Reviewer's report

Title: Regulation of Gene Expression in Ovarian Cancer Cells by Luteinizing Hormone Receptor Expression and Activation

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Reviewer: Ilpo Huhtaniemi

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General comments:
This is a very interesting and well-written manuscript describing the global gene expression response of a human ovarian cancer cell line expressing LH receptor. The data are, due to their nature, quite complex, however, providing exactly what gene array data should do, i.e. to function as a hypothesis-generating tool. The data are technically sound, and I have no comments on their statistical analysis. The hypothesis of gonadotropins being promoters of ovarian cancer has been around a long time, but in particular the clinical evidence is at best weak. There is experimental evidence from cell lines and genetically modified mice, but their relevance to the human situation is unknown. Hence, more information, like the one provided by the present study, is important. The weakness of the study is the artificial nature of the cell system. The cells express transfected LHR, but the presence of this receptor in normal and malignant ovarian surface epithelium is, if not questionable, at least highly variable.

Specific comments:
1. The working hypothesis of the study seems to be that gonadotropins might be tumorigenic in ovarian cancer. Do you in fact need this starting point. Could the working hypothesis be that gonadotropins may affect the function of OSE cells, and if this is the case, are the effects more likely pro- or anti-tumorigenic? One can find evidence in the literature for both, and this seems to be the message of the current manuscript, as well. The dogma has been that if there is an LH effect in OSE, it must be tumor-promoting. Could the situation be opposite? Gonadotropin action in gonadal somatic cells promotes their differentiation and specified functions. In the testis in particular, mature Leydig and Sertoli cells do not proliferate at all. Thus, it could be counterintuitive to propose that the acquisition of gonadotropin responsiveness is a sign of increased potential of malignant transformation. Hormone receptor negative tumors are in general more aggressive that the receptor positive and more differentiated ones. Could this be the case with ovarian cancer as well? The authors’ earlier study (ref. 14) seems to support this explanation?

2. What is the ovarian cancer relevance of these findings? LHR is ectopically expressed in these cells, but are the responses found specific for OSE, or would you make the same findings in any non-LHR expressing cell type after LHR transfection? A limited study with another cell line not expressing LHR (e.g. HEK
293) could elucidate this. More specifically, if you transfect LHR to another cell line and measure the gene expression before and following LH stimulation, would you find the same responses in gene expression? This could easily be done by qRT-PCR on a few gene products.

3. Along the same lines as above, are you studying the role of gonadotropin stimulation in ovarian cancer, or the effect of ectopic activation of the cAMP signaling pathway in general on gene expression and growth potential of any cell type? It might be useful to adjust the rationale of the study, taking this caveat into account.

4. Introduction gives a bit biased view about the current state of knowledge of the involvement of gonadotropins in the genesis of ovarian cancer. The majority of clinical data show no connection. You could also cite the data on gonadotropin ablation therapies on ovarian cancer, which are practically negative.

5. One intriguing finding was that the sheer expression of LHR without ligand stimulation evoked such a marked change in gene expression. Do you have an explanation to this? Does it mean that the receptor has some basal ligand-independent signaling activity?

**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