Author's response to reviews

Title: Pregnane X receptor is expressed in human breast carcinomas. Potential heterodimer formation between PXR and RXR-alpha

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Version: 2 Date: 11 April 2008

Author's response to reviews: see over
Dear Sir,

Herewith I am enclosing the revised version of the manuscript entitled PREGNANE X RECEPTOR IS EXPRESSED IN HUMAN BREAST CARCINOMAS. POTENTIAL HETERODIMERS FORMATION BETWEEN PXR AND RXR-α (I Conde, MVT Lobo, J Zamora, FJ González, E Alba, B Fraile, R Paniagua, MI Arenas), which has been made taking into account the referees’ reports.

We are enclosing text, figures, together with a letter of explanation of changes made following the referees´ concerns.

I hope that the revised manuscript merits now publication in BMC CANCER.
LETTER EXPLAINING THE CHANGES MADE FOLLOWING THE REVIEWERS’ COMMENTS

Reviewer 1

1. *They showed PXR to be associated with a postoperative recurrence in 38 patients.* …*PXR might be a postoperative prognostic factor, but not predictive factor.*

This item has been modified in both, the Conclusion of the Abstract and in the Discussion of the manuscript (Page 11, lines 28-30).

2. *The terms of first group and second group should be defined. How many patients underwent postoperative endocrine therapy or chemotherapy? Did all patients undergo only endocrine therapy?*

This paragraph has been rewritten (page 4, lines 17-28) the two patients’ groups are defined taking into account the appearance of recurrence. In the first group (without relapse), 19 patients received adjuvant therapy with tamoxifen and 20 patients were treated with tamoxifen and chemotherapy, 5 with chemotherapy only and 1 of them received radiotherapy. In the group 2, 3 patients received tamoxifen therapy and 19 chemotherapy and tamoxifen, 4 chemotherapy only and 5 adjuvant endocrine therapy without tamoxifen.

3. *Analysis for postoperative recurrence should be using survival curve analyses such as Kaplan Meier method with a log-rank test.*

This analysis has been done; see the Results section (page 8, lines 15, 19-21 and Figs. 3 and 4).

Reviewer 2

*Major Compulsory Revisions. The authors must significantly increase the size of the cases analyzed in order to give more strength to their data*

During the last month, we have got thirty nine new samples of infiltrative carcinomas and were included in this study, 25 of them were ductal infiltrative carcinomas and fourteen lobular infiltrative carcinomas. In this period, we have done immunohistochemistry with these samples, we have reviewed clinical records and samples were included in the new statistical analysis showed in the Results.

*Manuscript needs some language corrections before being published.*

The manuscript has been revised by an English-speaking colleague; however, we would thank for the editorial assistance.
Reviewer 3
This reviewer has done no comments.

Reviewer 4
1. In Table 2, authors described that the significant difference between PXR and cytoplasmic expression of RXR beta and gamma as well as between PXR and nuclear expression of RXR alpha. The authors should discuss it.
This item has been discussed; see discussion section, page 10, lines 10-33.
2. Authors described that the PXR expression was inversely correlated with the prognosis. Because PXR is thought to play some roles in the metabolism or endogenous steroid hormones and drugs, authors should mention the potential roles in the prognosis more.
Taking into account this suggestion, the discussion has been extended (page 11, lines 10-30).
3. In conclusion, author mentioned that PXR-RXR-alpha heterodimers might be involved in the development of endocrine therapies resistance. If so, the PXR and RXR-alpha expression in relapsed tissues after endocrine therapy should be examined.
Samples used in this study were primary tumours from patients who did not receive neither radiotherapy nor chemotherapy or neoadjuvant therapy before surgery. The recurrence observed in some of patients was metastasis in liver, bone or brain and in these cases they received a second line of treatment without surgery; therefore we couldn’t get new relapse samples. In addition, we have focused this study in the expression of these receptors before any treatment to check if they might be useful for prognosis.
4. The relationship between RXR expression and clinicopathological data in these cases should be examined to clarify the importance of RXR alpha in breast cancer prognosis.
This has been done; the results are expressed in Tables 4 and 5 and described on page 8, lines 16-21.
5. Some reports have described the localization of PXR might be affected in the presence or absence of ligands. The mechanism of different PXR localization among benign, carcinoma in situ and advanced tissues should be discussed. Also the additional experiments to clarify this mechanism should be studied using breast cancer cell lines.
The nuclear and cytoplasmic location of PXR is discussed on page 9, line 34 and page10, lines 1-5. The biological significance of this different location is yet unknown;
however, the present manuscript shows a retrospective study in human breast cancer, we reviewed the patients’ medical history after the immunohistochemistry analysis since our objective was to analyze the differences of PXR expression in breast tissues. Taking into account the results showed in this manuscript, we started to analyze the mechanisms of the PXR activation by different compounds in breast cancer cell lines; however, these experiments belong to a different set of objectives separated from this manuscript.

6. Manuscript needs some language corrections before being published

The manuscript has been revised by an English-speaking colleague; however, we would thank for the editorial assistance.