Author's response to reviews

Title: The expression of aldehyde dehydrogenase 1 (ALDH1) in ovarian carcinomas and its clinicopathological associations: a retrospective study

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Author's response to reviews: see over
Dear Editor,

Thank you for your letter of 18th March regarding our manuscript 1774236016158377 “The expression of aldehyde dehydrogenase 1 (ALDH1) in ovarian carcinomas and its clinicopathological associations: a retrospective study”. We all are grateful for the valuable comments and suggestions. Together, we have gone through the manuscript several times and discussed the comments and questions from the reviewers. Based on the comments and suggestions, we have performed additional immunohistochemistry using more markers. We have now finished the revision/correction work. We have the following answers to the comments mentioned by both the two reviewers and academic editor:

Reviewer 1

1. In this study, high expression of ALDH1, a marker for cancer stem cells, was associated with better prognosis in ovarian carcinoma cases. Similar results was shown by Bin Chang et at. (Modern pathology 2009). On the other hand, studies about cancer stem cells isolated as ALDH1+ cells (Am J Pathol, 2012, 180, 1159-1169) describe that high expression of ALDH1 is related to poorer prognosis. Are ALDH1-positive cancer cells indicate cancer stem cells in this study? I would like to recommend to perform double staining using other cancer stem cell markers including CD133, CD44 etc.

Answer: Although ALDH1 has been regarded as cancer stem cell (CSC) marker for ovarian cancer in some reports, not all ALDH1-positive tumor cells stand for CSCs. The main issue
involved in the contradictory reports in literature about CSC markers may be explained in the difference between in vitro data and in vivo data. Increasing evidence indicates that not all in vitro data can be verified in the in vivo experiments. For ALDH1, gene functional studies and cell line in vitro experiments support its CSC feature in a number of studies. However, our translational studies could not prove this as revealed in our present study, i.e., ALDH1-positive ovarian cancer cells do not represent CSCs. For example, in our study we find that there are 39 tumors with homogenously strong ALDH1 staining in their tumor cells, and similar finding was also reported in other studies [1, 2]. Generally, CSCs in a given tumor represent a small subpopulation of tumor cells, and it should not be possible that all the tumor cells in so many tumors are all CSCs. Instead, we agree with the notion that ALDH1, as a single marker, is not sufficient to identify CSCs [3], and different isoforms of ALDH1 may serve variable roles in CSCs [4].

We have been trying to follow or develop double staining immunohistochemistry in our laboratory. However, we find that double immunohistochemistry technology is often not an easy job to do, and the results using this technology should be explained with care, since optimizing two antibodies with similar specificity in this process can be troublesome. Although we do have published an article in this field [5], we chose immunohistochemistry of several antibodies in serially-cut sections to answer the reviewer’s question. We do have experience in CD133 immunohistochemistry. As we reported earlier in human lung cancer cell lines, strong CD133 positive cell line verified by flow cytometry and immunocytochemistry did not show CSC feature when these cells were xenotransplanted in NOD/SCID mice [6]. Also after that, we tried to perform CD133 immunohistochemistry
in large series of clinical samples without success, mainly because of usually much weaker CD133 expression revealed by immunohistochemistry in the paraffin embedded sections than flow cytometry in the isolated cells. However, after we received the comments/suggestions from the reviewers, we applied the mouse monoclonal anti human CD44 antibody from Dako to stain all the slides with strong ALDH1 staining (Allred score 7 and 8). It turned out that the ALDH1-positive cells were not all positive for CD44, and ALDH1-negative cells can be positive for CD44 (Fig.1S). The results were compatible with the existed opinions about CSC-related markers that no universal marker can be positive for all putative CSCs, and different markers may be combined to identify variable populations of so-called “CSCs”. It is therefore unneutral to nominate the ALDH1 positive tumor cells for CSCs in this study.

2. ALDH1 staining in stroma is a interesting findings. The image picture in Figure 1 reveal that ALDH1 was stained broadly in stroma of ovarian carcinoma. Which types of stroma cell show positive staining for ALDH1? To answer this question, I would like to perform double staining using stromal cell markers including SMA, LCA (CD45), CD68 etc.

Answer: To answer these questions, we re-performed immunohistochemistry on the serial-cut sections using different antibodies against FAP, CD68 and CD73, since we have been working with these antibodies. FAP is a marker for cancer associated fibroblasts. CD68 is a marker for monocytes/macrophages. CD73 is a marker for mesenchymal stem/stromal cells. We discovered that ALDH1 can be largely positive in most of the stroma (Allred score 7 and 8) in most of the cases and these stromal cells can be variably positive for the stromal cell markers. For example, ALDH1-positive stromal cells can be weakly positive for FAP in some cases, but strong positive for
other cases. Similarly, ALDH1-positive stromal cells were discovered positive for CD73 in some cases, but weakly, even negative in some other cases (Fig.2S, magnitude: ×200). For the macrophage marker CD68, we discovered that the ALDH1 positive stromal cells could be completely negative as shown on the Fig 2S, but could be positive in other cases, although the positivity was not so homogeneous as for FAP and CD73 in the stroma. Since our current study focused on the expression of ALDH1 in tumor cells and additional identification of ALDH1-positive stromal cells or subtyping of the stromal cells were not included in the revised manuscript. We do believe that ALDH1 expression in tumor stromal cells is worthy of further study, but detail subtyping of the stromal cells will be valuable, and that will be our next task in the ovarian cancer studies.

3. The authors used Allred scoring system for evaluation of IHC. A rational explanation is needed why they take this method.

Answer: Allred scoring system is a traditionally manual scoring system and widely used approach to evaluate immunohistochemical staining, which combines the percentage and intensity of positive cells. The Allred scoring system, together with immunoreactive score (IRS) and H-score, all manual scoring systems, were considered to be “gold standard” in IHC-data evaluation, and they were widely accepted and recommended by leading associations and organizations [7-10]. Although it was pointed out by some scientists that the Allred scoring system can be subjective compared to digital image analysis systems and may result in slight difference by digital systems or different pathologists, however, studies have shown that the automated and pathologist manual scoring systems may produce highly similar results [11-13]. Furthermore, currently available automated systems are too far from ideal: some programs are not able to isolate individual cells, and not capable for interpretation of morphological features [13, 14].

Reviewer 2

1. Known function of ALDH1 in normal stem cells, such as the biosynthesis of retinoic acid, which is a regulator of cellular proliferation, differentiation, and survival?

Answer: ALDH1 gene encodes a cytosolic isoform localized in the cytoplasm to catalyse dehydrogenation of aldehydes to their corresponding carboxylic acids. Yes, it has been
reported in normal stem cells that ALDH1 is involved in regulating cell differentiation [15, 16], proliferation and motility [17, 18]. Its regulation role in stem cells is particularly through the retinoid signaling pathway [19, 20]. It is also reported that inhibition of ALDH1-mediated retinoid signaling impairs human fetal islet cell differentiation and survival [16]. It is currently unknown about the reason for the contradictory findings in cancer and normal stem cell studies. But our current study, in addition to the normal stem cell studies in literature, may encourage the scientific community to re-evaluate its roles in cancer stem cell biology.

2. Speculation of the mechanisms of how ALDH1 expression contributes to better survival.

Answer: The potential explanations of how ALDH1 expression contributes to better survival in ovarian cancer patients may lay on the following three points: (1) Different isoforms of ALDH1 may play variable roles in CSCs [4], and therefore further characterization of the isoforms of ALDH in tumors will be necessary. (2) Ovarian cancers displayed a significantly reduced ALDH1 expression compared to benign tumors and normal ovary [21], unlike breast, lung or colon cancers, indicating a possibly different role of ALDH1 in ovarian cancer. Although this is hard to understand why such tumors have such a big contrast when its expression is considered, we should not exclude anything before more extensive studies on such a possibility are finished. (3) It is known that cancer stem cells share features of normal stem cells, and ALDH1 in normal stem cells has a function of activating cell differentiation through retinoid acid signaling pathway. It can’t be excluded in the ovarian cancer cells that ALDH exerts its role through the same molecular mechanism, by such contributing to the better survival in ovarian cancers, although other unknown molecular mechanisms should be explored.

3. The limitation of the present study, such as histological heterogeneity of ovarian cancers and appropriateness of the cut-off points in the evaluation of immunostaining.

Answer: The current study has several limitations. First of all, although Allred scoring system combines the percentage and intensity of positive cells, as a manual scoring system, it may induce a level of subjectivity, especially the cut-off points were always a matter of discussion. Second, histological heterogeneity of ovarian cancers was not able to be addressed in the present study.
References


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