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Original Article

The associations of biological parameters with the effects of radiotherapy and cisplatin-based concurrent chemoradiotherapy in uterine cervical cancer

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Abstract: Objectives: The purpose of this study was to determine the associations of the expressions of some important biomarkers that we have previously investigated with the clinic pathological characteristics and prognoses of patients with uterine cervical cancer in Taiwan. Methods: One hundred and three patients with cervical cancer were recruited consecutively between March 1999 and March 2006. Among them, 33 received radiotherapy or cisplatin-based concurrent chemoradiotherapy (CCRT) and 70 did not. We constructed cervical tissue microarrays and analyzed the associations of the immunoreactivities of human nonmetastatic clone 23 type 1 (nm23-H1), lipocalin 2, aldo-keto reductase family 1 member C3 (AKR1C3), ribonucleotide reductase M2, matrix metalloproteinase-2 and carbonate anhydrase 9 with clinicopathologcial variables and prognoses of cervical cancer patients. Results: Univariate analysis revealed that positive nm23-H1 and negative lipocalin 2 predict poorer survival in patients receiving radiotherapy or CCRT. However, these biomarkers were not associated with recurrence. Multivariate analysis showed that only positive lipocalin 2 is predictive of better survival in these patients (P=0.005). Deep stromal invasion was the only predictive parameter in patients not receiving radiotherapy or CCRT (P=0.036). Conclusion: Lipocalin 2 is applicable as a clinical target for cervical cancer patients receiving radiotherapy or CCRT in Taiwan.

Keywords: Lipocalin 2, human nonmetastatic clone 23 type 1, depth of stromal invasion, uterine cervical cancer, survival, radiotherapy, concurrent chemoradiotherapy

Introduction

Uterine cervical cancer is the 5th most common cancer among women in Taiwan. The age-standardized incidence rate was 11.86 per 100000 women in 2009, as reported by the Health Promotion Administration of the Ministry of Health and Welfare. From our previous studies, high immunoreactivity of human nonmetastatic clone 23 type 1 (nm23-H1) product in cancer tissue is predictive of poor overall survival for patients with uterine cervical cancer [1, 2]. Lipocalin 2 has also been found to be associated with overall survival of cervical cancer patients [2]. Nm23-H1, also known as NME1, is the first identified metastasis-associated gene [3]. Nucleoside diphosphate kinase (NDPK), the protein product of nm23-H1 gene, catalyzes nucleoside diphosphates into nucleoside triphosphates by phosphorylation [4, 5]. The Nm23 family protein consists of eight genes encoded for NDPK, nm23-H1, nm23-H2, nm23-H3, nm23-H4, nm23-H5, nm23-H6, nm23-H7, nm23-H8, and nm23-LV, derived from nm23-H1 [6-8]. Lipocalin 2 is a 25 kDa secretory protein that belongs to a diverse lipocalin family of small secreted lipocalin proteins, which generally bind to small hydrophobic ligands and soluble extracellular macromolecules [9, 10]. The lipocalin family has been implicated in a variety of functions, such as iron trafficking and induction of apoptosis [11, 12]. Lipocalin 2 has also been suggested to affect cell proliferation, regulation and differentiation and to be involved in cancer [13, 14].

In addition, we have investigated the roles of aldo-keto reductase family 1 member C3

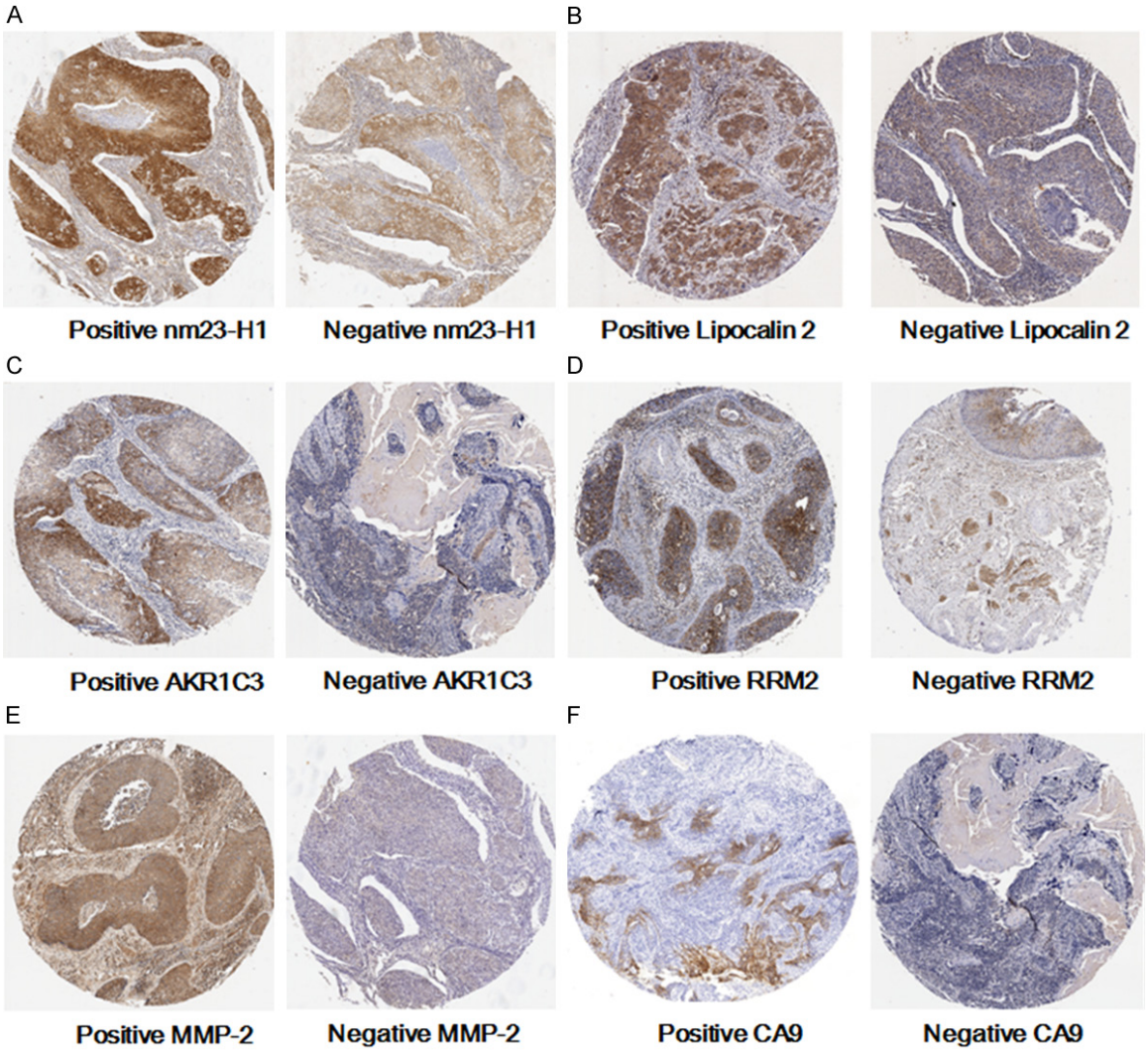

Figure 1. Positive and negative immunoreactivities of each biological parameter. (A) Human nonmetastatic clone 23 type 1 (nm23-H1); (B) Lipocalin 2 (C) aldo-keto reductase family 1 member C3 (AKR1C3); (D) Ribonucleotide reductase M2 (RRM2); (E) Metalloproteinase-2 (MMP-2); (F) Carbonic anhydrase 9 (CA9); Magnification: 40×.

(AKR1C3), ribonucleotide reductase M2 (RRM2) and matrix metalloproteinase-2 (MMP-2) in uterine cervical cancer and found that cervical cancer patients with cancer tissues exhibiting positive AKR1C3 immunoreactivities have higher recurrence probability and poorer survival [15]. Patients with positive RRM2 tend to have a higher probability of recurrence and significantly worse survival [16]. However, MMP-2 has not been reported to be predictive of recurrence in or survival of cervical cancer patients [17]. AKR1C3, also referred to as type 5 17β-hydroxysteroid dehydrogenase, belongs to the aldo-keto reductase enzymes of ~37 kDa and catalyzes the NAD(P)H-dependent reduction of carbonyl groups on a wide variety of substrates [18]. It has been demonstrated that low expression of AKR1C in patients with squamous cell esophageal carcinoma correlates with significantly lower incidence of tumor recurrence and better survival [19]. Ribonucleotide reductase (RNR) is an enzyme that catalyzes the formation of deoxyribonucleotides from ribonucleotides [20]. The cell cycle-dependent activity of RNR is regulated by the levels of RRM2, a small RNR subunit [21]. Moreover, we have studied the relationship between carbonic anhydrase 9 (CA9), the most active isoform of carbonic anhydrase in the hydration of carbon dioxide [22], and cervical cancer.

Radiotherapy and concurrent chemoradiotherapy (CCRT) are the main treatment modes for

Table 1. The correlations of biological parameters with clinicopathological characteristics of study subjects with uterine cervical cancer

Clinicopathologic Variables[a]	Nm23-H1		Lipocalin 2		AKR1C3		MMP-2		RRM2		CA9	
	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	(-)
Age (years; mean ± SD)	52.0±12.3	45.3±10.4	52.0±11.4	45.3±9.8	51.6±10.6	46.6±13.1	50.1±10.4	48.6±10.7	50.8±11.2	43.0-	60.7±7.6	49.5±11.0
P value	0.550		0.295		0.295		0.799		0.501		0.100	
Clinical stage												
I[b]	8	10	13	5	11	7	4	3	17	1	0	18
Others	8	6	13	1	15	0	6	2	15	0	3	12
P value	0.476		0.196		0.009[c]		0.608		1.000		0.083	
Pathologic types												
Squamous cell carcinoma[b]	16	13	24	5	25	5	10	4	29	1	3	27
Adenocarcinoma	0	3	2	1	1	2	0	1	3	0	0	3
P value	0.226		0.476		0.106		0.333		1.000		1.000	
Depth of stromal invasion												
< 1/2 depth[b]	3	6	7	2	5	4	2	0	8	1	1	8
> 1/2 depth	13	10	19	4	21	3	8	5	24	0	2	22
P value	0.433		1.000		0.068		0.524		0.273		1.000	
Tumor diameter												
< 4 cm[b]	8	9	12	5	13	4	4	2	16	1		16
> 4 cm	7	7	14	0	13	2	6	3	15	0	13	
P value	0.870		0.048[c]		0.659		1.000		0.348		0.589	
Tumor grade												
Well[b]	1	1	10	0	1	0	0	0	1	0	0	0
Moderate or poor	14	15	23	6	23	7	10	5	29	1	2	28
P value	1.000		1.000		1.000		-		1.000		0.097	
Parametrial invasion												
No invasion[b]	8	9	12	5	12	5	3	3	16	1	1	16
Invasion	8	7	14	1	2	0	7	2	0	0	2	14
P value	0.723		0.178		0.398		0.329		1.000		0.601	
Vaginal invasion												
No invasion[b]	14	10	19	5	18	6	8	3	24	0	1	23
Invasion	2	6	7	1	8	1	2	2	1	0	2	7
P value	0.220		1.000		0.642		0.560		0.273		0.174	
Pelvic lymph node metastasis												
Negative[b]	8	10	16	2	15	4	4	2	18	1	0	19
Positive	6	6	10	4	11	3	6	3	14	0	3	11
P value	0.476		0.365		1.000		1.000		1.000		0.067	

Statistical analysis: Chi-square or Fisher's exact test. [a]Some clinicopathological data could not be collected due to incomplete medical charts or records. [b]As a reference. [c]$P < 0.05$. Nm23-H1, human non-metastatic clone 23 type 1; AKR1C3, aldo-keto reductase family 1 member C3; RRM2, ribonucleotide reductase M2; MMP-2, metalloproteinase-2; CA9, carbonic anhydrase 9.

Table 2. Univariate analysis of the associations of biomarkers with cancer recurrence and survival in patients with uterine cervical cancer receiving radiotherapy or concurrent chemoradiotherapy

Parameters[a]	Recurrence		*p*	OR and 95% CI (risk of recurrence)	Survival		*p*	OR and 95% CI (risk of death)
	(+)	(-)			(+)	(-)		
Nm23-H1			0.220				0.037[c]	
Negative[b]	2	14		1.00	15	1		1.00
Positive	5	10		3.50 (0.44-42.11)	9	7		11.67 (1.10-559.89)
Lipocalin 2			0.562				0.023[c]	
Positive[b]	5	21		1.00	22	4		1.00
Negative	2	3		2.80 (0.18 to 31.16)	2	4		11.00 (1.04-145.10)
AKR1C3			0.161				0.642	
Negative[b]	0	7		1.00	6	1		1.00
Positive	7	17		∞ (0.44-∞)	18	8		2.67 (0.25- 137.76)
MMP-2			1.000				0.242	
Negative[b]	1	3		1.00	3	2		1.00
Positive	2	8		0.75 (0.03-58.83)	9	1		0.17 (0.00 to 4.90)
RRM2			1.000				1.000	
Negative[b]	0	1		1.00	1	0		1.00
Positive	7	23		unavailable	23	9		∞ (0.01-∞)
CA9			1.000				0.545	
Negative[b]	7	21		1.00	21	9		1.00
Positive	0	3		0.00 (0.00 -8.75)	3	0		0.00 (0.00-6.63)

Statistical analysis: Fisher's exact test. [a]Some clinicopathological data could not be collected due to incomplete medical charts or records. [b]As a reference. [c]$P < 0.05$. Recurrence: +, recurrence; -, no recurrence. Survival: +, survival; -, death. Nm23-H1, human nonmetastatic clone 23 type 1; AKR1C3, aldo-keto reductase family 1 member C3; RRM2, ribonucleotide reductase M2; MMP-2, metalloproteinase-2; CA9, carbonic anhydrase 9; OR: odds ratio; CI: confidence interval.

cervical cancer [23-26], especially when patients present with parametrial invasion, inadequate surgical vaginal margin, regional lymph node metastasis or local advanced stage. The concurrent chemotherapeutic drug prescribed at Chung Shan Medical University Hospital, Taiwan is cisplatin. The purpose of this study was to correlate biological parameters with the effects of radiotherapy and CCRT on cervical cancer patients treated at our hospital, by evaluating cancer recurrence and patient survival. Furthermore, we analyzed the significant factors for predicting the overall survival of patients receiving or not receiving radiotherapy or CCRT.

Materials and methods

Subjects

One hundred and three patients with cervical cancer were recruited consecutively between March 1999 and March 2006. They were staged clinically according to the International Federation of Gynecology and Obstetrics (FIGO) Classification. Standard treatment protocols of the Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taiwan were applied and recorded. Among these patients, 33 received adjuvant radiotherapy or CCRT. Eighteen patients presented with stage I cancer, including one with stage IA and 17 with stage IB. Eight patients presented with stage IIA cancer, 5 with stage IIB cancer and 2 with stage IIIB cancer. The prescription dose of radiotherapy was 80-85 Gy to Point A. The treated volume consisted of the gross tumor, the entire parametrium, the bilateral uterosacral ligaments, and the pelvic lymph node areas ,such as the presacral region, external iliac, internal iliac, obturator, and common iliac nodal stations. Meanwhile, 29 patients received concurrent chemotherapy with cisplatin. The dose of weekly cisplatin in CCRT was 40 mg/m^2. Seventy patients received neither radiotherapy nor CCRT. All patients were ethnically Taiwanese and lived in central Taiwan. The mean age of the patients was 50.6±11.1 years.

Biomarker expressions based on H score using tissue microarrays

Two formalin-fixed, paraffin-embedded tissue microarrays (MaxArray with tissue cores,

Table 3. Univariate and multivariate analyses of the influences of human nonmetastatic clone 23 type 1 (nm23-H1), lipocalin 2 and depth of stromal invasion on patient survival while considering time interval in patients with uterine cervical cancer receiving radiotherapy or concurrent chemoradiotherapy (N=33)

Parameters[a]	Case number	5-year survival rate (%)	Hazard ratio	95% confidence interval	*P* value
Univariate analysis					
Nm23-H1					0.077
Negative[b]	16	79.1	1.00	Reference	
Positive	16	92.3	5.38	0.65-45.45	
Lipocalin 2					0.0003[c]
Positive[b]	26	87.4	1.00	Reference	
Negative	6	20.0	9.48	2.10-43.0.1	
Depth of stromal invasion					0.060
< 1/2 depth[b]	9	100.0	1.00	Reference	
> 1/2 depth	24	63.4	35.71	0.06-∞	
Multivariate analysis					
Lipocalin 2					0.005[c]
Positive[b]	26	87.4	1.00	Reference	
Negative	6	20.0	8.89	1.95-40.6	

Statistical analysis: Kaplan-Meier method as well as univariate and multivariate Cox regression models. [a]Some clinicopathological data could not be collected due to incomplete medical charts or records. [b]As a reference. [c]$P < 0.05$.

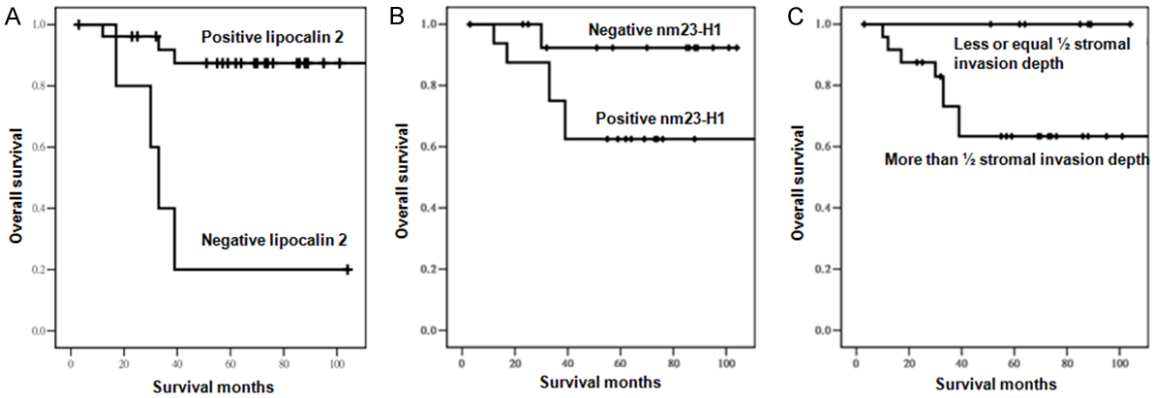

Figure 2. Kaplan-Meier curves for overall survival in patients with uterine cervical cancer, receiving radiotherapy or concurrent chemoradiotherapy, according to the immunoreactivities of lipocalin 2 and human nonmetastatic clone 23 type 1 (nm23-H1) in cancer tissue cores, as well as the depth of stromal invasion. (A) Patients with positive lipocalin 2 had significantly better survival (*P*=0.0003), compared with those with negative lipocalin 2. (B) Patients with positive nm23-H1 tended to have poorer survival (*P*=0.077), compared with those with negative nm23-H1. (C) Patients with cancer tissues in which there was > 1/2 depth of stromal invasion tended to have poorer survival (*P*=0.060), compared with those with cancer tissues in which there was < 1/2 depth of stromal invasion.

Zymed, CA, USA) were constructed using cancerous tissue cores of uterine cervix. All tissues were obtained from the histopathological archives of the Pathology Department, Chung Shan Medical University Hospital.

Tissue microarray sections were incubated with anti-nm23-H1 antibody (1:200 dilution; NM301 clone, mouse antihuman nm23-H1 monoclonal antibody; Novocastra, Newcastle, UK), antilipocalin 2 antibody (1:200 dilution; LCN2 antibody-neutrophil gelatinase-associated lipocalin (LCN2), goat antihuman polyclonal antibody-LSB2496; LifeSpan BioSciences, Seattle, Washington), anti-AKR1C3 rabbit polyclonal antibody (1:600 dilution; ab84327, Abcam Inc. MA,

USA), anti-MMP-2 antibody (1:100 dilution, mouse monoclonal antibody; Zymed, CA, USA), anti-RRM2 antibody (1:300 dilution; ab57653, Abcam Inc. MA, USA) and human CAIX antibody (1:200 dilution; Novocastra™ Liquid Mouse Monoclonal Antibody Carbonic Anhydrase IX, Newcastle, UK).

The immunoreactivity intensity was graded on a scale of 0-3 by calculating the mean of integrated optic density in a fixed field with a total pixel area of about 250 × 250 by the Pro Plus 6.1 image analysis program (Media Cybernetic, Inc., Silver Spring, MD) [2, 27]. The proportion of positive cells was assessed for each tissue core. Three images of representative areas were acquired and at least 300 cells per tissue core section were manually counted on a computer screen. The proportions of positive cells were categorized as follows: 0, less than one tenth; 0.1, less than one half; 0.5 and greater, 1. Finally, a semi-quantitative H score of nm23-H1, lipocalin 2, AKR1C3, MMP-2, RRM2 and CA9 immunoreactivities was determined by multiplying the four-tiered intensity score by the four-tiered proportion score of positive cells in the tumor. The minimum H score was 0 and the maximum H score was 3 [2, 27]. This study was approved by the Institutional Review Board of Chung Shan Medical University Hospital (CSMUH IRB; CS10019, CS12219 and CS12-133) and informed consent was obtained from each of the participants. The median H score of each biomarker was used as the cutoff level for separating positive and negative cancer tissues.

Statistical analysis

Chi-square or Fisher's exact tests were used to associate the expression of biological parameters such as nm23-H1, lipocalin 2, AKR1C3, MMP-2, RRM2 and CA9 with clinicopathological variables in cervical patients for univariate analysis. These methods were also used for the association of these biomarkers with the recurrence and survival events in patients with uterine cervical cancer while time interval was not included for analysis. *P* values and odds ratios were analyzed by WinPepi Software, version 10.0. The Kaplan-Meier method was applied for the cervical cancer patients who received or did not receive radiotherapy or CCRT based on the biomarkers expression or clinic pathological characteristics for the overall survival between primary surgery and death or the end of the study (May 31, 2012). Five-year survival rates were estimated by the Kaplan-Meier method in these patients. Multivariate (with a forward method) and univariate Cox regression models were respectively used to assess the prognostic value of biological and clinical parameters to calculate the hazard ratios and their 95% confidence intervals in multivariate and univariate analysis in the cervical cancer patients who received or did not receive radiotherapy or CCRT. The statistical analyses were performed using SPSS statistical software (version 11.0; SPSS, Inc., Chicago, IL). All statistical tests were two-sided and a *p* value of less than 0.05 was considered to be statistically significant.

Results

Cytoplasmic, nuclear or membrane staining for nm23-H1, lipocalin 2, AKR1C3, MMP-2, RRM2 and CA9 immunoreactivities was carried out. The median H scores of nm23-H1, lipocalin 2, AKR1C3, MMP-2, RRM2 and CA9 immunoreactivities were 2, 2, 0.5, 1.5, 1 and 0.1, respectively, and were used to separate positive and negative tissue cores (**Figure 1**).

The associations of these biologic parameters with clinic pathological variables of cervical patients are shown in **Table 1**. Among cervical cancer patients with cancer tissues exhibiting positive or negative expressions of the biomarkers there were no differences in clinic pathological characteristics except that cervical cancer patients with cancer tissues exhibiting positive AKR1C3 tended to present with > stage 1 (*P*=0.009) and those with cancer tissues exhibiting positive lipocalin 2 tended to have a cancer tissue diameter larger than 4 cm (*P*=0.048).

No clinic pathological variables were associated with cancer recurrence (clinical stage, *P*=0.412; pathologic type, *P*=1.000; depth of stromal invasion, *P*=0.077; tumor diameter, *P*=1.000; tumor grade, *P*=1.000; parametrial invasion, *P*=0.685; vaginal invasion, *P*=0.642; pelvic lymph node metastasis, *P*=0.413). When survival was included in the analysis, no clinic pathological variables were associated with patient survival (clinical stage, *P*=0.458; pathologic type, *P*=0545; tumor diameter, *P*=1.000; tumor grade, *P*=1.000; parametrial invasion,

Table 4. Univariate and multivariate analyses of the influences of clinicopathological and biological parameters on patient survival while considering time interval in patients with uterine cervical cancer not receiving radiotherapy or concurrent chemoradiotherapy (N=70)

Parameters[a]	Case number	5-year survival rate (%)	Hazard ratio	95% confidence interval	*P* value
Univariate analysis					
Nm23-H1					0.007[c]
Negative[b]	47	76.5	1.00	Reference	
Positive	19	91.1	4.08	1.65-12.35	
AKR1C3					0.034[c]
Negative[b]	30	92.9	1.00	Reference	
Positive	38	77.1	3.56	1.01-12.50	
RRM2					0.021[c]
Negative[b]	21	95.2	1.00	Reference	
Positive	47	79.1	7.52	0.99-56.94	
Depth of stromal invasion					0.0049[c]
< 1/2 depth[b]	34	93.7	1.00	Reference	
> 1/2 depth	33	74.6	5.15	1.47-18.30	
Tumor diameter					0.0029[c]
< 4 cm[b]	53	91.9	1.00	Reference	
> 4 cm	12	54.0	4.47	1.52-13.12	
Multivariate analysis					
Depth of stromal invasion					
< 1/2 depth[b]	34	93.7	1.00	Reference	0.036[c]
> 1/2 depth	33	74.6	5.25	1.12-24.68	

Statistical analysis: Kaplan-Meier method as well as univariate and multivariate Cox regression models. [a]Some clinicopathological data could not be collected due to incomplete medical charts or records. [b]As a reference. [c]$P < 0.05$.

P=1.000; vaginal invasion, *P*=1.000; pelvic lymph node metastasis, *P*=0.442) except depth of stromal invasion. This implied that cervical cancer patients with cancer tissues that exhibit deep stromal invasion have poorer survival (*P*=0.039).

Univariate analysis showed that positive nm23-H1 immunoreactivity in cancer tissues is associated with poorer survival in cervical cancer patients receiving radiotherapy or CCRT [*P*=0.037; odds ratio (OR), 11.67 (95% confidence interval (95% CI): 1.10-559.89); **Table 2**]. Positive lipocalin 2 expression appeared to lead to improved survival [*P*=0.023; OR, 0.09 (95% CI: 0.01-0.97)]. However, no biomarkers were associated with recurrence (**Table 2**).

To analyze the survival of patients receiving radiotherapy or CCRT, time interval and significant univariate factors (nm23-H1, lipocalin 2 and stromal invasion depth) were included. Patients with cancer tissues positive for nm23-H1 immunoreactivity or with deep stromal invasion tended to have poorer survival [*P*=0.077; hazard ratio (HR), 5.38 (95% CI: 0.65-45.45) and *P*=0.060; HR, 35.71 (95% CI: 0.06-∞), respectively; **Table 3**]. Only positive lipocalin 2 predicted better survival [*P*=0.0003; HR, 0.11 (95% CI: 0.02-0.48); **Figure 2**]. Furthermore, multivariate analysis revealed that positive lipocalin 2 immunoreactivity is the only independent parameter for predicting survival of cervical cancer patients receiving radiotherapy or CCRT [*P*=0.005; HR, 0.11 (95% CI: 0.02-0.48)].

Almost all cervical cancer patients with parametrial invasion, inadequate surgical vaginal margin or regional lymph node metastasis received radiotherapy or CCRT. Thus, these variables were not included in the analyses using Kaplain-Meier method or in the multivariate or univariate Cox regression models for the influence of clinic pathological variables on the survival of patients receiving radiotherapy or CCRT. Univariate analysis revealed that patients with positive nm23-H1, positive AKR1C3, deep

stromal invasion or large tumor diameter had higher hazard ratio for poor survival (HR: 4.08, 95% CI: 1.65-12.35; HR: 3.56, 95% CI: 1.01-12.50; HR: 5.15, 95% CI: 1.47-18.30 and HR: 4.47, 95% CI: 1.52-13.12, respectively) (**Table 4**). However, multivariate analysis indicated that deep stromal invasion is the only significant predictor of poor survival (*P*=0.036; HR: 5.25, 95% CI: 1.12-24.68).

Discussion

In this study, we found that negative lipocalin 2, positive nm23-H1 and deep stromal invasion are related to poorer survival of cervical cancer patients. None of our studied biomarkers or clinic pathological variables were related to recurrence after radiotherapy or CCRT.

Lipocalin 2 was not associated with clinic pathological variables but positive lipocalin 2 was related to larger tumor with a low level of significance (*P*=0.048). Multivariate analysis showed that only positive lipocalin 2 can predict better overall survival of cervical cancer patients receiving radiotherapy or CCRT. Bai ZL found no significant association between mRNA expression of repair cross-complementing group 1 (ERCC1), an important biomarker, and clinic pathological characteristics in patients with locally advanced cervical squamous cell carcinoma who underwent cisplatin-based CCRT [28]. They demonstrated that patients with low ERCC1 mRNA level have a significantly higher rate of complete response than patients with high ERCC1 mRNA level. Muallem MZ reported that increased expression of ERCC1 is associated with better survival of cervical cancer patients [29]. The 2-year overall survival was 68.6% in the group with low H scores for ERCC1 and gradually increased to 71.7% and 90.7% in the groups with intermediate and high H scores, respectively. However, the relationship of low ERCC1 expression with unfavorable outcomes of patients with locally advanced cervical cancer treated with platinum-based chemoradiotherapy could not be confirmed.

Lipocalin 2 immunoreactivity of cervical cancer tissue cores derived from surgical specimens was used to predict prognosis of patients receiving radiotherapy or CCRT. Lipocalin 2 in serum secreted form is easily analyzed and the levels of serum secreted lipocalin 2 in cervical cancer patients are significantly higher than in patients with cervical pre-invasive cancer and normal controls [30]. Lipocalin 2 expression is also low in high-grade dysplasia tissue. In our cervical cancer patients, increased lipocalin 2 was noted and cellular proliferation subsequently increased. This trend was consistent for the expressions of lipocalin 2 in tissue and serum. Lim R reported that tissue expression of lipocalin 2 in ovarian tumors changes with disease grade and this change is also reflected in serum levels [31]. Provatopoulou X found a significant and positive correlation between circulating lipocalin 2 levels and breast cancer disease severity [32]. Serum secreted lipocalin 2 deserves further investigation as a clinical prognostic tool for cervical cancer patients receiving radiotherapy or CCRT. Moreover, Harima Y suggested pretreatment serum apolipoprotein C-II as a biomarker for predicting and estimating the radiation treatment outcome of patients with cervical cancer [33].

Although the sample size was small, MMP-2 was investigated in our cervical cancer patients. Our findings were in agreement with those of Braicu EI who could not determine a predictive value for MMP-2 in monitoring patients with FIGO stage Ib-IIb cervical cancer undergoing chemoradiotherapy [34]. The stages of their patients were similar to those of our patients (stage I 18 patients, stage II 13 patients, and stage IIIB only 2 patients). However, they suggested that tissue inhibitor of metalloproteinase-2 (TIMP2), an inhibitor of MMP-2, and vascular endothelial growth factor A (VEGFA) are independent prognostic factors for overall survival. They also demonstrated that an increase of more than 500 pg/ml VEGFA and a decrease of more than 9% in the pre-therapeutic value of TIMP2 are significantly associated with a higher risk of early recurrence.

There is increased expression of RRM2 in cervical cancer [35], and when cervical cancer RRM2 is pharmacologically inhibited, a high rate of complete clinical response is observed after chemoradiotherapy. Radiation damages DNA by breaking chemical bonds, releasing electrons, and degrading atomic nuclei. Double-stranded DNA breaks are one specific type of damage caused by ionizing radiation. Kunos CA further elucidated the role of RRM2 in chemoradiotherapy DNA damage response [36] by investigating the immunohistochemical expression of RRM2 protein and subsequent cancer-

related outcomes in women diagnosed with clinical stage IB2. They found that uterine cervical cancers uniformly overexpress RNRM2, which is associated with a poor chemoradiotherapy response. However, our study did not demonstrate an association between RRM2 and patient prognosis.

In addition, we investigated the associations of the above biomarkers with the survival of patients not receiving radiotherapy or CCRT. We found that nm23-H1 and AKR1C3 are the significant biological parameters for predicting patient survival on univariate analysis. Bosnar MH performed DNA microarray analysis of nm23-H1 overexpressing CAL 27 cell oral squamous cell carcinoma and determined that the most severely altered pathways of nm23-H1 downstream genes are related to adhesion, invasion, and metastasis together with the TGFβ signaling pathway [37]. The up-regulation of AKR1C3 has also been reported to be associated with poorer prognosis for breast cancer patients [38, 39]. However, deep stromal invasion became a more significant factor after multivariate analysis in our study. This implies that biologic parameters are not as useful as deep stromal invasion for predicting survival of patients with early cervical cancer who do not need radiotherapy or CCRT. In contrast, the role of biologic parameters such as lipocalin 2 may be more important in patients with severe cervical cancer who require adjuvant radiation.

Identification of biomarkers that predict response to radiotherapy or CCRT allows patients to avoid toxicity associated with ineffective therapy. Tissue lipocalin 2 is the only significant biologic parameter that we have studied that is able to predict the survival of cervical cancer patients receiving radiotherapy or chemoradiotherapy. Serum secreted lipocalin 2 is suggested as a potential biomarker to predict the prognosis of these patients and deserves further investigation with larger sample size.

Disclosure of conflict of interest

None.

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