Author's response to reviews

Title: Cytosolic galectin-7 impairs p53 functions and induces chemo-resistance in breast cancer cells

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

Comments:
1.- As authors claim that increased cytosolic galectin-7 leads to increased resistance to chemotherapy, they should compare the chemosenstivity of different cells which they have represented in Figure 1D.

Response: We have not examined the resistance to apoptosis for all these cell lines because the heterogeneous lineage origin of these cells. For example, while some of these cells are derived from a biopsy of an adenocarcinoma of the breast, others are not tumorigenic and are derived from fibrotic tissue of the mammary gland.

Minor revisions
2- In Figure 3A, galectin-7 level in whole cell extract should be represented. In same Figure, Beta-tubulin blot should be repeated.

Response: A representative western blot showing galectin-7 level in whole cell extract is shown in figure 1D.

3- In result section authors have written that (page 6, last paragraph) inhibition of p53 co-related with decrease in p21 protein expression and CDKN1A mRNA (Figure 6, B-C), but in figure only p21 has been presented.

Response: The CDKN1A gene encodes the p21 protein.

Discretionary revisions
5- Effect of Galectin-7 and its mutant expression on cell growth should be studied, which will ensure that it just inhibits apoptosis without affecting cell proliferation.

Response: We have tested this possibility and added the new data in figure S5. A comment to that regards have been added in the discussion (lines 193-196).

6- Authors should study the half-life of cytosolic p53 protein in presence and absence of galectin-7 wild type as well as mutant form.

Response: This is an interesting issue that will need to be investigated in details in the future together with the possibility that gal-7 may also alter the active transport of p53 to the nucleus (as suggested by reviewer 2).

Reviewer 2:

Major points:
1) The authors claim that cytosolic galectin-7 inhibits nuclear translocation of p53 and promotes p53 degradation. However, they did not provide sufficient data to support those claims. In Fig. 7, the authors examined the expression levels of p53 in nuclear and cytosol of control and galectin-7 expressing cells and found that expression levels of p53 were low in galectin-7 expressing cells. But this result does not tell you whether galectin-7 inhibits p53 translocation or promotes p53
degradation or both. To distinguish those possibilities, p53 stability in presence or absence of galectin-7 should be measured. Perhaps, the author could also do a fractionation study using the cells described in Fig 7B. This may help determine whether there is a defect in p53 translocation in the galectin-7 expressing cells.

Response: We would like to thank the reviewer for this insightful comment. It is indeed possible that galectin-7 may also inhibit the translocation of p53 into the nucleus. To address this issue, the following comment has been added in the revised discussion (lines 201-204): « Another possibility that may explain lower levels of nuclear p53 protein and reduced p21 activation by galectin-7 is that galectin-7 may be part of a complex network of interrelated mechanisms that regulate the nucleocytoplasmic transport of p53 following cellular stress. These possibilities are currently under investigation.»

2) Some of the data presented in the manuscript need to be quantified. For example, the authors use low level of cleaved PARP-1 to justify the claim that R74S mutant is more potent than wild type galectin-7 in suppressing apoptosis (Fig 5c). However, it appears that expression level of b-actin loading control is also low in the R74S mutant cells. Similarly, the data presented in Fig 6A/B and Fig 7A could also use some quantifications.

Response: We agree with the reviewer that our data are too preliminary to claim that the R74S mutant is more potent than he wild-type. We have removed the sentence (line 142-143 in the original manuscript and lines 145-146 in the revised version) stating that: « In fact, MCF-7 cells expressing R74S were more resistant to apoptosis induced by etoposide and dox. ». In our opinion, this issue can only be solved in the future via thorough analysis (e.g. in other cell types and following exposure to other pro-apoptotic drugs, for example).

Minor points:
1) For fig 6C, a real time PCR analysis would be more convincing.

Response: We respectfully believe that this is not essential given that our mRNA analyses are supported by our analysis at the protein level (figure 6B).

2) A more detailed explanation of data presented on Fig 2A-D would be helpful for reader to understand this figure. For example, what are the two peaks in fig 2B means?

Response: We clarified the meaning of each experiment presented in Figures 2A-D by adding a few sentences to the results section (lines 112-117). A particular emphasis was focused on Figures 2B and 2D, which had not been clearly explained in the original version of the manuscript. We also added a few clarifications to the legend of Figure 2.