Reviewer's report

**Title:** Kisspeptin and GPR54 immunoreactivity in a cohort of 518 patients defines favourable prognosis and clear cell subtype in ovarian carcinoma

**Version:** 1  **Date:** 25 June 2007

**Reviewer:** Danny R Welch

Reviewer's report:

**OVERVIEW:** This manuscript utilizes a tissue microarray of >500 ovarian carcinoma samples to examine whether expression of kisspeptins and GPR54 are prognostic variables. The authors found that both kisspeptin and GPR54 expression are predictive of good prognosis (disease free and overall survival). Dual expression was most strongly associated with clear cell carcinoma subtype.

**CRITIQUE:** The authors present a well-designed study that asks an interesting question. So, significance of the work is high. Since ovarian cancer is so difficult clinically, the findings could be extremely helpful. The findings and interpretation are internally consistent. Except for one major concern and a handful of minor editorial issues, the manuscript merits publication.

Major concern – specificity of the antibodies is not demonstrated. Multiple laboratories have utilized poor reagents from a large number of commercial enterprises for KISS1/kisspeptins which have not been good quality. Since antibodies to GPCR's are so difficult to obtain, there is a question with those reagents as well. The authors recognize this and incorporated peptide competition; however, statements on pages 8-9 raise questions regarding antibody/antiserum specificity (i.e., blocking is not complete).

At minimum, there are multiple kisspeptins generated by cells (based upon cell culture models) and the blocking experiments need to show which are recognized by antibodies. Immunoblots showing exactly what is recognized are absolutely essential to convincing this reviewer that the reagents are suitable for making claims.

To the best of our knowledge, this report is the first to utilize anti-GPR-54 antibodies. In doing so, it is essential to show the characterization and provide properties (e.g., isotype, etc.)

Minor comments –

p4: Metastin was discovered by screening for binding to GPR54 (alternatively named in that paper). So the sentence beginning "Kisspeptins, encoded..." should be written to accurately reflect the situation.

The authors' PNAS paper reference should be updated

Detailed antigen retrieval methods need to be included in the manuscript since
not all antigen retrieval approaches work for all antibodies.

Use of 1:25 titre for the anti-GPR-54 antibody is troubling. Such a high titre is often fraught with ancillary bands in immunoblots.

Data should be shown for competition studies

The data should be discussed in the context of the paper earlier this year by Nash et al. whose data suggests that kisspeptin-GPR54 interaction may not be autocrine.

The Discussion should be shortened by 1/3 to 1/2.

The figure legends are sparse and do not provide sufficient information for a reader to understand the images without re-reading the text.

There are abbreviations mentioned in the text referring to figures (e.g., p10 and Figure 3). While relatively self-apparent, the manuscript should be made internally consistent with regard to utilization of the abbreviations or eliminate them.

Higher resolution insets that allow the reader to carefully look at subcellular distribution would be helpful. In particular, there is 'concern' that GPR54 is cytoplasmic rather than membrane-associated (Figure 2).