Reviewer's report

Title: STAMP alters the growth of transformed and ovarian cancer cells

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Reviewer: Kylie Louise Gorringe

Reviewer's report:

1. Is the question posed by the authors well defined?

Not entirely. It seems to be to further investigate the physiological role of STAMP. In general the paper doesn’t seem to connect the various sections into a cohesive whole. The last section in particular seems disconnected from the ovarian cancer data. However, I am not very familiar with the type of experiments in Figure 4, so perhaps I am missing the connection.

2. Are the methods appropriate and well described?

Yes

3. Are the data sound?

Major:

My main worry is with the QPCR data. It is not clear how the QPCR data has been analysed. The y axis in 2A is given as “relative amount” – relative to what? Also there are no error bars on these graphs – were the QPCRs replicated? This should be stated. In general, I would always do QPCR in triplicate, however I am not familiar with the Tissue Scan cDNA and validated Taqman assay used – perhaps these are sufficiently QC controlled to provide accurate results after 1 assay.

For the first set of Tissue Scan panels they do, I am not convinced it is appropriate to draw a conclusion from just 12 samples per tumour type. Given how variable the results are, I would much prefer to see the results for the wider tissue panels performed.

For the larger panels I would also suggest using a plot that shows individual sample data or a box-whisker plot to indicate the spread of values, rather than the average shown in Fig 2B. The use of standard error is not appropriate here, standard deviation should be shown as the samples are not replicates but drawn from a population.

There is a major problem, often underappreciated for ovarian cancer, which is that ovarian cancer subtypes are considered to be different diseases (see work by the group at BCC in Canada, eg Kobel et al., 2008., Plos Medicine and Gilks et al. 2008, Hum Pathol) and conclusions about expression and clinical outcome must take subtype into account. Grade should also be considered, as grade 1
serous are not the same as grade 2 & 3 serous. Similarly grade 3 endometrioid are likely to be different from grade 1 & 2. When considering stage, subtype is key, as erroneous conclusions can be drawn on stage, which are actually based on subtype – for example, most serous cases are diagnosed late, while most mucinous cases are diagnosed early. Immunohistochemical markers for example are usually consistent within a subtype over multiple stages, more so than between subtypes.

For the data shown in Figure 2B, I have a problem with the comparison with the normal samples provided in the panel. This tissue appears to be whole ovary, which is not an appropriate control for ovarian cancer, as it is mostly stromal cell types, whereas the tumours are epithelial in origin. Thus the difference observed may be a difference between stroma and epithelium rather than tumour and normal. A better control would be microdissected ovarian surface epithelial cells, though this is very difficult to obtain. An alternative would be fallopian tube epithelium, which may be a control suitable for the serous subtype. Even a comparison with benign or borderline epithelial tumours of the same subtype would be preferable. Alternatively, the data would be strengthened by some sort of in situ data showing protein or RNA expression differences between normal ovarian epithelium and tumour epithelium. At the very least the data should not be interpreted as it is at present.

Also, ovarian borderline tumours are not considered to be malignant and should be considered separately.

Minor:

In describing the very first experiment (Page 9) the authors give the cell counts for the two clones, but could they also give the count for the empty vector control for comparison?

When discussing the ovarian panel, the authors use the phrase “endometrio[i]d cancers” but presumably they mean ovarian cancers, as the subtypes of the ovarian cases in the panel is a mixture of subtypes.

What is “IGB”? I can’t find a definition for this term.

Discretionary:

I think the data in Table 1 would be better expressed as a bar graph, which would enable error bars to be shown to indicate the reproducibility of the values obtained by QPCR. Also I can’t see why for Table 1A all the cell lines were not done, when they are then used in the subsequent analysis. Why was Hey A8 chosen as the reference cell line?

I don’t really understand the normalisation of the QPCR done for Table 1B. Is the normalisation as written just (cell A Stamp siRNA)/(Hey Lamin siRNA) because the “cell A Lamin siRNA” cancels out? It is more usual to see a comparison of each cell line with a percentage knock-down relative to a control siRNA, although this is mentioned in the text. Using the SEM is not appropriate here (page 12, line
7) as the cell lines are not replicates but part of a population. Perhaps the range of percentage knockdown could be given, to show that all the cell lines were knocked down efficiently.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes. The authors should be complimented on defining their error bars.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
No. I don’t believe the interpretation of the correlation of STAMP mRNA and ovarian cancer is justified due to the use of whole ovary as a control. This contention could be supported by data mining of microarray data sets to see if other studies have detected this gene as overexpressed.

6. Are limitations of the work clearly stated?
No, I don’t think the authors are sufficiently familiar with some of the inherent difficulties associated with analysing ovarian cancer data, and these should be acknowledged in the discussion.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes

Discretionary:
Sometimes the choice of references could be improved.
E.g. ref 22 seems a bit odd, wouldn’t similar information be held in one of the 3 “Cancer statistics” reports cited? Or the FIGO annual report can be a useful resource for ovarian cancer stats(Heintz et al., 2006).
E.g. On page 6 the authors cite 2 papers to describe multiple pathways in ovarian cancer, but neither paper is about ovarian cancer.

8. Do the title and abstract accurately convey what has been found?
Yes.

9. Is the writing acceptable?
Yes.

Minor:
Page 10 line 6. Change “14-fold after being withdrawal” to “14-fold after withdrawal”
Page 24 legend to fig 2 “was determine as in A” to “was determined as in A”

Discretionary:
Page 5 line 4. The authors use the term “homologues”. This is a bit confusing here as the term is often used to compare genes between different species (i.e. orthologs). Perhaps this sentence could be rephrased or the term “paralogs” used.

Page 11 line 18. “expressed” used here is a bit confusing given it has a more biological connotation as well. Perhaps change to “shown” or something similar.

Page 11 line 21. change “that in the above control” to “that in the control”

Additional points (discretionary):

I wonder given the suggestion of interaction of STAMP with steroid hormones, whether the authors had looked at the ER/PR status of breast tumours in relation to STAMP expression. I don’t know if that information is available with the Tissue Scan data, but perhaps could be obtained from publicly available expression data sets.

**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