Reviewer's report

Title: FRA-1 protein overexpression is a feature of hyperplastic and neoplastic breast disorders

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Reviewer: Karin Milde-Langosch

The AP-1 transcription factor Fra-1 has obtained increasing interest in the last years, since its overexpression in tumor cells is often associated with increased motility, invasiveness and possibly metastasis of several tumor cell types. The study of Chiapetta et al. addresses the expression of Fra-1 in hyperplastic and neoplastic lesions of the female breast on a protein and RNA level. Using both approaches, the authors found high nuclear Fra-1 expression in all invasive tumors, whereas Fra-1 staining was weaker and/or cytoplasmic in hyperplasias and carcinoma in situ. These results are in contrast to those published earlier for breast cancer cell lines and tumors, where high Fra-1 expression was mainly observed in poorly differentiated, highly malignant tumor cells (Philipps et al., 1998; Bamberger et al., 1999; Zajchowski et al., 2001; Belguise et al., 2005). The reasons for these differences are not discussed.

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Major Compulsory Revisions

Regarding the points mentioned above, the results would be more convincing if the materials & methods and results sections would be more precise, and additional informations are partly necessary.

1. The clinical and histological characteristics of the 60 carcinomas (grading, stage, receptor status) should be given.
2. In contrast to earlier publications where different bands representing different phosphorylation states were shown in immunoblots, only one band was shown in Western blots in this study. In order to find out if this corresponds to the highly phosphorylated protein, the authors should give informations about the apparent molecular weight of the protein. In addition, comparison with Fra-1 expression in a cell line with known Fra-1 overexpression would help to clarify this issue.
3. The semiquantitative RT-PCR gave similar results compared with IHC. Yet, the published primer sequenced given in the manuscript are not from the Fra-1 cDNA sequence as published in Genbank, which sheds some doubt on these results. In addition, the authors state that Fra-1 expression in hyperplasias is lower as seen in Fig. 3. Yet, the bands in lane 3 and 4 of Fig. 3 are different to compare since the samples showed a different migration and a "smiling effect" even seen in the tubulin control.
4. In Table 1, it is not clear if only nuclear or nuclear and cytoplasmic staining were evaluated. A separate evaluation of both types of expression would be more informative.

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Minor Essential Revisions

1. In Fig. 3, 4 and 6, the position of the molecular weight markers should be indicated to show the position of the bands.
2. On page 10, the authors state that RT-PCR revealed Fra-1 gene amplification. Of course, this method can only detect RNA overexpression.
3. Page 6, line 7: "RT-PCR was performed according to ...". This description is not clear. The method should be described in more detail.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable
Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.