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The clinical significance of HERV-H LTR –associating 2 expression in cervical adenocarcinoma

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Abstract

HERV-H LTR –associating 2 (HHLA2) is a recently discovered member of the B7-family of immune checkpoint molecules that is overexpressed in several types of cancer. The aim of the present study was to investigate the expression of HHLA2 in cervical adenocarcinoma (AC) and the relationship between its expression and clinicopathological factors to assess its use as a potential marker for AC prognosis.

This study included 76 patients diagnosed with cervical AC. Their resected specimens were obtained and a tissue microarray was constructed. Expression of HHLA2 was detected by the immunohistochemistry. Based on the follow-up data, correlation of HHLA2 expression and clinicopathological features, including overall survival (OS) and disease-free survival, was evaluated. Furthermore, we investigated the correlation between the expression of HHLA2 and programmed death ligand 1 (PD-L1).

A total of 76 cases of invasive cervical AC were evaluated. High HHLA2 expression was detected in 62 cases (81.6%) and low HHLA2 expression was presented in 14 cases (18.4%). HHLA2 expression showed a significant negative correlation with lymph node metastasis ($P = .011$). Disease free survival was 75.0% and 49.0% in high-expression and the low expression group, respectively ($P = .057$). Although there was no statistical significance, an improved OS was observed in the high expression group (83.1% vs 64.9%, $P = .479$). Further, the expression of HHLA2 and PD-L1 correlated positively ($P = .005$). Thus, an improved OS was observed in the PD-L1 expression group (90.7% vs 66.2%, $P = .037$).

High expression of HHLA2 is related to tumor progression and prognosis in patients with cervical AC. Therefore, HHLA2 may be a potential biomarker for predicting prognosis of cervical AC.

Abbreviations: AC = adenocarcinoma, DFS = disease free survival, HHLA2 = HERV-H LTR –associating 2, OS = overall survival, PD-L1 = programmed death ligand 1, SCC = squamous cell carcinoma, TMA = tissue microarray.

Keywords: cervical adenocarcinoma, HERV-H LTR –associating 2, programmed death ligand 1, prognosis

1. Introduction

Cervical cancer is the fourth most common cancer in women. Although the proportion of cervical cancer deaths among all cancer-related deaths has decreased from 8.2% in 2008 to 7.5% in 2018, it is still the fourth leading cause of cancer death.[1] Although overall incidence of cervical cancer has declined, the incidence of cervical adenocarcinoma (AC) has been steadily increasing, particularly among women in their 20s and 30s.[2,3] In 1 study,[4] patients with AC were shown to have a poorer outcome than for those with cervical squamous cell carcinoma (SCC), with more incidences of distant recurrence. Thus, Marvin et al reported a lower sensitivity to radiotherapy (RT) and higher recurrence following RT in patients with cervical AC compared with those with cervical SCC.[5] Therefore, to improve the prognosis of cervical AC patients, novel therapeutic strategies need to be developed and established.

The B7/CD28 families of immune-regulatory ligands/receptors are well known for their potential roles in cancer pathogenesis. The B7 family consists of 3 phylogenetic subgroups; first, B7-1, B7-2, and ICOS-L; second, Programmed Death Ligand 1 (PD-L1) and 2 (PD-L2); and third, B7-H3, B7x, and HERV-H LTR-associating 2 (HHLA2).[6] These ligands are typically expressed on antigen presenting cells (APCs) and have the ability to modulate T-cell proliferation and function.[7] Immunotherapies targeting immune checkpoint have led to important clinical advances and provided novel treatment strategies against cancer.[8,9]

In cervical cancer, PD-L1 is expressed in approximately 35% of cervical SCC and 17% of cervical AC.[10] In cervical cancer, diffuse PD-L1 expression as compared to marginal PD-L1 expression on the interface between tumor and stroma, was associated with poor disease-free and diseases-specific survival rates.[11] However, higher numbers of infiltrating regulatory T cells in PD-L1 positive tumors showed positive association with better prognosis.[12] At present, pembrolizumab has been approved by the FDA for patients with recurrent or metastatic cervical cancer expressing PD-L1.[11]

HHLA2 was discovered in 1999 as a new member of the immunoglobulin (Ig) superfamily.[13] It is constitutively expressed on the surface of human monocytes and is induced on B cells.[6,14,15] HHLA2 binds to its putative receptor(s) on a variety of immune cells, including CD4 and CD8 T cells and antigen-presenting cells,[6] and it suppresses CD4 and CD8 T-cell function in the presence of T-cell receptor signaling.[13–15] HHLA2 is reported to have both, co-inhibitory[6] and co-stimulatory functions.[14]

Recent research has reported that HHLA2 has limited expression in normal tissues but is widely expressed in different human cancers including osteosarcoma, triple negative breast cancer, and colorectal cancer.[16–18] To date, HHLA2 expression in cervical AC has not been studied and the prognostic value of HHLA2 in patients with cervical cancer and the association between HHLA2 and clinicopathological characteristics remains unknown.

Therefore, the aim of the present study was to assess the expression of HHLA2 in AC and analyze the association between HHLA2 and clinicopathological characteristics. Furthermore, we investigated the correlation between the expression of HHLA2 and PD-L1.

2. Material and methods

2.1. Case selection and tissue microarray (TMA)

This retrospective study using patients' formalin-fixed paraffin blocks was approved by the Institutional Review Board of Busan Paik Hospital, Republic of Korea (IRB No. 17-0136). Written informed consent was obtained from all women who participated in the study. We collected formalin-fixed paraffin-embedded samples from 78 patients who underwent hysterectomy for cervical AC at Inje University Busan Paik Hospital (Busan, Republic of Korea) between 2005 and 2016. Sixty-five patients were diagnosed as usual type endocervical AC and 13 patients were diagnosed as other variants of endocervical AC. All hematoxylin and eosin stained slides were reviewed by 2 pathologists (HJ Cho and HY Park).

We made 2 sets of TMA blocks from a representative area of each tumor by using a 3-mm hole punch machine. In the process of making TMA, 2 cases of usual type endocervical AC were lost because of the drop out of the tissue cores. Thus, 76 cases of endocervical AC were included in the analysis.

2.2. HHLA2 and PD-L1 immunohistochemistry

For HHLA2 immunohistochemical staining, we used HHLA2 rabbit polyclonal antibody (Thermo Fisher Scientific, IL) at a 1:250 dilution on the Ventana Benchmark ULTRA system. We scored the intensity of the HHLA2 immunostain in the tumor membrane and/or cytoplasm as 0 (negative), 1+(weak), 2+(moderate), or 3+(strong), and divided the samples into low expression (0 or weak intensity) or high expression (moderate or strong intensity) groups.

For PD-L1 immunohistochemistry, we used anti-PD-L1 (SP263) rabbit monoclonal primary antibody (Ventana Medial Systems, Tucson, AZ, USA) with an OptiView DAB IHC Detection Kit followed by an OptiView Amplification Kit and analyzed the results on the Ventana Benchmark ULTRA system. We assessed PD-L1 expression in the tumor cell membrane from 0% to 100% in terms of tumor proportion score.

2.3. Data analysis

The data was analyzed for patient age at diagnosis, tumor histotype, depth of invasion (mm), tumor size, histologic grade, LVI, LN metastasis, pathologic staging as per the International Federation of Gynecology and Obstetrics guideline, HPV infection, Silva classification, PD-L1 expression, recurrence, and survival according to the expression of HHLA2.

Based on follow-up data, correlations between HHLA2 expression and clinicopathological features, including overall survival (OS) and disease-free survival, in cervical AC patients were evaluated.

2.4. Statistical analyses

Statistical analyses were performed using MedCalc version 14.8.1 (Frank Schoonjans, Ghent University, Belgium). Categorical variables were compared using chi-square test and Fisher exact test. The mean, median, and standard deviation were calculated for continuous variables, and were compared using the Mann-Whitney U test for two groups and the Kruskal-Wallis test for 3 or more unmatched groups. Kaplan-Meier plots and the log-rank test were used to compare the independent variables and
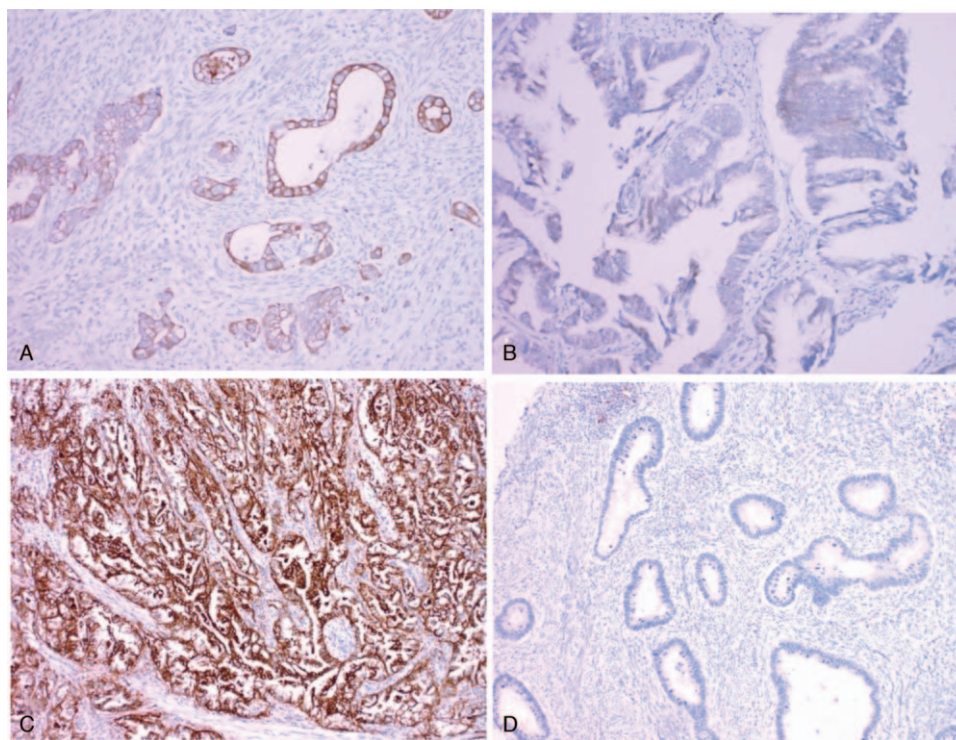
Figure 1. Immunohistochemical analysis with HHLA2 and PD-L1. (A) Strong (3+) and diffuse HHLA2 expression in the membranous pattern (B) Weak (1+) and patchy expression of HHLA2 (C) PD-L1-positive tumor proportion score 100% (D) PD-L1 tumor proportion score 0%. (A & B, HHLA2 immunohistochemical stain; C & D, PD-L1 stain; A, B & D, X200 magnification; C, x100 magnification).

survival. Univariate and multivariate Cox proportional hazard models were used to assess the relationship between the independent and dependent variables. Results with P value of less than .05 was considered statistically significant.

3. Results

3.1. HHLA2 and PD-L1 expression in cervical AC

HHLA2 protein expression was investigated by immunohistochemistry of 76 tumor tissues. HHLA2 protein was observed in both membranous and cytoplasmic expression in cancer cell. We assessed the membranous HHLA2 expression and HHLA2 staining intensity of cancer cells was graded as absent, week, intermediate or strong (Fig. 1A, B). The strong and intermediate groups were combined to 1 group that we labelled as high HHLA2 expression, and the week and absent groups were classified low HHLA2 expression group. In 97.4% of total tumors, HHLA2 expression was observed. Overall, 81.6% of tumors graded as high expression group and, as low expression group in 18.4% of tumors (Table 1). And PD-L1 expression in the tumor cell membrane from 0% to 100% in terms of tumor proportion score was assessed (Fig. 1C, D). PD-L1 expression showed in 36 patients (47.4%) and, 54.9% of patients with high HHLA2 expression were co-expressed PD-L1.

3.2. HHLA expression and clinicopathological characteristics

The correlation between HHLA2 expression and clinicopathologic characteristics in AC was analyzed. HHLA2 was expressed in the majority of AC patients. Based on the intensity of HHLA2 immunostain, 76 patients with AC were divided into a high-expression group (n=62) and a low-expression group (n=14) to investigate the correlation between HHLA2 expression and clinicopathological characteristics.

HHLA2 expression showed a significant negative correlation with LN metastasis ($P=.011$). However, HHLA2 expression did not show any significant correlation with age, HPV infection, stage, tumor grade, invasion depth, LVSI, tumor size, or Silva classification in patients with AC (Table 1).

3.3. Co-expression of HHLA2 and PD-L1

HHLA2 expression was associated with PD-L1 expression. Thirty-four patients (54.9%) in the high-expression group showed positive expression of PD-L1. Moreover, 12 patients (85.7%) in the low-expression group did not express PD-L1. There was significant positive correlation between HHLA2 expression and PD-L1 expression ($P=.006$) (Table 2).

This finding was further confirmed by using the Spearman correlation analysis to test the correlation between HHLA2 expression and the clinicopathological features. As shown in Table 2, the Spearman correlations of HHLA2 expression levels to LN metastasis and PD-L1 expression were -0.295 ($P=.009$) and 0.315 ($P=.005$), respectively. Collectively, the expression of HHLA2 was negatively correlated with LN metastasis, a prognostic factor but was positively correlated with PD-L1 expression.

3.4. Prognostic value of HHLA2 and PD-L1 expression

In our study, 14 (22.6%) of 62 patients with high HHLA2 expression had incidence of recurrence and 7 (50%) of 14

Table 1

Correlation between HHLA2 expression and clinicopathological characteristics of AC patients.

Characteristics	HHLA2 expression (n, %)		P value
	Low (14, 18.4)	High (62, 81.6)	
Age	46.1 ± 8.7	49.6 ± 9.7	.229
HPV infection	13 (18.6%)	57 (81.4%)	.811
No	3 (23.1)	15 (26.3)	
Yes	10 (76.9)	42 (73.7)	
Stage			.898
I	10 (71.4)	48 (77.4)	
II	4 (28.6)	14 (22.6)	
LN metastasis			.011*
No	6 (42.9)	48 (77.4)	
Yes	8 (57.1)	14 (22.6)	
Tumor grade			.191
1	4 (36.4)	31 (51.7)	
2	2 (18.2)	17 (28.3)	
3	5 (45.5)	12 (20.0)	
Invasion depth			.807
<1/3	3 (21.4)	14 (24.6)	
>1/3	11 (78.6)	43 (75.4)	
LVSI			.441
No	7 (50)	38 (61.3)	
Yes	7 (50)	24 (38.7)	
Tumor size			.573
<2 cm	2 (14.3)	13 (21.0)	
> 2 cm	12 (85.7)	49 (79.0)	
Silva classification			.915
A	3 (23.1)	17 (28.3)	
B	4 (30.8)	16 (26.7)	
C	6 (46.2)	27 (45.0)	
PD-L1 expression			.006*
NO	12 (85.7)	28 (45.1)	
Yes	2 (14.3)	34 (54.9)	

LVSI = lymphovascular space involvement, LN = lymph node.
* P < .05.

patients with low HHLA2 expression had incidence of recurrence. The recurrence rate was significantly lower in the high-expression group than in the low-expression group (P = .039). OS increase by approximately 9 months in the high expression group, but we did not observe a statistically significant difference (Table 3).

Since HHLA2 expression is positively correlated with PD-L1 expression, the recurrence and survival rates were analyzed according to the PD-L1 expression. Although recurrence rate was

Table 2

Spearman correlation analysis between HHLA2 expression and prognostic factors of AC.

Variables	HHLA2 expression	
	Spearman correlation	P value
Tumor grade	−0.180	.132
Depth of invasion	−0.029	.809
LN metastasis	−0.295	.009*
LVI	−0.089	.444
PD-L1 expression	0.315	.005*
Silva classification	−0.029	.804
Stage	−0.055	.639
Tumor size	−0.065	.576

HHLA2 = HERV-H LTR –associating 2, LVSI = lymphovascular space involvement, LN = lymph node.
* P < .05.

lower in the PD-L1 expression group than in the no expression group, there was no significant difference. However, survival was significantly higher in the PD-L1 expression group than in the no expression group (P = .032) (Table 3).

The overall survival (OS) rate was higher in the HHLA2 high-expression group than in the low-expression group, but there was no statistically significant difference (83.1% vs 64.9%, respectively; log-rank test, P = .479). The disease free survival (DFS) rate was higher in the HHLA2 high-expression group with borderline statistical significance (75.0% vs 49.0%, respectively; log-rank test, P = .057) (Fig. 2A).

The OS and DFS rates were 90.7% and 66.2%, respectively, in the PD-L1 expression group, whereas the OS and DFS rates were only 66.2% and 62.9%, respectively, in the no expression group (Fig. 2B). The OS rate was significantly higher in the PD-L1 expression group compared with that in the negative expression group (log-rank test, P = .037) (Fig. 2B).

Univariate and multivariate analyses of the Cox pro-portional hazard model on DFS and OS were performed to determine the prognostic value of HHLA2 expression and other clinicopathological variables. In univariate analysis, HHLA2 expression showed borderline significant correlation to the DFS of patients with AC (HR = 0.33, 95% CI = 0.11–1.03, P = .057). Meanwhile, LN metastasis (HR = 38.7, 95% CI = 13.05–114.93, P < .001), stage (HR = 20.8, 95% CI = 6.27–64.31, P < .001), Silva classification (HR = 2.18, 95% CI = 0.72–6.52, P = .002), and parametrial involvement (HR = 5.29, 95% CI 1.23–22.78, P = .025) were found to be associated with DFS (Table 4.1). In the multivariate analysis, LN metastasis was an independent prognostic factor for DFS of patients with cervical AC (HR = 6.42, 95% CI = 1.79–23.07, P = .004) (Table 4).

Univariate analysis revealed LN metastasis (HR = 15.07, 95% CI = 4.34–52.31, P < .0001), PD-L1 expression (HR = 0.32, 95% CI = 0.12–0.94, P = .037), stage (HR = 10.04, 95% CI = 2.48–40.63, P = .001), Silva classification (HR = 7.09, 95% CI = 1.68–29.87, P = .003), and parametrial involvement (HR = 8.83, 95% CI 1.49–52.26, P = .016) to be associated with OS. LN metastasis was an independent factor of OS (HR = 4.62, 95% CI = 1.08–19.73, P = .039), and PD-L1 expression showed borderline significance as an independent factor of OS (HR = 0.28, 95% CI = 0.07–1.10, P = .069) (Table 5).

4. Discussion

To the best of our knowledge, this is the first study to report the expression and clinical significance of HHLA2 in cervical AC. In our study, HHLA2 was expressed in the majority of cervical AC and usually co-expressed with PD-L1. The observed co-expression of HHLA2 and PD-L1 was similar to the pattern reported in osteosarcoma.[17] However, unlike osteosarcoma, HHLA2 and PD-L1 expression in cervical AC is associated with an improved prognosis.[17] Higher HHLA2 expression is associated with delayed cancer recurrence and PD-L1 expression is associated with prolonged survival. Moreover, PD-L1 expression showed borderline significance as an independent factor for prognosis in cervical AC.

In humans, HHLA2 expression has been investigated in several cancers.[16–20] High expression of HHLA2 was associated with worse clinical outcomes in breast cancer, colorectal carcinoma, and osteosarcoma.[16–18] Unlike these cancers, the pattern of HHLA2 expression in pancreatic[19] and gastric[20] cancer was similar to those of cervical AC in our study. Higher HHLA2
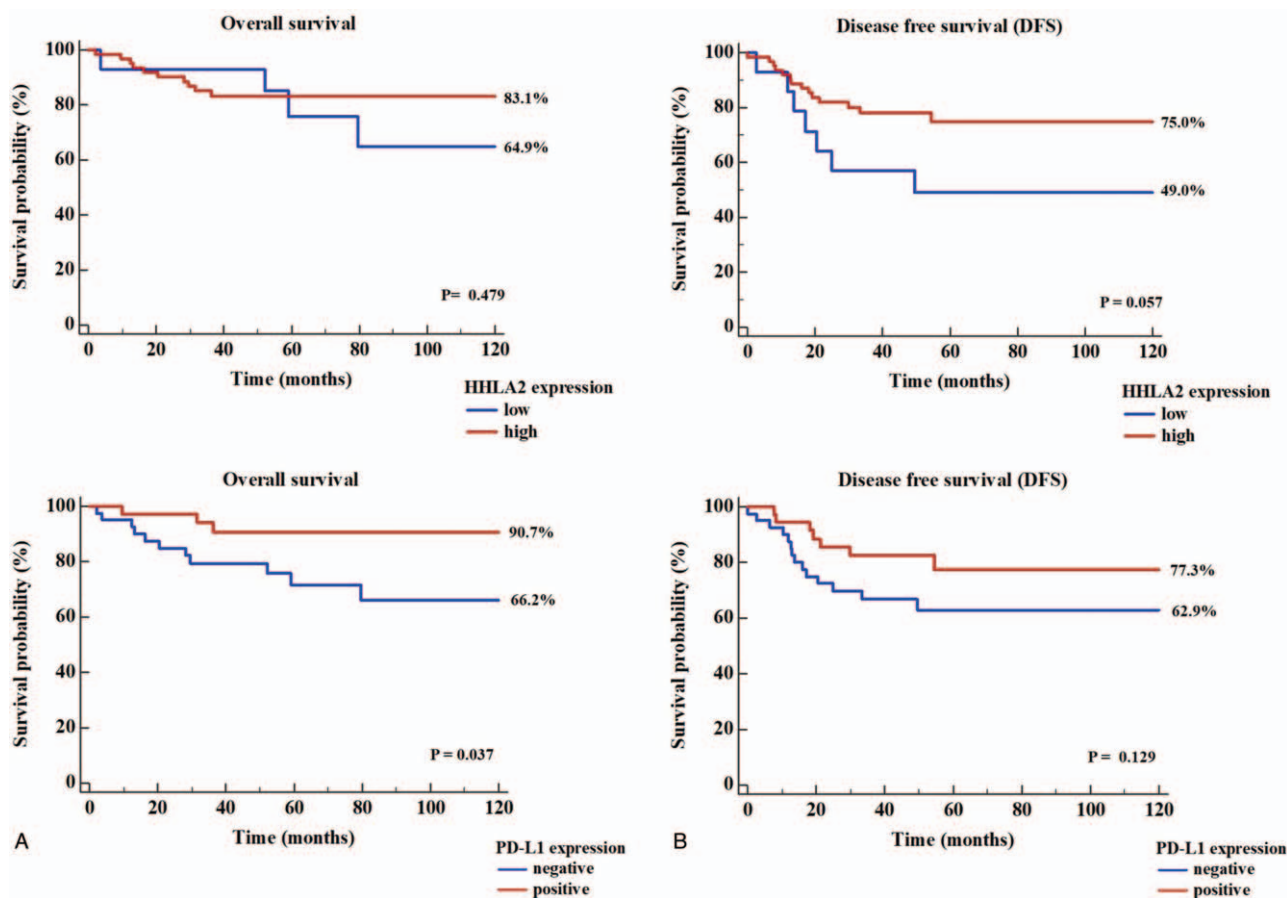
Figure 2. Prognosis according to HHLA2 expression and PD-L1 expression. (A) Overall survival (OS) and DFS according to HHLA2 expression. (A) OS rate was lower in patients with low expression of HHLA2 but there was no statistically significant difference (*P* = .479). (B) DFS was higher in patients with high HHLA2 expression but there was borderline statistical significance (*P* = 0.057). (B) OS and DFS according to PD-L1 expression. (A) OS rate was lower in patients with negative expression of PD-L1 and was higher with positive expression of PD-L1 (*P* = 0.037). (B) DFS was higher in patients with positive expression of PD-L1 expression but there was no statistically significant difference (*P* = .129).

expression in pancreatic cancer was significantly associated with delated cancer recurrence and improved survival.[19] In gastric cancer, the 5-year survival rate was significantly higher in patients with high HHLA2 expression compared to patients with low expression.[20]

In the present study, high HHLA2 expression was negatively correlated with LN metastasis and positively correlated with PD-L1 expression. Higher HHLA2 expression was also associated with lower recurrent rate. Although there was no statistical significance, an improved OS was observed in the high expression

group (83.1% vs 64.9%, *P* = .479). Further, PD-L1 expression was related with longer survival in cervical AC (90.7% vs 66.2%, *P* = .037), and although, PD-L1 expression group had lower recurrent rate, the results were not statistically significant.

Generally, PD-L1 is expressed in about 17%[10] of cases of cervical AC, but in our study, 47.4% of the cases of AC expressed PD-L1. PD-L1 expression on tumor cells is induced by cytokines, such as IFN-γ, secreted by tumor-infiltrating T cells.[21] Petrick et al[19] reported that tumors with high HHLA2 expression contained a higher numbers of tumor-infiltrating CD8 + T cells.

Table 3

Prognosis according to HHLA2 and PD-L1 expression.

	HHLA2 expression			PD-L1 expression		
	Low (n = 14, %)	High (n = 62, %)	P value	Negative (n = 40, %)	Positive (n = 36, %)	P value
Recur			.039*			.132
NO	7 (50.0)	48 (77.4)		26 (65.0)	29 (80.6)	
Yes	7 (50.0)	14 (22.6)		14 (35.0)	7 (19.4)	
Survival (months)	105.5 ± 21.2	116.3 ± 5.6	.281	101.9 ± 8.5	124.2 ± 5.5	.032*
NO	4 (28.6)	10 (16.1)		11 (27.5)	3 (8.3)	
Yes	10 (71.4)	52 (83.9)		29 (72.5)	33 (91.7)	

HHLA2 = HERV-H LTR –associating 2, PD-L1 = programmed death ligand 1.

Table 4

Univariate and multivariate analysis of disease free survival in AC using a Cox-regression model.

Factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
LN metastasis	38.7 (13.05–114.93)	< 0.001*	6.42 (1.79–23.07)	.004*
HHLA2 expression	0.33 (0.11–1.03)	0.057	1.07 (0.34–3.39)	.908
PD-L1 expression	0.51 (0.21–1.21)	0.129		
Tumor size	1.53 (0.54–4.34)	0.421		
Stage	20.8 (6.27–64.31)	< 0.001*	2.42 (0.81–7.21)	.110
Silva classification	2.18 (0.72–6.52)	0.002*	1.88 (0.71–4.94)	.202
Vaginal resection margin	1.71 (0.40–7.32)	0.467		
Parametrial invasion	5.29 (1.23–22.78)	0.025*	1.38 (0.34–5.69)	.654

AC = adenocarcinoma, CI = confidence interval, LN = lymph node.
* $P < .05$.

Futher, they suggested that HHLA2 expression in pancreatic and ampullary tumor cells can also be induced by tumor infiltrating T cells, and in fact may be regarded as a sign of an active immune response. This hypothesis find support in observation that increased HHLA2 expression is associated with improved survival.[19]

In the present study, HHLA2 and PD-L1 expression were associated with improved survival and prognosis in cervical AC. Although the analysis of tumor infiltrating T cells in cervical cancer was not performed, other reports have reported that tumor infiltrating cells are also expressed in cervical cancer.[22] Karim et al[22] showed more than half of TIL expressed PD-L1 and only 19% of tumor cells expressed PD-L1 in cervical cancers. In addition, the expression of PD-L1 did not show a direct impact on patient survival, but patients with a relative excess of infiltrating regulatory T cells had a better survival when the tumor was PD-L1 positive. TIL can also play a role in predicting response to anti-PD-L1 therapies,[23] so evaluating the extent and functional status of TIL can be complimentary to PD-L1 expression in cancer.

HHLA2 modulates T-cell functions through interaction with transmembrane and immunoglobulin domain containing 2 (TMIGD2) and an unknown second receptor involved with co-stimulation and co-inhibition in cancers.[24] HHLA2 expressed on APCs or tumor cells costimulates naïve T-cell proliferation and cytokine production through TMIGD2 via serine-threonine kinase AKT phosphorylation[14] and an unknown second receptor for HHLA2 on activated T cells exerts a coinhibitory function.[6]

Based on our results, we speculate that HHLA2 exerts a costimulatory effect on the T cell-mediated immune response via TMIGD2 in cervical AC patients. In our study, DFS was 75.0% and 49.0% in high-expression group and in the low- expression group, respectively ($P = .057$). Although there was no statistical significance, OS was improved in high-expression group (83.1% vs 64.9%, $P = .479$). Further, the expression of HHLA2 and PD-L1 was also positively correlated. Thus, OS in PD-L1 expression group was improved (90.7% vs. 66.2%, $P = .037$). Multivariate analysis demonstrated that PD-L1 expression showed borderline significance as an independent factor for OS (HR = 0.28, 95% CI = 0.07–1.10, $P = .069$).

There are some limitations in our study. The analysis of tumor infiltrating T cells in PD-L1 expression was not performed, and mechanism as co-stimulatory effect of HHLA2 was not evaluated. And, this study was conducted in a single institution and was based on a retrospective analysis of a small population. Validation studies with larger samples sizes are needed to confirm our results.

HHLA2 may as a prognostic factor with borderline significance but not an independent prognostic factor in our study. HHLA2 may be a potential biomarker for predicting prognosis in cervical AC. Although the immunological role of the HHLA2 signaling pathway is unclear, our results suggest that HHLA2 could be a co-stimulator of cervical AC.

In conclusion, the findings of this study demonstrate that HHLA2 expression is related to prognosis in patients with cervical AC, and may be a potential biomarker for predicting prognosis in patients with cervical AC.

Table 5

Univariate and multivariate analysis of overall survival in AC using a Cox-regression model.

Factors	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P-value	HR (95% CI)	P-value
LN metastasis	15.07 (4.34–52.31)	< .001*	4.62 (1.08–19.73)	.039*
HHLA2 expression	0.62 (0.17–2.28)	.479		
PD-L1 expression	0.32 (0.12–0.94)	.037*	0.28 (0.07–1.10)	.069
Tumor size	1.47 (0.40–5.38)	.558		
Stage	10.04 (2.48–40.63)	.001*	2.26 (0.61–8.45)	.224
Silva classification	7.09 (1.68–29.87)	.003*	2.94 (0.72–12.04)	.132
Vaginal resection margin	3.97 (0.66–23.66)	.130		
Parametrial invasion	8.83 (1.49–52.26)	.016*	0.72 (0.13–3.8)	.697

AC = adenocarcinoma, CI = confidence interval, LN = lymph node.
* $P < .05$.

Author contributions

JMB, HJC, IHC and DHJ designed the study; JMB searched database and reviewed studies; HJC and HYP reviewed the histologic specimen and studies; JMB, YNK, KBL, DSL, CHJ and MSS collected and analyzed data; JMB, DHK, DHI and BJM constructed the tables and figures; JMB, HJC and DHJ wrote the manuscript. All authors critically reviewed the manuscript and approved of the final draft, tables and figures. This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal.

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References

[1] Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health 2019;8:e191–203.

[2] Smith HO, Tiffany MF, Qualls CR, et al. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States—a 24-year population-based study. Gynecologic Oncol 2000;78:97–105.

[3] Hopkins MP, Morley GW. A comparison of adenocarcinoma and squamous cell carcinoma of the cervix. Obstet Gynecol 1991;77:912–7.

[4] Jung EJ, Byun JM, Kim YN, et al. Cervical adenocarcinoma has a poorer prognosis and a higher propensity for distant recurrence than squamous cell carcinoma. Int J Gynecol Cancer 2017;27:1228–36.

[5] Rotman M, Sedlis A, Piedmonte MR, et al. A phase III randomized trial of postoperative pelvic irradiation in Stage IB cervical carcinoma with poor prognostic features: follow-up of a gynecologic oncology group study. Int J Radiat Oncol Biol Phys 2006;65:169–76.

[6] Zhao R, Chinai JM, Buhl S, et al. HHLA2 is a member of the B7 family and inhibits human CD4 and CD8 T-cell function. Proc Natl Acad Sci U S A 2013;110:9879–84.

[7] Zang X, Allison JP. The B7 family and cancer therapy: costimulation and coinhibition. Clin Cancer Res 2007;13(18 Pt 1):5271–9.

[8] Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56–61.

[9] Pennock GK, Chow LQ. The evolving role of immune checkpoint inhibitors in cancer treatment. Oncologist 2015;20:812–22.

[10] Reddy OL, Shintaku PI, Moatamed NA. Programmed death-ligand 1 (PD-L1) is expressed in a significant number of the uterine cervical carcinomas. Diagn Pathol 2017;12:45.

[11] Heeren AM, Punt S, Bleeker MC, et al. Prognostic effect of different PD-L1 expression patterns in squamous cell carcinoma and adenocarcinoma of the cervix. Mod Pathol 2016;29:753–63.

[12] Saglam O, Conejo-Garcia J. PD-1/PD-L1 immune checkpoint inhibitors in advanced cervical cancer. Integr Cancer Sci Ther 2018;5:

[13] Mager DL, Hunter DG, Schertzer M, et al. Endogenous retroviruses provide the primary polyadenylation signal for two new human genes (HHLA2 and HHLA3). Genomics 1999;59:255–63.

[14] Zhu Y, Yao S, Iliopoulou BP, et al. B7-H5 costimulates human T cells via CD28H. Nat Commun 2013;4:2043.

[15] Flajnik MF, Tlapakova T, Criscitiello MF, et al. Evolution of the B7 family: co-evolution of B7H6 and NKp30, identification of a new B7 family member, B7H7, and of B7's historical relationship with the MHC. Immunogenetics 2012;64:571–90.

[16] Janakiram M, Chinai JM, Fineberg S, et al. Expression, clinical significance, and receptor identification of the newest B7 family member HHLA2 protein. Clin Cancer Res 2015;21:2359–66.

[17] Koirala P, Roth ME, Gill J, et al. HHLA2, a member of the B7 family, is expressed in human osteosarcoma and is associated with metastases and worse survival. Sci Rep 2016;6:31154.

[18] Zhu Z, Dong W. Overexpression of HHLA2, a member of the B7 family, is associated with worse survival in human colorectal carcinoma. Onco Targets Ther 2018;11:1563–70.

[19] Boor PPC, Sideras K, Biermann K, et al. HHLA2 is expressed in pancreatic and ampullary cancers and increased expression is associated with better post-surgical prognosis. Br J Cancer 2020;122:1211–8.

[20] Shimonosono M, Arigami T, Yanagita S, et al. The association of human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) expression with gastric cancer prognosis. Oncotarget 2018;9:22069–78.

[21] Ribas A, Hu-Lieskovan S. What does PD-L1 positive or negative mean? J Exp Med 2016;213:2835–40.

[22] Karim R, Jordanova ES, Piersma SJ, et al. Tumor-expressed B7-H1 and B7-DC in relation to PD-1+T-cell infiltration and survival of patients with cervical carcinoma. Clin Cancer Res 2009;15:6341–7.

[23] Festino L, Botti G, Lorigan P, et al. Cancer Treatment with Anti-PD-1/PD-L1 Agents: Is PD-L1 Expression a Biomarker for Patient Selection? Drugs 2016;76:925–45.

[24] Xiao Y, Freeman GJ. A New B7:CD28 family checkpoint target for cancer immunotherapy: HHLA2. Clin Cancer Res 2015;21:2201–3.