Author's response to reviews

Title: Differential expression of THOC1 and ALY mRNP biogenesis/export factors in human cancers

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

Please find attached the second revised version of manuscript number 2039112225369645: “Differential expression of THOC1 and ALY mRNP biogenesis/export factors in human cancers” by M. Domínguez-Sánchez, C. Sáez, MA Japón, A. Aguilera and myself. The comments of the referees have been attended appropriately and an explanation point by point to each referee’s comment follows below.

I would like to point out that only referee 3 raise some concerns about the manuscript that needs to be answered, which have to do with the statistical analysis. As we explain below, the Bonferroni method, suggested by this referee, cannot be applied to our study due to the specific hypothesis tested and the number of tests performed. This is a general problem for all these tests as it has been discussed by different authors. With respect to his/her consideration that the manuscript is of limited interest, I would like to recall that there are only two papers exploring the pattern of expression of THOC1 in cancer that are focused on breast and lung tissues (Guo et al, 2005, Cancer Res 65: 3011-3016), Yang et al 2008 Ann Clin Lab Sci 38: 105-112); whereas our analysis is more general and covers different tissues. Importantly, this is the first specific study on the expression pattern in cancer of the ALY mRNP factor. Although, there are data available from global analysis, as it is the case of immunohistochemical (IHC) analyses provided in the available human protein atlas, in the cases of THOC1 the results are not conclusive because there are
discrepancies between the different IHC reports. In the cases of ALY, our results differ from those of two protein atlas data. Although ALY is highly expressed in normal tissues and in a wide range of tumors, we have found a reduction in the levels of ALY associated with high-grade tumor samples. Thus, there are sufficient arguments to conclude that our study is relevant and novel, as referees 1 and 2 agree. As referee 1 acknowledges, we use different complementary approaches that let us to better assess the expression pattern of THOC1 and ALY.

We are convinced that the manuscript has improved substantially with the suggestions of the referees and we hope that the manuscript is now considered acceptable for publication. Thank you very much for your attention.

Yours sincerely,

Dr. Rosa Luna
Reviewer #1:

Level of interest: An article whose findings are important to those with closely related research interest.

Answer: Thank you very much. We appreciate the favorable reception of the work. As suggested by the referee we have included a new paragraph to temper the conclusions based in the IHC data limitations (see lines 322-325).
Reviewer #2:

Level of interest: An article whose findings are important to those with closely related research interest. "I think the manuscript attracts interest of those in related fields, and thus it is suitable for publication in BMC Cancer".

Answer: We thank the work of this referee that let us to improve the manuscript, and we appreciate the positive comments after the revision.
Reviewer #3

Level of interest: An article of limited interest.

Point 1 Even though the authors now cite two papers about a gene expression repository and the human protein atlas, they did not make an effort to incorporate these in their analysis or in discussion. I don’t think the current manuscript will be of value to the scientific community without taking these previous results into serious consideration.

Answer

We apologize if we did not explain this conveniently in the previous revised manuscript. As requested we have now included a more detailed comparative discussion between the previous IHC data from human protein atlas and our cancer tissue array (see lines 254-269 for THOC1; and lines 280-285, and 296-299 for ALY).

Nevertheless, we think that in the case of THOC1 the comparison does not provide reliable results, because there are two protein atlas IHC analyses (HPA019096 and HPA019687) with different conclusions due to the different protein levels detected in the tissues samples (approximately 50% of coincidence among the IHC reports).

In the case of ALY, the data of the two protein atlas available are quite similar, but their results show some differences respect to our data. These differences have been remarked in the manuscript as requested (see lines 280-285).

Moreover, our analysis covers more aspects, as it is included a relationship between the ALY expression pattern and the tumor grade, as indicated in the text (see lines 296-299)

Specific questions/suggestions:

Query: How do the observed mRNA level changes in THOC1, ALY differ from what was previously observed in other cancers, conditions, etc?

Answer: Performed as requested. We have compared our results with those of SAGE (Serial Analyses of Gene Expression) data for THOC1 and ALY available at the human Gene Compendium GeneCard http://www.genecards.org (see
lines 171-178 for THOC1 and lines 184-186 for ALY).

Query: It is not clear to me what is meant by the sentence: “However, in tumor tissues the samples used are too small to establish a reliable comparison.” Do the authors mean that sample size is too small? Or are they referring to the image size depicted at http://www.proteinatlas.org/?

Answer: Certainly the message was not properly explained. We wanted to say that in some cases, there were a small number of tumor samples in the analysis. However, as this is only the case for a few tumors we have decided to remove the sentence from the original version.

Query: Are there other proteins that are involved in mRNP biogenesis that exhibit similar patterns of IHC based on the data from the Human Protein Atlas?

Answer: Yes. For example the expression profile of two other related mRNPs, UAP56/Sub2 (a component of the conserved TREX complex) and NXF1/Mex67 (a conserved mRNA export factor that interacts physically and functionally with TREX), is similar to the expression pattern of ALY. This observation is now included in the Discussion, (see lines 373-380). Thank you very much for the suggestion.

Query: Similarly, based on data from the Human Protein Atlas, are there any other conditions/tissue types/cancer cell lines where THOC1, ALY expression was assessed using IHC that could help contextualize or generalize the results from the current study?

Answer: The Human Protein Atlas contains samples of 48 different types of normal tissues and 20 different types of cancer and shows that ALY is highly expressed in normal tissues and in a wide range of tumors as in our analysis, but with some differences. This has been discussed in the text following the referee’s suggestions (see lines 280-285).

Query: As I mentioned before, the study is entirely observational in nature. In cancer tissues/cell lines hundreds of genes are known to be differentially
expressed both at the level of mRNA and protein. Furthermore cancerous cell populations are highly heterogeneous. In any particular instance, the population of cancer cells will vary in their overall expression profiles and specifically their THOC1 and ALY expression. This is a major caveat of the current work and this point should not be ignored. This issue should be thoroughly discussed in the manuscript.

**Answer:** As requested we have included the points raised in the Discussion (See lines 322-325). Thank you very much for the point. Nevertheless, we would like to indicate that in any case this problem is a caveat for all studies addressing expression profiles of different tumors.

**Point 2** I appreciate the authors’ effort in providing appropriate statistical analyses to support their conclusions. As I mentioned in my previous review, the issue of the multiple hypothesis testing has to be considered. Unfortunately, the authors have decided to ignored this suggestion. Even though the two-tailed t-test is the appropriate statistical test, one cannot claim significance at the level of p < 0.05 given that at least ~19 hypotheses are tested for each protein of interest. At this significance threshold, one would observe on average one spurious statistically significant p-value even if there were absolutely no difference in expression. There are many ways to correct this problem. One popular and conservative way is to use the Bonferroni correction. In this case, one should require approximately a p < 0.003 to claim statistical significance. Given the nature of the study, a less conservative correction could be employed. Furthermore, the legend of supplementary table 2 has to rewritten to eliminate typos and language problems.

**Answer:** Thank you very much for the suggestion. Indeed, we considered your suggestion of performing the Bonferroni adjustment for multiple tests. However, this adjustment or its less conservative modification (Holm method) are not suitable for our experimental design. As it is well known the Bonferroni method is concerned with the general null hypothesis that all hypotheses are true simultaneously. However, Bonferroni method has numerous limitations, as
indicated by several authors (Perneger et al, 1998; Sankoh, 1997, Rothman, 1990). Thus, it cannot be used to assess the evidence for specific hypotheses when there is a pre-established hypothesis (Perneger et al, 1998; Rothman, 1990). As the weakness of the Bonferroni method is that the interpretation of a result depends on the numbers of tests performed, we cannot apply this adjustment to our study. Indeed, since in this method type I errors are decreased while type II errors are increased, truly important differences are deemed non-significant (Perneger, 1998). For this reason, and because the data evaluated are actual observations from nature (Rothman, 1990), we did not make Bonferroni adjustments.


- The legend of Supplementary table II has been corrected. Thank you very much.