Author's response to reviews

Title: Peripheral T-lymphocytes express WNT7A and its restoration in leukemia-derived lymphoblasts inhibits cell proliferation

Authors:

Alejandra B Ochoa-Hernández (bereniceqfb@hotmail.com)
Moisés Ramos-Solano (leboyfr@gmail.com)
Ivan D Meza-Canales (idavidmc@hotmail.com)
Beatriz García-Castro (gacas.bet@hotmail.com)
Mónica A Rosales-Reynoso (mareynoso@hotmail.com)
Judith A Rosales-Aviña (judithabisag1@gmail.com)
Esperanza Barrera-Chairez (vivibarrera2010@hotmail.com)
Pablo C Ortiz-Lazareno (paulcesar05@hotmail.com)
Georgina F Hernández-Flores (geodic1967@yahoo.com.mx)
Alejandro Bravo-Cuellar (abravocster@gmail.com)
Luis F Jave-Suarez (lfjave@yahoo.com)
Patricio Barros-Núñez (pbarros_gdl@yahoo.com.mx)
Adriana Aguilar-Lemarroy (adry.aguilar.lemarroy@gmail.com)

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Author’s response to reviews: see over

Editorial Board Members,

BMC Cancer

Dear BMC Cancer Board Members,

We have again revised our manuscript (Peripheral T-lymphocytes express WNT7A and its restoration in leukemia-derived lymphoblasts inhibits cell proliferation) according to the latest comments and suggestions raised by reviewers Teresa Adell and David Loose. Additionally, this version was also proofread by a professional native English speaker. We think that this version now includes all comments and suggestions generated by the reviewers and hope for a positive answer from you.

Many thanks in advance,

Adriana Aguilar-Lemarroy, PhD,
Corresponding Author
Sierra Mojada No. 800,
Col. Independencia,
44340, Guadalajara, Jalisco,
México
Phone: (+52) (33) 3617 0060, ext. 31926
Fax: (+52) (33) 3654 0854
E-mail: adry.aguilar.lemarroy@gmail.com
Response to Reviewers

We are grateful for the comments that all of the reviewers made. We have worked on our manuscript again, taking all of the additional observations into consideration. As suggested, this final version of the manuscript was revised by a professional native English speaker.

**Referee 1:**

*I just would suggest the authors to fuse the graphics A and B from figure 6, because they are a bit repetitive. In just one graphic it would be easier to visualize the result.*

Response: We cannot fuse these results because they were made independently and were not similarly analyzed. The difference between both is that in “A”, quantification was calculated by normalizing all samples only with the value obtained in Jurkat-pLVX cells. In contrast, in B, quantification was calculated by normalizing LiCl-treated cells with their corresponding Jurkat-pLVX or Jurkat-pLVX-WNT7a non-treated cells.

We have modified the Figure legend to emphasize this difference better.
Referee 3:

Criticism regarding selection of GAPDH and RPL32.
The data in supplemental Figure 2 do support the notion that at least in PBMC RPL32 is a suitable reference gene, where is the corresponding data for GAPDH? The data in supplemental Figure 1 are somewhat in support of the author’s notion that these 2 reference genes are suitable. However, both GAPDH and RPL change by at least 1Ct following DOX treatment. Sometimes there is no perfect reference for every treatment in every cell line. The authors should discuss this in the manuscript by simply indicating that SOME, but certainly not all, of the changes in WNT7a expression may be caused by changes in reference gene expression.
The citation cited in the cover letter, in fact argues AGAINST, the selected genes as reference:
“Following PHA/PMA treatment, the B2M gene was unresponsive, and the 18S rRNA, GAPDH and TBP genes showed only minimal changes in expression levels. These results indicate that the 18S rRNA and B2M genes are useful as internal controls in RTPCR with human lymphoblastoid cells because they have generally constant levels of expression across cell lines following exposure to ionizing radiation as well as to PHA/PMA.”
Response: On checking all data back to each of the q-RT-PCR experiments, I realized that in fact the majority of the experiments were performed not only using GAPDH and RPL32 as reference genes, but also with RPS18, and the graphics that we show in most of the figures is the average of all three with standard deviation.

The order of the experiments in this paper is different from the order that we previously used. We have first performed the qRT-PCR experiments in the cell lines, then in patients, and finally, in the sorted cells and PBMC treated with PHA. Because we observed that RPL32 and RPS18 gave very similar results in the last experiments using PHA, we no longer used GAPDH as reference gene, employing only RPL32 and RPS18. It is important to note that at least two reference genes were used in all assays.

Many apologies for this omission! We have now verified each figure legend and the Methods and Results section to insert the reference genes used for each experiment in adequate fashion. Additionally, we also inserted this point (including the citation) in the Discussion section, as suggested by the reviewer.

**Minor Criticisms**

#2. Please include the information provided in the cover letter in the Methods section "we used the maximum volume permitted (8µL) in
the kit (SuperScript III first-Strand Synthesis System) for the cDNA synthesis.”
This makes it clear that 8ul is not a typographic error.

Response: We have now included this information in the Methods section as suggested.