Reviewer's report

**Title:** Characterization of aldehyde dehydrogenase isozymes in ovarian cancer tissues and sphere cultures

**Version:** 1  **Date:** 17 May 2012

**Reviewer:** Kylie Louise Gorringe

**Reviewer's report:**

The authors undertake an observational study of the expression of ALDH isozymes in ovarian cancer. The experiments undertaken are appropriate and well performed, and it is particularly refreshing to have the statistical methods clearly stated. The manuscript is well written.

The authors appropriately have taken care to consider ovarian cancer as a heterogenous group of tumours, however for some of their analysis this has meant that the number of cases in some subgroups is low, reducing the ability to detect differences; this limitation of the study should be noted in the discussion.

**Major compulsory**

1. In the introduction, a figure of 12-15% for mucinous tumours is given. If referring to invasive tumours, this is now considered to be an overestimate as in older case series many ovarian mucinous tumours are thought to be misdiagnosed metastases from other organs (McCluggage&Wilkinson, Histopathology 2005, 47, 231–247). In the mucinous cases in this study, what steps were taken to attempt to obtain only primary mucinous ovarian tumours?

2. What were the histological subtypes of the benign and borderline cases included in the study? If they are all serous, for example, is it then valid to compare them to all other subtypes? If they are a mixture of histologies, is it valid to consider them as a single group given their different cells of origin and genetic alterations (e.g. see review by Bell, Modern Pathology (2005) 18, S19–S32.)

3. What is the power to detect subtype-specific differences by IHC? Apart from ALDH1A1, the number of cases in the minor histotypes is limited, and subtle changes may not be detected. Perhaps this could be noted in the discussion.

4. Were there any differences noted between the grades of invasive tumours? It is becoming increasingly accepted, for example, that high grade serous (2&3) is different to low grade serous with respect to genetic and immunohistochemical profiles (e.g. TP53 mutations, and Kobel PLoS Med 5(12):e232.) and similarly grade 3 endometrioid is more akin to high grade serous than to low grade endometrioid. There should be sufficient serous cases to address this for ALDH1A1, if not the other proteins or subtypes.

**Minor essential**

1. Were the QPCR and IHC assays performed on any matched samples? If so,
were the individual results correlated?

2. What were the cancer cell lines used in the study? Were these well known lines or in-house established? This would be essential information for other researchers as there are many data on the well known lines that could be integrated with the results here, and it would assist in the reproduction of results e.g. as controls.

3. Similarly, which cell lines were used in the Aldefluor assay? It is stated that representative experiments are shown in figure 5, how many times were these repeated for each cell line?

Discretionary

1. It is interesting that there is a trend for the benign cases to show increased stromal expression of ALDH1A1. A recent study found that 33% of benign serous tumours diagnosed as cystadenomas had genetic alterations in the stromal component suggestive that they were in fact fibromas (Hunter S et al., 2011, Clin Cancer Res. 17(23):7273-82). This might be something for the researchers to consider in their future studies if they were able to obtain more benign cases.

2. In the legend to Figure 3, it would be helpful to note the number of cases of each subtype so that the reader doesn’t have to flick back to the methods (e.g. “S, serous ovarian tumors (n=19)”)

3. The legend to Figure 5 is unclear. Please state the difference between the DEAB and ALDH graphs. Which cell line was used for each of the shown experiments?

4. Have the authors considered mining expression microarray datasets for additional mRNA data? E.g. Tothill (2005) included low and high grade serous as well as endometrioid cases, Anglesio (2011) compared serous and clear cell samples, and Haverty (2009) and Ramakrishna (2010) each included a small number of mucinous and clear cell cases along with serous and endometrioid. Addition of these data might strengthen the differences noted between subtypes given the low number of cases in the QPCR data.

5. Results/Discussion page 9 2nd to last line “…Figure 5 are parallel the results” perhaps should be “Figure 5 are parallel to the results”

6. It might be helpful for anyone trying to reproduce the results if the IHC scoring was described in more detail with respect to the % of positive cells scored as “1” and “2”.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests