Reviewer's report

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

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Reviewer: Brigitte Schlegelberger

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General

The authors have investigated the expression of the Forkhead transcription factor FOXM1 that plays a role in cell proliferation and has been reported to be elevated in other tumours in human breast cancer. Using semiquantitative real-time PCR and immunohistochemistry, FOXM1 turned out to be upregulated in primary breast cancer compared to normal breast tissue and in malignant compared to benign mammary cell lines. This may imply that FOXM1 overexpression is associated with enhanced proliferation and invasiveness of cancer cells. Furthermore, the authors demonstrate a positive correlation of FOXM1 and HER2 expression. In a univariate analysis, FOXM1 overexpression was associated with an unfavourable prognosis. The authors conclude that FOXM1 may represent a novel breast tumour marker with prognostic significance and a potential drug target since FOXM1 inhibitors are available.

The topic is of high relevance for cancer research. The methods were performed accurately and the results are presented convincingly.

Major Compulsory Revisions

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.

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.

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.

Minor Essential Revisions
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?

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 tissue and in significantly more breast cancer cases than HER2. This argument should be omitted or at least weakened.

Discretionary Revisions
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…”.

2. The discussion can be shortened by about one third.

What next?: Accept after minor essential revisions

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.