Original Research

Correlation of immunohistochemistry and silver *in situ* hybridization for the assessment of c-MET in uterine cervical cancer patients treated with radical hysterectomy

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Summary

Objectives: The aim of this study was to compare c-MET protein overexpression and gene copy number (GCN) in uterine cervical cancer and to assess their prognostic significance. *Methods:* c-MET protein expression and GCN status were determined using immunohistochemistry (IHC) and silver *in situ* hybridization (SISH), respectively, in 117 cervical cancers comprising 83 squamous cell carcinomas (SCCs), 23 adenocarcinomas (ACs), 7 adenosquamous cell carcinomas (ASCCs), and 4 other types. *Results:* Forty-five of 117 (38.5%) cervical cancer patients had c-MET protein overexpression (IHC 2+ in 40 cases and IHC 3+ in 5 cases). The frequency of overexpression was 31.3% in SCCs, 73.9% in ACs, 14.3% in ASCCs and 25.0% in other types. IHC 3+ c-MET protein overexpression was observed only in ACs and correlated with worse overall survival (OS) ($p = 0.001$) and progression-free survival (PFS) ($p < 0.001$). High polysomy (HP) of chromosome 7 and gene amplification (GA) were found in 6 (5.1%) and 0 of the 117 cervical cancers, respectively. Of the 6 HP cases, 3 were SCCs and 3 were ACs. GCN could not be determined in 16 (13.7%) of the 117 cases. HP cases showed a trend for worse prognosis than cases with negative c-MET SISH, but this did not reach statistical significance (OS, $p = 0.307$; PFS, $p = 0.184$). Nonetheless, c-MET protein overexpression and increased GCN were significantly correlated ($r = 0.228$, $p = 0.022$). *Conclusions:* c-MET, evaluated using IHC and GCN, may be a prognostic biomarker of poor prognosis in patients with cervical AC.

Key words: Cervical cancer; C-MET; Immunohistochemistry; Silver *in situ* hybridization; Prognosis.

Introduction

Cervical cancer is one of the major causes of morbidity and mortality worldwide, with an estimated 569,847 newly diagnosed cases and 311,365 deaths in 2018. The age-standardized incidence and mortality rates in 2018 were 13.1 and 6.9 per 100,000 people, respectively [1]. In South Korea alone, it is estimated there were 3,566 new cases and 10.8 age-standardized occurrences per 100,000 people in 2016 [2]. While radiation therapy (RT) can be used at all stages of the disease, surgery is limited to patients with stage I to IIA disease [3]. Although salvage treatment advances, almost all patients who have a recurrent disease show poor prognosis [4-7].

c-MET signaling has been concerned in several human cancers and is important in both tumorigenesis and metastasis [8, 9]. *MET* is located on chromosome 7q31 and encodes a ligand-binding domain, a regulatory juxtamembrane domain, and a receptor tyrosine kinase domain [10, 11]. Upon binding by its ligand, hepatocyte growth factor/scatter factor (HGF/SF), MET is dimerized and directs cellular activity through Ras/mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and

signal transducer and activator of transcription (STAT) signaling pathways [10, 11]. Baykal *et al.* demonstrated overexpression of c-MET in 60% of stage IB (International Federation of Gynecology and Obstetrics, FIGO) cervical cancer patients treated primarily with surgery [12]. Tsai *et al.* reported that high c-MET expression might play an important role in the progression of cervical adenocarcinoma [13]. Refaat *et al.* proposed that c-MET overexpression could also be a prognostic biomarker and therapeutic target for locoregional metastatic cervical cancer patients treated with concurrent chemoradiation therapy (CCRT) [4]. A meta-analysis showed that c-MET is a potential diagnostic and prognostic marker of cervical cancer [14]. In epithelial ovarian cancers, c-MET overexpression detected using immunohistochemistry (IHC) was associated with better prognosis, whereas high c-*MET* gene copy number (GCN) was a significant factor for poor prognosis [15].

Although c-MET has been reported as a prognostic biomarker in cervical cancer patients in several previous studies, it was evaluated using only IHC. Previous studies have reported c-MET overexpression in 30.4% [12] and 60.0% [13] of cases and correlation with worse prognosis in

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both studies. Nonetheless, effective targeted therapies for c-MET have yet to be reported. For therapeutic targeting, c-MET overexpression must be identified more accurately. Addition of GCN evaluation could provide more accurate data on the association between c-MET and prognosis. If the c-MET biomarker can identify patients with a high risk of recurrence after primary treatment, individualized treatments could then be directed at these patients.

The present study of uterine cervical cancer patients investigated the prognostic significance of GCN evaluated using silver *in situ* hybridization (SISH), and of c-MET protein expression evaluated using IHC.

Materials and Methods

Patients

We reviewed patients who underwent standard treatment for uterine cervical cancer at Konkuk University Medical Center from August 2005 to August 2018. Specimens collected from a punch biopsy or from an electrosurgical conization (LEEP)/cold knife conization were insufficient to manufacture a tissue microarray (TMA) for SISH. We therefore included patients with FIGO stage IB1 to IIA2 who underwent radical hysterectomy based on National Comprehensive Cancer Network (NCCN) guidelines. Patients who underwent neoadjuvant chemotherapy before hysterectomy or who underwent primary CCRT were excluded from the analysis, leaving 117 patients eligible for the study. All specimens were selected from formalin-fixed, paraffin-embedded (FFPE) cancer tissue blocks. The tumors were reviewed by 3 pathologists (WYK, HKP, and JHP) and classified according to the criteria of the World Health Organization [16]. Clinicopathological characteristics including patient age, stage of disease, regional lymph node status, resection margin status, parametrial invasion, clinical response to therapy, progression-free survival (PFS), and overall survival (OS) were assessed. PFS was defined as the duration from date of initial treatment to disease progression or last follow-up. OS was defined as the duration from date of initial treatment to the date of either the last follow-up or death. Follow-up was calculated from date of initial treatment to date of either the last follow-up or death [15]. Thirty-six (30.8%) of 117 patients died due to tumor burden or were lost to follow-up during the study period. Enrolled cases were restaged in accordance to the 2009 FIGO staging system.

Konkuk University Hospital institutional review board reviewed and approved this study protocol (KUH1040069).

Tissue samples and construction of tissue microarray

The TMA was constructed using representative tissue samples from 117 uterine cervical cancers. Briefly, core tissues (3 mm in diameter) were obtained from FFPE tissue donor blocks using tissue cylinders and arranged in a new recipient TMA block. Corresponding histopathology in each TMA spot was reviewed and confirmed by WYK after staining with hematoxylin and eosin [15].

Immunohistochemistry

Three-μm-thick sectioned TMA slides were subjected to an autoimmunostainer (BenchMark ULTRA, Ventana Medical Systems, Tucson, AZ, USA). The primary antibody used was a rabbit anti-Total c-MET (SP44) monoclonal antibody (Ventana Medical Systems). An OptiView DAB detection kit (Ventana Medical Systems) was used to visualize the immunoreaction. MET-positive lung cancer tissues were used as positive controls and immunostained in parallel [15]. Tissue sections omitting the primary antibody were used as negative controls. In addition, pericarcinoma tissues of each case were checked for internal negative tissue control. No staining of MET was identified in pericarcinoma tissues.

The IHC results were independently evaluated by two pathologists (WYK and SHP). Discordant cases were resolved by discussion and determination in a common session using a multiview microscope. Following DAKO HercepTest guidelines (DAKO, Glostrup, Denmark), a four-tiered semiquantitative approach was used to generate a score for MET expression (0, 1+, 2+, or 4+) [15, 17]. Scores of 2+ and 3+ were considered positive for c-MET overexpression.

Silver in situ hybridization

Automated dual-color SISH was performed on a Ventana BenchMark GX system (Ventana Medical Systems) to assess alterations in *c-MET* GCN. To detect and visualize the c-*MET* gene, a dinitrophenyl-labeled *MET* DNA Probe (Ventana Medical Systems) and an UltraView SISH Detection Kit (Ventana Medical Systems) were used. The centromere of chromosome 7 was identified with a digoxigenin (DIG)-labeled chromosome 7 probe (Ventana Medical Systems) and the UltraView Red DIG Detection Kit (Ventana Medical Systems) [15, 16].

The SISH signals were a dark brown dot for c-*MET* and a red dot for chromosome 7. These were quantified manually by counting signals in at least 100 tumor nuclei per core using a light microscope. In the case of clustered multiple signals, a small cluster was counted as 6 signals and a large cluster as 12 signals according to the interpretive guide for Ventana INFORM HER2 DNA probe staining of breast carcinoma (Ventana Medical Systems). The SISH results were independently evaluated by two pathologists (WYK and HKP) and discordant results were resolved in a common session using a multiview microscope [15].

Using the University of Colorado Cancer Center (UCCC) criteria for the epidermal growth factor receptor gene [15, 17, 18], c-*MET* gene status was classified into six groups based on the number of copies (disomy, low trisomy, high trisomy, low polysomy, high polysomy (HP), or gene amplification (GA)). HP and GA were considered to be SISH positive.

Table 1. — *Clinical characteristics of the patients with cervical cancer enrolled*

Parameter	Results (n = 117)
Median age (range), y	49.0 (24-77)
Histotype, n (%)	
Squamous cell carcinoma	83 (70.9)
Adenocarcinoma	23 (19.7)
Endocervical adenocarcinoma, usual type	14
Mucinous adenocarcinoma, gastric type	4
Mucinous adenocarcinoma, NOS	3
Serous adenocarcinoma	1
Clear cell adenocarcinoma	1
Adenosquamous cell carcinoma	7 (6.0)
others	4 (3.4)
FIGO stage, n (%)	
IB1 / IB2	89 (76.1) / 9 (7.7)
IIA1 / IIA2	8 (6.8) / 9 (7.7)
IVA* / IVB*	1 (0.9) / 1 (0.9)
Lymph node metastasis, n (%)	
NR[†]	6 (5.1)
No	77 (65.8)
Yes	34 (29.1)
Positive resection margin, n (%)	
No	104 (88.9)
Yes	13 (11.1)
Parametrial invasion, n (%)	
NR	7 (6.0)
No	96 (82.1)
Yes	14 (12.0)
Lymph-vascular space invasion, n (%)	
Indeterminate	1 (0.9)
No	61 (52.1)
Yes	55 (47.0)

*Misdiagnosed as a bladder cancer and an ovarian cancer; [†]Diagnosed after simple hysterectomy; NR: no result.

Statistical Analysis

A Jonckheere–Terpstra test was applied to assess correlations between c-MET protein overexpression or *c-MET* GCN and patient prognosis, and a least significance test using ranks was applied for multiple comparisons among the histotype groups. The prognostic significance of SISH and IHC results and of clinicopathologic factors was analyzed using Cox proportional analysis. The Spearman rank test was used to analyze correlations between SISH and IHC results. Survival curves were obtained via the Kaplan–Meier method and the significance of differences between these curves was determined with a log-rank test using IBM® SPSS® statistics 22.0 software (IBM SPSS Statistics, Chicago, IL, USA). A p-value < 0.05 was considered statistically significant.

Results

Clinical data

Three patients were excluded due to treatment with neoadjuvant chemotherapy. No patients were excluded due to refusal of standard treatment. The median follow-up period was 45 months (range, 0-150) and the median age of patients was 49.0 years (range, 24-77). Eighty-three (70.9%) patients were diagnosed as having squamous cell carcinoma (SCC). Twenty three (19.7%) patients were diagnosed with adenocarcinoma (AC), comprising histologically of 14 usual type endocervical AC, 7 mucinous AC (4 gastric type and 3 not otherwise specified types), one serous AC and one clear cell AC. Seven (6.0%) cases were diagnosed as adenosquamous cell carcinoma (ASCC). The remaining cases were 2 small cell neuroendocrine carcinomas, 1 atypical carcinoid tumor and 1 AC admixed with neuroendocrine carcinoma. Patients with stage IB, IIA and IV disease comprised 83.8%, 14.5% and 1.8% of cases, respectively. Two stage IV cases (one SCC and another AC) were initially misdiagnosed as having urinary bladder cancer and ovarian cancer during surgery. Although these 2 patients had not undergone radical hysterectomy, they were nevertheless included during manufacture of the TMA. Table 1 presents the clinicopathological characteristics of the enrolled cases. Of the 117 patients, 38 (32.5%) received postoperative concurrent cisplatin-based chemotherapy with external beam RT due to high-risk factors for recurrence (i.e. positive lymph nodes, positive resection margins, parametrial invasion). During the study period, 15 cases had recurrent disease and another 4 died of disease. Patients with different histological subtypes showed no significant differences in OS or PFS ($p = 0.743, p = 0.718$; data not shown).

c-MET protein expression

c-MET protein expression was assessed using IHC in 117 cases. Seventy-two (61.5%) were scored as 0/1+, 40 (34.2%) as 2+ and 5 (4.3%) as 3+ (Figure 1A2-C2, Table 2). One stage IV SCC was scored as 2+ and another stage IV AC was scored as 3+. There was a statistically significant difference in c-MET expression across the histological subtypes ($p = 0.007$; Table 2), with IHC 2+/3+ cases more frequent in the AC cases (74.5%) compared to only 31.3% of the SCC cases. IHC 3+ was detected only in AC cases. Patients classified as having c-MET overexpression (IHC 2+/3+) did not show significant differences in OS and PFS compared to those deemed not to have overexpression (IHC 0/1+) ($p = 0.958$ and $p = 0.799$; Figure 2A and 2B). However, patients with c-MET IHC 3+ showed significantly worse OS and PFS compared to patients with IHC 0/1+/2+ ($p = 0.001$ and $p < 0.001$; Figure 2C and 2D). Of the 23 AC cases, IHC 3+ was correlated with significantly worse OS and PFS ($p = 0.005, p = 0.006$; data not shown).
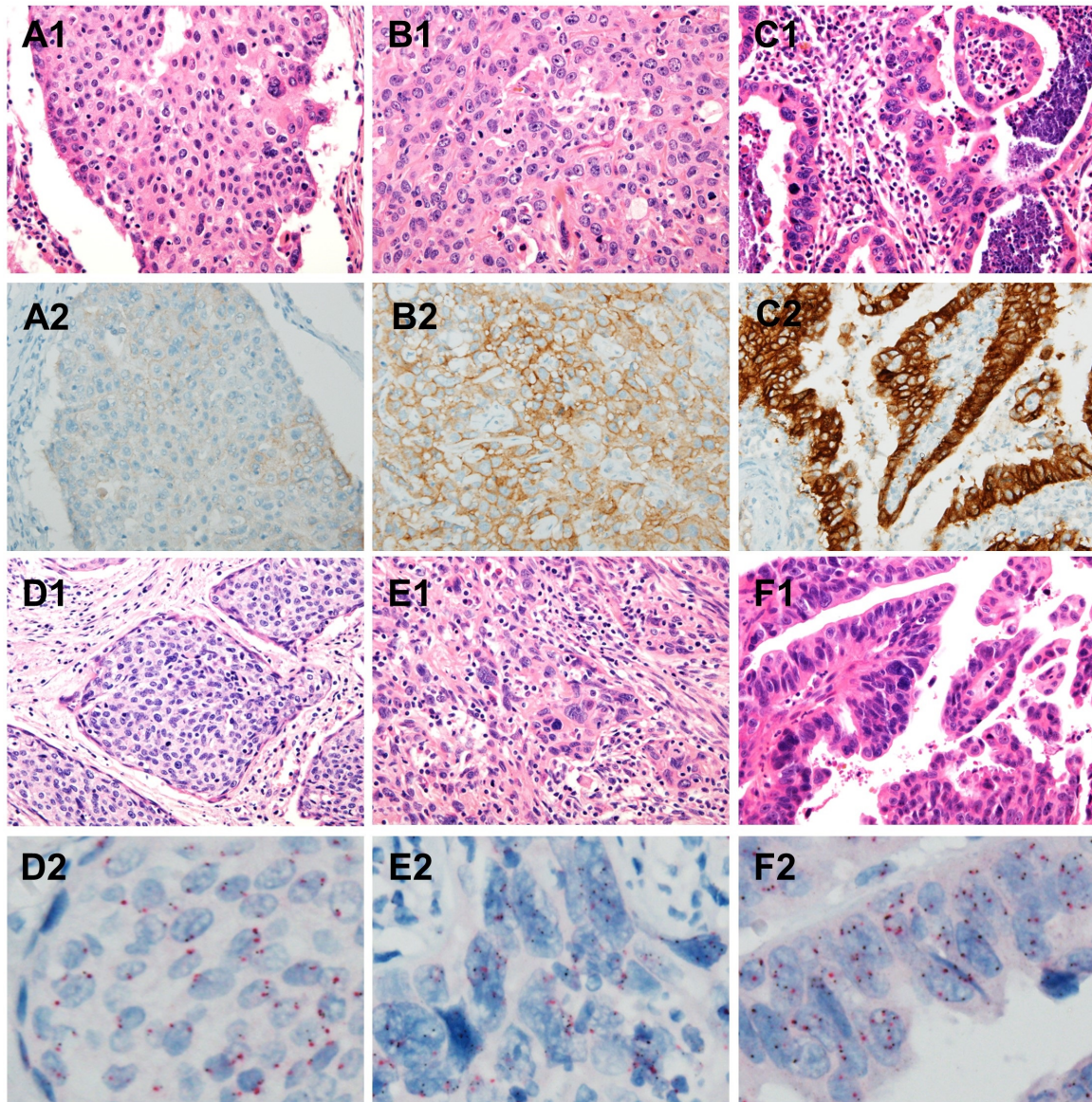
Figure 1. — Representative micrographs of hematoxylin and eosin (H&E) staining, c-MET IHC and SISH results in cervical carcinomas. (A-C) Three images of cervical carcinomas exhibiting IHC scores of 1+ (A2, squamous cell carcinoma), 2+ (B2, squamous cell carcinoma), and 3+ (C2, usual type endocervical adenocarcinoma). A1, B1, and C1 show H&E-stained sections of cases A, B, and C, respectively (original magnification ×400). (D-F) Representative c-MET SISH images of disomy in squamous cell carcinoma (D2), high polysomy in squamous cell carcinoma (E2) and high polysomy in serous adenocarcinoma (F2) (original magnification ×600). D1, E1, and F1 show H&E-stained sections of cases D, E, and F, respectively (original magnification ×400).

c-MET gene copy number

c-MET GCN was evaluated in 101 of 117 cases using SISH, with 16 cases (13.7%) classified as 'no result.' In total, 95 (94.1%) patients were scored as disomy/low trisomy/low polysomy and only 6 (5.9%) with HP (Figure 1, D2-F2, Table 2). High trisomy and GA were not detected. One stage IV SCC case showed disomy and another stage IV AC showed HP. A positive *c-MET* SISH result for HP was detected in 3 SCC cases (3.6%) and in 3 AC cases (13.0%) (Table 2). All 3 AC cases with HP were IHC 3 +, while the 3 SCC cases with HP were IHC 1+,1+, and 2+. There were no statistically significant differences in *c-MET* GCN across the histological subtypes (*p* = 0.199; Table 2). Although patients with high *c-MET* GCN tended to exhibit worse OS and PFS compared to patients with negative *c-MET* SISH results, these differences did not reach statistical significance (*p* = 0.307 and *p* = 0.184; Figure 2E and 2F). AC patients with HP did not show worse OS and PFS than AC patients with negative *c-MET* SISH (*p* = 0.336, *p* = 0.126; data not shown).
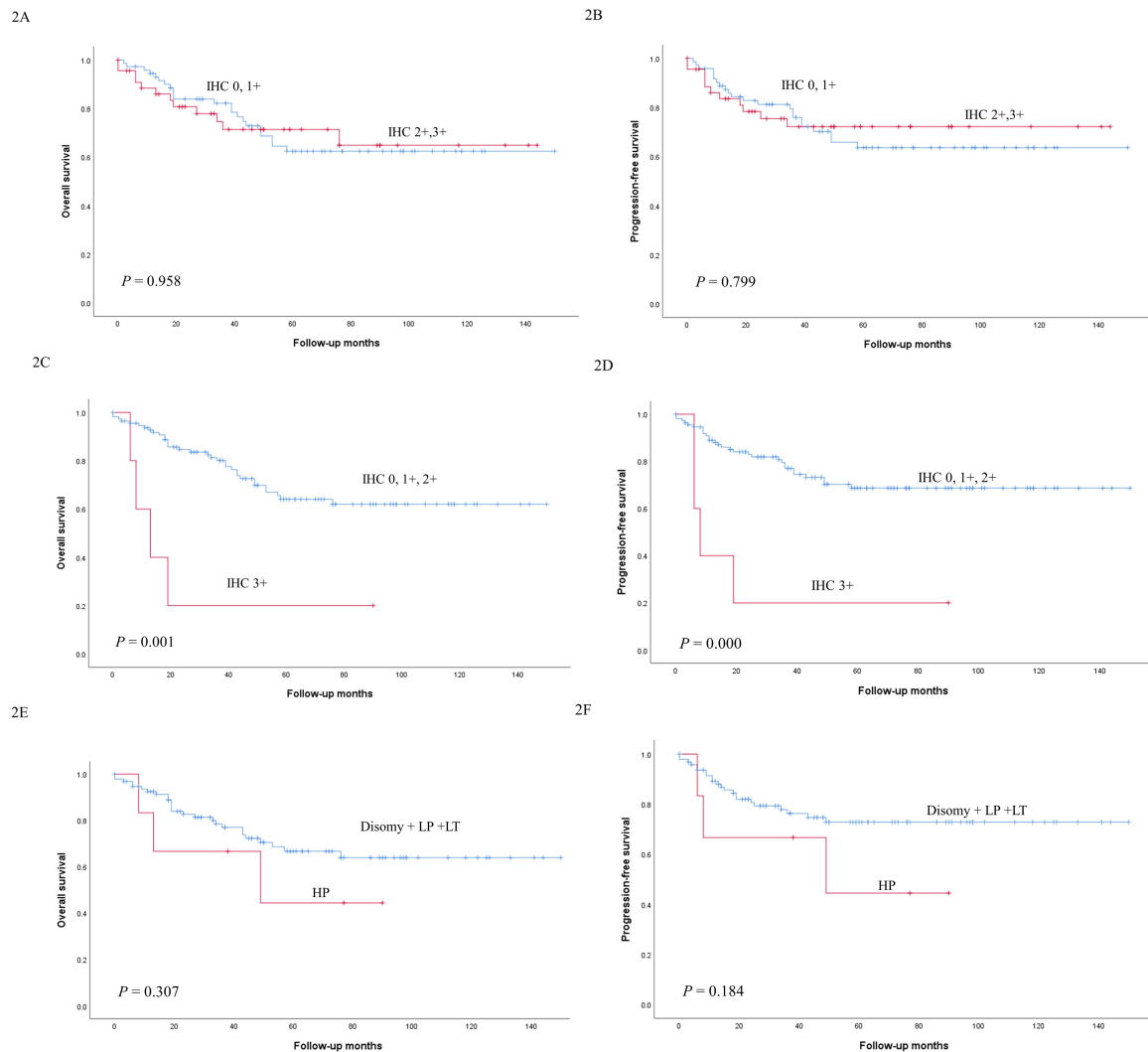
2A
2B

2C
2D

2E
2F

Figure 2. — Kaplan–Meier curves showing overall and progression-free survival of patients with uterine cervical cancer stratified by c-MET overexpression detected by immunohistochemistry (A-D) and positivity of silver *in situ* hybridization, indicating c-MET gene copy number alteration (E, F). Overall and progression-free survival times were decreased in patients with IHC 3+ c-MET overexpression (C, D). In contrast, no differences in overall and progression-free survival times were found with increasing gene copy number (E, F).

Table 2. — *MET overexpression and copy number alterations in cervical cancer*

	MET IHC				MET SISH				
	Negative	Positive			Negative	Positive			
Histotype	0 or 1+	2+	3+	p	DS, LT, or LP	HP	A	NR	p
SCC	57 (68.7)	26 (31.3)	0	0.007	70 (84.3)	3 (3.6)	0	10 (12.0)	0.199
AC	6 (26.1)	12 (52.8)	5 (21.7)		17 (73.9)	3 (13.0)	0	3 (13.0)	
ASCC	6 (85.7)	1 (14.3)	0		5 (71.4)	0	0	2 (28.6)	
Others	3 (75.0)	1 (25.0)	0		3 (75.0)	0	0	1 (25.0)	
Total (%)	72 (61.5)	40 (34.2)	5 (4.3)		95 (94.1)	6 (5.9)	0	16 (13.7)	

SCC, squamous cell carcinoma; AC, adenocarcinoma; ASCC, adenosquamous cell carcinoma; DS, disomy; LT, low trisomy; LP, low polysomy; HP, high polysomy; GA, gene amplification; NR, no result.

Table 3. — *Cox proportional analyses of the association between prognostic variables and overall survival in cervical cancer*

	Univariate analysis	Multivariate analysis	
	p value	Hazard ratio [95% CI]	*p* value
FIGO stage < IIb	0.000	0.03 [0.00-0.25]	0.002
Histotypes	0.743	NA	
Lymph node metastasis	0.231	NA	
Parametrial invasion	0.001	0.84 [0.26-2.75]	0.777
Positive resection margin	0.151	NA	
Negative LVSI	0.011	0.40 [0.20-0.83]	0.013
MET IHC 2+/3+	0.958	NA	
MET IHC 0/1+/2++	0.001	0.10 [0.02-0.64]	0.015
SISH positive	0.302	NA	

LVSI, lympho-vascular space invasion; IHC, immunohistochemistry; SISH, silver in situ hybridization; HP, high polysomy; NA, not applicable.

Correlation between c-MET protein expression and gene copy number

Following exclusion of the 16 cases classified as 'no result', there was a positive and significant association between c-MET protein overexpression and increased GCN (r = 0.228, *p* = 0.022). Of the 16 'no result' cases, 10 (10/83, 12.0%) were SCCs, 3 (3/23, 13.0%) were ACs, 2 (2/7, 28.6%) were ASCCs, and 1 (1/4, 25.0%) was another type.

Prognostic significance of c-MET protein expression and of clinicopathologic indicators

In univariate analysis, the significant factors associated with decreased OS were high FIGO stage ($P < 0.001$), parametrial invasion ($p = 0.001$), lympho-vascular space invasion (LVSI) ($p = 0.011$), and IHC 3+ c-MET protein expression ($p = 0.001$) (Table 3). Multivariate analysis using Cox proportional analysis revealed that high FIGO stage, LVSI and IHC 3+ c-MET protein expression were independently associated with worse OS: FIGO stage < IIb ($p = 0.002$; hazard ratio [HR] = 0.03; 95% confidence interval [CI], 0.00-0.25), negative LVSI ($p = 0.013$; HR = 0.40; 95% CI, 0.20-0.83) and IHC 0/1+/ 2+ c-MET expression ($p = 0.015$; HR = 0.10; 95% CI, 0.02-0.64) (Table 3). Table 4 presents the clinicopathological features of AC cases determined to be IHC 3+. All 3 increased *c-MET* GCN cases showed IHC 3+ overexpression.

Discussion

Targeted therapies have been highlighted recently [7, 19]. Tailored treatments for individual patients would clearly lead to better treatment outcomes. In the present study, we showed that c-MET protein overexpression (IHC 3+) was independently associated with poor prognosis in patients with uterine cervical AC treated by surgery. Although *c-MET* GCN did not show significant correlation with OS or PFS, we demonstrated a significant correlation between c-MET protein overexpression using IHC and in-

creased *c-MET* GCN using SISH.

To our knowledge, this is the first study using SISH in cervical cancer to evaluate *c-MET* GCN. Since previous studies have suggested that *MET* GA has prognostic impact for several different human malignancies [10, 15, 19], we hypothesized that SISH would add further accuracy to the prediction of outcome for cervical cancer. Fluorescent- or silver- ISH technology has been shown to give accurate assessment of GCN in tumor cells within tissue sections [20-27]. Therefore, Southern blot analysis and qPCR has been gradually replaced by ISH methods [16]. We used SISH instead of FISH to quantify GCN because this evaluation can be performed using conventional light microscopy and an automated platform while preserving cell morphology. Additionally, SISH is more suited to routine clinical practice because the slides are stable and can be reevaluated several years after staining [15, 17, 27]. However, *c-MET* GCN could not be assessed here in 16 (13.7%) cases using SISH and hence these were classified as "no result" (NR). One of the reasons for the high NR rate may be due to the occurrence of DNA degradation in old paraffin blocks. Nuovo *et al.* suggested that *in situ*-based signals for DNA and microRNA in FFPE tissues are significantly reduced over time [28]. The authors theorized this was due to degradation of the target through gradual loss of the phosphodiester bonds in DNA and RNA and of the peptide bonds in proteins [28]. Nonetheless, they suggested the reduction in signal could be limited by preparing the sections immediately before testing, as carried out in the present study. Despite using the same archival blocks for the IHC analyses, all samples gave a result. This finding indicates that GCN evaluation using older paraffin blocks is likely to be less reliable than IHC-based testing for protein expression. Another reason for negative results with *c-MET* GCN testing may be the amount of cancer tissue. Our previous study using SISH on epithelial ovarian cancer showed NR in only 1.9% (2/104) of cases [15]. Moreover, the TMA used in this previous study was made using the same methods and by the same

Table 4. — *Clinicopathological data of adenocarcinoma cases determined to have an immunohistochemistry score of 3+*

Histotype	Age at diagnosis (yo)	Stage	PFS (Mo)	OS (Mo)	IHC	SISH
AC*	54	IB1	19	19	3+	Disomy
AC†	45	IB1	90	90	3+	High polysomy
AC*	56	IB1	6	6	3+	Low trisomy
AC*	39	IB1	8	8	3+	High polysomy
AC‡	49	IVB	6	13	3+	High polysomy

*AC, adenocarcinoma; PFS, progression-free survival; OS, overall survival; * endocervical adenocarcinoma, usual type; † mucinous adenocarcinoma, NOS; ‡ serous adenocarcinoma.*

team as in the present study, and the epithelial ovarian cancer blocks were as old as the cervical cancer blocks. Although cervical cancer patients treated by RT and conization were excluded from this study due to insufficient volume of cancer tissue, some FFPE blocks from radical hysterectomy cases may have been less suitable for TMA compared to cytoreduced epithelial ovarian cancer cases.

This study found significantly more positive c-MET overexpression (IHC 2+/3+) in cervical AC compared to SCC ($p = 0.007$). Interestingly, the 5 IHC 3+ cases were all ACs. Moreover, patients with c-MET IHC 3+ showed significantly worse OS and PFS compared to those with IHC 0/1+/2+ ($p = 0.001$ and $p < 0.001$; Figure 2C and 2D). Previous studies reported c-MET overexpression in 30.4% and 60.0% of cases [12, 13], which also correlated with worse prognosis. However, in the present study we found that IHC 3+ c-MET overexpression accounted for only 4.3% of all cervical cancer cases. These differences in the frequency of positive cases may be due to the more recent and therefore perhaps more specific antibodies used for IHC in our study. The autoimmunostainer used in our study may also provide higher sensitivity and specificity than in previous studies. Finally, different scoring criteria for IHC may also explain the different IHC+ frequencies. The present IHC data were classified using a 4-tiered system, whereas previous studies used 3- or 2-tiered classification systems.

Multivariate analysis revealed independent association between high FIGO staging, positive LVSI, IHC 3+ and worse prognosis (Table 3). However, high-risk factors for the recurrence of cervical cancer did not impact prognosis. In the clinic, adjuvant CCRT after surgery is recommended for patients with at least one high-risk factor for recurrence. LVSI with large tumor size, or LVSI with deep cervical stromal invasion are criterion for adjuvant RT. Although LVSI is an intermediate risk factor for recurrence, patients without other combined intermediate risk factors are not obligated to undergo adjuvant RT. In real world practice, LVSI without other intermediate risk factors may have an impact on prognosis. According to the present results, c-MET overexpression with IHC 3+ should be highlighted as an independent risk factor for recurrence in cervical AC cases. Therapy that utilizes anti-MET agents may therefore produce superior outcomes in selected AC patients.

The limitations of this study are firstly that it was retro-

spective, although the study population is likely to be representative of cervical cancer patients who receive radical hysterectomy. Nearly all patients who underwent this treatment at a single institute since its' opening were enrolled, thus limiting the extent of selection bias. Secondly, the significant correlation between c-MET protein overexpression and increased *c-MET* GCN was observed after excluding 16 *c-MET* GCN cases (13.7%) classified as 'NR'. This technical limitation with SISH may have biased the study and evaluation of GCN with another method may have led to different findings. Thirdly, although the two stage IV cases are unlikely to have changed the conclusion, they could bias the results of the study. If the one AC case with IHC 3+ and high polysomy was an earlier FIGO stage, the patient would probably still have had poorer prognosis due to their high c-MET protein expression and GCN. Nonetheless, the two stage IV cases could still have affected the survival analysis. Finally, this work was conducted on patients who had been treated by radical hysterectomy, thus allowing supply of sufficient tissue for the TMA. The inclusion of patients from all stages may have altered the findings of this study.

In conclusion, IHC 3+ c-MET overexpression in surgically treated cervical cancer patients was significantly correlated with poor PFS and OS and was detected only in adenocarcinomas. Although *c-MET* GCN alone was not associated with prognosis, high GCN was significantly correlated with high c-MET protein expression. Further studies of c-MET overexpression and *c-MET* GCN in cervical cancer patients from all stages are required to confirm their correlation and prognostic significance.

Acknowledgments

Conflict of Interest

No conflicts of interest were declared.

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