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 metallopeptinase (MMP)-2

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Reviewer: Antimo Migliaccio

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The paper by Wu et al. shows that the GnRH-II agonist promotes the endometrial cancer cell motility by stimulating ERK 1/2 and JNK phosphorylation and a MAPK-dependent activation of metallo-proteases 2 (MMP-2). As a consequence GnRH-II could contribute to development of an invasive and more aggressive phenotype of endometrial cancer. This is an interesting finding as GnRH II has been demonstrated to be endowed with an antiproliferative effect due to its ability to activate GADD45-alpha, thereby inducing endometrial cancer cell apoptosis. For this reason GnRH-II agonists have been also considered as potential antineoplastic drugs.

However, I think that there are many points to be addressed.

1- By a conceptual point of view, I think that the Authors should investigate whether GnRH-II, under their experimental setting, beside to stimulate cell motility, have an effect on apoptosis and/or cell proliferation.

2 - As regards the experimental data, in Fig. 1, the Authors show that GnRH-II stimulates cell migration and invasion. This figure would benefit from data obtained from cytoskeleton analysis using phalloidin and wound scratch assay. Again, GnRH-II concentrations higher than 1 µM could be used and shown, to identify the optimal effective concentration. Furthermore, in Fig. 1A the Authors should explain why “3T3 fibroblast-conditioned medium was added in the lower chamber as a chemo attractant” to assess the cell migration (page 17 – invasion and migration assays). 3T3 fibroblasts could produce both ECM and degrading enzymes as well as growth factors.

3 - In Figs. 3B and 3C the Authors should describe the experimental approach used. Do the Authors used a fluorescent probe to identify siRNA transfected cells? If not, data in panels B and C should be, at least corrected for transfection efficiency or even repeated by using a fluorescent marker in co-transfection experiment (i. e. Alexa-Fluor ). In addition, the Author should explain why in the Figs. 1A and 1B cells were allowed to migrate for 24 hrs, while in Figs. 3B and 3C were kept for 72 hrs. This time frame seems too long to be used in a siRNA transient transfection.

4 - In the experiments shown in Fig. 5, the highest MMP-2 expression was
observed at a GnRH-II concentration of 1 nM, whereas the maximal cell migration/invasion was observed with concentrations of 1 µM. How the Authors explain this difference? What’s the GnRH concentration used in the experiments shown in Fig. 5C (1 nM or 1 µM)?

5 - Finally, there is another point calling for an explanation: in the experiments shown in Fig. 5C the number of migrated cells is expressed as number of fields. What does it mean? Usually the number of migrating cells is expressed as a percent increase over the control or number of cells passing into lower chamber.

In conclusion I think that this paper should be considered for publication only once the above points are addressed.

Level of interest: An article of importance in its field

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'