

ITGA7, CD133, ALDH1 are inter-correlated, and linked with poor differentiation, lymph node metastasis as well as worse survival in surgical cervical cancer

Na Yuan¹ , Lei Wang², Qiang Xi¹, Niandong Zou¹, Xianyu Zhang¹, Xiurong Lu¹ and Zhilin Zhang¹

¹Department of Radiotherapy, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China

²Department of Severe Care Unit, The First Affiliated Hospital of Hebei North University, Zhangjiakou, China

Abstract

Aim: Integrin alpha 7 (ITGA7) regulates cancer stemness and metastasis in several malignancies, while its role in cervical cancer is obscure. Therefore, the current study aimed to investigate the correlation among ITGA7, cluster of differentiation 133 (CD133), and aldehyde dehydrogenase isoform 1 (ALDH1), as well as their relation to tumor features and survival in cervical cancer patients.

Methods: A total of 133 surgical cervical cancer patients were enrolled. Tumor ITGA7, CD133, and ALDH1 expressions were determined by immunohistochemistry (IHC). Furthermore, the clinicopathological features, disease-free survival (DFS), and overall survival (OS) were collected.

Results: ITGA7 expression positively related to CD133 expression ($p = 0.040$) and ALDH1 expression ($p < 0.001$). Besides, ITGA7 ($p = 0.001$), CD133 ($p = 0.016$), and ALDH1 ($p = 0.009$) high expressions linked with poor tumor differentiation; meanwhile, ITGA7 ($p = 0.010$) and ALDH1 ($p = 0.004$) high expressions correlated with more prevalence of lymph node metastasis. However, ITGA7, CD133, or ALDH1 expression was not associated with other clinicopathological features. Inspiringly, it was worth noting that ITGA7 ($p = 0.009$), CD133 ($p = 0.041$), and ALDH1 ($p = 0.035$) high expressions predicted unfavorable DFS; meanwhile, both ITGA7 ($p = 0.021$) and ALDH1 ($p = 0.023$) high expressions but not CD133 expression ($p = 0.169$) forecasted exasperated OS.

Conclusion: ITGA7, CD133, ALDH1 are inter-correlated, and linked with poor differentiation, lymph node metastasis as well as worse survival in surgical cervical cancer.

Key words: aldehyde dehydrogenase isoform 1, cervical cancer, cluster of differentiation 133, integrin alpha 7, prognosis.

Introduction

Cervical cancer, usually caused by human papilloma virus (HPV) infection, is the fourth leading cause of cancer death in women, with an estimated prevalence of 604 000 new cases and 342 000 deaths worldwide

in 2020.^{1–4} In cervical cancer, neoplasm metastasis and relapse are the most common reasons for exacerbated prognosis as well as high mortality.⁵ Typically, it has been well established that cancer stem cell (CSC) plays a critical role in not only promoting tumor metastasis and relapse, but also enhancing

Received: October 22 2021.

Accepted: January 16 2022.

Correspondence

Na Yuan, Department of Radiotherapy, The First Affiliated Hospital of Hebei North University, 12 Chang Qing Road, Qiao Xi District, Zhangjiakou 075061, China.

Email: an16391486@163.com

© 2022 The Authors. *Journal of Obstetrics and Gynaecology Research* published by John Wiley & Sons Australia, Ltd 1011 on behalf of Japan Society of Obstetrics and Gynecology.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

multidrug resistance.⁶ So far, a series of CSC surface markers have been identified to monitor the relapse, metastasis, and survival prognostication in cervical cancer which might assist in realizing individualized therapy in the future.^{7,8}

Integrin alpha 7 (ITGA7) is localized on chromosome 12p13 and consists of at least 27 exons spanning a region of about 22.5 kb, which acts as an oncogene and plays a critical role in facilitating the stemness in multiple carcinomas.^{9–13} For instance, prior studies disclose that ITGA7 facilitates the proliferation or metastasis of tumor cells and promotes the activity of CSC in renal cell carcinoma, non-small-cell lung cancer and tongue squamous cell carcinoma (SCC).^{11–13} In a more in-depth research, ITGA7 upregulates CSC properties through the activation of the focal adhesion kinase (FAK)-mediated signaling pathways in esophageal SCC.⁹ Whereas, its clinical relevance in cervical cancer still needs further exploration. Interestingly, previous studies show that CD133 serves as a biomarker for CSC properties and prognosis in cervical cancer.^{14,15} Beyond that, ALDH1 is also linked with the CSC properties and serves as a prognostic indicator in cervical cancer.¹⁶ Furthermore, another study illuminates that highly expressed ALDH1 is associated with enhanced radioresistant in cervical cancer model via regulating CSC properties.¹⁷ Since ITGA7, CD133, and ALDH1 are biomarkers for CSC properties, thus we hypothesize that they might be intercorrelated. Therefore, the current study aimed to explore the correlation of ITGA7 with CD133 and ALDH1, clinical features as well as survival profile in cervical cancer patients.

Methods

Patients

A total of 133 cervical cancer patients underwent surgical resection in The First Affiliated Hospital of Hebei North University from January 2016 to December 2020 were retrospectively reviewed in this study. The screening criteria for all patients were: (i) diagnosis of cervical cancer according to the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines, Version 2.2015¹⁸; (ii) age ≥ 18 years old; (iii) International Federation of Gynecology and Obstetrics (FIGO) stage I–IIA; (iv) with complete clinical features (including age, HPV status, histological type, differentiation, tumor size, lymph node metastasis, and FIGO stage) and survival data

(including disease-free survival [DFS] and overall survival [OS]); (v) with available cancer specimens to perform IHC assay. In addition, according to the medical documents, the patients were ineligible for the study if they met the criteria as follows: (i) had history of other cancers or malignancies; (ii) relapsed cervical cancer; (iii) received chemotherapy, radiotherapy, or targeted therapy before resection. This study was approved by the Institutional Review Board (W2021029) with omitted informed consents.

Data collection

The clinical data were collected, including age, HPV status, histological type, differentiation, tumor size, lymph node metastasis (preoperative lymph node metastasis was examined by images of computed tomography [CT] and magnetic resonance imaging [MRI]; it was finally confirmed by postoperative pathological examination and used to guide subsequent treatment.), and FIGO stage (classified by 2009 FIGO Staging System).¹⁹ Besides, the survival data were also obtained from the medical records to calculate DFS and OS, and the final date for survival data collection was February 2021.

Treatment

The cervical cancer patients received surgical resection, and the surgery regimen was performed for the corresponding patients with different FIGO stage: (i) FIGO stage IA patients: total hysterectomy or partial hysterectomy plus pelvic lymph node resection; (ii) FIGO stage IB/IIA patients: radical hysterectomy plus pelvic lymph node resection, with or without para-aortic lymph node sampling.

IHC assay

Cervical cancer specimens of all patients, which were fixed in formalin and embedded in paraffin (FFEP), were collected to assess the expressions of ITGA7, CD133, and ALDH1 by IHC assay. The mouse monoclonal anti-ITGA7 antibody (1:50 dilution, Santa Cruz Biotechnology), the mouse monoclonal anti-CD133 antibody (1:50 dilution, Miltenyi biotec), and the mouse monoclonal anti-ALDH1 antibody (1:100 dilution, Abcam; Cambridge, UK) were applied as primary antibody. The goat anti-mouse IgG (H&L) (Abcam) with 1:2000 dilution was used as secondary antibody.^{13,20,21} After staining, the protein expression was calculated using a semiquantitative scoring method. Briefly, in every slide of specimens, five selected high-power fields (HPF, $\times 400$) were

independently counted by two pathologists without prior knowledge of the clinicopathological information to evaluate the intensity and density of cells. Staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong), while staining density was scored as 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%). The product of staining intensity score and staining density score was the final IHC score, which was classified as low expression (final IHC score ≤ 3) and high expression (final IHC score > 3).

Statistical analysis

Correlations between categorical variable was evaluated by chi-square test, and correlations between dichotomous variable and ordinal variable were evaluated by Mann–Whitney *U* test. Kaplan–Meier curves and log-rank test were used to display the correlation between the survival data and ITGA7/CD133/ALDH1 expression. Besides, Cox's proportional hazards regression analysis was applied for prognostic factor analysis, and hazard ratio with 95% confidence intervals (CI) were displayed using forest plot. Statistical significance was concluded if a *p* value < 0.05 was presented in the corresponding analysis. SPSS V.19.0 software (IBM Corp., Armonk, New York, USA) and GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, California, USA) were used for above statistical analyses and graph plotting.

Results

Clinical features

The age of analyzed cervical cancer patients was 51.2 ± 10.4 years (Table 1). Besides, 28 (21.1%) patients and 105 (78.9%) patients were HPV negative and positive, separately. In terms of histological type, there were 8 (6.0%) patients with adenosquamous carcinoma (ASC), 21 (15.8%) patients with adenocarcinoma (ADC), and 104 (78.2%) patients with SCC. Concerning the differentiation status, 42 (31.6%) patients were well differentiated, 49 (36.8%) patients were moderate differentiated, and 42 (31.6%) patients were poor differentiated. Regarding tumor size, 66 (49.6%) patients had tumor less than 4 cm meanwhile 67 (50.4%) patients had tumor larger than or equal to 4 cm. In addition, 28 (21.1%) patients had lymph node metastasis, while 105 (78.9%) patients did not. In regards to FIGO stage, 15 (11.3%), 20 (15.0%), 16 (12.0%), 34 (25.6%), 15 (11.3%), and

Table 1 Clinical features

Items	Cervical cancer patients (<i>N</i> = 133)
Age (years), mean \pm SD	51.2 \pm 10.4
HPV status, no. (%)	
Negative	28 (21.1)
Positive	105 (78.9)
Histological type, no. (%)	
ASC	8 (6.0)
ADC	21 (15.8)
SCC	104 (78.2)
Differentiation, no. (%)	
Well	42 (31.6)
Moderate	49 (36.8)
Poor	42 (31.6)
Tumor size, no. (%)	
< 4 cm	66 (49.6)
≥ 4 cm	67 (50.4)
Lymph node metastasis, no. (%)	
No	105 (78.9)
Yes	28 (21.1)
FIGO stage, no. (%)	
IA1	15 (11.3)
IA2	20 (15.0)
IB1	16 (12.0)
IB2	34 (25.6)
IIA1	15 (11.3)
IIA2	33 (24.8)

Abbreviations: ADC, adenocarcinoma; ASC, adenosquamous carcinoma; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; SCC, squamous cell carcinoma.

33 (24.8%) patients were with stage IA1, IA2, IB1, IB2, IIA1, and IIA2, separately. Meanwhile, the frequency of lymph node metastasis for them were 0.0%, 5.0%, 18.8%, 11.8%, 46.7%, and 39.4%, respectively (Table S1, Supporting Information).

Expression of ITGA7, CD133, ALDH1 and their intercorrelation

Expression of ITGA7, CD133, and ALDH1 was measured by IHC assay (Figure 1a). Besides, the data showed that 57(42.9%), 26 (19.5%), 35 (26.3%), and 13 (11.3%) patients had the ITGA7 IHC score of 0–3, 4–6, 7–9, and 10–12, respectively (Figure 1b). Additionally, it was revealed that 95(71.4%), 24 (18.1%), 10 (7.5%), and 4 (3.0%) patients had the CD133 IHC score of 0–3, 4–6, 7–9, and 10–12, respectively. Moreover, it was illustrated that 39(29.3%), 30 (22.6%), 48 (36.1%), and 16 (12.0%) patients had the ALDH1 IHC score of 0–3, 4–6, 7–9, and 10–12, respectively.

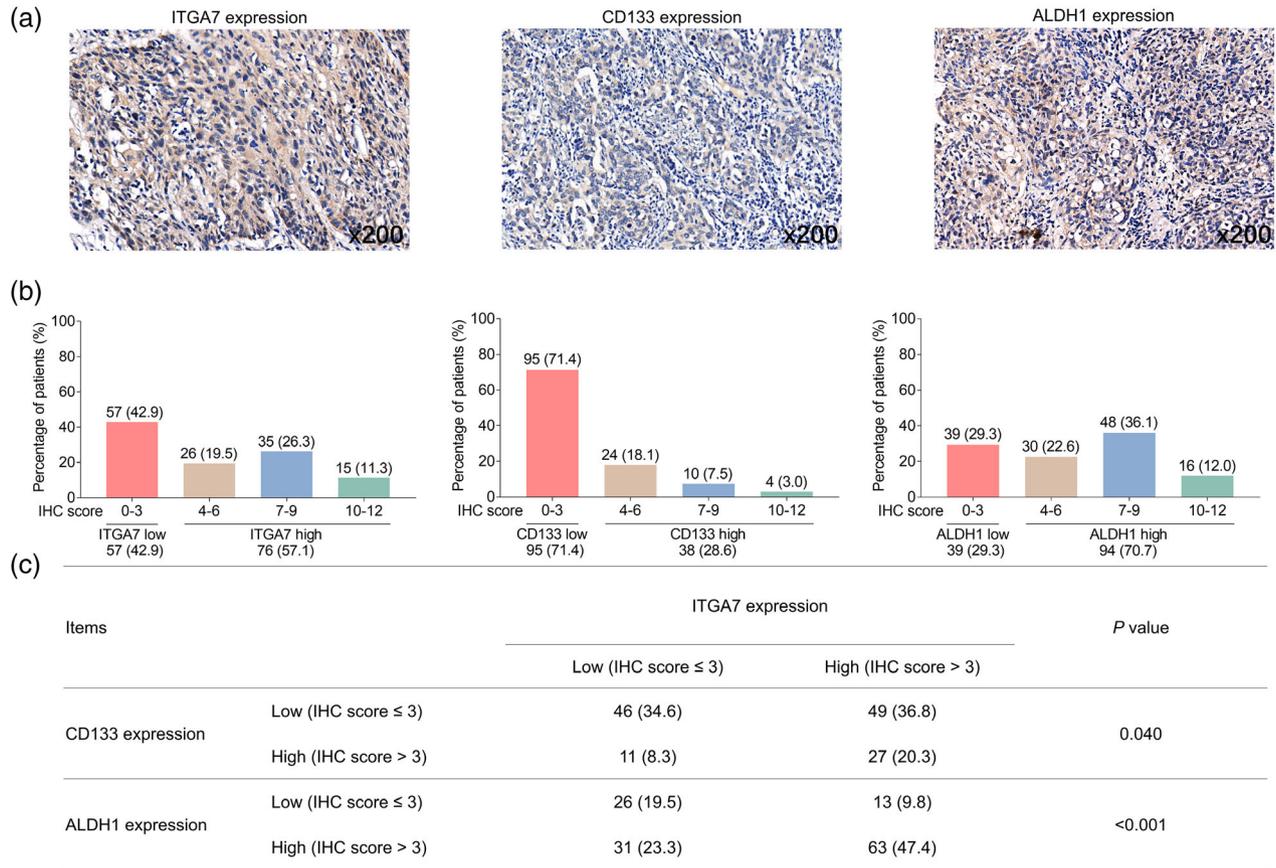


Figure 1 Level of ITGA7, CD133, and ALDH1 (by chi-square test). Expression of ITGA7, CD133, and ALDH1 by IHC assay (a); distribution of ITGA7, CD133, and ALDH1 expressions in cervical cancer patients (b); correlation of ITGA7 expression with CD133 and ALDH1 expression (c). ALDH1, aldehyde dehydrogenase 1; CD133, cluster of differentiation 133; ITGA7, integrin subunit alpha 7; IHC, immunohistochemistry

It was worth noticing that ITGA7 expression was positively correlated with CD133 expression ($p = 0.040$) and ALDH1 expression ($p < 0.001$) (Figure 1c).

Correlation of ITGA7, CD133, and ALDH1 expressions with clinical features

High expression of ITGA7 ($p = 0.001$), CD133 ($p = 0.016$), and ALDH1 ($p = 0.009$) was associated with poor differentiation (Table 2). Besides, high expression of ITGA7 ($p = 0.010$) and ALDH1 ($p = 0.004$) was correlated with the occurrence of lymph node metastasis. Moreover, high expression of ITGA7 ($p = 0.004$), CD133 ($p = 0.013$), and ALDH1 ($p = 0.031$) was linked with higher FIGO stage. Whereas, expression of ITGA7, CD133, and ALDH1 was not correlated with other characteristics such as

age, HPV status, histological type, or tumor size (all $p > 0.05$).

Correlation of ITGA7, CD133, and ALDH1 expressions with accumulating DFS and OS

ITGA7 high ($p = 0.009$), CD133 high ($p = 0.041$), and ALDH1 high ($p = 0.035$) expressions were correlated with worse accumulating DFS (Figure 2a-c). Besides, ITGA7 high ($p = 0.021$) and ALDH1 high ($p = 0.023$) were also correlated with worse accumulating OS, but CD133 high was not related to accumulating OS ($p = 0.068$) (Figure 3a-c). Additionally, the combination of ITGA7, CD133, and ALDH1 also showed value in predicting survival for cervical cancer patients. In detail, patients with at least one expression high (ITGA7, ALDH1, or CD133) had poorer accumulating DFS compared with those with no expressions high ($p = 0.005$, Figure S1a). Similarly,

Table 2 Correlations between ITGA7/CD133/ALDH1 expression and clinical features

Items	ITGA7 expression			CD133 expression			ALDH1 expression		
	Low	High	<i>p</i> -Value	Low	High	<i>p</i> -Value	Low	High	<i>p</i> -Value
Age, no. (%)			0.801			0.474			0.820
<50 years	25 (41.7)	35 (58.3)		41 (68.3)	19 (31.7)		17 (28.3)	43 (71.7)	
≥50 years	32 (43.8)	41 (56.2)		54 (74.0)	19 (26.0)		22 (30.1)	51 (69.9)	
HPV status, no. (%)			0.197			0.346			0.403
Negative	9 (32.1)	19 (67.9)		22 (78.6)	6 (21.4)		10 (35.7)	18 (64.3)	
Positive	48 (45.7)	57 (54.3)		73 (69.5)	32 (30.5)		29 (27.6)	76 (72.4)	
Histological type, no. (%)			0.168			0.541			0.785
ASC	2 (25.0)	6 (75.0)		6 (75.0)	2 (25.0)		2 (25.0)	6 (75.0)	
ADC	6 (28.6)	15 (71.4)		17 (81.0)	4 (19.0)		5 (23.8)	16 (76.2)	
SCC	49 (47.1)	55 (52.9)		72 (69.2)	32 (30.8)		32 (30.8)	72 (69.2)	
Differentiation, no. (%)			0.001			0.016			0.009
Well	26 (61.9)	16 (38.1)		35 (83.3)	7 (16.7)		17 (40.5)	25 (59.5)	
Moderate	20 (40.8)	29 (59.2)		35 (71.4)	14 (28.6)		16 (32.7)	33 (67.3)	
Poor	11 (26.2)	31 (73.8)		25 (59.5)	17 (40.5)		6 (14.3)	36 (85.7)	
Tumor size, no. (%)			0.099			0.062			0.165
<4 cm	33 (50.0)	33 (50.0)		52 (78.8)	14 (21.2)		23 (34.8)	43 (65.2)	
≥4 cm	24 (35.8)	43 (64.2)		43 (64.2)	24 (35.8)		16 (23.9)	51 (76.1)	
Lymph node metastasis, no. (%)			0.010			0.158			0.004
No	51 (48.6)	54 (51.4)		78 (74.3)	27 (25.7)		37 (35.2)	68 (64.8)	
Yes	6 (21.4)	22 (78.6)		17 (60.7)	11 (39.3)		2 (7.1)	26 (92.9)	
FIGO stage, no. (%)			0.004			0.013			0.031
IA1	11 (73.3)	4 (26.7)		14 (93.3)	1 (6.7)		8 (53.3)	7 (46.7)	
IA2	13 (65.0)	7 (35.0)		16 (80.0)	4 (20.0)		8 (40.0)	12 (60.0)	
IB1	3 (18.8)	13 (81.3)		10 (62.5)	6 (37.5)		3 (18.8)	13 (81.3)	
IB2	14 (41.2)	20 (58.8)		25 (73.5)	9 (26.5)		9 (26.5)	25 (73.5)	
IIA1	6 (40.0)	9 (60.0)		12 (80.0)	3 (20.0)		4 (26.7)	11 (73.3)	
IIA2	10 (30.3)	23 (69.7)		18 (54.5)	15 (45.5)		7 (21.2)	26 (78.8)	

Abbreviations: ADC, adenocarcinoma; ALDH1, aldehyde dehydrogenase 1; ASC, adenosquamous carcinoma; CD133, cluster of differentiation 133; FIGO, International Federation of Gynecology and Obstetrics; ITGA7, integrin subunit alpha 7; HPV, human papillomavirus; SCC, squamous cell carcinoma and the bold values represents data is a statistical significance.

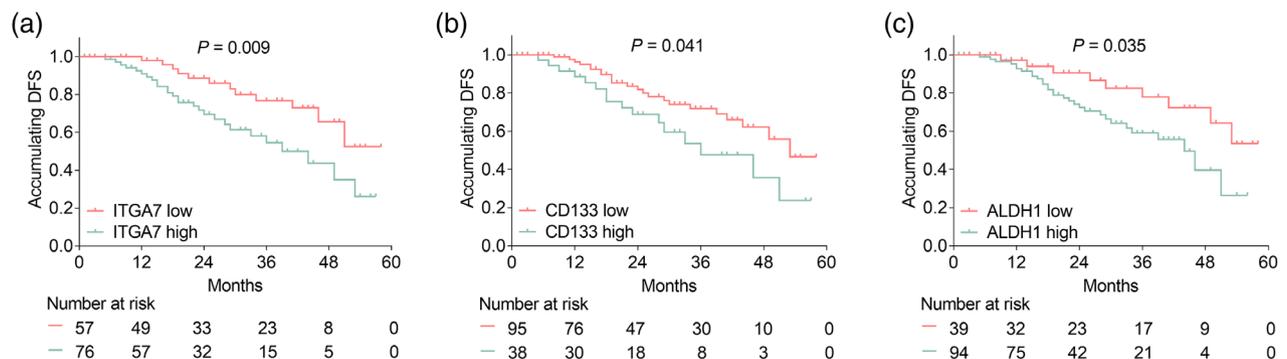


Figure 2 Correlation of ITGA7, CD133, and ALDH1 expressions with accumulating DFS (by log-rank test). Association of ITGA7 (a), CD133 (b), and ALDH1 (c) expressions with accumulating DFS. ALDH1: aldehyde dehydrogenase 1; CD133: cluster of differentiation 133; DFS: disease-free survival; ITGA7: integrin subunit alpha 7

patients with at least two expressions high also had shorter accumulating DFS compared with those with less than two expressions high ($p = 0.001$,

Figure S1b). Whereas, there was no difference in accumulating DFS between patients with three expressions high and those with less than three expressions

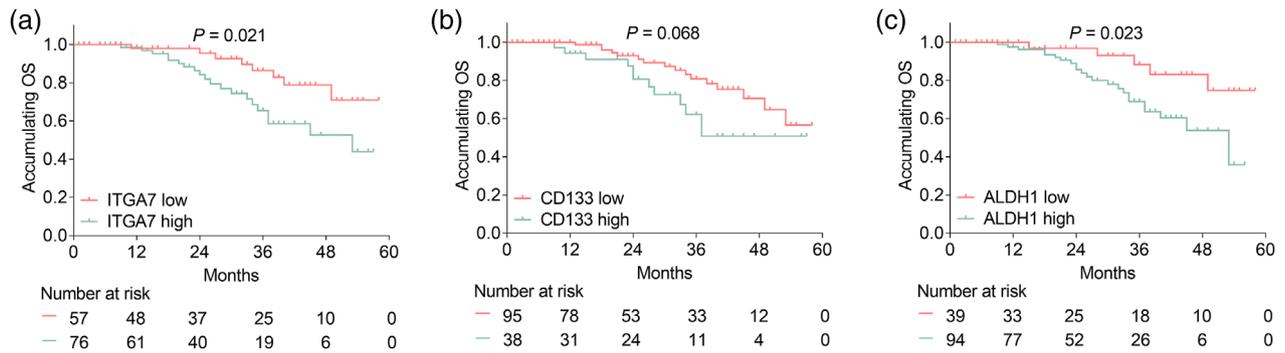


Figure 3 Correlation of ITGA7, CD133, and ALDH1 expressions with accumulating OS (by log-rank test). Association of ITGA7 (a), CD133 (b), and ALDH1 (c) expressions with accumulating OS. ALDH1, aldehyde dehydrogenase 1; CD133, cluster of differentiation 133; ITGA7, integrin subunit alpha 7; OS, overall survival

high ($p = 0.322$, Figure S1c). Besides, the similar trends were also observed in analyzing accumulating OS (Figure S2a-c).

Additionally, multivariate Cox's proportional hazards regression analyses showed that none of ITGA7, CD133, and ALDH1 was independent prognostic factor for DFS or OS in cervical cancer patients (Tables S2 and S3).

Discussion

ITGA7 is reported to be positively linked with the activation of CSCs in multiple carcinomas including tongue SCC, esophageal SCC, renal cell carcinoma as well as non-small-cell lung cancer.^{9,11–13} Whereas, the role of ITGA7 in regulating the stemness of cervical cancer cells thus further acting as a potential biomarker for the prognosis in cervical cancer patients remains elusive. Interestingly, in terms of cervical cancer, previous studies illustrate that CD133 and ALDH1 are associated with tumor stemness and further relate to the poor prognosis in cervical cancer.^{14,15,22–25} To be specific, CD133 is reported to facilitate the proliferation of CSC and initiate tumor growth in cervical cancer pathology.^{14,15,22,23} Similarly, it is revealed that ALDH1 is highly expressed in CSC cells and further serves as a biomarker indicating poor prognosis in cervical cancer.^{22,25} In the current study, it was revealed that ITGA7 expression was positively correlated with expression of CD133 and ALDH1. Possible reason would be that ITGA7 might serve as an oncogene, which inhibits the cell apoptosis as well as induces the cell metastasis and invasion via activating FAK/Akt signaling or binding with S100 calcium-binding protein, besides ITGA7 is

proved to be linked with CSC activity in many other neoplasms.^{9–13,26} In the meantime, CD133 and ALDH1 are tumor initiating genes and also serve as CSC surface markers according to the evidence mentioned above.^{24,25}

Therefore, ITGA7 expression is positively correlated with the expression of CD133 and ALDH1.

Preceding studies illustrate that ITGA7 overexpression correlates with advanced clinicopathological properties in diverse carcinomas.^{9,27,28} Some specific examples are as follows: in rectal cancer patients, ITGA7 overexpression correlates with higher pathological grade, larger tumor size, and advanced T stage²⁷; in breast cancer, ITGA7 overexpression is associated with higher TNM stage and pathological grade²⁸; what is more, another study illustrates that elevated expression of ITGA7 in OSCC tissues links with poor differentiation and higher risk of lymph node metastasis.⁹ In terms of CD133 and ALDH1, their overexpression also correlates with advanced clinicopathological properties in multiple cancers.^{14,22,24,29,30} In the present study, overexpression of ITGA7 and ALDH1 was correlated with poor differentiation and higher occurrence of lymph node metastasis. Whereas CD133 overexpression was only associated with poor differentiation. Underlying explanation could be that: Given that ITGA7 and ALDH1 facilitate the proliferation of CSC in multiple carcinomas.^{9,11–13,24} Besides, it is widely acknowledged that CSC has a tumor-initiating capacity and plays a vital role in promoting metastasis. Therefore, overexpression of ITGA7 and ALDH1 is correlated with higher occurrence of lymph node metastasis in cervical cancer patients.⁷ ITGA7, CD133, and ALDH1 mediate the CSC properties in multiple carcinomas.^{9,11–14,22,24,29,30} Therefore, overexpression of

ITGA7, CD133, and ALDH1 might be correlated with poor differentiation in cervical cancer pathology.

So far, former studies have revealed the correlation of ITGA7 expression with prognosis for various neoplasms.^{12,13} One study reveals that highly expressed ITGA7 is correlated with shorter event-free-survival and OS in non-small-cell lung cancer patients.¹³ Another study uncovers that ITGA7 links with worse OS in tongue SCC.¹² In the present study, overexpression of ITGA7 and ALDH1 was correlated with shorter accumulating DFS and OS whereas CD133 overexpression only correlated with DFS but not OS. Possible explanations could be that (1) ITGA7 and ALDH1 are linked with worse clinicopathological features such as higher occurrence of lymph node metastasis and poor differentiation in cervical cancer. Moreover, worse clinicopathological features might contribute to an unsatisfying survival profile. Consequently, the overexpression of ITGA7 and ALDH1 is correlated with a worse survival profile. (2) The proportion of CD133 positive cases is relatively small in our study, meanwhile there are few end-point events (death) during the follow up. Therefore, the statistical power is subsequently reduced. Hence for this purpose, CD133 overexpression is merely slightly correlated with OS.

There were some limitations in this study: (1) as a single-center study, a selection bias might exist, which could influence the applicability of the study findings. (2) As a cohort study, confounding bias should not be neglected which affected the assessment of prognostic value in terms of ITGA7, CD133, and ALDH1. (3) The underlying mechanism of ITGA7 in pathogenesis of cervical cancer needed to be validated in the future.

To sum up, ITGA7, CD133, and ALDH1 are not only inter-correlated, but also linked with poor differentiation, higher occurrence of lymph node metastasis as well as worse DFS or OS in cervical cancer patients, which implies that ITGA7, CD133, and ALDH1 could serve as prognostic indicators for cervical cancer patients' management.

Acknowledgments

This study was supported by Important Project of Medicine and Science Study in Hebei, China (20221352).

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;**71**(3):209–49.
2. Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Glob Health*. 2020;**8**(2):e191–203.
3. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. *Lancet*. 2019;**393**(10167):169–82.
4. Vu M, Yu J, Awolude OA, Chuang L. Cervical cancer worldwide. *Curr Probl Cancer*. 2018;**42**(5):457–65.
5. Paavonen J. Human papillomavirus infection and the development of cervical cancer and related genital neoplasias. *Int J Infect Dis*. 2007;**11**(Suppl 2):S3–9.
6. Mendoza-Almanza G, Ortiz-Sanchez E, Rocha-Zavaleta L, Rivas-Santiago C, Esparza-Ibarra E, Olmos J. Cervical cancer stem cells and other leading factors associated with cervical cancer development. *Oncol Lett*. 2019;**18**(4):3423–32.
7. Huang R, Rofstad EK. Cancer stem cells (CSCs), cervical CSCs and targeted therapies. *Oncotarget*. 2017;**8**(21):35351–67.
8. Boulton JE. Grey scale ultrasound appearances in hepatocellular carcinoma. *Clin Radiol*. 1979;**30**(5):547–52.
9. Ming XY, Fu L, Zhang LY, Qin YR, Cao TT, Chan KW, et al. Integrin alpha7 is a functional cancer stem cell surface marker in oesophageal squamous cell carcinoma. *Nat Commun*. 2016;**7**:13568.
10. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002;**110**(6):673–87.
11. Chen Y, Wang Y, Cai Z, Fan X, Zhang Y. Integrin alpha7 is overexpressed and correlates with higher pathological grade, increased T stage, advanced TNM stage as well as worse survival in clear cell renal cell carcinoma patients: a retrospective study. *J Clin Lab Anal*. 2020;**34**(1):e23034.
12. Lv Z, Yang Y, Yang C. Integrin alpha7 correlates with worse clinical features and prognosis, and its knockdown inhibits cell proliferation and stemness in tongue squamous cell carcinoma. *Int J Oncol*. 2020;**56**(1):69–84.
13. Xia D, Chen B, Yang X. Correlation of integrin alpha 7 with clinicopathological characteristics and survival profiles, as well as its regulatory role in cell proliferation, apoptosis, and stemness in non-small-cell lung cancer. *J Clin Lab Anal*. 2019;**33**(8):e22973.
14. Javed S, Sharma BK, Sood S, Sharma S, Bagga R, Bhattacharyya S, et al. Significance of CD133 positive cells in four novel HPV-16 positive cervical cancer-derived cell lines and biopsies of invasive cervical cancer. *BMC Cancer*. 2018;**18**(1):357.

15. Leung CON, Deng W, Ye TM, Ngan HYS, Tsao SW, Cheung ANY, et al. MicroRNA-135a-induced formation of CD133+ subpopulation with cancer stem cell properties in cervical cancer. *Carcinogenesis*. 2020;**41**(11):1592–604.
16. Sudhalkar N, Rathod NP, Mathews A, Chopra S, Sriram H, Shrivastava SK, et al. Potential role of cancer stem cells as biomarkers and therapeutic targets in cervical cancer. *Cancer Rep (Hoboken)*. 2019;**2**(2):e1144.
17. Yao T, Weng X, Yao Y, Huang C, Li J, Peng Y, et al. ALDH1-positive cells exhibited a radioresistant phenotype that was enhanced with hypoxia in cervical cancer. *BMC Cancer*. 2020;**20**(1):891.
18. Koh WJ, Greer BE, Abu-Rustum NR, et al. Cervical cancer, version 2.2015. *J Natl Compr Cancer Netw*. 2015;**13**(4):395–404; quiz.
19. Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. *Int J Gynaecol Obstet*. 2009;**105**(2):107–8.
20. Ferrandina G, Martinelli E, Petrillo M, Prisco MG, Zannoni G, Sioletic S, et al. CD133 antigen expression in ovarian cancer. *BMC Cancer*. 2009;**9**:221.
21. Kim SJ, Kim YS, Jang ED, Seo KJ, Kim JS. Prognostic impact and clinicopathological correlation of CD133 and ALDH1 expression in invasive breast cancer. *J Breast Cancer*. 2015;**18**(4):347–55.
22. Liu CT, Xin Y, Tong CY, et al. Production of interleukin4 in CD133+ cervical cancer stem cells promotes resistance to apoptosis and initiates tumor growth. *Mol Med Rep*. 2016;**13**(6):5068–76.
23. Kwon T, Bak Y, Ham SY, Yu DY, Yoon DY. A1E reduces stemness and self-renewal in HPV 16-positive cervical cancer stem cells. *BMC Complement Altern Med*. 2016;**16**:42.
24. Liu C, Zhang Y, Liang S, Ying Y. Aldehyde dehydrogenase 1, a target of miR-222, is expressed at elevated levels in cervical cancer. *Exp Ther Med*. 2020;**19**(3):1673–80.
25. Wang L, Liu Y, Zhou Y, Wang J, Tu L, Sun Z, et al. Zoledronic acid inhibits the growth of cancer stem cell derived from cervical cancer cell by attenuating their stemness phenotype and inducing apoptosis and cell cycle arrest through the Erk1/2 and Akt pathways. *J Exp Clin Cancer Res*. 2019;**38**(1):93.
26. Hsu YL, Hung JY, Liang YY, Lin YS, Tsai MJ, Chou SH, et al. S100P interacts with integrin alpha7 and increases cancer cell migration and invasion in lung cancer. *Oncotarget*. 2015;**6**(30):29585–98.
27. Huang J, Tian Y, Chen J. Overexpression of integrin alpha7 correlates with advanced disease condition and poor prognosis in rectal cancer patients. *Int J Clin Exp Pathol*. 2018;**11**(9):4630–6.
28. Bai X, Gao C, Zhang L, Yang S. Integrin alpha7 high expression correlates with deteriorative tumor features and worse overall survival, and its knockdown inhibits cell proliferation and invasion but increases apoptosis in breast cancer. *J Clin Lab Anal*. 2019;**33**(8):e22979.
29. Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. *Oncotarget*. 2016;**7**(10):11018–32.
30. Wei D, Peng JJ, Gao H, Zhang T, Tan Y, Hu YH. ALDH1 expression and the prognosis of lung cancer: a systematic review and meta-analysis. *Heart Lung Circ*. 2015;**24**(8):780–8.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Association of co-expression of ITGA7, CD133, and ALDH1 with DFS (by log-rank test). Comparison of DFS between patients with at least one expression high and patients with no one expression high (a); Comparison of DFS between patients with at least two expressions high and patients with less than two expressions high (b); Comparison of DFS between patients with three expressions high and patients with less than three expressions high (c). ITGA7: integrin subunit alpha 7; CD133: cluster of differentiation 133; ALDH1: aldehyde dehydrogenase 1; DFS: disease-free survival

Figure S2 Association of co-expression of ITGA7, CD133, and ALDH1 with OS (by log-rank test). Comparison of OS between patients with at least one expression high and patients with no one expression high (a); Comparison of OS between patients with at least two expressions high and patients with less than two expressions high (b); Comparison of OS between patients with three expressions high and patients with less than three expressions high (c). ITGA7: integrin subunit alpha 7; CD133: cluster of differentiation 133; ALDH1: aldehyde dehydrogenase 1; OS: overall survival

Table S1 Lymph node metastasis of patients. FIGO, International Federation of Gynecology and Obstetrics

Table S2 Cox's proportional hazards regression analysis for DFS. ADC, adenocarcinoma; ALDH1, aldehyde dehydrogenase 1; ASC, adenosquamous carcinoma; CD133, cluster of differentiation 133; CI, confidence interval; DFS, disease-free survival; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; HR, hazard ratio; ITGA7, integrin subunit alpha 7; SCC, squamous cell carcinoma

Table S3 Cox's proportional hazards regression analysis for OS. ADC, adenocarcinoma; ASC, adenosquamous carcinoma; CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; HPV, human papillomavirus; HR, hazard ratio; OS, overall survival; SCC, squamous cell carcinoma