**Author’s response to reviews**

**Title:** Leptin-induced ER--Positive Breast Cancer Cell Viability and Migration is mediated by Suppressing CCN5-Signaling via Activating JAK/AKT/STAT-pathway

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To

Editorial Board Members

BMC Cancer

Subject: Revised Manuscript- Leptin-induced ER--Positive Breast Cancer Cell Viability and Migration is mediated by Suppressing CCN5-Signaling via Activating JAK/AKT/STAT pathway by Inamul Haque, Arnab Ghosh, Seth Acup, Snigdha Banerjee, Kakali Dhar, Amitabha Ray, Sandipto Sarkar, Suman Kambhampati and Sushanta K. Banerjee

Dear Members:

We appreciate your timely review of our manuscript and the opportunity to revise, correct and improve the manuscript for second time. The reviewers’ vital comments and suggestions have been considered carefully, and we addressed each one in the attached pages as well as in the revised manuscript (yellow left bracket).
We believe that the quality of the manuscript has been significantly enhanced by these modifications.

Once again, we appreciate the efforts and time you and the reviewers have spent evaluating this manuscript. We look forward to your comments on this revised manuscript.

Sincerely

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Reviewer reports/ Responses

Young Suk Jo (Reviewer 2): RE: Leptin-induced ER-Positive Breast Cancer Cell Growth and Migration is mediated by Suppressing CCN5-Signaling via Activating JAK/Stat-Akt pathway
1. Figure 2 indicated leptin-induced CCN5 suppression in transcription level. If so, the authors can show dose- and time-dependent repression of CCN5 by leptin treatment as shown in Figure 1.

RESPONSE: The dose dependent data are included in the revised manuscript (See Figures 2A and B)

2. New experiments (Figure 6A) have shown that leptin induced phosphorylation of STAT3, AKT and ERK in ER-positive breast cell lines. How about ER-negative cell lines such as MDA-MB-231? In addition, the authors need to show the change of CCN5 levels in ER-negative cell lines by leptin treatment.

3.

RESPONSE: Since CCN5 is not expressed in MDA-MB-231 cells, examine the impact of leptin on CCN5 in MDA-MB-231 cells is not a realistic approach. However, initial step of these experiments, we tested the effect of leptin on CCN5 in various breast cancer cell lines including MDA-MB-231 cells and we found no effect of leptin on CCN5 in MDA-MB-231 cells (data not included).

4. Figure 6B, the authors used various inhibitors such as AG490 (JAK2 inhibitor), Wortmannin and U0126. Again, the authors did not show any control experiment to show the proper action of these inhibitors. The authors at least need to show the phosphorylation status of STAT3, AKT and ERK under inhibitor treatments.

RESPONSE: Data included in the revised manuscript (Figure 6C)

5. Based on the results from Figure 6, the authors concluded that the leptin action might be operational via JAK/STAT-PI3/AKT signaling pathway. However, I could not understand how STAT can be upstream molecule of PI3K/AKT, because STAT is a transcription factor and PI3K/AKT is cytosolic kinases. In fact, JAK2 directly activates PI3K/AKT, independently from STAT.
6. Furthermore, the authors also need to show the relationship of EMT-related genes such as E-cadherin, vimentin and snail with leptin-induced activation of STAT3, AKT and ERK signaling. Naturally, new experiments using ER-negative cell lines should be added as control experiments.


Since CCN5 is not expressed in MDA-MB-231 cells and leptin cannot alter CCN5 in MDA-MB-231 cells, we did not include MDA-MB-231 as a control experiment.

7. The authors' final conclusion is that leptin-induced CCN5 repression through JAK/STAT-Akt pathway promotes cell growth and migration in ER-positive breast cell. To support their conclusion, the authors should present negative effect of leptin in ER-negative breast cell lines on CCN5, EMT-related genes or JAK/STAT-Akt pathway and explain the mechanism how this different response could be generated in both cell systems.

RESPONSE: Previously, we have shown that CCN5 is not expressed in MDA-MB-231 cells and we also found that leptin has no impact (positive or negative) on CCN5 in this cell line. Therefore, the study of the effect of leptin on CCN5 in MDA-MB-231 cells is not a realistic approach.

8. For cell proliferation and migration assays, the authors need to perform rescue experiments to see the effect of hrCCN5 on various inhibitors such as AG490, wortmannin and U0126, using ER-positive and ER-negative cell lines.
RESPONSE: Since it has been reported previously by us and others that CCN5 induced inhibition of cell viability and migration can be regulated by Akt or ERK pathway, inclusion of the similar data in this manuscript is thus unnecessary. The experiments proposed by the reviewer are excellent but will not serve the purpose of the goal of the present experiments. Our objectives of these studies are (1) to find out the role of CCN5 in leptin pathobiology and (2) how leptin regulates CCN5. The completion of the experiments proposed by the reviewer will uncover the downstream mechanisms of CCN5, which has already been published and is not the goal of the present manuscript. Thus, inclusion of this data deviate us from our goal and make the manuscript unfocused. Hope the reviewer will agree with us.

Again, use of ER-negative cell lines, which expressed no CCN5, is not a logistic approach for this study. Hope the reviewer will agree with us and accept the logic.