AMIGO2 expression as a predictor of recurrence in cervical cancer with intermediate risk

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Abstract. Patients with recurrent cervical cancer have limited treatment options and are often considered to be incurable. Since the expression of amphoterin-induced gene and open reading frame 2 (AMIGO2) in clinical samples is a prognostic factor for colorectal cancer and gastric cancer, the present aimed to elucidate whether it is also a prognostic factor for cervical cancer. Patients with primary cervical cancer who underwent radical hysterectomy or radical trachelectomy at our institution (Faculty of Medicine, Tottori University, Yonago, Japan) between September 2005 and October 2016 were retrospectively collected. Immunohistochemical analysis using a specific antibody against AMIGO2 was performed on 101 tumor samples, and the clinical characteristics, disease-free survival (DFS) and overall survival (OS) of the patients were examined. Patients in the AMIGO2-high group had a shorter 5-year DFS and OS than those in the AMIGO2-low group (P<0.001). Furthermore, AMIGO2 was an independent prognostic factor for DFS in multivariate analysis (P=0.0012). Patients in the AMIGO2-high group exhibited obvious recurrence compared with those in the AMIGO2-low group in the high-(P=0.03) and intermediate-risk groups (P=0.003). Positive lymph node metastasis, and parametrial, stromal and lymph vascular space invasion were significantly more common in AMIGO2-high patients. Taken together, AMIGO2 expression may be a predictive marker of recurrence for cervical cancer. In particular, it may be an indicator to determine the need for postoperative adjuvant therapy in intermediate-risk group patients.

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Introduction

Cervical cancer is the fourth most common malignancy in women worldwide. It was estimated that there were 604,000 new diagnostic cases and 342,000 deaths in 2020. Approximately 90% of new incidences and deaths worldwide in 2020 occurred in low- and middle-income countries. In high-income countries, the mortality rate is low because of the human papillomavirus (HPV) vaccine, routine screening, and adequate treatment (1). However, the prognosis of patients with advanced or recurrent cervical cancer remains unacceptable.

The standard treatment for cervical cancer is surgery or radiotherapy, and is selected according to the clinical stage and treatment strategy. It is likely to differ between Western and Asian countries. In Japan, 53.3, 24.3, 11.5, and 10.9% of patients had stage I, II, III, and IV cervical cancer, respectively. A total of 61.5% of patients received surgery alone or surgery followed by concurrent chemoradiotherapy (CCRT) (2). In Europe and the United States, radiotherapy is a common treatment for patients with the International Federation of Gynecology and Obstetrics (FIGO) stage II cervical cancer, whereas in Japan, approximately half of these patients undergo surgery or neoadjuvant chemotherapy followed by surgery (3). Patients undergoing surgery are required to receive adjuvant therapy based on recurrent risk classification. Recurrent risk factors include pelvic lymph node metastasis, parametrial invasion, stromal invasion, lymph-vascular space invasion (LVSI), and tumor size. Recurrent risk classification (high, intermediate, and low risk) is decided based on these risk factors. The definition of intermediate risk is based on the Sedlis criteria: 1) greater than one-third stromal invasion, 2) capillary lymphatic space involvement, or 3) cervical tumor diameters ≥4 cm (4). Other studies have demonstrated the necessity of considering tumor histology (5,6) and close or positive surgical margins (7,8). Therefore, the definition of intermediate risk has not been strictly described. Generally, low-risk patients do not require adjuvant therapy. However, high-risk patients are recommended postoperative treatment. Furthermore, the most recent National Comprehensive Cancer Network guidelines advocate adjuvant therapy for intermediate-risk patients (9). However, the treatment for intermediate-risk

cases is not standardized and varies across institutions. The question of whether all cases with intermediate risk should receive adjuvant therapy is controversial and remains an open question. Therefore, there is a need to explore markers for adjuvant therapy in intermediate-risk groups, and this was the focus of this study.

The prognosis for FIGO stage I-II cervical cancer is relatively favorable; however, some patients experience early relapse. Moreover, recurrent cases have limited treatment options and are often considered to be incurable. Therefore, if future recurrence in these stages can be predicted, options for postoperative adjuvant therapy can be increased.

Recently, our group revealed that the protein, Amphoterin-induced gene and open reading frame 2/adhesion molecule with IgG domain 2 (AMIGO2) is a prognostic factor for colorectal and gastric cancer in clinical samples (10,11). However, the association between AMIGO2 levels and cervical cancer remains unclear. In this study, using immunohistochemical analysis of the cervical tumor samples and clinical evaluation of the patients, we aimed to determine whether AMIGO2 can predict recurrence and whether AMIGO2 is associated with prognostic factors in cervical cancer. Currently, postoperative adjuvant treatment is standard for the high-risk group, but postoperative treatment for the intermediate-risk group varies from institution to institution, and there is no appropriate marker for adjuvant treatment. The results of this study clearly showed that AMIGO2 expression is a marker for postoperative adjuvant therapy in the intermediate-risk group. Therefore, we believe that our study would aid in determining appropriate treatment strategies for intermediate-risk group.

Materials and methods

Patient samples. The present study was approved by the institutional review board of Tottori University Hospital (IRB number: 21A152). Patients who underwent radical hysterectomy or radical trachelectomy for preoperative diagnosis of early cervical cancer (clinical T1N0M0 or T2N0M0) at Tottori University between 2005 and 2016 were included (12). The clinical samples used in this study were not the patients who were finally diagnosed with early-stage cervical cancer, but those who underwent radical hysterectomy or radical trachelectomy for preoperative early-stage cervical cancer. We chose this target population because of the possibility of investigating the effect of lymph node metastasis by AMIGO2 expression in the same T classification. All patients provided opt-out consent in accordance with institutional guidelines. Immunohistochemical analysis was performed on 101 samples. Recurrent risk was classified as low, intermediate, or high, based on the postoperative pathological diagnosis. High risk is associated with positive pelvic lymph node metastasis, and parametrial invasion. Intermediate risk is determined by 'greater than one-third stromal invasion,' 'capillary lymphatic space involvement,' or 'cervical tumor diameters ≥4 cm,' according to the Sedlis criteria (4). Low risk involves conditions that are excluded from high and intermediate risk. All high-risk patients, except one who refused treatment, received standard clinical treatment (adjuvant CCRT; pelvic radiotherapy as a total

of 45-50 Gy/5-6 weeks and chemotherapy with cisplatin, 40 mg/m2/week) (9) Low- and intermediate-risk patients (none of whom received adjuvant CCRT) were carefully monitored. Clinical characteristics, recurrence, form of recurrence, disease-free survival (DFS), and overall survival (OS) were retrospectively investigated based on clinical records. Form of recurrence was divided into distant metastasis and local recurrence including vaginal vault or pelvic relapse.

Immunohistochemistry. The cervical cancer samples were fixed in formalin and embedded in paraffin. Serial sections were cut at 4 μm, deparaffinized in xylene for 15 min, and rehydrated through a graded alcohol series for 15 min. The sections were autoclaved for 10 min in 10 mM citrate buffer (pH 6.0) in a microwave and were incubated in 3% hydrogen peroxidase (Nichirei Biosciences Inc., 426042, Tokyo, Japan) to block endogenous peroxidases for 20 min to prevent non-specific antigen binding. The slides were subsequently incubated with rat anti-AMIGO2 antibody (rTNK1Bo12a, 1:1,000 dilution) or mouse anti-Podoplanin antibody (D2-40, Nichirei Biosciences Inc., 426042, Tokyo, Japan) overnight at 4°C (13). The sections were then incubated with goat polyclonal anti-rat IgG horseradish peroxidase-conjugated antibody (Abcam, ab98425, Cambridge, United Kingdom, 1:300 dilution) or biotin-labeled rabbit polyclonal anti-mouse IgG antibody, horseradish peroxidase-conjugated streptavidin (Nichirei Biosciences Inc.) at 20°C for 20 min, respectively. Secondary staining was performed using a peroxidase substrate kit (Vector Laboratories, SK-4105, Burlingame, CA, USA), and the sections were counterstained with hematoxylin. A positive rate of >70 and ≤70% was defined as high expression of AMIGO2 (AMIGO2-High group) and low expression of AMIGO2 (AMIGO2-Low group), respectively, using a microscope (Leica, DM500, Wetzlar, Germany).

Statistical analysis. Patient survival distribution was determined using the Kaplan-Meier method and log-rank test. Categorical variables were analyzed using Fisher's exact test. Univariate and multivariate analyses were performed using the Cox proportional hazard model. Statistical significance was set at P<0.05. Statistical analyses were performed using EZR software version 1.54, 2020 (14).

Results

Immunohistochemical detection of AMIGO2 expression and clinicopathological factors in cervical cancer. To confirm the expression of AMIGO2 in cervical cancer, we performed immunohistochemical analysis using AMIGO2-specific antibodies. Cervical carcinomas were histologically classified into squamous cell carcinoma (SCC) and adenocarcinoma including adenosquamous carcinoma (non-SCC), and analyzed for AMIGO2 expression (Fig. 1); however, no association was found between the histological type and AMIGO2 expression (Table I). In all histological types, AMIGO2 expression was higher in cancer cells and lower in the surrounding stroma; AMIGO2 protein was localized in both the nucleus and cytoplasm (Fig. 1A and C). Based
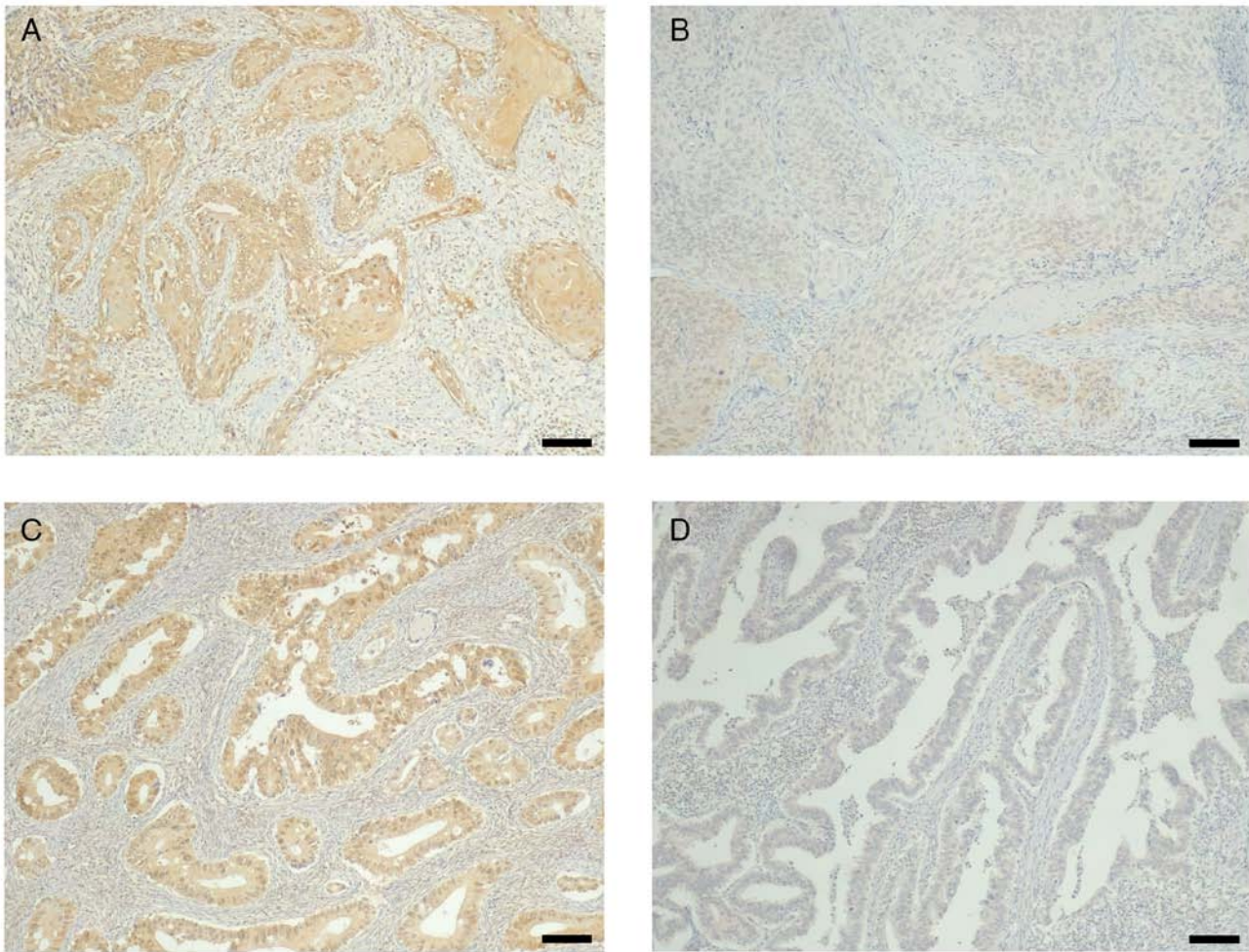
Figure 1. Immunohistochemical staining for AMIGO2 expression in cervical cancer tissues. Typical (A and B) cervical squamous cell carcinomas and (C and D) adenocarcinomas are presented. (A and C) High expression (≥70% staining intensity) and (B and D) low expression (<70% staining intensity) of AMIGO2 are shown. Scale bar, 100 μm. AMIGO2, amphoterin-induced gene and open reading frame 2.

on a cutoff of 70% AMIGO2-positive cell fraction in cancer cells, cases were classified as AMIGO2-High (Fig. 1A and C) and AMIGO2-Low groups (Fig. 1B and D), regardless of the histological type.

Of the 101 cervical cancer samples evaluated, 39.6% (40/101) were in the AMIGO2-High group and 60.4% (61/101) in the AMIGO2-Low group. The correlation between clinicopathological variables and AMIGO2 expression in tumor tissues is shown in Table I. Approximately one-fifth of patients (20/101; 19.8%) upstaged to FIGO stage IIIC1p because of lymph node metastasis. The AMIGO2-High group had a higher frequency of bulky tumors (≥4 cm, 8/40; 20.0%) than the AMIGO2-Low group (2/61; 3.3%). Furthermore, lymph node metastases (15/40; 37.5% vs. 5/61; 8.2%), parametrial invasion (8/40; 20.0% vs. 1/61; 1.6%), stromal invasion (22/40; 55.0% vs. 19/61; 31.1%), and LVSI-positive cases (23/40; 57.5% vs. 16/61; 26.2%) were all significantly higher in the AMIGO2-High group (P<0.05). In contrast, age and histological type did not differ between the two groups.

To analyze for AMIGO2 expression in tumor cells invading lymphatic vessels, immunohistochemistry was performed using only two cases that were evaluable. Although the expression intensity of AMIGO2 was different in the two

cases, the expression was confirmed in almost all invasive tumor cells (Fig. S1). Despite the high incidence, due to the small number of cases available for analysis, it was not possible to conclude whether invasive cancer cells have a significantly higher incidence.

AMIGO2 expression as a predictor of cervical cancer recurrence. Kaplan-Meier survival analysis and log-rank tests were performed to examine the relationship between AMIGO2 expression and prognosis in cervical cancer. Patients in the AMIGO2-High group had shorter 5 year-DFS than those in the AMIGO2-Low group [59% {95% confidence interval (CI) 42-73%} vs. 95% (95% CI 85-98%) (P<0.001)] (Fig. 2A). Furthermore, at the 5-year OS, patients in the AMIGO2-High group had clearly worse survival than those in the AMIGO2-Low group [80% (95% CI 63-89%) vs. 100% {95% CI not available (NA)-NA} (P=0.0011)] (Fig. 2B).

Next, we investigated (including AMIGO2 expression) the independent risk factors for DFS and OS, respectively: in univariate analysis of DFS, lymph node metastasis, tumor size, parametrial invasion, stromal invasion, LVSI and AMIGO2 were considered prognostic factors. Furthermore, in multivariate analysis, AMIGO2 expression was the only

Table I. AMIGO2 expression and clinicopathological factors in 101 cervical cancer patients.

Parameter	AMIGO2-High (n=40)	AMIGO2-Low (n=61)	P-value
Age, years (range, 22-71 years)			0.79
<60	32	51	
≥60	8	10	
Histology			0.2
SCC	30	37	
Non-SCC	10	24	
Tumor size			0.011
<4 cm	32	59	
≥4 cm	8	2	
Lymph node metastasis			<0.001
+	15	5	
-	25	56	
Parametrial invasion			0.0024
+	8	1	
-	32	60	
Stromal invasion			0.023
+	22	19	
-	18	42	
LVSI			0.0032
+	23	16	
-	17	45	

AMIGO2, amphoterin-induced gene and open reading frame 2; SCC, squamous cell carcinoma; LVSI, lymph-vascular space invasion.
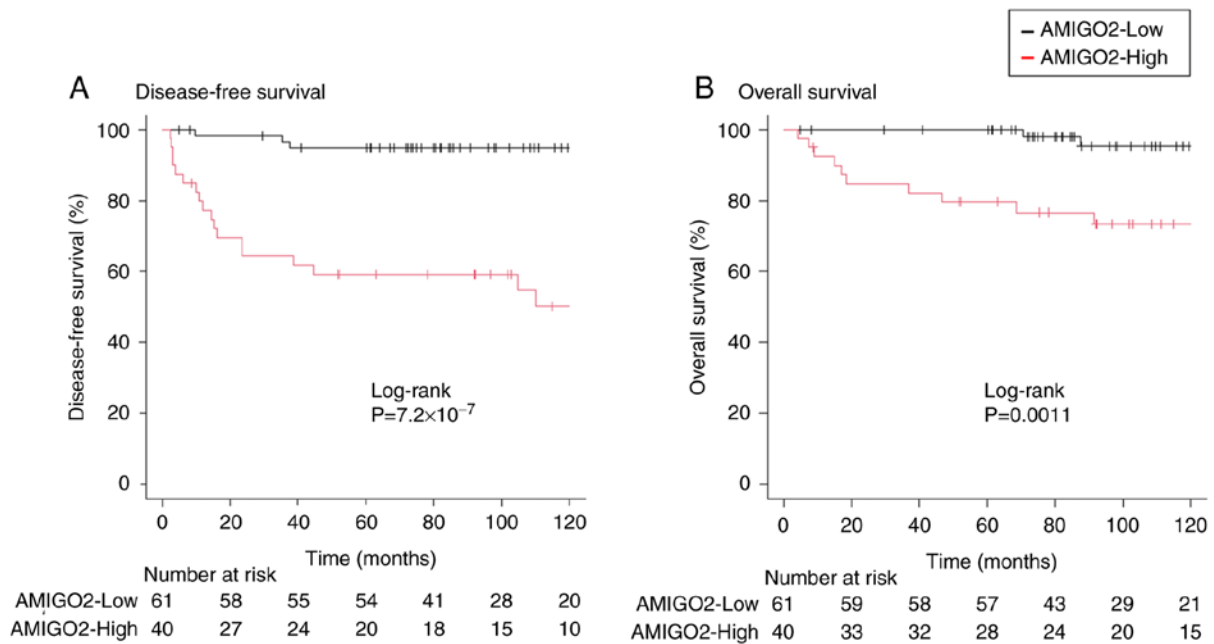

Figure 2. AMIGO2 expression and survival in cervical cancer patients. Cumulative survival rates were assessed using the Kaplan-Meier method. Differences were analyzed using the log-rank test. (A) Disease-free survival and (B) overall survival are shown. AMIGO2, amphoterin-induced gene and open reading frame 2.

independent prognostic factor for DFS (Table II). When the analysis was limited to the intermediate-risk group, AMIGO2 expression was also the only independent prognostic factor for DFS (Table SI). In the univariate analysis of OS, the same

Table II. Multivariate analysis for disease-free survival.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age						
≥60 years vs. <60 years	0.5	0.12-2.1	0.35			
Histology						
Non-SCC vs. SCC	0.76	0.3-1.9	0.57			
Tumor size						
≥4 cm vs. <4 cm	3.97	1.5-11	0.0074	1.7	0.58-5.1	0.33
Lymph node metastasis[a]						
Positive vs. negative	3.4	1.4-8.1	0.0055	0.79	0.28-2.3	0.66
Parametrial invasion						
Positive vs. negative	4.4	1.6-12	0.004	1.56	0.54-4.5	0.41
Stromal invasion						
Positive vs. negative	3.1	1.2-7.6	0.02	0.98	0.31-3.2	0.97
LVSI						
Positive vs. negative	4.83	1.9-13	0.001	3.2	0.85-12	0.085
AMIGO2 expression						
High vs. low	11.5	3.4-39	<0.001	8.3	2.3-30	0.0012

AMIGO2, amphoterin-induced gene and open reading frame 2; HR, hazard ratio; CI, confidence interval; LVSI, lymph-vascular space invasion; SCC, squamous cell carcinoma. [a]Lymph node metastasis positive (Stage III) vs. negative (Stage I + II).

variable was an independent prognostic factor for DFS. On the other hand, multivariate analysis revealed no statistically independent prognostic factors for OS, including AMIGO2 (Table III). It was suggested that this is probably due in part to the small number of cases. However, since the P value was 0.08, the lowest value compared to the other factors, it was considered that there was a trend, although not statistically significant.

Patients with cervical cancer have been postoperatively classified into three risk categories (high, intermediate, and low risk) based on the risk factor assessment (pelvic lymph node metastasis, parametric invasion, stromal invasion, LVSI, and tumor size). Therefore, Kaplan-Meier curves for 5-year DFS were generated for each risk category. As shown in Fig. 3, in the high- and intermediate-risk groups, AMIGO2-High patients had a significantly shorter 5-year DFS than AMIGO2-Low patients (Fig. 3A and B). In contrast, there was no association between AMIGO2 expression and prognosis in the low-risk group (Fig. 3C). In contrast, AMIGO2 expression did not affect OS according to risk classification (data not shown). Finally, when we examined the correlation between AMIGO2 expression and the site of recurrence by classifying distant metastasis and local recurrence, we found no difference in the recurrence patterns based on AMIGO2 expression (Table SII).

Discussion

The present study verified the relationship between AMIGO2 expression and clinicopathological factors using clinical specimens of cervical cancer in the present study. Our data clearly demonstrated that high AMIGO2 expression is a prognostic factor for cervical cancer. Notably, AMIGO2 expression can be used as an indicator to predict the recurrence of intermediate-risk cervical cancer, suggesting that AMIGO2 as a predictive marker could contribute to the treatment strategy for the patient. To our knowledge, this is the first report showing AMIGO2 expression as a molecular marker that can predict recurrence risk in cervical cancer.

Our previous studies i) identified AMIGO2 as a driver gene for liver metastases using a mouse cell line (15), ii) extrapolated its function in mouse cells to that in human cancer cell lines (16), and iii) indicated that high AMIGO2 expression acted as a driver for liver metastases in human gastric and colorectal cancers and resulted in poor prognosis (10,11). Additionally, Liu *et al* (17) reported that AMIGO2 is related to peritoneal dissemination in *an in vivo* model using human ovarian cancer cells. Thus, enhanced AMIGO2 expression may play a crucial role in cancer prognosis by activating metastasis in various cancers. The present study showed that higher expression of AMIGO2 is associated with the recurrence of human cervical cancer, suggesting that this molecule is involved in the progression of cervical cancer as well as the above-mentioned cancer types.

In this study, high AMIGO2 expression in cervical cancer was associated with poor prognosis in DFS and OS, with a particularly strong association with DFS. A prognostic analysis of early-stage cervical cancer cases similar to the present study reported that 5-year OS and DFS were 83 and

Table III. Multivariate analysis for overall survival.

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age						
≥60 years vs. <60 years	0.48	0.06-3.7	0.48			
Histology						
Non-SCC vs. SCC	0.89	0.27-3.0	0.85			
Tumor size						
≥4 cm vs. <4 cm	8.9	2.8-28	<0.001	3.6	0.98-13	0.053
Lymph node metastasis[a]						
Positive vs. negative	6.2	1.9-20	0.0018	2.3	0.42-12	0.34
Parametrial invasion						
Positive vs. negative	4.4	1.2-16	0.03	1.5	0.39-6.1	0.54
Stromal invasion						
Positive vs. negative	4.7	1.3-17	0.02	2.8	0.44-18	0.27
LVSI						
Positive vs. negative	3.6	1.1-12	0.04	0.5	0.06-4.1	0.52
AMIGO2 expression						
High vs. low	8.3	1.8-38	0.006	4.3	0.84-22	0.08

AMIGO2, amphoterin-induced gene and open reading frame 2; HR, hazard ratio; CI, confidence interval; LVSI, lymph-vascular space invasion; SCC, squamous cell carcinoma. [a]Lymph node metastasis positive (Stage III) vs. negative (Stage I + II).

74%, respectively (18). Compared to these results, the 5-year OS and DFS in this study were worse for AMIGO-High and better for AMIGO-Low. Thus, the results of the present study strongly suggest that AMIGO2 expression is a very useful prognostic marker in human early-stage cervical cancer. The multivariate analysis data strongly suggested that among all the factors, AMIGO2 expression was the only independent predictor of cervical cancer prognosis, strongly suggesting its usefulness as a predictive marker of recurrence. Interestingly, the present study indicated that the AMIGO2-High group had a considerably shorter 5-year DFS than the AMIGO2-Low intermediate-risk group. The criteria for postoperative adjuvant therapy for these intermediate-risk patients have not been defined yet. Moreover, the Japanese Society of Gynecologic Oncology and the Korean Gynecologic Oncology Group reported that 37.3 and 26% of intermediate-risk patients did not receive adjuvant therapy, respectively (5,19). Previously, our institution also had not administered adjuvant therapy for intermediate-risk cases. This was because our previous research demonstrated that deep stromal invasion can be excluded from the list of criteria for adjuvant radiotherapy (20). However, the present study revealed that the intermediate-risk group with high AMIGO2 expression exhibits high metastasis (Fig. 3). Thus, the intermediate-risk group with high expression of AMIGO2 should undergo adequate adjuvant therapy as well as the high-risk group.

Lymph node involvement was newly incorporated into staging and defined as stage IIIC in FIGO 2018. This is because positive lymph node metastasis is strongly associated with poor outcomes and low survival. Previous work from our lab on the prognosis of patients with cervical cancer, also showed that those with lymph node metastases had poor prognosis (21). In contrast, the present study showed that lymph node metastasis was not an independent prognostic factor. This was due to the small number of lymph node-positive cases (n=20), which was considered a limitation of the study. In this study, instead of comparing the presence or absence of lymph node metastasis between the same stages, different stages were used for comparison. The reason for this is due to the patient background of the population diagnosed with early-stage cervical cancer (clinical T1N0M0 or T2N0M0) at preoperative diagnosis. The incidence of lymph node metastasis was higher in the AMIGO2-High group than in the AMIGO2-Low group, and there was a clear difference in the proportion of FIGO stage IIIC1 patients, suggesting that AMIGO2 expression may affect lymph node metastasis and may be associated with the prognosis of cervical cancer. Furthermore, it would be possible to predict the prognosis by examining AMIGO2 expression preoperatively.

In conclusion, this study revealed that AMIGO2 expression is an independent prognostic factor in cervical cancer recurrence, suggesting that AMIGO2 expression is a marker for cervical cancer recurrence. In patients who were classified as intermediate-risk postoperatively, the high AMIGO2-expressing group was shown to be at higher risk of recurrence, and it was recommended that these patients be considered for postoperative treatment as a high-risk group.
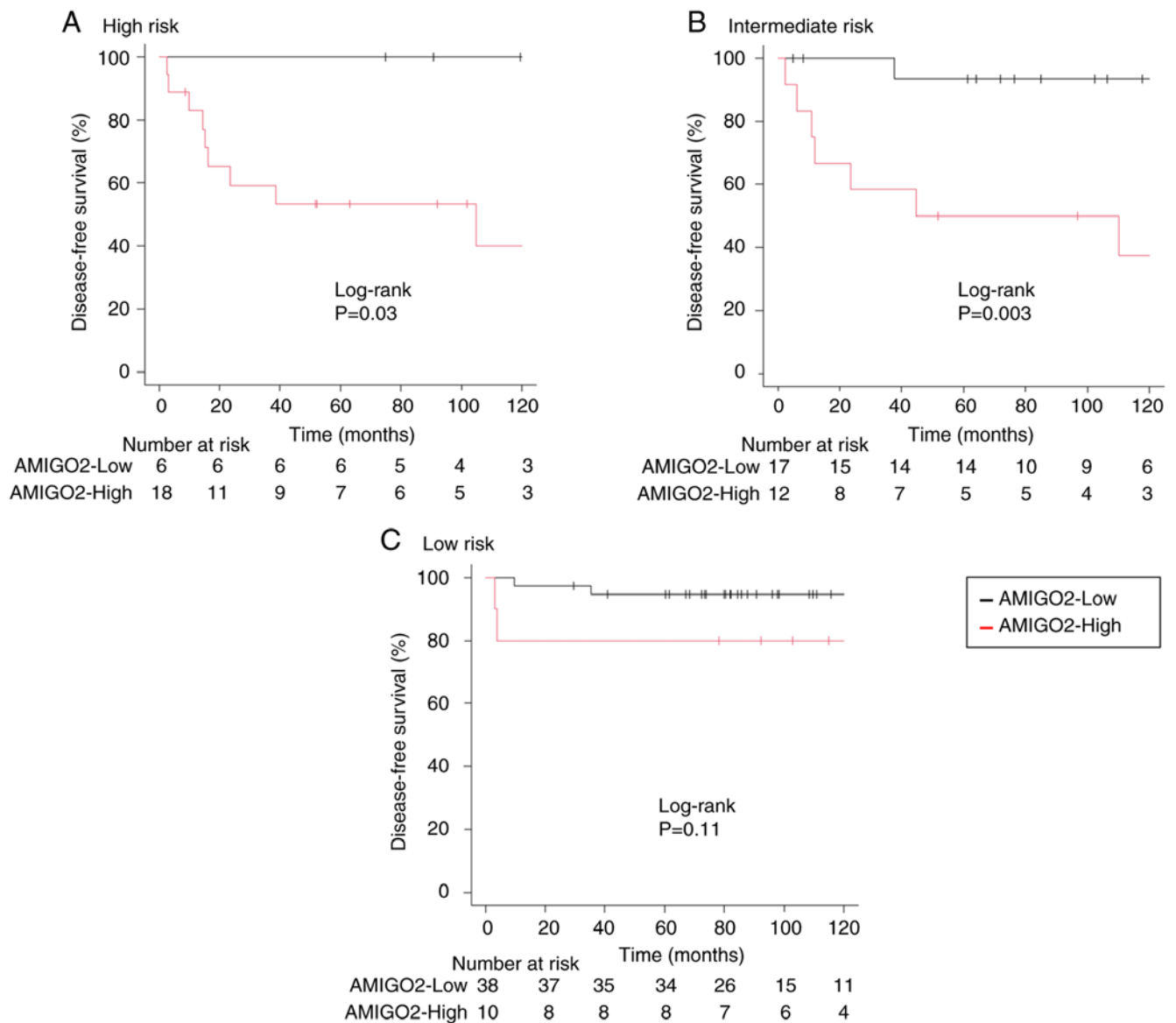
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Figure 3. AMIGO2 expression determines the three risk categories into which postoperative cervical cancer patients are classified. Disease-free survival of cervical cancer with (A) high, (B) intermediate and (C) low postoperative recurrence risk. AMIGO2, amphoterin-induced gene and open reading frame 2.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YI, SS, MO and FO established the study design and analytical concept. YI, MO and DO collected and analyzed clinical data. YI, RI, HS and MO performed histological analysis. YI and MO confirm the authenticity of all the raw data. YI, MO, SS, HK, FT and FO interpreted the data. HK, SS, FO and FT acquired the funding. YI and SS were major contributors in writing the manuscript. RI, HS, MO and FO made critical revisions to the manuscript. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by the institutional review board of Tottori University Hospital (IRB no. 21A152). All patients provided opt-out consent in accordance with institutional guidelines.

Patient consent for publication

The requirement for patient consent for publication was waived due to the retrospective nature of the study.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.

2. Nagase S, Ohta T, Takahashi F, Yamagami W and Yaegashi N; Board Members of the 2020 Committee on Gynecologic Oncology of the Japan Society of Obstetrics and Gynecology: Annual report of the Committee on Gynecologic Oncology, the Japan Society of Obstetrics and Gynecology: Annual patient report for 2018 and annual treatment report for 2013. J Obstet Gynaecol Res 48: 541-552, 2022.

3. Shigeta S, Shida M, Nagase S, Ikeda M, Takahashi F, Shibata T, Yamagami W, Katabuchi H, Yaegashi N, Aoki D and Mikami M: Epidemiological guideline influence on the therapeutic trend and patient outcome of uterine cervical cancer in Japan: Japan society of gynecologic oncology guideline evaluation committee project. Gynecol Oncol 159: 248-255, 2020.

4. Sedlis A, Bundy BN, Rotman MZ, Lentz SS, Muderspach LI and Zaino RJ: A randomized trial of pelvic radiation therapy versus no further therapy in selected patients with stage IB carcinoma of the cervix after radical hysterectomy and pelvic lymphad-enectomy: A Gynecologic Oncology Group Study. Gynecol Oncol 73: 177-183, 1999.

5. Ryu SY, Kim MH, Nam BH, Lee TS, Song ES, Park CY, Kim JW, Kim YB, Ryu HS, Park SY, *et al*: Intermediate-risk grouping of cervical cancer patients treated with radical hysterectomy: A Korean Gynecologic Oncology Group study. Br J Cancer 110: 278-285, 2014.

6. Noh JM, Park W, Kim YS, Kim JY, Kim HJ, Kim J, Kim JH, Yoon MS, Choi JH, Yoon WS, *et al*: Comparison of clinical outcomes of adenocarcinoma and adenosquamous carcinoma in uterine cervical cancer patients receiving surgical resection followed by radiotherapy: A multicenter retrospective study (KROG 13-10). Gynecol Oncol 132: 618-623, 2014.

7. Diaz ES, Aoyama C, Baquing MA, Beavis A, Silva E, Holschneider C and Cass I: Predictors of residual carcinoma or carcinoma-in-situ at hysterectomy following cervical conization with positive margins. Gynecol Oncol 132: 76-80, 2014.

8. Estape RE, Angioli R, Madrigal M, Janicek M, Gomez C, Penalver M and Averette H: Close vaginal margins as a prognostic factor after radical hysterectomy. Gynecol Oncol 68: 229-232, 1998.

9. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Cervical Cancer. Version 1. National Comprehensive Cancer Network, 2023

10. Tanio A, Saito H, Amisaki M, Hara K, Sugezawa K, Uejima C, Tada Y, Kihara K, Yamamoto M, Nosaka K, *et al*: AMIGO2 as a novel indicator of liver metastasis in patients with colorectal cancer. Oncol Lett 21: 278, 2021.

11. Goto K, Morimoto M, Osaki M, Tanio A, Izutsu R, Fujiwara Y and Okada F: The impact of AMIGO2 on prognosis and hepatic metastasis in gastric cancer patients. BMC Cancer 22: 280, 2022.

12. Olawaiye AB, Baker TP, Washington MK and Mutch DG: The new (Version 9) American Joint Committee on Cancer tumor, node, metastasis staging for cervical cancer. CA Cancer J Clin 71: 287-298, 2021.

13. Goto K, Osaki M, Izutsu R, Tanaka H, Sasaki R, Tanio A, Satofuka H, Kazuki Y, Yamamoto M, Kugoh H, *et al*: Establishment of an antibody specific for AMIGO2 improves immunohistochemical evaluation of liver metastases and clinical outcomes in patients with colorectal cancer. Diagn Pathol 17: 16, 2022.

14. Kanda Y: Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 48: 452-458, 2013.

15. Kanda Y, Osaki M, Onuma K, Sonoda A, Kobayashi M, Hamada J, Nicolson GL, Ochiya T and Okada F: Amigo2-upregulation in tumour cells facilitates their attachment to liver endothelial cells resulting in liver metastases. Sci Rep 7: 43567, 2017.

16. Izutsu R, Osaki M, Jehung JP, Seong HK and Okada F: Liver Metastasis formation is defined by AMIGO2 expression via adhesion to hepatic endothelial cells in human gastric and colorectal cancer cells. Pathol Res Pract 237: 154015, 2022.

17. Liu Y, Yang J, Shi Z, Tan X, Jin N, O'Brien C, Ott C, Grisoli A, Lee E, Volk K, *et al*: In vivo selection of highly metastatic human ovarian cancer sublines reveals role for AMIGO2 in intra-peritoneal metastatic regulation. Cancer Lett 503: 163-173, 2021.

18. Landoni F, Maneo A, Colombo A, Placa F, Milani R, Perego P, Favini G, Ferri L and Mangioni C: Randamised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. Lancet 350: 535-540, 1997.

19. Shigeta S, Shimada M, Tsuji K, Nagai T, Tanase Y, Matsuo K, Kamiura S, Iwata T, Yokota H and Mikami M: Risk assessment in the patients with uterine cervical cancer harboring intermediate risk factors after radical hysterectomy: A multicenter, retrospective analysis by the Japanese Gynecologic Oncology Group. Int J Clin Oncol 27: 1507-1515, 2022.

20. Shimada M, Kigawa J, Takahashi M, Minagawa Y, Okada M, Kanamori Y, Itamochi H, Oishi T, Iba T and Terakawa N: Stromal invasion of the cervix can be excluded from the criteria for using adjuvant radiotherapy following radical surgery for patients with cervical cancer. Gynecol Oncol 93: 628-631, 2004.

21. Osaku D, Komatsu H, Okawa M, Iida Y, Sato S, Oishi T and Harada T: Re-classification of uterine cervical cancer cases treated with radical hysterectomy based on the 2018 FIGO staging system. Taiwan J Obstet Gynecol 60: 1054-1058, 2021.