Author's response to reviews

Title: The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism.

Authors:

Fernando J Reyes-Zurita (ferjes@ugr.es)
Gisela Pachón-Peña (giselapachon@ub.edu)
Daneida Lizárraga (D.Lizarraga@grat.unimaas.nl)
Eva E Rufino-Palomares (evaevae@ugr.es)
Marta Cascante (martacascante@ub.edu)
José A Lupiáñez (jlcar@ugr.es)

Version: 3 Date: 17 January 2011

Author's response to reviews: see over
Reply to Reviewer #1

**Title:** The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism.

**Version:** 2  **Date:** 13 April 2010
**Reviewer:** Sung-Kwon Moon
**Reviewer’s report:**

Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions.

As far as Reviewer #1 is concerned, he doesn’t specify in his last report exactly what his/her main criticism is. We believe that in our previous answers and modifications to our text we sufficiently satisfied his queries and demands, which is backed up by the comments of the other three reviewers.

Nevertheless, we have included sentences in several places in the text to indicate that we are fully aware that further work must be done to confirm the general validity of our findings, work which we are carrying out at the present moment.

In the Discussion section, for example:

“The apoptotic mitochondrial pathway is thus triggered, producing mitochondrial disruption and the activation of caspase-9, which finally leads to the activation of caspases -3, -8 and -7. **Further studies inhibiting these routes in different colon-cancer cell types will be needed to confirm the proposed mechanism and justify its general validity, studies which we are engaged in at the moment.**

Finally, if his main criticism hinges upon our statistical analyses we would be very grateful if he would send us the results of the parallel analysis that he has made (according to the comments in his report), together with comments on any of our data which he might find wrong, so that we can repeat our calculations.

We should point out that none of the other three reviewers has raised any objection to our statistics and thus we are slightly mystified by this reviewer’s hinted objections.
Reply to Reviewer #2

Title: The natural triterpene maslinic acid induces apoptosis in HT29 colon cancer cells by a JNK-p53-dependent mechanism.

Version: 2 Date: 16 November 2010
Reviewer: Manuel Perucho

I agree with referee #1 that the authors have not addressed in a direct manner the objections raised by the referee. However, I also think that overall, the revised manuscript is an improved version over the original, and that the criticisms by referee #1, while correct, they are not essential for the main message of the paper. Also, the comment that this paper is just a continuation of the previously published study by the authors is somewhat incorrect because the new paper contains significant new additional data.

My main concern with the paper is the use of a single tumor cell line in which are based all the conclusions. It is not sound to generalize on potential inhibitory effects of a particular drug on human colon cancer based only on the data derived from in vitro studies with a single tumor cell line.

The authors should address this issue and mention the need to extend the studies to additional cell lines to see the degree of generalization of the findings.

We are happy to see that this reviewer has a more positive attitude to our paper than that of Reviewer #1 and that he doesn’t subscribe to the idea that we are merely repeating information from our previous paper here.

Although we have described the anti-tumoral effect on Caco-2 cells in a previous paper (ref.) and, as mentioned in this paper, we are currently investigating these effects in vivo in Apc-min mice, with promising initial results, we have included the following sentences in the Abstract, Introduction and Discussion sections.

Abstract:

Thus we propose a plausible sequential molecular mechanism for the expression of the different proteins responsible for the intrinsic mitochondrial apoptotic pathway. Nevertheless, further studies with other cell lines will be needed to confirm the general nature of these findings.

Introduction:

Finally, we determined the probable molecular mechanism via which MA induces its cytotoxic effects in HT29 cells. In this way have seen how maslinic acid activates p53 and thus helps to trigger apoptosis. We determined how, in HT29, MA activates MAP kinases, including JNKs and thus p53, by which means they increase their functional activity. We further found that MA’s capacity to trigger apoptosis depends upon JNK and p53 via the induction of Bax, the inhibition of Bcl-2, the release of cytochrome-c, the activation of caspase-9 and finally that of caspases -3, -7 and -8. Further studies with different colon-cancer cell lines will be needed to validate the general nature of the proposed mechanism.
Discussion:

The apoptotic mitochondrial pathway is thus triggered, producing mitochondrial disruption and the activation of caspase-9, which finally leads to the activation of caspases -3, -8 and -7. Further studies inhibiting these routes in different colon-cancer cell types will be needed to confirm the proposed mechanism and justify its general validity.