**Reviewer's report**

**Title:** The role of the REG4 gene and its encoding product in ovarian epithelial carcinoma

**Version:** 2  
**Date:** 27 January 2015

**Reviewer:** Rebecca T Marquez

**Reviewer's report:**

**Major Compulsory Revisions:**

1. Your study suggests significant differences in histotype associated with Reg4 expression. According to your cell line data, ES-2 cells (clear cell carcinomas) do not have upregulation of REG4. Can you further detail the “miscellaneous subtypes” you examined for Table 2. Are any of these clear cell? Because Huang et al, has already shown that mucinous tumors have an increase in REG4. This paper would be strengthened by showing a novel finding where clear cell cancers have low REG4 and correlate this will survival rates comparing clear cell and mucinous cancers.

2. This study would be strengthened by measuring REG4 in metastatic lesions in order to confirm the author’s conclusion that REG4 expression correlates with poor outcome. This is especially important since there are not differences in REG4 expression between stage I and stageIV cancers and is less expressed in tumors versus benign.

**Minor Essential Revisions:**

1. Figure 1 is too tight. Especially figure 1d and 1e are overlapping. 1b needs labels on western. Figure 1c needs to have the amount of REG4 protein in legend in increasing order to match graph. page 13 line 245. 50nM/ml needs to be changed to 50 nM. nanomolar concentration is nanomoles/ml. Also occurs in figure legend. Change throughout manuscript.

2. Figure legends and methods need to be in more detail. Please include the definition of controls. Please describe how the experiment was carried out in more detail in the results section. i.e. what was compared.

3. Figure 3. What is CTR(SKOV3)? How did you perform your mock treatment? Why is there such a difference in migration between CTR and Mock? please make mock image brighter. This does not appear to be reflected in the migration index. The labels are spelled wrong for migration index graph. I believe it would be better to separate the graphs based on the similar experiment that is being performed. i.e. mock and Reg4 protein should be next to each other since these are what is being compared. Make separate figures or place a space between these two pairs. Discuss in text in more detail.

4. It is not necessary to list the “n” for each experiment in the results section or
the figure labels. Instead please move this to the figure legend for easy reference.

5. Figure 4. please state the statistical tests you performed in the figure legend and what is being compared. Also, please discuss why benign lesions have a higher Reg4 expression than cancer. Are these differences statistically significant? please rewrite the results section describing this figure. for Example, page 14 line 260. “Expression of REG4 mRNA were also higher in mucinous benign tumors and carcinomas compared to serous ones (P < 0.05; Figure 4B). are the mucinous combined benign tumors and cancers? if so, these should be separated. Please clarify.

6. Figure 5. Please include a magnified image in order to identify the difference in cytoplasmic staining and an arrow to point out the differences. Make the scale legend readable and include what the size is in the figure legend.

7. Table 1,2 and 3. Please indicate what the bold text indicates. It appears to indicate statistically significant p-values, however in table 3, the the correlation with cumulative survival is 0.084 which is not statistically significant, however, it is bold. Table 1 and 3 need to be detailed by histotype. Table 1 needs to state that it is measuring REG4 protein expression and not gene expression. Also, use same descriptions in table as in the definition of p-values. this is confusing.

8. Conclusion. Please explain in more detail what “phenotype-related complexes” ( page 18 line 348) are. More detail about REG4 mechanism would be helpful. How does it regulate gene expression?

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 interests