Reviewer's report

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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Reviewer: Young Suk Jo

Reviewer's report:

RE: Leptin-induced ER-Positive Breast Cancer Cell Growth and Migration is mediated by Suppressing CCN5-Signaling via Activating JAK/Stat-Akt pathway

In this revised manuscript, the authors have added some new experimental results to characterize the leptin-induced signal transduction in their cell-based assay systems. However, the data is still incomplete and confused. Moreover, the conclusion is not supported by their own data.

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.

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. 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.

4. 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.

5. 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.

6. 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.

7. 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.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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