Author's response to reviews

Title: ALDH1A1 overexpression is associated with the progression and prognosis in gastric cancer

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Author's response to reviews: see over
Dear Dr. Stuti Shroff and Annie Lyn Bravo:

MS: 1303549647133328

Title: ALDH1A1 overexpression is associated with the progression and prognosis in gastric cancer

We would like to thank BMC Cancer for giving us the opportunity to revise our manuscript.

We thank the reviewers for their careful read and thoughtful comments on previous draft. We have carefully taken their comments into consideration in preparing our revision, which has resulted in a paper that is clearer, more compelling, and broader.

The following summarizes how we responded to reviewers’ comments.

Thanks for all the help.

Best wishes,

Wei-sheng Luo, MD

Corresponding Author
Major Compulsory Revisions

Q1:

In table 1, the tumors have been classified depending on tumor site as upper, middle and lower. How were these determined? Tumors located within 5 cm of the gastroesophageal junction are staged as esophageal primary tumors, were they included in this study? In other studies, distal tumors have a worse prognosis than proximal gastric cancers.

Response:

We determined the tumor site as follow: Cardia and fundus ventriculi was classified as upper, corpus ventriculi was classified as middle, and pars pylorica was classified as lower.

Tumors located within 5 cm of the gastroesophageal junction above were excluded in our study, we only screened the tumors located in the gastroesophageal junction and within 5 cm of the gastroesophageal junction below.

According to the reviewer’s suggestion, we have studied lots of research about gastric cancer. And we agree with the reviewer that tumor location, in some studies, is a predictive factor for prognosis of gastric cancer. For example, Shin NR et al. reported that tumor site had independent prognostic value in the multivariate analysis of gastric cancer. (Shin NR et al. Overexpression of Snail is associated with lymph
node metastasis and poor prognosis in patients with gastric cancer. BMC Cancer. 2012; 12:521) However, in some other studies, it also reported that tumor site had no prognostic value in gastric cancer, which have similar results with our data. For instance, Ye XT et al. found that tumor site had no value for both RFS and OS of patients with gastric cancer. (Ye XT et al. Overexpression of NUAK1 is associated with disease-free survival and overall survival in patients with gastric cancer. Med Oncol. 2014; 31:61)

Q2:

The images in Figure 1 staining score 1, 2 and 3, and figure 3 should be retaken at a higher resolution. The expression of ALDH1A1 in the cytoplasmic compartment of the cell is not obvious. Expression of ALDH1A1 is compared to MMP-9 with an intent to demonstrate invasive potential of individual tumor cells. Were the staining/scoring criteria used in this study, the same as was used in the MMP-9 studies by Chu et al.?

Response:

Thank you for the reviewer’s suggestion, we have retaken new images with higher resolution in new Figure 1 and new Figure 3. In new Figure 1, it contains 8 microphotographs, 4 at low magnification (upper panels) and 4 at high magnification (lower panels). In addition, we also substituted the legend of figure 1 that “Gastric
cancer tissue illustrating the range of intensities of HSP60 immunostaining from 0 to 3. The scale bar represents 50 µm.” for that “Gastric cancer tissue illustrating the range of intensities of ALDH1A1 immunostaining from 0 to 3. The lower panels represent magnified pictures of boxed area in the corresponding upper panels. The scale bar represents 50 µm” in the figure legend. (Line 2-4 of Page 22)

We did not use the same staining/scoring criteria which was used in the MMP-9 studies by Chu et al. We used the scoring criteria as follow: ALDH1A1 and MMP-9 staining intensities were rated on a scale of 0-3 according to the percentage of positive tumor (0, < 5% positive cells; 1, 5-10%; 2, 11-50%; or 3, > 50%). The expression is very low for 0, low for 1, moderate for 2 and high for 3. ALDH1A1 and MMP-9 expression were classified as negative for scores ≤ 1 and positive for scores ≥ 2. And we found that MMP-9 was significantly associated with depth of invasion and lymph node metastasis, in addition, MMP-9 played an important role in gastric cancer prognosis (data not show). Which was similar with the results reported by Chu et al..

Minor Essential Revisions

Q1:

There are several grammatical and spelling errors in the manuscript, these need to be corrected before resubmission.
Response:

Thank you for the reviewers, and we have revised the grammatical errors in submission accordingly.

Q2:

In the methods section of the paper, under the subheading "follow-up" the authors mention recurrence was confirmed by tumor marker levels for a variety of tumor markers. Were all of these assessed in each patient or were different markers examined in different patients.

Response:

The recurrence was confirmed by imageology and tumor markers levels in our study.

(Line 19-21 of Page 8) There were different markers examined in different patients. However, in our study, we always defined recurrence of gastric cancer mainly according to the image data, and tumor markers were used as assistant markers in confirming recurrence.

Q3:

One of the inclusion criteria for patients' in this study was that the patients underwent "radical" surgery, how is radical surgery defined and was the extent of surgery similar
for tumors at various locations in the stomach?

Response:

In our study, the gastric cancer radical surgery required to extirpate all the tumor and the reminder of the tumor (Ro), together with the great omentum and with regional extended lymphadenectomy. To extirpate the tumor it is necessary to be done gastric resection or total gastrectomy, depending on the localization, the extension of the tumor and the histological type, followed by the reestablish the digestive transit. The lymphadenectomy required lymph node extirpation (D1, D2, D3) accordingly the 3 lymph node stations-N1, N2, N3, depending on the localization of the tumor and the stage of the disease.

Q4:

Though the role and rationale for using MMP-9 expression to ALDH1A1 expression has been discussed in the discussion section, there is no mention of it in the introduction; the role of MMP-9 should also be mentioned in the introduction.

Response:

The reviewer’s suggestion is constructive for our manuscript. It is necessary to add a paragraph of describing why we researched ALDH1A1 in combination with MMP-9
in the introduction. Therefore, we have added the statement that “In addition, it has been reported that MMP-9 plays an important role in gastric cancer recurrence and prognosis. Therefore, we also investigated the relationship of ALDH1A1 and MMP-9 protein in gastric cancer” in the introduction. (Line 13-16 of Page 6)

Q5:

What is the MMP-9 expression index, how is it determined?

Response:

Degradation of extracellular matrix (ECM) was a signal for the beginning of invasion and metastasis, and MMPs are important molecules involved in ECM degradation during invasion and metastasis. In addition, it has been reported that MMP-9 plays an important role in gastric cancer recurrence and prognosis.( Chu D et al. Matrix metalloproteinase-9 is associated with disease-free survival and overall survival in patients with gastric cancer. *Int J Cancer* 2011, 129: 887-895) Therefore, we used MMP-9 as index, and investigated the influence of tumor invasiveness on the prognostic value of ALDH1A1 expression in gastric cancer. The MMP-9 expression index is determined as follow: MMP-9 staining intensities were rated on a scale of 0-3 according to the percentage of positive tumor (0, < 5% positive cells; 1, 5-10%; 2, 11-50%; or 3, > 50%). The expression is very low for 0, low for 1, moderate for 2 and high for 3. MMP-9 expression was classified as negative for scores ≤ 1 and positive
for scores ≥ 2. When patients with MMP-9-negative expression, we classified them to the low invasiveness group, and when patients with MMP-9-positive expression, we classified them to the high invasiveness group.

Q6:

The correlation of ALDH1A1 expression and stage of disease in gastric cancer was not shown in the manuscript, and it should be added in the results. Other studies have demonstrated that lymphovascular invasion and perineural invasion are independent prognosticators of poor outcome in patients with gastric carcinoma, was expression of ALDH1A1 assessed in the context of these variables with regards to univariate and multivariate analyses. Was there any correlation between histologic grade of the tumor and ALDH1A1 expression? Is ALDH1A1 expressed more frequently in poorly differentiated/undifferentiated adenocarcinomas?

Response:

The reviewers’ suggestion is constructive for our manuscript. We have added the results in our manuscript, and found that the expression of ALDH1A1 was correlated with the stage of disease in gastric cancer. (Page 19, New Table 1)

Because that the clinical data was provided by the pathology department, and the pathology department did not examine the lymphovascular invasion and perineural
invasion in gastric cancer samples, we could not find the relevant data. We assessed the expression of ALDH1A1 in univariate and multivariate analyses without these variables. The reviewer’s suggestion is important for our research, we will improve the relevant data in our future work, which will make our study more persuasive.

In our study, we found that the ALDH1A1 expression was not significantly difference in histologic differentiation. Although it had the tendency that ALDH1A1 was expressed more frequently in well or moderate differentiated adenocarcinomas.

(Page 19, Table 1)

Q7:

Previous work that examined levels of ALDH1 expression in gastric cancer and correlated it clinically (e.g. Wakamatsu, 2012) should have been acknowledged and the present study described and discussed in the context of previous work."

Response:

The reviewer’s suggestion is considerable for our manuscript. We have added the discussion of “Wakamatsu et al. revealed that ALDH1 was overexpression and had positively correlated with depth invasion and TNM stage in gastric cancer, moreover, ALDH1 positivity was significantly higher in diffuse-type lymph node metastasis than that in the primary tumor” in the discussion. (Line 7-10 of Page 14) We also
substituted the statement of “ALDH1 was highly expressed in depth of invasion, especially in T3 and T4 carcinomas” for that “ALDH1 was highly expressed in depth of invasion, especially in T3 and T4 carcinomas, which was consistent with previously reported results”. (Line 12-14 of Page 15)