Reviewer’s report

Title: Identification of differentially expressed genes using annealing control primer system in stage III serous ovarian carcinoma

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Reviewer: Jeremy Chien

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Manuscript Summary: In this manuscript, Kim et al described the identification of differentially expressed genes (DEGs) in serous carcinomas of the ovary. They used the annealing control primer (ACP) assays to compare differences in gene expression between normal ovary and late-stage serous carcinomas. A total of 114 DEGs were identified using ACP-base assays, and 38 of those were used in the quantitative PCR confirmatory study. Supervised cluster analysis and survival analysis were performed to correlate with DEG expression and clinical outcomes.

Major compulsory revision: One of the serous limitations with current study is the use of normal ovary as the control for differential expression of genes in high-grade, late-stage serous ovarian cancer. Normal ovary contains various tissue types, e.g., ovarian surface epithelium, stroma, granulosa cells, theca cells, and germ cells. Current study design is therefore unable to determine whether differential gene expression observed in serous tumor is the result of aberrant transcriptional regulation or the result of different tissue types. It is therefore recommended to use normal ovarian surface epithelium as a control. In addition, emerging evidence indicates that fallopian epithelium may also represent another point of origin for high-grade serous ovarian cancer, and as such it should be included as a control. When fallopian epithelium is used a control, it is also advisable to take into account the hormonal cycle under which the tissue was collected, given that gene expression of histologically normal fallopian epithelium in luteal phase is more similar to serous cancer than the expression between fallopian epithelium in follicular phase and serous ovarian cancer. Without knowing these exact cell type and the hormonal status of the normal ovary used in this study, it is unclear whether differential gene expression is the result of 1) transcriptional deregulation in cancer, 2) tissue-specific differences, 3) hormonal differences, or 4) a combination of all of the above. Therefore, the biological significance of genes identified in this study is not clear.

Specific critiques:

1. Figure 3 is difficult to follow. Such complicated data can be best represented by heat map diagram. It is also striking to see that some samples (such as 5, 10, and 14) has, in general, lower expression of candidate genes. Please comment on the quality of these samples.

2. Figure 4 may also be shown in heat map. Otherwise, please provide legends for each symbol used in the graphs.
3. Figure 5-7, although DDAH2 and TCEAL2 expression was associated with survival outcome in univariate analysis, no significant association was observed when adjusted for chemoresistant phenotype. Please comment on possible explantion for this discrepency. In addition, it is advisable to include other clinical variables, such as optimal debulking status and performance status as these factors also influent overall survival.

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**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 intrest.