Prognostic significance of uPA and uPAR expression in patients with cervical cancer undergoing radiotherapy

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Abstract. Cervical cancer remains a major health threat. Urokinase serves as a marker of metastatic tumors. The present study aimed to determine whether the expression levels of urokinase plasminogen activator (uPA) and urokinase plasminogen activator receptor (uPAR), before and during the course of radiotherapy, serve as prognostic markers for patients with cervical cancer. Cervical tumor tissue biopsies were collected from 72 patients before radiotherapy and after the completion of external beam radiotherapy (EBRT) before intracavitary brachytherapy. The levels of uPA and uPAR were determined using ELISA assays. The significance of the associations between the protein expression levels and the clinical outcomes of patients was determined. Although irradiation enhanced uPA and uPAR expression in cervical cancer cell lines, average uPA levels significantly decreased in tumors, and uPAR levels significantly increased after EBRT. The levels of uPA increased in 12 patients and decreased in 26 patients; and those of uPAR increased in 13 patients and decreased in two patients. Cox regression analysis revealed that increased expression of uPAR was significantly associated with 5-year overall survival rate [hazard ratio (HR), 3.65; 95% confidence interval (CI), 1.18-11.30]. However, the levels of both proteins before radiotherapy failed to predict clinical outcomes. Other significant predictive factors were partial response (HR 7.22; 95% CI 1.17-44.73) and disease progression (HR, 13.41; 95% CI, 1.17-153.07). These findings indicated that increased expression of uPAR in cervical tumor tissue during radiotherapy may serve as a prognostic marker for patients with cervical cancer.

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Introduction

A major obstacle to cancer treatment is the adaptive resistance of tumor cells, and alterations in the regulation of gene expression caused by therapeutic stress may lead to treatment failure (1). Several mechanisms, including upregulation of gene expression and activation of signal transducers, defend cancer cells against radiation to confer a survival advantage. These events induce the expression of genes encoding molecules that promote survival, repair DNA damage, induce inflammation and inhibit apoptosis (2,3).

The expression of genes that encode components of the urokinase system is associated with the diagnosis and prognosis of cancer of the breast, gastrointestinal tract, colon and rectum, esophagus, kidneys, endometrium and ovaries (4,5). The urokinase system comprises of urokinase-type plasminogen activator (uPA), tissue plasminogen activator (tPA), urokinase plasminogen activator receptor (uPAR) and the plasminogen activator inhibitors (PAI)-1 and -2 (6). Similar to matrix metalloproteinases, urokinase principally induces lysis of the basement membrane and the extracellular matrix during tissue remodeling, which confers upon urokinase multifunctional roles in neoplastic and malignant transformation, tumor angiogenesis, tumor progression and metastasis (7).

The transcription of the gene encoding uPA, which mediates metastasis, is activated by transcription factors such as AP1, erythroblast transformation specific-1 (Ets-1) and Ets-2 (8). Furthermore, uPA is involved in tissue remodeling (9). The expression of its receptor uPAR (also known as CD87), a glycosyl phosphatidylinositol-anchor protein, is regulated by transcription factors such as Sp1, NF-κB, TCF, hypoxia-inducible factor-1α and forkhead box protein (Fox) M1 (6,10). The main functions of uPAR involve the regulation of extracellular proteolysis and cell-extracellular matrix interactions (11). Upregulation of the expression of uPAR and uPA enhances tumor progression as well as the aggressiveness of numerous cancer cell types such as lung, breast and stomach cancer (12). Furthermore, uPAs serve as biomarkers and targets for treatment of human malignancies such as breast and pancreatic cancer (13). For example, the expression levels of uPA and PAI-1 are recommended by the American Society

of Clinical Oncology as markers for selecting a regimen to treat patients with node-negative breast cancer and to predict metastasis (14).

Cervical cancer remains a major public health problem for women in developing countries, where it has caused ~270,000 deaths annually worldwide as of 2018 (15). In total, ~5% of all human cancer cases are caused by infection with human papillomavirus (HPV), and most are cancer cases are of the cervix (16). Despite the development and availability of HPV vaccines, oncogenic HPVs are detected by cervical cancer screening programs (17). Expression of uPA and PAI-1 correlates with the prognosis of patients with cervical cancer, and HPV16 E6 induces cancer cells to migrate following the activation of uPA (18,19). Furthermore, uPA serves as a prognostic marker for the metastatic potential of cervical cancer (20).

The present study therefore reasoned that investigation of the components of the uPA system and their relationship with resistance to therapy may improve the outcomes of patients with cervical cancer. To provide support for this hypothesis, whether irradiation enhanced the expression of uPA and uPAR in patient tissue samples and cell lines and whether such changes predicted patients' clinical outcomes were investigated.

Materials and methods

Antibodies and reagents. Antibodies against uPAR, Ets-1 and FoxM1 (cat. nos. 12863, 14069 and 5436, respectively) were purchased from Cell Signaling Technology, Inc., anti-GAPDH (cat. no. MA5-15738) was purchased from Thermo Fisher Scientific, Inc. and anti-uPA (cat. no. MAB7776) was purchased from EMD Millipore. Secondary antibodies, goat anti-mouse IgG peroxidase conjugated (cat. no. AP124P) and goat anti-rabbit IgG peroxidase conjugated (cat. no. AP132P), were purchased from EMD Millipore. RIPA cell lysis buffer and ECL solution were purchased from Merck KGaA. The working dilution for uPAR, Ets-1 and FoxM1 antibodies was 1:1,000, 1:500 for uPA antibody and 1:15,000 for GAPDH antibody. The dilution used for both secondary antibodies was 1:15,000.

Cell lines and irradiation. The human cervical carcinoma cell lines SiHa (HPV16-positive), HeLa (HPV18-positive) and C33A (HPV-negative) were purchased from the American Type Culture Collection and maintained in DMEM medium supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, and 100 mg/ml streptomycin in a humidified incubator at 37°C (5% CO_2). When exponentially proliferating cells in culture dishes reached 70-80% confluence, they were exposed at room temperature to 5 Gray ionizing radiation (6 MV) generated using a linear accelerator (Varian Medical Systems, Inc.); source-to-surface distance, 100 cm; field size=25x25 cm². The sham-irradiated cell lysates in Fig. 1 were collected at 24 h after irradiation. Irradiated cell lysates were collected at 4, 8, 16, 24 and 48 h after irradiation and subjected to immunoblotting.

Immunoblotting. Whole cell lysates from sham and irradiated cell lines were prepared using RIPA cell lysis buffer were briefly sonicated, at 20 kHz on ice for 30 sec, using an ultrasonic sonicator (Thermo Fisher Scientific, Inc.). Protein concentrations were quantified using the bicinchoninic acid (BCA) method. Next, the proteins in the lysates were separated using 10% gel SDS-PAGE electrophoresis and then electrophoretically transferred onto nitrocellulose membranes, which were probed with the aforementioned antibodies listed above at 4°C overnight. GAPDH served as a loading control. Immunocomplexes were detected using secondary antibodies conjugated to horseradish peroxidase for 1 h at room temperature, and a western blot imaging system (Synoptics Ltd) that employs ECL (Immobilon Forte Western HRP substrate; Merck KGaA) was used to visualize the bands. Densitometry was determined using ImageJ version 1.53 g (National Institutes of Health). Immunoblotting was performed in triplicate.

Patients and sample preparation. Patients diagnosed with cervical cancer (n=153) who underwent initial external beam radiation therapy (EBRT) between April 2014 and August 2017 at the Department of Radiation Oncology, Chulabhorn Hospital (Bangkok, Thailand), were considered for inclusion in the present study. The inclusion criteria were as follows: i) Patients pathologically diagnosed with cervical cancer [stages IB-IVA according to the International Federation of Gynecology and Obstetrics (FIGO) 2009] (21), ii) tissue specimens collected at baseline (before treatment with EBRT) and during radiotherapy (before 1st brachytherapy), and iii) clinical data available at initial diagnosis. The exclusion criteria were incomplete sample collection and inadequate amount of tissue sample. The inclusion criteria were met by 72 patients who were included in the data analyses. The primary outcomes of the study were 5-year disease-free survival rate (DFS) and 5-year overall survival rate (OS), defined as the date of radiation therapy until April 2020. DFS refers to patients who survived without signs or recurrence of cervical cancer, and OS refers to those who survived until April 2020. The follow-up was performed at the Department of Radiation Oncology, Chulabhorn Hospital. The patients were followed-up every 3 months after the completion of treatment in the first 2 years, every 4 months in year 3-4 and every 6 months thereafter.

Tumor samples and ELISAs. Cervical tumor biopsies were obtained from patients with their informed consent before their first radiation treatment and during the 16th to 22nd fractions of EBRT at the conventional dose of 2 Gy per fraction, before the first brachytherapy session. Tumor tissue samples were stored at -80°C and then lysed with RIPA lysis buffer and sonicated on ice at 40 kHz for one minute each. ELISAs of uPA and uPAR levels were performed using human uPA and uPAR kits (cat. nos. ab119611 and ab119612, respectively) in accordance with the manufacturer's instructions (Abcam). Color intensity at 450 nm was determined using an EnSight multimode plate reader (PerkinElmer, Inc.). Each ELISA reaction included 40 μg of tissue lysate. The concentrations of uPA and uPAR were calculated according to standard curves. An increase of uPA and uPAR ≥1.0 ng per 40 μg of total protein of irradiated vs pretreatment tissues was defined as increased expression, and a reduction of uPA and uPAR by ≥1.0 ng per 40 μg of total protein was defined as decreased expression. ELISAs were
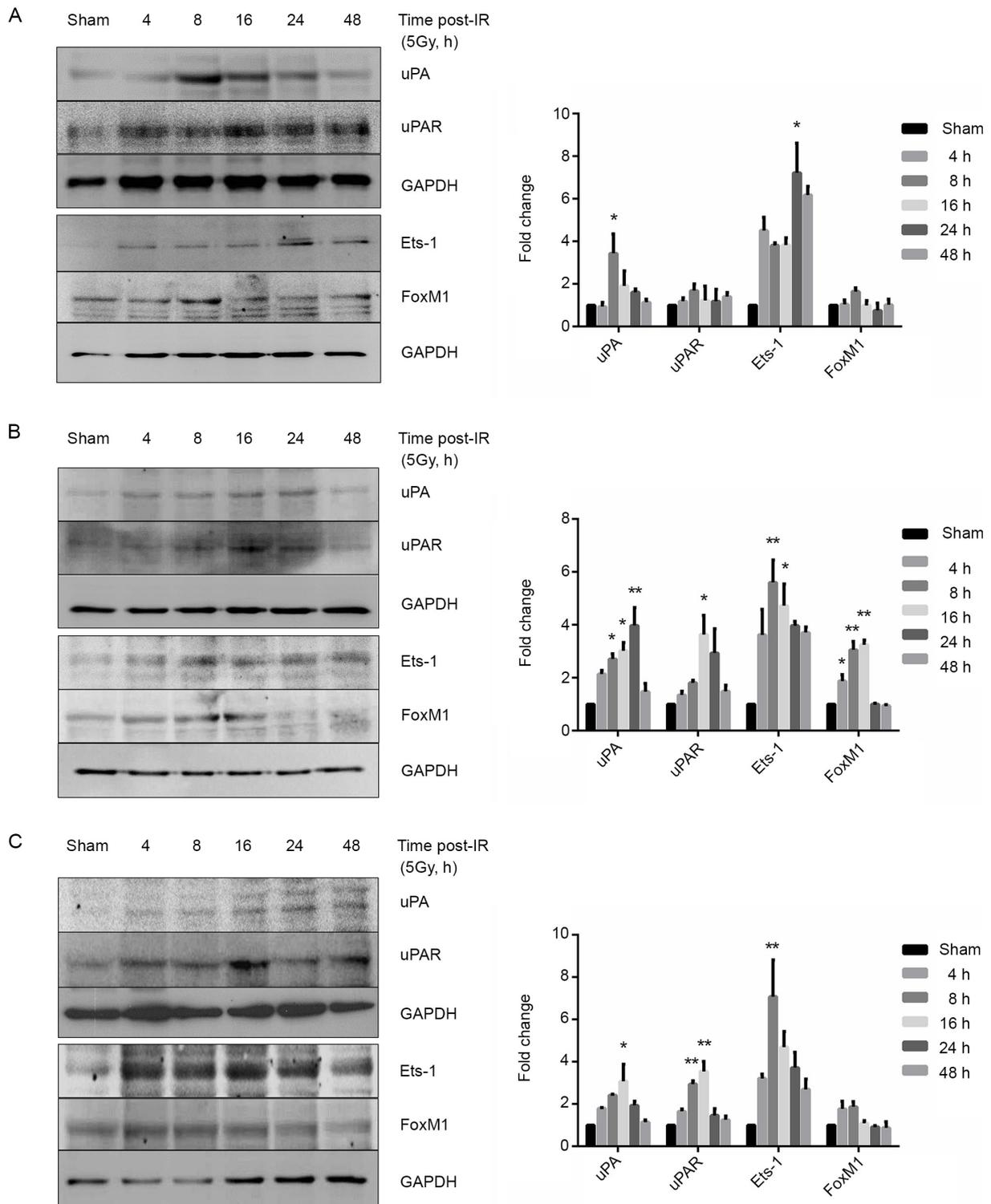
Figure 1. Immunoblotting analysis of uPA, uPAR and their target transcription factors after 5 Gy irradiation of (A) SiHa, (B) HeLa and (C) C33A cells. [*]P<0.05 and [**]P<0.01 vs sham. uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; FoxM1, forkhead box protein M1; IR, irradiation.

performed in duplicate. The median levels for uPA and uPAR pretreatment before and after completion of EBRT were used as cut-off values for survival analysis. For uPA, the median values were 2.64 ng and 1.79 ng/40 μg of tissue sample protein for pretreatment and after EBRT, respectively. For uPAR, the median values were 1.04 ng and 1.36 ng/40 μg of tissue sample protein for pretreatment and after EBRT, respectively.

Statistical analysis. Continuous variables are presented the median and range, and categorical variables are shown as frequencies and percentages (unless otherwise shown). Statistical comparisons between continuous variables were undertaken using one-way ANOVA with Tukey's post hoc test. A paired t-test was used to compare protein expression levels. DFS and OS were evaluated using the Kaplan-Meier

method with a log-rank test. The Cox proportional hazards model was used for univariate analysis of covariates as well as for multivariate analysis. P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using STATA version 12.1 software (StataCorp LLC).

Results

Exposure to ionizing radiation upregulates uPA expression in cervical cancer cell lines. To determine whether the expression of uPA and its receptor uPAR were regulated by ionizing radiation, the human cervical cancer cell lines were irradiated (5 Gy) as follows: SiHa (HPV16-Positive), HeLa (HPV18-positive) and C33A (HPV-negative). As shown in Fig. 1, immunoblotting analysis revealed that the expression of uPA and uPAR was increased in irradiated cells after 8-24 h. uPA was significantly increased at 8 h, 8-24 h and 16 h post-irradiation for SiHa, HeLa and C33A cells, respectively. uPAR expression levels were significantly upregulated at 8 h and 8-16 h after irradiation in HeLa and C33A cells but not in SiHa cells. Radiation exposure also generally increased the expression level of uPA-regulated transcription factor Ets-1 in all three cell lines after 4-48 h, but only significantly upregulated the protein after 24 h, 8-16 h and 8 h for SiHa, HeLa and C33A cells, respectively. Furthermore, the uPAR-regulated transcription factor FoxM1 was significantly upregulated by ionizing radiation in HeLa cells after 8-16 h, but not in the other cell lines.

Patient characteristics and responses to treatment. It was next determined whether the expression of uPA and uPAR was upregulated in patients receiving radiotherapy. Patient demographic data are shown in Table I. Their mean age was 53.5 years (range, 25-85 years), 73.61% had squamous cell carcinoma (38.89% with FIGO Stage IIB). In total, ~67% of patients were HPV-positive, 76.39% received concurrent chemoradiotherapy followed by intracavitary radiotherapy, 65.28% were administered cisplatin-based chemotherapy and 16.67% were administered carboplatin. The patients received a median dose of 89.45 Gy (equivalent dose$_2$) with median radiation treatment for 43 days.

All patients were treated and regularly underwent follow-up examinations. As shown in Table II, ~70.8% experienced a complete response (undetectable tumors) and the median survival time was 35 months (range, 2-60 months). Cervical tumor biopsies were performed during their 16th to 22nd fractions of pelvic irradiation at the conventional dose of 2 Gy per fraction, before the first brachytherapy session. The uPA and uPAR levels remained largely unchanged (47.22 and 79.17% of the patients, respectively), whereas radiotherapy led to an increase in uPA and uPAR levels in 16.67 and 18.06% of patients, respectively. Furthermore, uPA and uPAR levels in 36.11 and 2.78% decreased, respectively. For uPA, 34 patients had no change in expression, 26 patients had decreased expression and 12 patients had increased expression. For uPAR, 57 patients had no change in expression, two patients had decreased expression and 13 patients had increased expression. These results showed that uPA expression generally decreased after EBRT (P=0.02), although uPAR expression increased

Table I. Characteristics of 72 patients with cervical cancer.

Variables	Value
Age at diagnosis, median (range) years	53.5 (25-85)
Histological type, n (%)	
Squamous cell carcinoma	53 (73.61)
Neuroendocrine carcinoma	4 (5.55)
Adenosquamous carcinoma	12 (16.67)
Adenocarcinoma	3 (4.17)
FIGO staging, n (%)	
IB2- IIA	17 (23.61)
IIB	28 (38.89)
IIIA-IIIB	25 (34.72)
IVA	2 (2.78)
HPV status, n (%)	
Negative	8 (11.11)
Positive	48 (66.67)
Not available	16 (22.22)
Treatment, n (%)	
CCRT + ICRT	55 (76.39)
CCRT + ICRT + Adjuvant treatment	13 (18.06)
EBRT + ICRT	3 (4.17)
EBRT + ICRT + Adjuvant treatment	1 (1.39)
Chemotherapy regimen, n (%)	
No chemotherapy	4 (5.56)
Cisplatin	47 (65.28)
Carboplatin	12 (16.67)
Combination	9 (12.50)
Number of days for radiation treatments, median (range) days	43 (12-87)
EBRT + Brachytherapy, median (range), Gy	89.45 (56-99.6)
ICRT course fractions, range, days	3-5
Cycle of chemotherapy, range, cycles	0-7
Technique of EBRT, n (%)	
AP/PA	21 (29.17)
4-field box	44 (61.11)
VMAT	2 (2.78)
Combination	5 (6.94)

CCRT, concurrent radiotherapy; ICRT, intracavitary radiotherapy; EBRT, external beam radiation therapy; AP/PA, anterior-posterior/posterior-anterior; VMAT, volumetric modulated arc therapy; EQD, equivalent dose; FIGO, Federation of Gynecology and Obstetrics.

(P=0.001) (Fig. 2). Notably, the changes in uPA and uPAR expression in HPV-positive patients followed a similar trend (Fig. S1), suggesting that HPV plays a role in the regulation of the expression of uPA and uPAR.

Factors influencing therapeutic outcome. The results shown in Table III indicated that treatment response was a major predictive factor of outcomes. Among the 72 patients, 13 died, 59 survived and four experienced recurrence (Table II). The

Table II. Responses to treatment of 72 patients with cervical cancer who underwent radiation therapy.

Variables	Value
Patient treatment outcome, n (%)	
Survived	59 (81.94)
Deceased	13 (18.06)
Experienced recurrence	4 (5.56)
Treatment response, n (%)	
Complete response	51 (70.83)
Partial response	8 (11.11)
Progressive disease	6 (8.33)
N/A	7 (9.72)
uPA levels, n (%)	
No change	34 (47.22)
Decreased	26 (36.11)
Increased	12 (16.67)
uPAR levels, n (%)	
No change	57 (79.17)
Decreased	2 (2.78)
Increased	13 (18.06)
Overall survival time, median (range) months	35 (2-60)

uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor.

DFS of patients with progressive disease was significantly shorter (HR, 56.59; 95% CI, 13.45-238.09) as well as their OS [HR, 13.41; 95% CI, 1.17-153.07 (univariate analysis); and HR, 47.16; 95% CI, 3.11-714.63 (multivariate analysis)]. The patients with a partial response to therapy experienced shorter OS (HR, 7.22; 95% CI, 1.17-44.73).

Although the level of uPA expression was not significantly associated with OS (HR, 2.68; 95% CI, 0.71-10.06), increased uPAR expression was predictive. Univariate and multivariate analyses indicated that the upregulation of uPAR levels in cervical tumor tissue may serve as a prognostic factor for predicting OS [HR, 3.65; 95% CI, 1.18-11.30 (univariate analysis); and HR, 6.72; 95% CI, 1.71-26.37 (multivariate analysis)]. The data shown in Fig. 3 support the conclusion that increased uPAR expression may serve as a prognostic factor for OS but not DFS. Neither the absolute levels of expression nor the differences of either protein were a significant predictor of DFS. Together, these findings supported the conclusion that the levels of uPA and uPAR before and after EBRT were not significantly associated with DFS or OS (Figs. S2-5).

Discussion

Irradiation of tumor cells activates numerous genes encoding products that contribute to tumor invasion and metastasis, which frequently shortens survival time (22). For example, irradiation activates uPA and uPAR expression and causes meningiomas to grow *in vivo* (23). Consistent with these findings, the present *in vitro* data showed that uPA and uPAR expression was upregulated by radiation, which may be explained by their transcriptional activation by Ets-1 and FoxM1, respectively (10,24). Radiation-induced activation of NF-κB activation may account for upregulation of uPA and uPAR as well (25,26). In contrast to this, the current data acquired using tumor samples differed from these results regarding uPA expression, particularly because the protocol mimicked that employed in the clinic (five fractions per week). This may be attributed to the presence of HPV, which confers radiosensitivity (27), and patients who were HPV-positive appeared to have reduced uPA levels but enhanced uPAR levels after receiving radiotherapy. The HPV E6 oncoprotein targets and reduces the expression of microRNA (miR)-(miR-23b) and miR-34a, which negatively regulates uPA expression (18,28,29). These miRs are induced in irradiated tumor tissue samples (30,31). Thus, fractionated irradiation may enhance miR-23b and miR-34a expression and subsequently downregulate uPA expression.

HPV status and genotypes may affect the outcome of treatment because the prognosis of patients who are HPV-negative with cervical cancer is worse compared with those with HPV infection (32). Furthermore, HPV titers may predict the radiation response of cervical tumors (33). Similarly, HPV-positive head and neck tumors are more sensitive to conventional radiation or chemo- therapies (34,35). Higher radiosensitivity is caused by defective DNA damage repair, particularly due to double-stand breaks (36). An improved understanding of the interactions between HPV and therapeutics will hopefully contribute to the development of optimal individualized treatments. Another plausible reason for the downregulation of uPA after EBRT is activation of c-Myc by radiation (37,38).

Although the expression of uPA and PAI-1 in cervical tumor tissue was previously suggested to serve as a prognostic marker for stage II cervical cancer (19), the induced expression of uPA in response to radiotherapy was not significantly related to a patient DFS or OS in the present study. Furthermore, the level of uPA in cervical tumor tissue has little prognostic value (39). Nevertheless, evidence indicates that the urokinase system is closely associated with stem cell-like properties of numerous types of cancer cell, such as activated STAT3 in lung cancer and CD24⁻/CD44⁺ breast cancer (40). Accumulating evidence also indicates an association between the urokinase system and EMT, involving tumor cell depolarization into a mesenchymal phenotype characterized by high motility and enhanced resistance to cell death (41). Inhibition or downregulation of uPA, uPAR or both, inhibits hypoxia-mediated EMT (42). Additionally, uPA is known for its functions in tumor cell invasion, its involvement in TGF-β activation as well as its participation in EMT (43). Thus, uPA may contribute to the formation of distant metastasis and the characteristics of the tumor microenvironment, which further studies should be looking into.

The present study demonstrated that uPAR expression may serve to predict prognosis because of its contributions to metastasis and cell survival through expression of mesenchymal genes (44). Furthermore, uPAR affects DNA damage repair through activation of Chk1 and Rad51 and contributes to multi-drug resistance mechanisms through its interaction with proteins such as vitronectin, integrins and EGFR (45,46). The current study further highlights that increased expression of uPAR in

Table III. Univariate and multivariate Cox proportional hazard regression analyses of DFS and OS.

| Variable | DFS | | OS | | | |
| | Univariate analysis | | Univariate analysis | | Multivariate analysis | |
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years						
≤50	Ref.	-	Ref.	-		
>50	0.58 (0.22-1.49)	0.256	0.81 (0.27-2.42)	0.707		
FIGO stage						
IB2- IIA	Ref.	-	Ref.	-		
IIB	2.25 (0.47-10.83)	0.312	1.48 (0.29-7.66)	0.637		
IIIA-IIIB	3.25 (0.67-15.70)	0.142	2.93 (0.59-14.62)	0.190		
IVA	6.37 (0.58-70.55)	0.131	0.00	>0.999		
Histological type						
Squamous cell carcinoma	Ref.	-	Ref.			
Neuroendocrine carcinoma	1.23 (0.16-9.59)	0.840	0.00	>0.999		
Other	1.66 (0.57-4.78)	0.351	1.70 (0.52-5.55)	0.379		
Treatment response					Ref.	-
Complete	Ref.	-	Ref.	-	6.07 (0.90-41.10)	0.064
Partial	2.41 (0.50-11.66)	0.273	7.22 (1.17-44.73)	0.034	47.16 (3.11-714.63)	0.005
Progressive disease	56.59 (13.45-238.09)	<0.001	13.41 (1.17-153.07)	0.037	58.58 (11.47-299.34)	<0.001
N/A	3.68 (0.76-17.92)	0.107	40.97 (9.27-181.04)	<0.001		
uPA levels						
No change	Ref.	-	Ref.	-		
Decreased	0.54 (0.16-1.74)	0.299	0.93 (0.25-3.48)	0.918		
Increased	1.39 (0.43-4.53)	0.585	2.68 (0.71-10.06)	0.145		
uPAR levels						
No change	Ref.	-	Ref.	-	Ref.	-
Decreased	2.32 (0.30-17.76)	0.419	0.00	>0.999	0.00	>0.999
Increased	1.12 (0.32-3.96)	0.855	3.65 (1.18-11.30)	0.025	6.72 (1.71-26.37)	0.006

Comparisons made against Ref. group. HR, hazard ratio; CI, confidence interval; Ref., reference; DFS, disease-free survival; OS, overall survival.
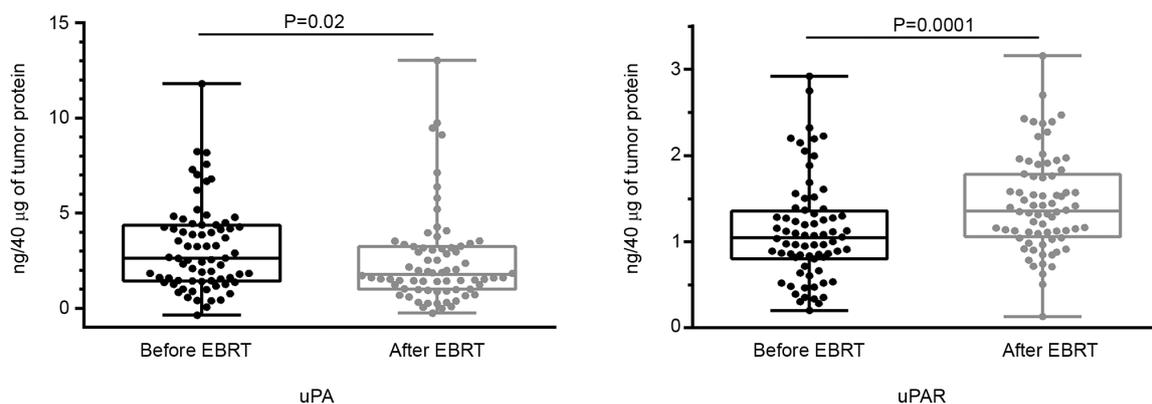

Figure 2. ELISAs of the levels of uPA and uPAR in tumor tissue samples collected from patients with cervical cancer before and after EBRT. uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; EBRT, external beam radiation therapy.

cervical tumor tissue, particularly during treatment, may serve as a prognostic factor that predicts survival. In accordance with the present findings, circulating soluble uPAR serves as a marker for diagnosis and prognosis of cervical cancer (47). Thus, uPAR should be considered a marker for predicting the prognosis of patients with cervical cancer as serum levels of uPAR are
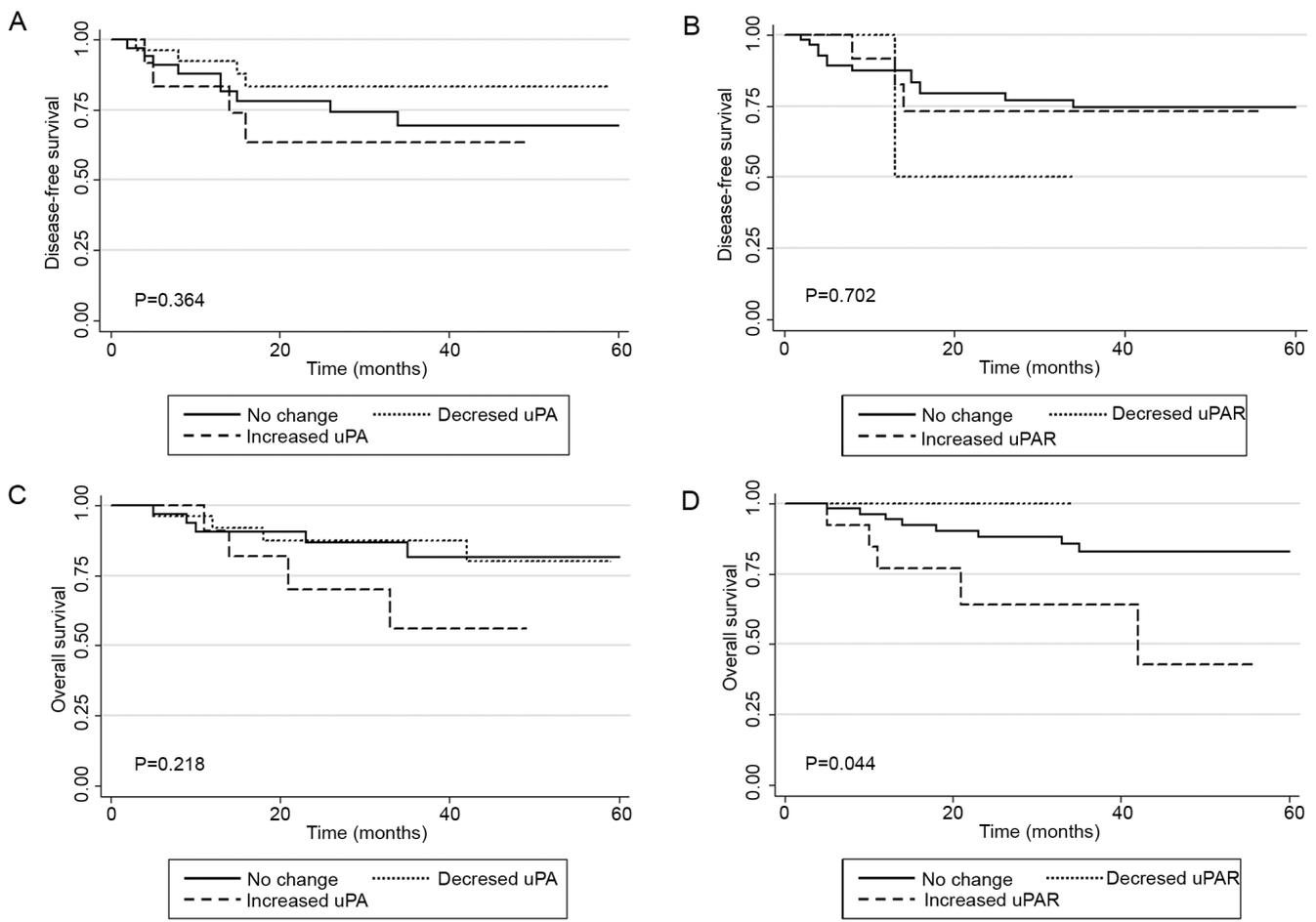
Figure 3. Kaplan-Meier analysis of disease-free survival of patients with no change, decreased, or increased (A) uPA levels or (B) uPAR levels and overall survival of the patients with no change, decreased or increased (C) uPA levels and (D) uPAR levels. uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor.

associated with treatment outcomes of patients with breast, colon, rectal and ovarian cancer (48,49). Acquiring post-EBRT tissue biopsies is relatively invasive, and future studies should focus on determining whether less invasive assays, such as those that measure serum uPAR, are as sensitive and specific as those that measure tissue uPAR. The lack of serum uPA and uPAR level measurements is a potential limitation of the current study. In the meantime, for those patients who poorly respond to radiotherapy, the authors hypothesize that post-EBRT biopsies can be used to determine the levels of uPAR as well as those of other prognostic markers. uPAR should therefore be considered to select the most effective treatment strategy.

The current study reported that the expression of uPA and uPAR was unsuitable as a prognostic marker for patients with cervical cancer, which may be explained by the proteolytic activities of uPA and uPAR that are often associated with tumor cell migration and metastasis, which then contributed to a radioresistant phenotype (50). In contrast to this, uPAR expression may reflect the induction of the epithelial mesenchymal transition (EMT) upon activation of the ERK1/2 signaling pathway (51). Nevertheless, the results were based on the limited availability of number of patients (n=72) who underwent EBRT and were voluntary to participate in the study. Several other factors should be considered when comparing the results from different studies, such as genotype

and HPV status. A larger number of subjects will therefore be required to validate the present findings.

With the emergence of high-throughput sequencing and advances in bioinformatics, computational methods are used to analyze genotypes, transcriptomes, proteomes and metabolomes to identify specific diagnostic and prognostic markers of cancer (52). For example, a mutation profiling study found that mutations in the gene encoding fibroblast growth factor receptor are associated with poor progression-free survival rate in patients with cervical cancer (53). System biology approaches may therefore prove useful and provide new directions in the pursuit of identifying new markers for cancer diagnosis and therapy.

In summary, although the expression levels of uPA and uPAR were altered by irradiation, only the upregulation in uPAR expression in tissue samples predicted the OS of patients with cervical cancer. Targeting uPAR expressed in cervical tumors may therefore contribute to more effective therapeutic strategies.

Acknowledgments

Availability of data and materials

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

Authors' contributions

DN conceptualized the study and wrote the manuscript. DN, SC and KL designed the study. DN and PC performed the *in vitro* experiments and collected the data. PC, SB and SC collected and evaluated the clinical data and conducted the statistical analyses. DN and SC confirmed the authenticity of the raw data. SC and KL supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Committee on Human Rights Related to Research Involving Human Subjects, Chulabhorn Research Institute, (Bangkok, Thailand) approved the study (approval no. 04/2557). Written informed consent was provided by each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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