

Dear Editor,

We deeply appreciate the work and efforts made by the Editors and Reviewers in reviewing our manuscript. We have made every effort to correct the manuscript accordingly. We truly believe that our manuscript has now been strengthened. However, as you will notice in our rebuttal points, we were not able to completely change the manuscript per Reviewer #2's comments. The revised version of the manuscript highlights the changes and additions made according to the reviewer's criticisms. Please find below our response to the specific comments

Rebuttal to Reviewers' comments:

Reviewer 1:

"This is an interesting study. The authors show convincing results to support their conclusions."

1. There is a few grammatical errors

R:/ We truly appreciate the positive evaluation of the Reviewer on the manuscript. We have now revised the text and made corrections of English and word spelling check. Changes made are highlighted in the revised version of the manuscript.

2. I would suggest combining Fig.1 and Fig 2.

R:/ We would like to please the Reviewer by combining Fig 1 and 2. However, due to the quantity of data showing numerous blots and densitometry graphics, it is very difficult to present a combined Fig without seriously affecting its understanding by the readers.

Specific comments:

1. Fig 1. How did they establish the doses of leptin used in their study? Are these biologically relevant levels? If not, experiments should be done with more biologically relevant concentrations.

R:/ As it is described in the section M&M, subsection Cell Cultures (page 7), the concentrations of leptin used in this investigation are biologically relevant. The revised version of the MS now shows that leptin levels used correspond to those currently found in overweight, obese and morbid obese patients.

2. Please, indicate the IC50 data in the result section entitled "Leptin reduces..."

R:/ In the revised version of the MS is now included the IC50 data for PTX, PTX+leptin and PTX+leptin+IONP-LPrA2.

Reviewer 2:

"..the results are convincing and it sounds interesting"

1. In the section "Notch signaling is involved in leptin-induced EmCa cancer cell invasion", the authors need to provide the data and images about invasion changes of different groups.

R:/ Our published studies [see Ref. #16] have already showed data and images suggesting that Notch signaling is involved in leptin-induced EmCa cell invasion. In the previous studies Notch signaling was completely blocked by using an inhibitor (DAPT) of gamma-secretase, which is essential for the activation of membrane-bound Notch receptors. In the present study, we have further assessed that functional Notch signaling is required for leptin's action on cell invasion. Moreover, it was found that the more aggressive An3Ca cells (type II EmCa) were higher affected by Notch silencing. However, no significant differences were found by silencing specific Notch receptors. This information is included in the revised version of the MS.

2. “Why four kinds of EMCa cells were done in the part about “Leptin induces Notch protein in EmCa cells” and only two cell lines in the part about “Leptin induces mRNA expression in EmCa cells”

R:/ For the first time we have addressed the question whether leptin induces Notch in EmCa cells. We provide data from 4 representative cell lines that include type I and type II EmCa, which strongly suggest Notch receptor, ligand and target proteins are induced by the adipokine leptin. Because it is known that not always protein expression and mRNA levels correlate, we investigated in a representative type I and type II EmCa cell line how leptin could affect mRNA levels of Notch receptors, ligands and targets.

Reviewer 3:

..”The manuscript is well-written with adequate experimental data...The content of the text is satisfactory enough and the figures are informative”.

R:/ We are very pleased to find out that the Reviewer has satisfactorily evaluated our MS.

All changes and corrections/additions are highlighted in the revised version of the manuscript.