Reviewer's report

Title: Gonadotropin-releasing hormone type II (GnRH-II) agonist regulates the invasiveness of endometrial cancer cells through the GnRH-I receptor and mitogen-activated protein kinase (MAPK)-dependent activation of matrix metalloproteinase (MMP)-2

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Reviewer: Lucia Anna Anna Stivala

Reviewer's report:

In this manuscript, the authors provide some evidence showing that GnRH-II agonist promotes cell motility and invasion of two endometrial cancer cells through the GnRH-I receptor activation.

The findings are important to understand the role of GnRH-Rs in the invasiveness of hormone-dependent cancers. However, some experimental designs and the way the data are presented compromised the quality of this manuscript as outlined below. Thus, I do not recommend in this form the manuscript for publication in BMC cancer.

Major Compulsory Revisions

1. Most of the indicated western blot experiments, including gel zymography, do not have their quantification and data are not from different experiments.

2. In the figure 1A, the quality of the representative figures on cell migration and invasion is not sufficient. It is hard to interpret the dose-dependent effect, which does not seem apparently to match with the chart data on the left. In addition, the measurement units are not accurately written.

3. In which cell line siRNA of GnRH-I receptor has been done? Only one blotting is shown, while in the results the authors report that they have knocked down GnRH-I receptor in both cell lines. More importantly, this immunoblot appears to be the same already published by the authors in a previous paper (see Wu et al., Cancer Res 2009, 69, 4204, fig 3A), and still the same is shown in the following figure 3A.

4. The significance of the figure 2B in the context of work is unclear. It has been already reported that GnRH-I receptor is present in endometrial tumours, and it has also been hypothesized a correlation between its expression and tumour invasiveness. It would make sense if the authors could be able to associate the GnRH receptors expression with cancer grading.

5 - In figure 3A, siRNA suppress almost completely GnRH-I receptor expression, but cell migration and invasion appear to be reduced and not completely blocked. What's about the involvement of the type II GnRH receptor? The authors have to mention about that in the discussion.
6. MMP-2 protein levels and activity showed in figure 5 have to be completed. Insufficient the description of result quantification, as well as the number of experiments performed (at least three are required).

In addition, MMP-9 is also up-regulated in tumours, as well as by treatment with GnRH agonist.

The authors claim that cell migration and invasion of endometrial cancer cells depend on MMP-2 activation. But this motility has not been completely abolished by the MMP-2 inhibitor, thus the author should explore also the involvement of MMP-9 in their cell system.

Minor Essential Revisions

1. Abstract is not well organized, the aim should be moved from methods to the background, and the methods should be re-written.

2. In figures 4 and 5C both chart legends are illegible.

3. Why the author use SKOV-3 cancer cells in figure 2A as positive control? They do not have GnRH-I receptor, if anything "negative" control. No information about this cell line is present in materials and methods.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'