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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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 two main 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 al. (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 generally accepted as cancer stem cell (CSC) marker for ovarian cancer, not all ALDH1-positive tumor cells stand for CSCs. Because homogenously...
strong staining were observed in thirty-nine cases in this study, and similar phenomenon was found in other studies [1, 2], however, it is incompatible with the basic CSC theory that CSCs are a small subpopulation in tumor tissue. We agree that ALDH1, as a single marker, is not sufficient to identify CSCs [3], and different isoforms of ALDH may serve variable roles in CSCs [4]. We found in previous laboratory studies from our group that CD44 was more difficult to be positively stained by immunohistochemistry than flow cytometry, and the situation for CD133 was even worse. Mouse monoclonal anti human CD44 antibody from Dako was used to stain 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. ALDH1 did not appear to be co-expressed with the CSC markers CD44, CD117 and CD133 by IHC [5].

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 storomal cell markers including SMA, LCA (CD45), CD68 etc.

Answer: In our study, ALDH1 can be largely positive in most of the stroma (Allred score 7 and 8) in some cases. These cases were further stained with variable markers including FAP for cancer associated fibroblasts, CD68 for monocytes/macrophages, CD73 for mesenchymal
stem/stromal cells. It turned out that the ALDH1-positive stromal cells can be weakly positive for FAP, and strongly positive for CD73 (Fig.2S), which can be explained, because mesenchymal stem/stromal cells may have fibroblast-like phenotype. CD68 positive and ALDH1 positive stromal cells can also be observed. Since the current study found that the expression of ALDH1 in tumor cells is rather interesting and therefore more focused on its expression in tumor cells, the additional identification of ALDH1-positive stromal cells were not included in the revised manuscript. The ALDH1 expression in tumor stromal cells is worthy of further study.

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 [6-9]. 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 [10-12]. 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 [12, 13].

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. It should be noted that ALDH1 is involved in positively regulating cellular differentiation [14, 15], proliferation and motility [16, 17]. Its regulation role in stem cells is particularly through the retinoid signaling pathway [18, 19]. Inhibition of ALDH1-mediated retinoid signalling impairs human fetal islet cell differentiation and survival [15].

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 ALDH may play variable roles in CSCs [4]. Different colonies of ALDH1 antibody may react with diverse proteins and thus lead to distinct results. (2) Ovarian cancer displayed a significantly reduced ALDH1 expression compared to benign tumors and normal ovary [5], unlike breast, lung or colon cancers, indicating a possibly different role of ALDH1 in ovarian cancer. (3) The exact roles of the CSC related marker ALDH1 are still poorly understood, due to either a current lack of understanding of its biological functions or the lack of the correlating information of varied isoforms, splicing variants or substrates to stem cell function [21].

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 compared to digital image analysis systems. Second, histological heterogeneity of ovarian cancers was not able to be addressed in the present study. Finally,
The treatment of the patients enrolled was not uniform and it is not available to be included in multivariate analysis.

References


Sincerely yours,

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