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Kallikrein 5 overexpression is associated with poor prognosis in uterine cervical cancer

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ABSTRACT

Objective: Kallikrein 5 (KLK5), which is frequently observed in normal cervico-vaginal fluid, is known to be related to prognosis in several solid tumors. We investigated the prognostic significance of KLK5 in uterine cervical cancer using tumor tissue microarray and immunohistochemistry staining.


Methods: We analyzed samples of 165 patients with uterine cervical cancer who received definitive radiation therapy between 2004 and 2012. We divided patients into two groups stratified by their KLK5 activity by immunohistochemistry staining: negative/weak (0–1+) (n=120 patients) and moderate/strong (2–3+) group (n=45 patients). Patient and tumor characteristics, patterns of failure, and survival outcomes were compared. Univariable and multivariable analyses were performed to identify prognostic factors.

Results: Patients with KLK5 2–3+ were younger (median: 52 vs. 60 years) and had frequent paraaortic lymph node involvement (40.0% vs. 18.3%) than those with KLK5 0–1+. With a median follow-up of 60.8 (interquartile range, 47.5–77.9) months, patients with KLK5 2–3+ had inferior 5-year locoregional recurrence-free survival and distant metastasis-free survival of 61.7% (vs. 77.5% in KLK5 0–1+ group) and 59.4% (vs. 72.8% in the KLK5 0–1+ group), respectively (all p<0.05). KLK5 2–3+ expression retained its significance after adjusting for other well-known prognostic factors of tumor size and stage in multivariable analysis.


Conclusions: KLK5 overexpression is associated with the aggressiveness of cervical cancer and may underlie the diminished response to conventional treatments. Therefore, KLK5 could be a reliable prognostic factor in cervical cancer.


Keywords: Uterine Cervical Cancer; Kallikreins; Radiation Therapy; Prognosis

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

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INTRODUCTION

Cervical cancer ranks second in terms of incidence globally and seventh in Korea [1], with approximately 10 times lower rates in Western Europe and North America [2,3]. Although the incidence rate of cervical cancer has been declining worldwide over the last several decades [3,4], it is increasing in younger Korean populations [4,5].

Radiation therapy (RT) is the main treatment for both advanced (III–IVA) and early (IB–IIB) stages of cervical cancer [6]. Technological advances in RT including image-guided brachytherapy and radio-sensitizing drugs have significantly improved treatment outcomes, with local control rates > 90% and 5-year overall survival rates of 80% [7,8]. However, some patients do not respond to conventional treatments or relapse shortly afterwards. Some biologic markers, such as tumor-related leukocytosis [9], receptors (EPHA4, ENDRA, and NR2C1), micro RNAs (miR-203, and miR-30b) [10], transcription factors (E2F, E2F4, ETS1, and CULT1) [11], and other proteins (PARP1, CDK1, WNK1, and CRYAB) [12] as well as several clinicopathologic factors including Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage, tumor size, and lymph node status have been suggested to identify patients who are at the greatest risk of recurrence.

As a family of 15 genes on chromosome 19q13.4, kallikreins (KLKs) are trypsin- or chymotrypsin like proteases that are expressed in not only human cervico-vaginal fluid [13,14] but also other physiologic conditions including skin desquamation [15], and brain development [16]. Under normal physiological conditions, proteolytic cascades associated with KLKs is known to be responsible for corneocytes desquamation within skin layers [15] and antimicrobial effects by cleavage of the antimicrobial human cathelicidin protein hCAP-18 in cervico-vaginal fluid [17,18]. On the contrary, many previous reports regarding KLKs advocated that KLKs could regulate both tumor progression and suppression in various aspects [19]. Among various contributions of KLK family in tumor growth, KLK5 itself has several roles in cancer progression. Firstly, KLK5 promotes tumor proliferative signaling with an activation of growth-factor signaling pathway, such as insulin-like growth factor signaling [20,21]. KLK5 is also known to cleave extracellular matrix components, collagen, fibronectin, and laminin which could provoke cancer invasion and metastasis [20]. Lastly, KLK5 also degrades and activates cathelicidin-derived antimicrobial peptide, a key component of innate immunity, resulting in cancer cell immune evasion [17,22]. Taken together, KLK5 might be closely relevant to tumorigenic condition which brings an attention as a potential therapeutic target.

There is little evidence on the prognostic value of KLKs in cervical cancer, [14] although there are several reports regarding the prognostic value of KLKs in other solid tumors, such as prostate, breast, ovarian cancer, and hepatocellular carcinoma [23–26]. In addition, KLK5 is expected to have a prognostic value in ovarian cancer, another female reproductive tract malignancy [27] and prostate cancer [21]. To this end, in the present study, we carried out immunohistochemical analysis of clinical specimens using tissue microarray to identify the role of KLK5 in tumor aggressiveness and prognosis of uterine cervical cancer.

MATERIALS AND METHODS

1. Tumor specimens

Paraffin-embedded specimens from 165 patients with uterine cervical carcinoma who received definitive RT were obtained from Yonsei Cancer Center archives. Inclusion criteria

were histologically confirmed cervical carcinoma and RT to intact uterus between January 2004 and December 2012. Patients 1) who had recurrent or metastatic disease, 2) who had a history of other malignancies, or 3) who had missing follow-up data were excluded. The protocol was approved by the Institutional Review Board (No. 4-2015-0454) or human research ethics committee at each participating center and complied with country-specific regulatory requirements. The study was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was waived because the revision of the Bioethics and Safety Act of Korea was endorsed in 2013.

2. Treatment

All patients were treated with external beam RT and high-dose rate intracavitary brachytherapy. Detailed procedures of external beam RT and intracavitary brachytherapy were described previously. [7,9,28,29] One hundred and thirty-one patients without lymph node involvement received whole pelvis RT; 14 patients with paraaortic lymph node involvement were treated with extended-field RT covering up to T11/L2 or T12/L1 [28], and 20 patients received a semi-extended-field RT from the superior border to the upper margin of L2. Regarding intracavitary brachytherapy, a total dose of 30 Gy in 6 fractions (88 patients) or 10 fractions (50 patients) was most commonly prescribed at point A. After weekly pelvic examination, midline block at 27–36 Gy was applied in 100 patients [7]. After 2000, concurrent chemo-RT was routinely performed [29].

3. Tissue microarray construction

Resected tumor specimens were fixed in 10% buffered formalin and embedded in paraffin according to a standard protocol [30]. All available hematoxylin and eosin-stained slides were reviewed, and the most representative tumor areas without necrosis were marked on the slides. Formalin-Fixed Paraffin-Embedded blocks for tissues corresponding to the marked areas were sampled using the Quick-Ray Tissue Microarrayer (Unitma, Seongnam, Korea).

4. Immunohistochemistry

Immunohistochemical analysis was performed using a compact polymer method (Bond Intense Detection Kit; Leica Biosystems, Newcastle-upon-Tyne, UK). The 4- μ m-thick, FFPE sections were deparaffinised and dehydrated with xylene and then rehydrated in a graded series of alcohol. Endogenous peroxidase activity was quenched by incubation in 0.3% hydrogen peroxide and methanol for 20 minutes. Following a rinse in phosphate-buffered saline, sections in citrate buffer (0.01 M, pH 6.0) were irradiated in a microwave oven for 20 min and allowed to cool at room temperature. The sections were incubated with polyclonal anti-KLK5 antibody (1:100; R&D Systems, Minneapolis, MN, USA), and immunoreactivity was visualized using the Peroxidase/DAB EnVision+ Detection System (Dako, Carpinteria, CA, USA). The sections were counterstained with haematoxylin and covered with cover slips for observation under a microscope. The positive control was normal skin tissue; the negative control was prepared by substituting non-immune serum for the primary antibody, and no staining was detected [31].

Immunoreactivity was independently scored by 2 board-certified pathologists who were blinded to clinicopathological information. The staining positivity in tumor cells was scored on a scale of 0–3 as follows: 0, negative; 1, weak; 2, moderate; and 3, intense. The percentage of positive tumor cells was classified into four categories: 1, <10%; 2, 10%–24%; 3, 25%–49%; and 4, \geq 50%. The final score was calculated by multiplying the intensity and percentage scores. Immunoreactivity was then classified as negative (score=0), weakly

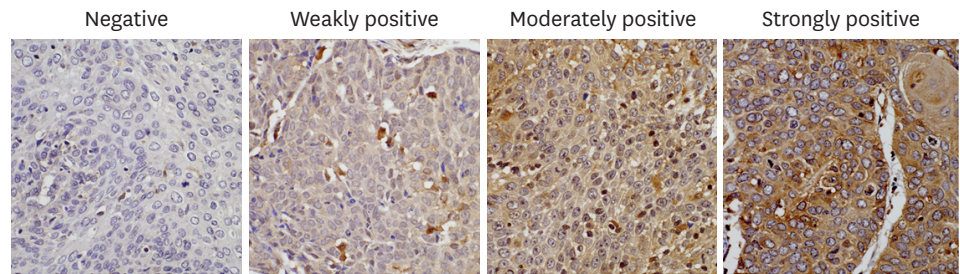


Fig. 1. Expression of human KLK5 in uterine cervical cancer tissue detected by tissue microarray and immunohistochemistry. KLK5, kallikrein 5.

positive (score=1 or 2), moderately positive (score=3–6), and strongly positive (score=8–12) (**Fig. 1**). Disagreements between the two pathologists were resolved by discussion. For further analysis, we categorized patients into 2 groups regarding immunoreactivity; 0–1+ group (negative to weakly positive) and 2–3+ group (moderately to strongly positive).

5. Statistical analysis

Either χ^2 test or Mann-Whitney U test was employed to compare the categorical or continuous data between 2 groups. The response rate was defined as the proportion of responders (complete and partial response) among all patients. Locoregional recurrence-free survival (LRFS) and distant metastasis-free survival (DMFS) were determined from the date of initial diagnosis to the date of each event or death from any cause (whichever came first). Survival was estimated with the Kaplan-Meier method, and comparisons were performed with the log-rank test. The Cox proportional hazards model was used to analyze the significance of prognostic factors and bootstrapping method with 1,000 resampling was performed to confirm covariate independence in multivariable analysis. Statistical significance was set at a 2-sided p-value <0.05. Statistical analyses were performed using SPSS version 25.0.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The study population comprised 165 patients (median age, 57) with stage IA–IVA uterine cervical carcinoma, and the baseline characteristics are summarized in **Table 1**. Most patients (n=147, 84.1%) were histologically diagnosed with squamous cell carcinoma. With a median tumor size of 4.5 (interquartile range [IQR], 3.0–6.0) cm, half of the patients had FIGO stage III–IVA disease. Patients received definitive RT with (n=142, 86.1%) or without (n=23, 13.9%) concurrent platinum-based chemotherapy. Among 165 patients, most patients were categorized into the KLK5 0–1+ group (n=120, 72.7%), and 45 patients (27.3%) were categorized into the KLK5 2–3+ group. Patients in the KLK5 2–3+ group were younger (median 52 years), had frequent paraaortic lymph node involvement at diagnosis (n=18, 40.0%), and were more frequently treated with concurrent chemo-RT (n=43, 95.6%). Additionally, 18 patients (40.0%) in the KLK5 2–3+ group presented with tumor-related leukocytosis defined as leukocyte exceeding 9,000/ μ L at 2 separate occasions without infection compared to the 22 patients (18.3%) in the KLK5 0–1+ group that exhibited tumor-related leukocytosis at baseline (p=0.004). Moreover, 6 patients with KLK5 3+ had a larger tumor size (median, 6.6 cm), and 5 of them presented with advanced disease of FIGO stage III–IVA.

Table 1. Baseline characteristics stratified by KLK5 expression intensity based on immunohistochemistry staining

Variables	Total (n=165)	KLK5 0-1+ (n=120)	KLK5 2-3+ (n=45)	p
Age (yr)	57 [48-67]	60 [49-68]	52 [46-61]	0.008
Hb (mg/dL)				0.085
<10	35 (21.2)	21 (17.5)	14 (31.1)	
≥10	130 (78.8)	99 (82.5)	31 (68.9)	
Histology				0.782
SCC	147 (89.1)	106 (88.4)	41 (91.1)	
ADC	13 (7.9)	9 (7.5)	4 (8.9)	
ADSCC	4 (2.4)	4 (3.3)	0 (0.0)	
Clear cell	1 (0.6)	1 (0.8)	0 (0.0)	
Differentiation				0.913
WD	7 (4.2)	5 (4.2)	2 (4.4)	
MD	13 (7.9)	9 (7.5)	4 (8.9)	
PD	15 (9.1)	12 (10.0)	3 (6.7)	
Not available	130 (78.8)	94 (78.3)	36 (80.0)	
PET evaluation				0.158
Yes	139 (84.2)	98 (81.7)	41 (91.1)	
Size (cm)	4.5 [3.0-6.0]	4.0 [3.0-5.7]	5.0 [3.4-6.2]	0.113
<4	97 (58.8)	75 (62.5)	22 (48.9)	0.155
≥4	68 (41.2)	45 (37.5)	23 (51.1)	
FIGO stage*				0.863
I-II	79 (47.9)	58 (48.3)	21 (46.7)	
III-IV	86 (52.1)	62 (51.7)	24 (53.3)	
LN metastasis				0.015
No	90 (54.5)	68 (56.7)	22 (48.9)	
Pelvic	58 (35.2)	45 (37.5)	13 (28.9)	
Paraortic	17 (10.3)	7 (5.8)	10 (22.2)	
TRL				0.004
No	125 (75.8)	98 (81.7)	27 (60.0)	
Yes	40 (24.2)	22 (18.3)	18 (40.0)	
Treatment				0.041
RT alone	23 (13.9)	21 (17.5)	2 (4.4)	
CCRT	142 (86.1)	99 (82.5)	43 (95.6)	

Values are presented as number of patients (%) or median [interquartile range].

ADC, adenocarcinoma; ADSCC, adenosquamous cell carcinoma; CCRT, concurrent chemoradiation therapy; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; Hb, hemoglobin; KLK5, kallikrein 5; LN, lymph node; MD, moderately differentiated; PD, poorly differentiated; PET, positron emission tomography; RT, radiation therapy; SCC, squamous cell carcinoma; TRL, tumor-related leukocytosis; WD, well differentiated.

*FIGO stage refers to the revised 2018 FIGO staging.

There was no difference in the rate of complete response after RT between the KLK5 0-1+ and 2-3+ groups (95.8% vs. 91.1%, $p = 0.258$). However, tumors in the KLK5 3+ group showed the lowest complete response rate (66.7%) than those in the KLK5 0-1+ and KLK5 2+ group (95.8% and 94.9%, respectively, $p=0.036$). Detailed information on patterns of failures is summarized in **Fig. 2**. The overall progression was more frequently observed in the KLK5 2-3+ group (53.3%) than in the KLK5 0-1+ group (24.2%, $p<0.001$). There were 40 patients (75.5% of disease progression in the entire cohort) who developed distant metastases. Specifically, patients with tumor expressing KLK5 2-3+ had a higher risk of distant metastasis than patients in the KLK5 0-1+ group (37.8% vs. 19.2%, $p=0.021$). Twenty-one patients of the entire cohort experienced local or regional recurrences. Local recurrences were observed in 7 patients (5.8%, **Fig. 2A**) in the KLK5 0-1+ group and 5 patients (11.1%, **Fig. 2B**) in the KLK5 2-3+ group; regional recurrences were observed in 4 patients (3.3%, **Fig. 2A**) in the KLK5 0-1+ group and 8 patients (17.8%, **Fig. 2B**) in the KLK5 2-3+ group (all $p<0.05$).

With a median follow-up period of 60.8 (IQR, 47.5-77.9) months, 5-year LRF5 and DMFS rates for the entire cohort were 73.1% and 69.0%, respectively. There was a significant difference

KLK5 and poor prognosis in cervical cancer

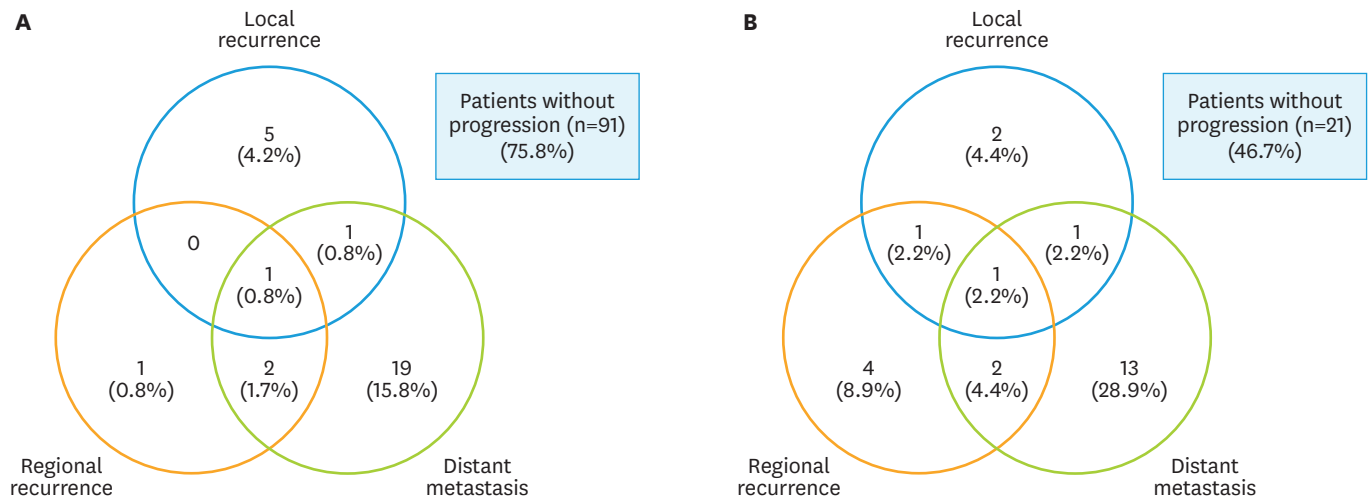


Fig. 2. Patterns of failure in the KLK5 0-1+ (A) and KLK5 2-3+ (B) groups. KLK5, kallikrein 5.

in LRFS between the 2 groups (5-year LRFS: KLK5 0-1+ and 2-3+ group, 77.5% and 61.7%, respectively, **Fig. 3A**). KLK5 2-3+ expression was a significant unfavorable factor for LRFS after adjusting for age, hemoglobin level, size, stage, lymph node involvement, treatment modality, and response after treatment (hazard ratio [HR]=2.15; 95% confidence interval [CI]=1.10-4.20; p=0.026; **Table 2**). A similar trend was also observed in the DMFS of the patients in the 2 groups; the KLK5 2-3+ group had inferior 5-year DMFS outcomes (59.4%) compared to the KLK5 0-1+ group (72.8%, **Fig. 3B**). In addition, the inferior DMFS outcomes of the KLK5 2-3+ group were observed consistently in both FIGO I-II (71.4% vs. 77.0% in the KLK5 0-1+ group) and FIGO III-IV (48.7% vs. 69.1% in the KLK5 0-1+ group) stages (**Supplementary Fig. 1**). Multivariable analysis demonstrated that KLK5 overexpression was a significant independent predictor of DMFS (HR=1.81; 95% CI=1.29-3.34; p=0.021, **Table 3**) along with response after treatment.

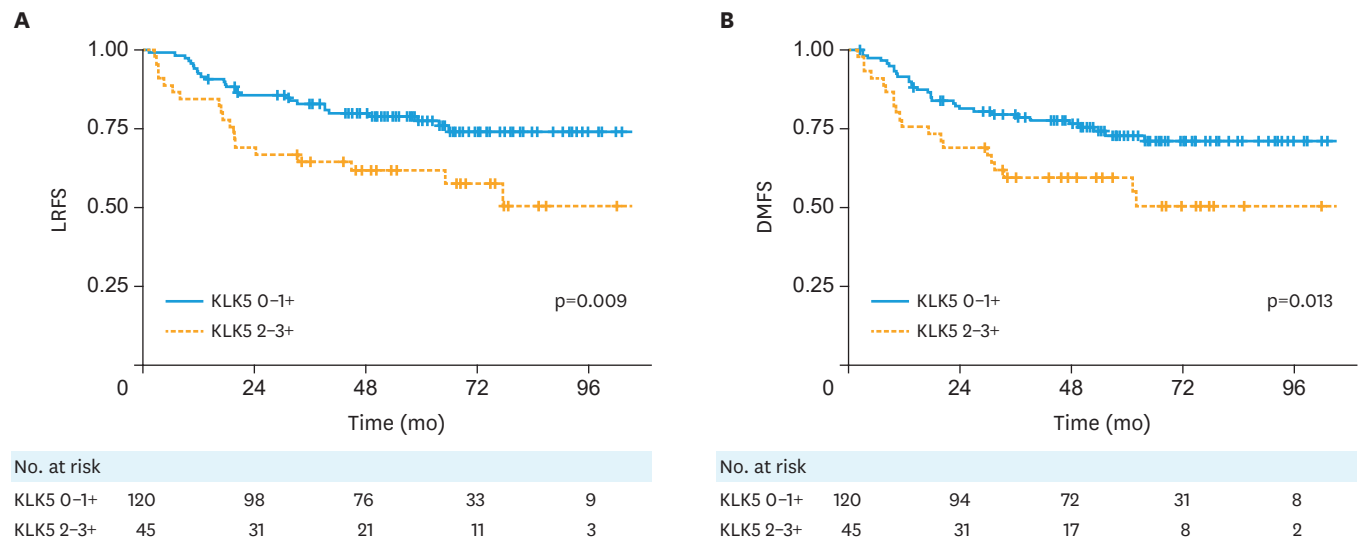


Fig. 3. (A) LRFS and (B) DMFS stratified by KLK5 immunoreactivity. DMFS, distant metastasis-free survival; KLK5, kallikrein 5; LRFS, locoregional recurrence-free survival.

Table 2. Unadjusted and adjusted HR and 95% CI for locoregional free survival in 165 patients with uterine cervical cancer

Variables	HR [†]	95% CI	p	HR [‡]	95% CI	p
Age (yr)						
<57 vs. ≥57	0.49	0.27–0.91	0.023	0.50	0.24–1.03	0.061
Hb (mg/dL)						
≥10 vs. <10	1.49	0.77–2.89	0.233	1.12	0.53–2.38	0.771
Tumour size (cm)						
<4 vs. ≥4	1.67	0.94–2.98	0.083	0.82	0.38–1.74	0.600
Histologic grade*						
WD/MD vs. PD	0.98	0.41–2.38	0.970			
FIGO stage						
I–II vs. III–IVA	2.02	1.10–3.71	0.023	5.22	1.71–15.97	0.004
LN metastasis						
No vs. Yes	1.41	0.79–2.51	0.248	0.75	0.25–1.17	0.121
Treatment						
RT vs. CCRT	0.75	0.35–1.60	0.450	0.52	0.22–1.23	0.137
Response after treatment						
CR vs. non-CR	28.17	11.84–66.98	<0.001	15.67	5.91–41.59	<0.001
KLK5						
0–1+ vs. 2–3+	2.14	1.19–3.85	0.011	2.15	1.10–4.20	0.026

The foreparts of the parentheses were set as the reference groups in the multivariable analysis.

CCRT, concurrent chemoradiation therapy; CI, confidence interval; CR, complete response; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; Hb, haemoglobin; HR, hazard ratio; KLK5, kallikrein 5; LN, lymph node; MD, moderately differentiated; PD, poorly differentiated; RT, radiation therapy; WD, well differentiated.

*Only available for 35 patients in the entire cohort; [†]Unadjusted; [‡]Mutually adjusted for seven variables after bootstrapping with 1,000 resamples.

Table 3. Unadjusted and adjusted HR and 95% CI for distant metastasis-free survival in 165 patients with uterine cervical cancer

Variables	HR [†]	95% CI	p	HR [‡]	95% CI	p
Age (yr)						
<57 vs. ≥57	0.50	0.28–0.89	0.018	0.52	0.27–1.01	0.053
Hb (mg/dL)						
≥10 vs. <10	1.38	0.74–2.60	0.315	1.37	0.65–2.91	0.410
Tumour size (cm)						
<4 vs. ≥4	1.97	1.13–3.42	0.016	1.68	0.85–3.30	0.134
Histologic grade*						
WD/MD vs. PD	1.10	0.42–2.87	0.849			
FIGO stage						
I–II vs. III–IVA	1.49	0.85–2.61	0.160	1.60	0.50–5.20	0.431
LN metastasis						
No vs. Yes	1.26	0.73–2.18	0.410	0.67	0.24–1.89	0.448
Treatment						
RT vs. CCRT	0.86	0.40–1.83	0.696	0.48	0.21–1.12	0.090
Response after treatment						
CR vs. non-CR	5.51	2.30–13.23	<0.001	2.97	1.08–8.20	0.035
KLK5						
0–1+ vs. 2–3+	2.01	1.15–3.53	0.015	1.81	1.29–3.34	0.021

The foreparts of the parentheses were set as the reference groups in the multivariable analysis.

CCRT, concurrent chemoradiation therapy; CI, confidence interval; CR, complete response; FIGO, Fédération Internationale de Gynécologie et d'Obstétrique; Hb, haemoglobin; HR, hazard ratio; KLK5, kallikrein 5; LN, lymph node; MD, moderately differentiated; PD, poorly differentiated; RT, radiation therapy; WD, well differentiated.

*Only available for 35 patients in the entire cohort; [†]Unadjusted; [‡]Mutually adjusted for seven variables after bootstrapping with 1,000 resamples.

DISCUSSION

Histologically, RT has been the mainstay of treatment for patients with cervical cancer due to its radio-sensitivity. However, the unknown biological differences in radio-resistance of non-responders who suffered from subsequent recurrences have been an obstacle for a cure. In the current study, we have demonstrated that KLK5 overexpression (KLK5 2–3+) detected in 27.2% of patients through a tissue microarray cohort was associated with poor RT response, resulting in a frequent locoregional recurrence and distant metastasis. Taken together, KLK5 overexpression could be a potential prognostic factor.

Certain KLKs including KLK5 are co-expressed in human cultured cervical cancer cell lines [13,14]. A proteolytic cascade of KLK5 elicits an activation of tumor proliferating signaling, enhancing cancer invasion or metastasis, and inducing immune evasion of cancer cell [19]. As an initiator for other KLKs, KLK5 has been identified as a preferential activator of protease-activated receptor-2 (PAR-2) [32,33], a receptor that is upregulated in cervical cancer and contributes to cancer proliferation [34]. Although we could not evaluate the expression of PAR-2 in the current cohort, further investigations incorporating KLK5 and PAR-2 in cervix cancer should be needed to clarify the relationship. In addition to extracellular matrix cleavage of KLK5 resulting in cancer invasion and metastasis [20,21], Mukai et al. [26] reported that KLK5 mediates oncogenic downstream signal through proteolytic activation of hepatocyte growth factor activator, which activates hepatocyte growth factor/scatter factor and tyrosine kinase MET signaling, which in turn, initiates invasion and metastasis [35]. Given the association between KLK5 and carcinogenesis/cancer progression [19], we can speculate that KLK5 overexpression could be related to aggressive tumor behavior in cervical cancer. Despite the limited number of patients in the KLK5 3+ group, the patients presented with advanced and larger tumor size and consequently resulted in lower rate of complete response after RT than those in the KLK5 0–2+ group.

The mechanistic link between KLKs and immune evasion has only recently begun to emerge. KLKs can affect the innate immune response preferable to tumor progression as extrinsic complement-related factors [36] and activators of transforming growth factor [37]. Specifically, KLK5 alters the tumor microenvironment by inducing the activation of the cathelicidin antimicrobial peptide LL-37, which stimulates neutrophils, monocytes, and T-lymphocytes [17,22]. Thus, continuous leukocyte recruitment to KLK5-overexpressing tumors could be characterized by changes in blood leukocyte content and hypermetabolic bone marrow in PET images. We previously reported that tumor-related leukocytosis in uterine cervical cancer is a distinct clinical entity presenting with an advanced stage at a younger age that does not respond well to standard treatments [9]. In that study, 16.2% of patients (n=398) with stage IA–IVA cervical cancer (n=2,456) who received definitive RT exhibited tumor-related leukocytosis, accompanied by an increased risk of locoregional recurrence, distant failure, and poor overall survival. In the current study, we also observed frequent tumor-related leukocytosis in KLK5 2–3+ group (40.0%) compared to KLK5 0–1+ group (18.3%). Although the exact contribution of KLK5 in tumor-related leukocytosis in tumor microenvironment needs to be explored, this indicates that the poor prognosis of tumor-related leukocytosis might be one of the phenotypes associated with KLK5 expression.

KLK5 was recently suggested as a potential biomarker in ovarian cancer [27]; in a study of 52 patients, higher KLK5 levels in serum and ascites were associated with worse progression-free survival. In addition, KLK5 protein was only detected in patients with ovarian cancer, but not in

normal female subjects and patients with benign ovarian tumors. While no currently available drugs target KLK5, recent researches have taken preliminary steps toward identifying selective pharmacological inhibitors of this protease. Based on our findings, we anticipate that direct/indirect targeting of KLK by selective synthetic KLK inhibitors and modulators will be an effective therapeutic strategy that can be combined with standard chemo-radiotherapy [38] or immune checkpoint inhibitors, which is especially promising considering the important role of KLKs in the immune response. As in the case of prostate-specific membrane antigen targeted by antitumor vaccines [39], KLK serine proteases are potential targets for immunotherapy.

Our study has several limitations. First, owing to the limited number of available tissue microarray, there are issues regarding selection bias and statistical significance of the results. However, to our knowledge, this study has the largest clinical data accompanied with tissue microarray assay thus far on cervical cancer treated with RT. In addition, we only evaluated the immunoreactivity of KLK5 among KLKs. Given that KLK5 activation is the first step in activating multiple KLKs, identifying KLK5s with immunohistochemistry staining may be an efficient and effective method for stratifying patients at higher risk for poor survival. Lastly, lack of surgical specimen in the current analysis might not be able to provide the information on tumor heterogeneity. However, biopsy specimen could provide essential information in the current study since we aimed to identify the role of KLK5 in not only tumor characteristics but also RT response.

In summary, we demonstrated that KLK5 directly affects an aggressive tumor behavior and the poor treatment response observed in a subset of cervical cancer patients. KLK5 overexpression detected by immunohistochemistry can identify high-risk cervical cancer patients who require more intensive treatment in clinical trials, although further studies are needed to clarify the involvement of KLK5 in tumor progression and to assess its value as a biomarker for predicting prognosis or treatment response or as a therapeutic target.

SUPPLEMENTARY MATERIAL

Supplementary Fig. 1

DMFS in the FIGO stage I–II (A) and III–IV (B) groups stratified by KLK5 immunoreactivity.

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REFERENCES

1. Jung KW, Won YJ, Kong HJ, Lee ES; Community of Population-Based Regional Cancer Registries. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2015. *Cancer Res Treat* 2018;50:303-16. [PUBMED](#) | [CROSSREF](#)
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;65:5-29. [PUBMED](#) | [CROSSREF](#)
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424. [PUBMED](#) | [CROSSREF](#)
4. Moon EK, Oh CM, Won YJ, Lee JK, Jung KW, Cho H, et al. Trends and age-period-cohort effects on the incidence and mortality rate of cervical cancer in Korea. *Cancer Res Treat* 2017;49:526-33. [PUBMED](#) | [CROSSREF](#)

5. Park Y, Vongdala C, Kim J, Ki M. Changing trends in the incidence (1999–2011) and mortality (1983–2013) of cervical cancer in the Republic of Korea. *Epidemiol Health* 2015;37:e2015024.
[PUBMED](#) | [CROSSREF](#)
6. Chemoradiotherapy for Cervical Cancer Meta-Analysis Collaboration. Reducing uncertainties about the effects of chemoradiotherapy for cervical cancer: a systematic review and meta-analysis of individual patient data from 18 randomized trials. *J Clin Oncol* 2008;26:5802-12.
[PUBMED](#) | [CROSSREF](#)
7. Kim KH, Kim S, Kim GE, Koom WS, Kim SW, Nam EJ, et al. De-escalation of the cumulative central radiation dose according to the tumor response can reduce rectal toxicity without compromising the treatment outcome in patients with uterine cervical cancer. *Gynecol Oncol* 2015;139:439-46.
[PUBMED](#) | [CROSSREF](#)
8. Pötter R, Tanderup K, Kirisits C, de Leeuw A, Kirchheiner K, Nout R, et al. The EMBRACE II study: the outcome and prospect of two decades of evolution within the GEC-ESTRO GYN working group and the EMBRACE studies. *Clin Transl Radiat Oncol* 2018;9:48-60.
[PUBMED](#) | [CROSSREF](#)
9. Cho Y, Kim KH, Yoon HI, Kim GE, Kim YB. Tumor-related leukocytosis is associated with poor radiation response and clinical outcome in uterine cervical cancer patients. *Ann Oncol* 2016;27:2067-74.
[PUBMED](#) | [CROSSREF](#)
10. Sharma G, Agarwal SM. Identification of critical microRNA gene targets in cervical cancer using network properties. *MicroRNA* 2014;3:37-44.
[PUBMED](#) | [CROSSREF](#)
11. Srivastava P, Mangal M, Agarwal SM. Understanding the transcriptional regulation of cervix cancer using microarray gene expression data and promoter sequence analysis of a curated gene set. *Gene* 2014;535:233-8.
[PUBMED](#) | [CROSSREF](#)
12. Kori M, Yalcin Arga K. Potential biomarkers and therapeutic targets in cervical cancer: Insights from the meta-analysis of transcriptomics data within network biomedicine perspective. *PLoS One* 2018;13:e0200717.
[PUBMED](#) | [CROSSREF](#)
13. Shaw JL, Diamandis EP. Distribution of 15 human kallikreins in tissues and biological fluids. *Clin Chem* 2007;53:1423-32.
[PUBMED](#) | [CROSSREF](#)
14. Shaw JL, Diamandis EP. A potential role for tissue kallikrein-related peptidases in human cervico-vaginal physiology. *Biol Chem* 2008;389:681-8.
[PUBMED](#) | [CROSSREF](#)
15. Caubet C, Jonca N, Brattsand M, Guerrin M, Bernard D, Schmidt R, et al. Degradation of corneodesmosome proteins by two serine proteases of the kallikrein family, SCTE/KLK5/hK5 and SCCE/KLK7/hK7. *J Invest Dermatol* 2004;122:1235-44.
[PUBMED](#) | [CROSSREF](#)
16. Yousef GM, Kishi T, Diamandis EP. Role of kallikrein enzymes in the central nervous system. *Clin Chim Acta* 2003;329:1-8.
[PUBMED](#) | [CROSSREF](#)
17. Yamasaki K, Schaubert J, Coda A, Lin H, Dorschner RA, Schechter NM, et al. Kallikrein-mediated proteolysis regulates the antimicrobial effects of cathelicidins in skin. *FASEB J* 2006;20:2068-80.
[PUBMED](#) | [CROSSREF](#)
18. Frohm Nilsson M, Sandstedt B, Sørensen O, Weber G, Borregaard N, Ståhle-Bäckdahl M. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. *Infect Immun* 1999;67:2561-6.
[PUBMED](#) | [CROSSREF](#)
19. Filippou PS, Karagiannis GS, Musrap N, Diamandis EP. Kallikrein-related peptidases (KLKs) and the hallmarks of cancer. *Crit Rev Clin Lab Sci* 2016;53:277-91.
[PUBMED](#) | [CROSSREF](#)
20. Michael IP, Sotiropoulou G, Pampalakis G, Magklara A, Ghosh M, Wasney G, et al. Biochemical and enzymatic characterization of human kallikrein 5 (hK5), a novel serine protease potentially involved in cancer progression. *J Biol Chem* 2005;280:14628-35.
[PUBMED](#) | [CROSSREF](#)
21. Michael IP, Pampalakis G, Mikolajczyk SD, Malm J, Sotiropoulou G, Diamandis EP. Human tissue kallikrein 5 is a member of a proteolytic cascade pathway involved in seminal clot liquefaction and potentially in prostate cancer progression. *J Biol Chem* 2006;281:12743-50.
[PUBMED](#) | [CROSSREF](#)

22. Yamasaki K, Di Nardo A, Bardan A, Murakami M, Ohtake T, Coda A, et al. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med* 2007;13:975-80.
[PUBMED](#) | [CROSSREF](#)
23. Dhar S, Bhargava R, Yunes M, Li B, Goyal J, Naber SP, et al. Analysis of normal epithelial cell specific-1 (NES1)/kallikrein 10 mRNA expression by in situ hybridization, a novel marker for breast cancer. *Clin Cancer Res* 2001;7:3393-8.
[PUBMED](#)
24. Stege R, Grande M, Carlström K, Tribukait B, Poussette A. Prognostic significance of tissue prostate-specific antigen in endocrine-treated prostate carcinomas. *Clin Cancer Res* 2000;6:160-5.
[PUBMED](#)
25. Avgeris M, Mavridis K, Scorilas A. Kallikrein-related peptidase genes as promising biomarkers for prognosis and monitoring of human malignancies. *Biol Chem* 2010;391:505-11.
[PUBMED](#) | [CROSSREF](#)
26. Mukai S, Fukushima T, Naka D, Tanaka H, Osada Y, Kataoka H. Activation of hepatocyte growth factor activator zymogen (pro-HGFA) by human kallikrein 1-related peptidases. *FEBS J* 2008;275:1003-17.
[PUBMED](#) | [CROSSREF](#)
27. Dorn J, Magdolen V, Gkazepis A, Gerte T, Harlozinska A, Sedlaczek P, et al. Circulating biomarker tissue kallikrein-related peptidase KLK5 impacts ovarian cancer patients' survival. *Ann Oncol* 2011;22:1783-90.
[PUBMED](#) | [CROSSREF](#)
28. Choi J, Yoon HI, Lee J, Keum KC, Kim GE, Kim YB. Optimal extent of prophylactic irradiation of paraaortic lymph nodes in patients with uterine cervical cancer. *PLoS One* 2015;10:e0145158.
[PUBMED](#) | [CROSSREF](#)
29. Kim YB, Cho JH, Keum KC, Lee CG, Seong J, Suh CO, et al. Concurrent chemoradiotherapy followed by adjuvant chemotherapy in uterine cervical cancer patients with high-risk factors. *Gynecol Oncol* 2007;104:58-63.
[PUBMED](#) | [CROSSREF](#)
30. Kampf C, Olsson I, Ryberg U, Sjöstedt E, Pontén F. Production of tissue microarrays, immunohistochemistry staining and digitalization within the human protein atlas. *J Vis Exp* 2012;(63):3620.
[PUBMED](#) | [CROSSREF](#)
31. Meyerholz DK, Beck AP. Principles and approaches for reproducible scoring of tissue stains in research. *Lab Invest* 2018;98:844-55.
[PUBMED](#) | [CROSSREF](#)
32. Hollenberg MD, Oikonomopoulou K, Hansen KK, Saifeddine M, Ramachandran R, Diamandis EP. Kallikreins and proteinase-mediated signaling: proteinase-activated receptors (PARs) and the pathophysiology of inflammatory diseases and cancer. *Biol Chem* 2008;389:643-51.
[PUBMED](#) | [CROSSREF](#)
33. Oikonomopoulou K, Hansen KK, Saifeddine M, Tea I, Blaber M, Blaber SI, et al. Proteinase-activated receptors, targets for kallikrein signaling. *J Biol Chem* 2006;281:32095-112.
[PUBMED](#) | [CROSSREF](#)
34. Sánchez-Hernández PE, Ramirez-Dueñas MG, Albarran-Somoza B, García-Iglesias T, del Toro-Arreola A, Franco-Topete R, et al. Protease-activated receptor-2 (PAR-2) in cervical cancer proliferation. *Gynecol Oncol* 2008;108:19-26.
[PUBMED](#) | [CROSSREF](#)
35. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G. Targeting MET in cancer: rationale and progress. *Nat Rev Cancer* 2012;12:89-103.
[PUBMED](#) | [CROSSREF](#)
36. Oikonomopoulou K, DeAngelis RA, Chen H, Diamandis EP, Hollenberg MD, Ricklin D, et al. Induction of complement C3a receptor responses by kallikrein-related peptidase 14. *J Immunol* 2013;191:3858-66.
[PUBMED](#) | [CROSSREF](#)
37. Pampalakis G, Sotiropoulou G. Tissue kallikrein proteolytic cascade pathways in normal physiology and cancer. *Biochim Biophys Acta* 2007;1776:22-31.
[PUBMED](#) | [CROSSREF](#)
38. Prassas I, Eissa A, Poda G, Diamandis EP. Unleashing the therapeutic potential of human kallikrein-related serine proteases. *Nat Rev Drug Discov* 2015;14:183-202.
[PUBMED](#) | [CROSSREF](#)
39. Cereda V, Formica V, Menghi A, Pellicori S, Roselli M. Kallikrein-related peptidases targeted therapies in prostate cancer: perspectives and challenges. *Expert Opin Investig Drugs* 2015;24:929-47.
[PUBMED](#) | [CROSSREF](#)