Reviewer’s report

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

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Reviewer: Jennifer Byrne

Reviewer’s report:

This manuscript describes aldehyde dehydrogenase isozyme expression in ovarian cancer tissues and sphere cultures. Much of the work presented is solid and the experiments appear to have been carefully performed. However, numerous details need to be corrected, as will be outlined in the "minor essential revisions" section below. More significantly, the data shown in numerous figures and tables need to be more completely and accurately described, both within the figures and tables themselves, and in the text. Without this information, it is difficult to assess the significance of the results presented.

Major Compulsory Revisions

1. Insufficient information was supplied for the ovarian tissue samples examined. A table should be included describing the clinical and histopathological characteristics of the cohort(s) examined, which could be included as supplementary data. It was unclear whether the results shown in Fig. 1 (IHC results obtained using different antibodies) showed tissue samples from the same set of patients. It was also unclear whether samples examined for gene expression analyses (Fig. 3) were from the same or a different cohort.

2. I found it difficult to understand some of the data presented in Tables 1-4. Confidence intervals were specified for some but not all variables. Instead, these columns included “ref.” which was not defined. Some confidence intervals were described as “NS” (not significant), which did not make sense. A confidence interval is a description of data distribution, not the result of a statistical test.

3. The names of ovarian cancer cell lines analysed need to be provided throughout. It is not sufficient to cite a previous study in the Methods. Only a single cell line of endometrioid sub-type was examined (Fig. 4). Broad conclusions cannot be drawn from examining a single cell line and this should be clearly acknowledged.

4. In Fig. 3, the number of specimens in each histological subtype category needs to be indicated, either within the figure or the legend. Gene names need to be shown in italics. The significance of the lines extending from boxes (95% confidence intervals), and the central tendency shown as a horizontal line within the boxes (medians?), both need to be explained.

5. In the results section of the abstract (page 2) it was stated that “the expression
of ALDH enzymes was induced in ovarian cancer cells growing as sphere suspension”. This was not supported by the results shown in Fig. 4, as this was not true for all histological subtypes examined. There were numerous issues with the data shown in Fig. 4. No names of cell lines were provided, so it was not clear which results were obtained for which individual cell line, and then which subset of these cell lines was shown in Fig. 4B. This is critical in terms of the ability of future researchers to attempt to replicate these findings.

6. The representative results shown in Fig. 5 were impossible to interpret. The writing on individual graphs is so small that it cannot be read. The significance of gated regions was not explained. As cell lines were not identified, it was impossible to compare these ALDH activity results with those of Western blot analyses, as stated on the 3rd and 2nd last lines of page 9, and also lines 3-5, page 10. If analyses were performed on multiple occasions for individual cell lines, it would be better to show the combined data in graphs comparing sphere and 2D culture cells.

Minor Essential Revisions

1. The abstract (page 2, methods section) refers to "correlated" when no correlations seem to have been performed.

2. Page 7, lines 7 and 15: please indicate which Table contains data for which ALDH isozyme.

3. Page 8, lines 11, 12 and throughout manuscript: Please show references to genotype (DNA, mRNA) in italics and phenotype (protein) in ordinary text.

4. Page 8, line 12: Please note that a boxplot is a method of representing data, not a form of analysis.

5. Page 8, lines 14, 15: The statement “The RNA expression patterns resemble the protein expression of ALDH1A1” needs to be clarified. Which expression patterns exactly? How were they judged to be similar?

6. Page 8, 4th line from end: The statement “most of the ovarian cancer cell lines had elevated expression of ALDH isozymes…” needs to be clarified. In the case of ALDH1A3, most cancer cell lines in fact showed similar expression levels to controls.

7. Page 8, last line, page 9, first line: The statement “While endometrioid and mucinous…” needs to be modified, as only one endometrioid cell line was examined, and the mucinous cell lines did not in fact show “extraordinarily high” ALDH1A1 expression, relative to the endometrioid cell line. The word “detectable” should also be inserted in front of “protein expression” (end of sentence).

8. Page 9, line 12: The statement regarding ALDH7A1 expression in the endometrioid cell line is incorrect, as there was no “slight increase” apparent in spheres versus 2D cultured cells in Fig. 4B.
9. Page 9, lines 13, 14: The statement regarding ALDH1A1 expression being “more robust” in spheres versus 2D cultured cells is similarly incorrect, as this was not true for all cell lines analysed.

10. Fig. 4 legend, page 24, line 7: Four control samples are shown in Fig. 4A, but only one is referred to in the legend.

11. Fig 2: Please define the magnification(s) used. From the data shown, different magnifications may have been used for different images.

12. Fig. 4: No molecular weights are shown for the species detected. It was not explained why the ALDH3A2 antibody detects products of different molecular weights and what these products are. It was also not explained why ALDH1A1 was detected in both mucinous cell lines examined from 2D cultures in Fig. 4A but not in Fig. 4B.

13. Fig. 5: Please use panel identifiers (eg A, B, C, etc) in any revised version.

14. Throughout: the manuscript should be carefully reread for grammatical errors and missing words, which were numerous, but for the sake of time, have not been individually highlighted in this report.

Discretionary Revisions

1. Fig. 2B: It is unclear why so many images showing essentially the same result were included. Some images are also much darker than others and may not reproduce well. Sufficient data are likely shown in Fig. 2A.

2. Fig. 4A: Is it possible that any of the antibodies used cross-react with other species (eg. could the ALDH7A1 antibody cross-react with ALDH1A1)?

3. For readers not familiar with this area, it may be useful to include a dendrogram to describe the relationships between all the ALDH isozymes analysed.

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

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

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

Declaration of competing interests:

I declare that I have no competing interests