Author's response to reviews

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

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Author's response to reviews: see over
To: Prof. Deborah Saltman

Editorial Director of BMC Cancer

Subject: Manuscript MS: 1346135773111559

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

Authors: Chiappetta et al.

Dear Prof. Deborah Saltman,

Thank you for your letter dated September 1st 2006 concerning the above-mentioned manuscript that we submitted to BMC Cancer. We have been pleased to hear that this manuscript may be acceptable for publication after the modifications required by the reviewers.

We have added novel data and revised the text according to the suggestions of reviewers (see item-by-item response to the reviewers).

Looking forward to hearing from you,

yours sincerely

Alfredo Fusco,

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Item by item response to the reviewers:

REVIEWER 1:

1. We have added in the “Material and Methods” section (page 5) the clinical and histological characteristics of the 60 carcinomas used in this study. They were: 51 patients (85%) presented with stage I and 9 patients (15%) with stage II tumours. 70% of the tumours were hormonal receptor-positive tumours and 30% were hormonal receptor-negative. The Elston-Ellis grade determined at diagnosis was I in 12 patients (20%), II in 38 (63%) and III in 10 patients (17%).

2. and 3. We have repeated the Western Blot analysis (Figure 3) and we have added the molecular weight to allow the calculation of the molecular size of the protein. Moreover, we have added a lane with the proteins extracted from the MDA-MB231 cells which overexpress the FRA-1 protein, as positive control, to verify the antibody specificity. In this analysis, the “smiling” effect observed in the previously experiment, was not present.

As far as the presence of a single band in all the analyzed samples is concerned, this result would suggest that Fra-1 phosphorylation status does not change. We have described this result at page 9.

3. We reported the correct FRA-1 cDNA sequence in the “Material and Methods” section (page 6). We apologize for this mistake due to an erroneous “cut and paste”.

4. As requested by this reviewer, we have modified the Table 1, adding the differential subcellular localization of FRA-1 protein.
Minor revisions

1) As requested, the position of the molecular weight markers has been indicated in Figures 3, 4, and 6.

2) We agree with this Reviewer that RT-PCR amplification revealed FRA-1 gene overexpression, and so we have modified the text at page 10.

3) We have described in more detail the RT-PCR method at page 6.

REVIEWER 2:

1. We tested the Fra-1 antibody specificity with protein extracted from MDA-MB231 cell line which overexpress the FRA-1 protein (Figure 3). Moreover, the specific FRA-1 protein bands were identified by apparent molecular weight relative to standard protein as shown in Figure 3. We have added this information at page 6 (Western Blot) and at page 9 (FRA-1 overexpression in breast tumour samples). We detected a specific single FRA-1 band with an apparent molecular weight of 38 kDa in the carcinoma samples and in breast hyperplastic lesions: we have described these results at page 9.

2. About the results shown in the Figures 2B and 2D, we have changed the “high cytoplasmic staining” definition with “constant cytoplasmic staining” concerning the fibroadenoma immunoreactivity in “Discussion” section. As discussed, the difference about fibroadenomas and atypical hyperplasias is that the FRA-1 immunolocalization was essentially present in the nuclei of the atypical lesions.

3. We agree with this Reviewer that RT-PCR amplification revealed only FRA-1 gene overexpression with respect to the normal breast tissue. Therefore, we have modified the text at page 9. Moreover, we have added in the “Material and Methods” section the number of the cycles used for the amplification.
4. The housekeeping gene used to normalize the quantitative PCR was $g6pd$ instead of $gapdh$. $g6pd$ with respect to $gapdh$ has a lower expression level, and, therefore, it was chosen in order to normalize this type of assay. However, since the $fra-1$ specific mRNA is practically undetectable in the normal breast tissue, any housekeeping gene used would have been found in a higher quantity. Since more the amount of template is high, less cycles serve to detect a gene, and so we decided to show Ct directly because this makes clearer that $fra-1$ expression is present only in dysplastic and neoplastic tissues, whereas it is undetectable in normal tissue. If we state the amplification of $fra-1$ in fold-change, it could be thought that in the normal tissue there is a minimal expression of $fra-1$ gene. Conversely, we would like to underline that $fra-1$ specific mRNA is absent in the normal tissue. Moreover, we have performed the statistic TEST T (p-value) analysis in order to evaluate whether the difference in Ct values between carcinoma group and fibroadenoma and dysplasia group was significant: the result was 2.36E-05 indicating that the difference is significant.

MINOR REVISIONS:

We have modified the text according to the suggestion of this Reviewer.