA samples only samples of sufficient quality (Ct value <27 for the realtime analysis of GAPDH) were included.
Therefore, there is no drop-out in the analysis of this collection. The major function of this RNA collection is to unequivocally demonstrate up- or downregulation of potentially novel tumour markers in breast tumour tissue compared to morphologically well defined normal breast tissue. The collection has not the function of defining marker expression in breast cancer subgroups. This is done by using the breast cancer tissue microarray.

Comment 2:

The authors discuss the positive correlation between the FOXM1 expression and HER2 status, and hypothesise a potential role of FOXM1 in directly activating the HER2 promoter leading to the overexpression of HER2. However, FOXM1 was overexpressed in a significant number of normal breast tissues and in significantly more breast cancer cases than HER2. This argument should be omitted or at least weakened.

Response 2:

Our statement in the “Results” (page 9): “Considering the whole tissue microarray 87% (187/204) of the breast carcinomas expressed nuclear FOXM1 versus 42% (19/46) of normal breast tissue specimens” may have been misinterpreted. FOXM1 is not “overexpressed” in normal breast tissue. The percentage given above involves any nuclear staining (IRS≥1) in normal breast tissue and only expresses if there is a staining or not.

In accordance with the low FOXM1 expression in normal breast tissue, the median nuclear immunoreactive score in normal breast tissue was zero (median IRS=0), while in breast carcinomas the median IRS was three. We have added this sentence to the Results part.

Discretionary Revisions

Comment 1:
The manuscript should be accurately proof-read for typographical errors, e.g. page 3: “…of a breast tumours hormone receptor status…”, page 6: “…all reactions were performed in triplicates.” or page 13: “There studies were based on…”.

Response 1:
The manuscript has been accurately proof-read.

Reviewer 2: Mong Hong Lee

Comment 1:
Figure: The two curves present here look OK but the p value of 0.110 is not of high enough significance to be relevant. I feel that the authors have shown that FOXM1 overexpression occurs in breast cancer, but if the p value is correct, whether FoxM1 overexpression leads to poor survival prognosis is questionable.

Response 1:
In the “Abstract” we replaced the last sentence in the results part “Univariate survival analysis showed an association between FOXM1 protein expression and unfavourable prognosis” by the sentence “Univariate survival analysis showed a tendency between FOXM1 protein expression and unfavourable prognosis (P = 0.110)”.

On page 10 (“Results”) we dropped the headline “Unfavourable prognosis for patients with FOXM1 overexpression” because the p-value of 0.110 is not significant enough. The following text after the dropped headline is attached to the previous result part with the headline “Correlation between nuclear FOXM1 and clinicopathological patient parameters”.

On page 12 (“Discussion”) we replaced “clear tendency” by “tendency” in the third line.
Taking into consideration the retrospective nature of our study we replaced the sentence “Larger confirmation studies will be done to evaluate the prognostic relevance of FOXM1 in breast cancer” on page 12 (“Discussion”) by the sentence “These data have to be confirmed in a prospective multi-centre trial including treatment information on FOXM1 positive and negative patients before FOXM1 overexpression in breast cancer can be considered as an established prognostic marker potentially useful for stratification of patient treatment”.