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Aldehyde Dehydrogenase 1 Expression Predicts Chemoresistance and Poor Clinical Outcomes in Patients with Locally Advanced Cervical Cancer Treated with Neoadjuvant Chemotherapy Prior to Radical Hysterectomy

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ABSTRACT

Background. Neoadjuvant chemotherapy (NAC) is an important treatment strategy for cervical cancer; however, few predictive markers of the response to NAC exist. Aldehyde dehydrogenase 1 (ALDH1), a cancer stem cell marker, is associated with chemoresistance in a variety of cancers. This study attempted to investigate the value of ALDH1 as a predictive marker of chemosensitivity and its prognostic value in cervical cancer patients treated with NAC.

Methods. Immunohistochemistry was used to evaluate ALDH1 expression in matched pre- and post-NAC tumor samples from 52 patients with cervical cancer. Kaplan–Meier analysis and a Cox proportional hazards regression model were applied to determine overall survival (OS) and disease-free survival (DFS).

Results. Fourteen patients (26.9 %) had ALDH1-positive tumors pre-NAC, and ALDH1 expression pre-NAC was significantly associated with a low clinical chemotherapy response rate and clinical non-response. Twenty-two patients (42.3 %) had ALDH1-positive tumors post-NAC, and ALDH1 expression post-NAC was associated with poor DFS and OS (both $p = 0.004$). Multivariate analysis revealed that ALDH1 expression post-NAC was an independent prognostic factor for OS (hazard ratio 3.513; $p = 0.033$). Moreover, we observed that ALDH1 expression was increased after NAC in 18 patients (36.7 %). Increased levels of ALDH1 expression after NAC predicted poor DFS and OS ($p = 0.013$ and $p = 0.08$, respectively).

Conclusions. Our findings suggest that ALDH1 expression pre-NAC may be a predictive marker for response to NAC, and ALDH1 expression post-NAC could be a prognostic marker for cervical cancer.

Cervical cancer is the second most frequently diagnosed malignancy in women in developing countries[1]. According to the International Federation of Gynecology and Obstetrics (FIGO) guidelines, concurrent chemoradiotherapy (CCRT) is the primary standard treatment for locally advanced cervical cancer [2] but is associated with a high incidence of long-term complications such as sexual dysfunction and ovarian dysfunction.[3,4] Neoadjuvant chemotherapy (NAC) followed by radical surgery has been proposed as a valid alternative to CCRT; [5,6] however, NAC is ineffective in approximately[1] 0–50 % of patients with locally advanced cervical cancer.[7–9] Therefore, to avoid potential therapy-related complications and inappropriate delays in surgical treatment, it would be advantageous to

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identify chemosensitive tumors before initiating NAC. To date, only a few markers have been described that can predict response to NAC.

Aldehyde dehydrogenase 1 (ALDH1), one of 19 human ALDH isoforms, is responsible for catalyzing the conversion of retinol to retinoic acid.[10] Recently, ALDH1 was recognized as a reliable marker of cancer stem cells (CSCs),[11–14] and ALDH1-positive cancer cells have been demonstrated to be chemoresistant from a variety of tumors, including breast, rectal, and esophageal carcinomas.[15–17] These results suggest that the expression of ALDH1 by tumor cells may predict a poor response to chemotherapy; however, the potential of ALDH1 as a predictive marker of the response to NAC has not been investigated in patients with cervical cancer.

Hererin, we evaluated the potential of ALDH1 as a predictive marker of chemoresistance in patients with locally advanced cervical cancer by investigating the association between ALDH1 expression pre-NAC and the response to NAC. We also assessed the prognostic value of ALDH1 expression in patients with locally advanced cervical cancer treated with NAC.

PATIENTS AND METHODS

Patients and Tissue Samples

From January 2003 to June 2008, patients diagnosed with cervical cancer and registered at Sun Yat-sen Memorial Hospital, Sun Yat-sen University, were considered for the study; 52 patients with stage IB2–IIB disease for whom matching biopsies (taken pre- and post-NAC following surgery) were available for pathological and immunohistochemical analysis were included. Their clinicopathological characteristics are summarized in Table 1. All specimens were anonymously coded in accordance with local ethical guidelines (as stipulated by the Declaration of Helsinki). The study protocol was approved by the University Review Board. Follow-up (median 71 months; range 3–123 months) was as previously described.[8,9]

Treatment and Response

Patients received two or three courses of cisplatin-based chemotherapy. During the inclusion period, patients were enrolled on one of two common regimens: (i) PF: 75 mg/m^2 cisplatin or carboplatin area under concentration-time curve (AUC)4–5 intravenously on day 1, 750 mg/m^2 fluorouracil on days 1–5 with an interval of 21 days; and (ii) TP: 135 mg/m^2 paclitaxel plus 75 mg/m^2 cisplatin or carboplatin AUC4–5 intravenously with an interval of 21 days. All patients subsequently underwent a type III radical hysterectomy with systematic pelvic lymphadenectomy plus para-aortic lymphadenectomy, if indicated, within 3 weeks of finishing chemotherapy. Postoperative adjuvant therapy was administered to patients with risk factors for recurrence, according to the FIGO guidelines. Chemotherapeutic response was assessed before chemotherapy and 2 weeks after the last cycle of chemotherapy by bimanual gynecological examination, colposcopy, transvaginal ultrasound and/or magnetic resonance imaging (MRI). Clinical response to NAC was evaluated based on the Response Evaluation Criteria in Solid Tumors (RECIST) criteria,[18] as follows: complete resolution of the tumor (CR); partial response (PR), i.e. >50 % decrease in the tumor volume; stable disease (SD), i.e. <50 % decrease or a <25 % increase in the tumor volume; and progressive disease (PD), i.e. >25 % increase in the tumor volume. Pathological response was determined by the final postoperative pathological analysis. Patients were classified as responders (CR or PR) and non-responders (SD or PD).[19]

Immunohistochemistry and Evaluation

Immunohistochemistry was performed using an anti-ALDH1 antibody (BD Biosciences, Franklin Lakes, NJ, USA) following a previously described standard method.[20] Paraffin sections of normal human liver tissue were used as a positive control, and the primary antibody was replaced with phosphate buffered saline (PBS) for the negative control. Cytoplasmic staining of tumor cells was considered when scoring ALDH1-positive cells; stromal and vascular staining was not evaluated. Immunostaining was evaluated using a scoring system for ALDH1 as follows:[21] 0, negative staining in all tumor cells; 1+, weak positive or focal positive staining of ≤10 % cells; 2+, moderate positive staining of >10 to ≤50 % cells; 3+, strong positive staining of >50 % cells; ALDH1 expression was considered positive if the score was ≥2 (electronic supplementary Fig. S1).

Statistical Analysis

Statistical analysis was performed using SPSS software, version 13.0 (SPSS Inc., Chicago, IL, USA). Associations between the clinicopathologic characteristics and the pattern of ALDH1 expression pre- and post-NAC were examined using the Pearson's χ^2 test. Multiple logistic regression models were used to identify predictors of response to NAC. Survival rates were calculated using the Kaplan–Meier method and compared using the log-rank test. Changes in the ALDH1 immunohistochemical score after NAC were assessed using the Wilcoxon signed-rank test. Univariate and multivariate survival analyses were performed using the Cox regression model for disease-free survival (DFS) and overall survival (OS). A forward stepwise procedure was used to identify independent

TABLE 1 Associations between expression of ALDH1 pre- and post-NAC and clinicopathologic characteristics of locally advanced cervical cancer (stages IB2–IIB)

Variable	Cases		ALDH1 pre-NAC			ALDH1 post-NAC		
	N	(%)	Negative	Positive	*p* value[a]	Negative	Positive	*p* value[a]
Age (years)			38	14		27	22	
≤43[b]	26	50.0	19	7	NS	14	11	NS
>43	26	50.0	19	7		13	11	
FIGO stage								
IB2	28	53.8	21	7	NS	18	9	0.071
IIA/IIB	24	46.2	17	7		9	13	
Histologic subtype								
Squamous	42	80.8	32	10	NS	21	18	NS
Non-squamous	10	19.2	6	4		6	4	
Tumor grade								
G1/G2	35	67.3	28	7	NS	14	21	0.094
G3	17	32.7	10	7		11	6	
Tumor size prior to NAC (cm)								
≤5[c]	30	57.7	23	7	NS	17	10	NS
>5	22	42.3	15	7		10	12	
Clinical response to NAC								
Responder (CR/PR)	34	65.4	29	5	**0.006**	18	13	NS
Non-responder (SD)	18	34.6	9	9		9	9	
Tumor size after NAC (cm)								
≤3.5[d]	27	51.9	24	3	**0.008**	16	8	NS
>3.5	25	48.1	14	11		11	14	
Lymph node metastasis								
Negative	27	51.9	20	7	NS	19	6	**0.003**
Positive	25	48.1	18	7		8	16	
Parametrial invasion								
Negative	45	86.5	32	13	NS	26	17	**0.043**
Positive	7	13.5	6	1		1	5	
Surgical margin involved								
No	50	96.2	37	13	NS	26	21	NS
Yes	2	3.8	1	1		1	1	
Lymphovascular invasion								
Negative	34	65.4	29	5	0.006	18	15	NS
Positive	18	34.6	9	9		9	7	
Cervical stromal invasion								
≤one-third	17	32.7	13	4	NS	12	5	NS
>one-third	35	67.3	25	10		15	17	
Recurrence								
No	34	65.4	26	8	NS	22	9	**0.003**
Yes	18	34.6	12	6		5	13	
Cancer-related death								
No	36	69.2	28	8	NS	23	10	**0.003**
Yes	16	30.8	10	6		4	12	
NAC regimen								
TP	16	30.8	12	4	NS	10	5	NS

TABLE 1 continued

Variable	Cases		ALDH1 pre-NAC			ALDH1 post-NAC		
	N	(%)	Negative	Positive	*p* value[a]	Negative	Positive	*p* value[a]
PF	36	69.2	26	10		17	17	

ALDH1 aldehyde dehydrogenase 1, *NAC* neoadjuvant chemotherapy, *NS* not significant, *FIGO* International Federation of Gynecology and Obstetrics, *CR* complete response, *PR* partial response, *SD* stable disease, *TP* taxol + cisplatin/carboplatin, *PF* cisplatin/carboplatin + fluorouracil

[a] The *p* value was determined using the χ^2 test. Significant *p* values are shown in bold

[b] Range 27–63 years, median 43 years

[c] Diameters ranging from 3–9 cm, median 5 cm

[d] Diameters ranging from 0–6 cm, median 3.5 cm

variables in the multivariate analysis. *p* values ≤ 0.05 indicated statistical significance.

RESULTS

Clinical Response of Patients with Cervical Cancer to Neoadjuvant Chemotherapy (NAC)

Of the 52 patients, 16 (30.8 %) received the TP regimen and 36 (69.2 %) received the PF regimen (Table 1). Overall, 34 patients (65.4 %) responded to NAC, including 5 CR and 29 PR, and 18 patients (34.6 %) were non-responders, all of whom had SD. Samples from the five patients with CR were evaluated by postoperative pathological examination: three patients were confirmed to have a pathologically complete response (pCR), whereas the other two had residual tumors <3 mm.

Aldehyde Dehydrogenase 1 (ALDH1) Expression Pre- and Post-NAC and its Association with the Clinicopathologic Features of Cervical Cancer

Immunohistochemical analysis was performed on 52 paired samples collected pre- and post-NAC. ALDH1 staining was mainly localized to the cytoplasm of the tumor cells, with faint expression observed in the surrounding stromal and vascular areas (electronic supplementary Fig. S1). The associations between the expression of ALDH1 pre- and post-NAC and the clinicopathologic features of the patients are listed in Table 1. Of the pre-NAC biopsies, 14 (26.9 %) were ALDH1-positive and 38 (73.1 %) were ALDH1-negative. Positive ALDH1 staining pre-NAC was associated with a higher rate of lympho-vascular space invasion ($p = 0.006$), a poor clinical response to NAC, and a lower reduction in tumor size after NAC ($p = 0.006$ and $p = 0.008$, respectively). The post-NAC biopsies of 22/49 (42.3 %) patients who did not achieve pCR had positive ALDH1 expression. Moreover, post-NAC expression of ALDH1 was associated with lymph node metastasis and parametrial invasion ($p = 0.003$ and $p = 0.043$, respectively).

ALDH1 Expression Pre-NAC is Predictive of Response to NAC

To determine pretreatment predictors of the response to NAC, we assessed pretreatment clinical features (tumor stage, tumor grade, histologic subtype, and tumor size pretreatment) and ALDH1 expression pre-NAC. Patients with negative ALDH1 expression pre-NAC and those with 'earlier' tumor stage (IB2 vs. IIA/IIB) were significantly more responsive to NAC ($p = 0.006$ and $p = 0.031$, respectively). Logistic regression analysis showed that both ALDH1 expression pre-NAC [$p = 0.017$; odds ratio (OR) 6.264; 95 % CI 1.385–28.322] and tumor stage ($p = 0.041$; OR 4.193; 95 % CI 1.063–16.539) were independent predictors of response to NAC (Table 2).

Association Between ALDH1 Expression Pre- and Post-NAC

An increase in ALDH1 expression after NAC has been reported in patients with breast and rectal cancer.[15,16] To investigate whether expression of ALDH1 is affected by NAC in cervical cancer, we assessed ALDH1 expression pre- and post-NAC in the 49 patients who did not achieve pCR (among 52 patients, three with pCR were not included). Images of ALDH1 staining for representative cases are shown in Fig. 1a. The grade of ALDH1 expression increased after NAC in 18/49 (36.7 %) patients ($p = 0.037$; Fig. 1b) [including 13 with negative pre-NAC staining who had positive staining post-NAC, and five with positive pre-NAC who had increase in tumor grade], remained stable in 22 (44.9 %) patients, and decreased in nine (18.4 %) patients. Among the 13 patients with negative pre-NAC staining and positive post-NAC staining,

TABLE 2 Expression of ALDH1 pre-NAC and pretreatment factors as predictors for responsiveness to NAC in stages IB2–IIB cervical cancer

Variable	Response to NAC		*p* value[a]	*p* value[b]
	Responder	Non-responder		
ALDH1 pre-NAC				
Negative	29	9	**0.006**	**0.017**
Positive	5	9		
Histologic subtype				
Squamous	28	14	0.690	0.971
Non-squamous	6	4		
FIGO stage				
IB2	22	6	**0.031**	**0.041**
IIA/IIB	12	12		
Tumor grade				
G1/G2	24	11	0.448	0.796
G3	10	7		
Tumor size before NAC (cm)				
≤5	21	9	0.414	0.822
>5	13	9		

ALDH1 aldehyde dehydrogenase 1, *NAC* neoadjuvant chemotherapy, *FIGO* International Federation of Gynecology and Obstetrics

[a] The *p* value was determined using the χ^2 test. Significant *p* values are shown in bold

[b] Logistic regression analysis with ALDH1 expression prior to NAC and pretreatment factors as covariates

nine were clinical responders and four were non-responders. Increased ALDH1 expression after NAC was associated with a higher rate of lymph node metastasis and parametrial invasion ($p = 0.002$ and $p = 0.011$, respectively; electronic supplementary Table S1).

Prognostic Value of ALDH1 Expression Pre- and Post-NAC

During the median follow-up of 71 months (range 3–123), 18/52 (34.6 %) patients relapsed, of whom 16 (88.9 %) died due to their disease. Two patients with local recurrent vaginal cervical cancer were alive after surgery and adjuvant CCRT. The 5-year OS and DFS rates were 69.2 and 65.4 %, respectively. Although not significant, patients with positive ALDH1 expression pre-NAC had poorer 5-year DFS (57.1 vs. 68.4 %; $p = 0.343$) [electronic supplementary Fig. S2a] and 5-year OS (57.1 vs. 73.7 %; $p = 0.186$) [electronic supplementary Fig. S2b] than patients with negative ALDH1 expression pre-NAC. Responders to NAC had longer 5-year DFS ($p = 0.001$) [electronic supplementary Fig. S2c] and OS ($p = 0.015$) [electronic supplementary Fig. S2d] than non-responders. Positive ALDH1 expression post-NAC was associated with poorer 5-year DFS ($p = 0.004$) [electronic supplementary Fig. S2e], and OS ($p = 0.004$) [electronic supplementary Fig. S2f] than negative ALDH1 expression post-NAC. In-

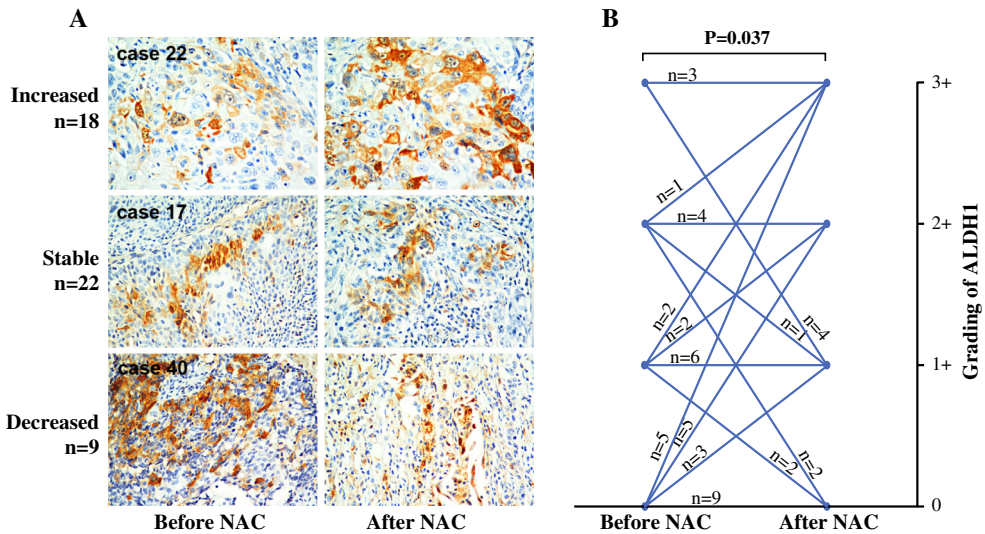

FIG. 1 Comparison of ALDH1 expression pre- and post-NAC. **a** ALDH1 immunostaining pre- and post-NAC for three representative cases: Case 22, indicating increased staining from 1+ to 2+; Case 17, which shows stable ALDH1 immunostaining at 1+; and Case 40, indicating decreased staining from 3+ to 1+ (original magnification ×400). **b** The Wilcoxon signed-rank test was used to analyze the changes in ALDH1 expression after NAC. Of the 49 paired samples not achieving pCR before or after NAC, ALDH1 expression significantly increased after NAC in 18 patients ($p = 0.037$), remained stable in 22 patients, and decreased in 9 patients. *ALDH1* aldehyde dehydrogenase 1, *NAC* neoadjuvant chemotherapy, *pCR* pathologically complete response

creased expression of ALDH1 after NAC was associated with poorer 5-year DFS ($p = 0.013$) [electronic supplementary Fig. S2g] and OS, although this effect was not significant for OS (50.0 % vs. 77.4 %; $p = 0.08$) [electronic supplementary Fig. S2h].

In the univariate analysis, tumor size pre-NAC (>5 cm), non-response to NAC, lymph node metastasis, positive ALDH1 expression post-NAC, and increased ALDH1 expression after NAC correlated with poor 5-year DFS. Non-response to NAC emerged as an independent negative prognostic factor for 5-year DFS in the multivariate analysis [$p = 0.003$; hazard ratio (HR) 5.072; 95 % CI 1.781–14.976]. For 5-year OS, tumor size pre-NAC, response to NAC, and ALDH1 expression post-NAC were entered into the multivariate analysis; ALDH1 expression post-NAC was the only significant independent variable ($p = 0.033$; HR 3.513; 95 % CI 1.109–11.250) [Table 3].

DISCUSSION

NAC is widely used in the treatment of locally advanced cervical cancer in many developing countries, including China; however, few predictors of the response to NAC exist.[19,22–25] The present study demonstrates that ALDH1 expression pre-NAC is an independent predictor of clinical response to NAC in patients with locally advanced cervical cancer (FIGO stages IB2–IIB). In addition, ALDH1 expression significantly increased after NAC, and ALDH1 expression post-NAC and an increase in ALDH1 expression after NAC were associated with a poorer outcome in patients with cervical cancer. These results provide new evidence of a correlation between ALDH1 positivity and chemoresistance in cervical cancer.

In the current study, patients with a high proportion of ALDH1-positive tumor cells had a poorer response to

TABLE 3 Univariate and multivariate analysis of factors associated with disease-free survival and overall survival for patients with stages IB2–IIB cervical cancer who received NAC prior to radical surgery

Variable	5-Year disease-free survival[c]				5-Year overall survival[c]			
	Univariate	Multivariate[d]			Univariate	Multivariate[d]		
	p value[a]	*p* value[a]	HR[b]	95 % CI	*p* value[a]	*p* value[a]	HR[b]	95 % CI
Age (years) (>43 vs. ≤43)	0.569	NA			0.843	NA		
FIGO stage (IIA/IIB vs. IB2)	0.161	NA			0.361	NA		
Histologic subtype (non-squamous vs. squamous)	0.963	NA			0.964	NA		
Tumor grade (poor vs. well/moderate)	0.730	NA			0.963	NA		
Tumor size before NAC (>5 cm vs. ≤5 cm)	**0.017**	0.094	2.506	0.854–7.350	**0.023**	0.184	2.095	0.704–6.236
Tumor size after NAC (>3.5 cm vs. ≤3.5 cm)	0.675	NA			0.355	NA		
NAC regimen (PF vs. TP)	0.819	NA			0.501	NA		
Response to NAC (non-responder vs. responder)	**0.003**	**0.003**	**5.072**	**1.781–14.976**	**0.022**	0.120	2.225	0.813–6.092
Lymph node metastasis (positive vs. negative)	**0.027**	0.472	0.589	0.139–2.492	0.091	NA		
Parametrial invasion (positive vs. negative)	0.235	NA			0.517	NA		
Surgical margin involved (positive vs. negative)	0.119	NA			0.516	NA		
Lymphovascular invasion (positive vs. negative)	0.550	NA			0.836	NA		
Cervical stromal invasion (>1/3 vs. ≤1/3)	0.109	NA			0.068	NA		
ALDH1 pre-NAC (positive vs. negative)	0.351	NA			0.195	NA		
ALDH1 post-NAC (positive vs. negative)	**0.009**	0.288	2.149	0.524–8.812	**0.009**	**0.033**	**3.513**	**1.109–11.250**
ALDH1 increased after NAC (increased vs. non-increased)	**0.019**	0.065	3.288	0.927–11.662	0.101	NA		

NAC neoadjuvant chemotherapy, *HR* hazard ratio, *CI* confidence interval, *NA* not applicable, *FIGO* International Federation of Gynecology and Obstetrics, *PF* cisplatin/carboplatin + fluorouracil, *TP* taxol + cisplatin/carboplatin, *ALDH1* aldehyde dehydrogenase 1

[a] Significant *p* values are shown in bold font

[b] HR >1 indicates risk for recurrence/death increased; HR <1 indicates risk for recurrence/death decreased

[c] Univariate and multivariate analyses and Cox proportional hazards regression model

[d] Variables associated with survival by univariate analysis were adopted as covariates in multivariate analyses

NAC. This observation suggests that pretreatment screening of ALDH1 expression may provide helpful information for decision making as patients with ALDH1-positive tumors may be less likely to benefit from NAC. High ALDH1 expression has been associated with resistance to chemotherapy in breast, rectal and esophageal carcinomas.[15–17] Moreover, recent reports showed that ALDH1 was expressed at high levels in cisplatin-resistant cervical cancer cell lines.[12,14]Therefore, ALDH1-positive tumor cells in pre-NAC biopsies may be predictive of chemoresistance to NAC. Pre-NAC ALDH1 expression could be used as a reliable marker to identify patients who could benefit most from NAC.

The biochemical link between ALDH1-positive cells and resistance to chemotherapy is not clearly understood.[26] As a cytosolic enzyme, ALDH1 plays a significant role in oxidizing toxic aldehydes and other potentially harmful chemical components and drugs, such as cyclophosphamide in hematopoietic cell lines.[27] Furthermore, ALDH1 is a marker of CSCs, and ALDH1-positive cells are thought to either be inherently chemoresistant or acquire chemoresistance via clonal evolution during chemotherapy.[26,28] Based on our evaluation of paired cervical cancer tissues obtained pre- and post-chemotherapy, we propose that both intrinsic and acquired characteristics are involved in the refractory behavior of ALDH1-positive tumor cells to NAC.

In pre-NAC specimens, positive ALDH1 expression was significantly associated with lymphovascular space invasion and, more importantly, was significantly associated with non-response to NAC. This may indicate that ALDH1-positive tumor cells are intrinsically chemoresistant. Previously, we and other researchers demonstrated that ALDH1 is a reliable marker of cervical CSCs.[11,13,14] Although present in very small numbers, CSCs are thought to be inherently chemoresistant.[29] We speculate that ALDH1-positive tumors are chemoresistant as they may contain a higher proportion of CSCs.

In the post-NAC samples, positive ALDH1 expression was significantly associated with lymph node metastasis and parametrial invasion. Only positive ALDH1 expression post-NAC remained an independent prognostic indicator in the multivariate survival analysis. Similar to this study, Sakakibara et al. found that breast cancer patients with residual tumors containing ALDH1-positive cells post-NAC had a poorer prognosis than patients with ALDH1-negative cells or no residual tumor.[30] This indicates that it may be useful to evaluate ALDH1 expression post-NAC in patients with cervical cancer, even in patients whose tumors are ALDH1-negative pre-NAC. For those patients with positive ALDH1 staining post-NAC, closer follow-up is needed. We speculate that anti-ALDH1 treatments may bring some benefits;[26] however, more evidence is needed from further preclinical and clinical studies.

Furthermore, when we compared ALDH1 expression pre-NAC and post-NAC, we found that ALDH1 expression significantly increased after NAC, and 13 patients with negative ALDH1 staining pre-NAC had positive ALDH1 staining post-NAC. ALDH1-positive cells after NAC may, to some extent, imply acquired chemoresistance. Chemotherapy can selectively enrich ALDH1-positive CSCs in breast and colorectal cancers.[31,32] Similarly, the upregulation of multidrug resistance protein 1 (MDR1/P-gp/ABCB1) and ATP-binding cassette sub-family G member 2 (BCRP/ABCG2) can occur after chemotherapy for breast cancer.[31] Increased ALDH1 expression after NAC was associated with poorer clinical outcomes and significantly higher rates of tumor-related death and recurrence in this study. As a result, NAC should not be recommended for those patients with positive pre-NAC ALDH1 staining.

Although ALDH1-positive tumor cells are chemoresistant, 5 of the 14 patients with positive pre-NAC markers were clinical responders in our study. Furthermore, nine patients with ALDH1-positive tumor cells had a reduced grade of ALDH1 staining after NAC, which showed that they were sensitive to chemotherapy.[30] This discrepancy may be partially explained by the specificity of ALDH1 in marking CSCs, since CSC markers may detect not only stem cells exclusively but also a larger tumor cell population with similar expression of stem cell markers.[16]

There were several limitations to this present study. As a result of the limited number of samples, we failed to observe any significant effect of pre-NAC ALDH1 expression on DFS or OS. Moreover, the potential bias of adjuvant postoperative treatment on DFS and OS may have been present in our research.

CONCLUSIONS

This study demonstrates that assessment of the CSC marker ALDH1 prior to treatment could provide valuable information to help identify patients with cervical cancer who are likely to respond to NAC. Expression of ALDH1 significantly increased after NAC, and ALDH1 expression post-NAC, and increased expression of ALDH1 after NAC, were associated with poorer clinical outcomes. A prospective multicenter study with a larger sample size is warranted to further confirm these findings.

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CONFLICT OF INTEREST Qingsheng Xie, Jinxiao Liang, Qunxian Rao, Xiaofei Xie, Ruixin Li, Yunyun Liu, Hui Zhou, Jingjing Han, Tingting Yao, and Zhongqiu Lin declare that they have no actual or potential competing financial interests.

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