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Original Article

Overexpression of Karyopherin α2 in small cell carcinoma of the cervix correlates with poor prognosis

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Abstract: Background: Cervical small cell carcinoma (SCCC) is uncommon and little is known about its molecular markers. Karyopherin α2 (KPNA2) has been demonstrated in a variety of malignancies. Our objective was to determine whether the KPNA2 level is predictive of clinical outcome in patients with SCCC. Methods: We detected KPNA2 expression by immunohistochemistry in SCCC tumors from 62 patients. The staining results were evaluated by H-score. The correlation among KPNA2 expression level, clinical characteristics, and prognosis was analyzed. Results: KPNA2 expression was detected in tumor tissue from 55 patients with SCCC (55/62, 89%). High KPNA2 expression correlated significantly with International Federation of Gynecology and Obstetrics staging (P=0.035), tumor size (P=0.019), poorer overall survival (OS) (P=0.008), and poorer disease-free survival (P=0.004) compared to low KPNA2 expression. Multivariate analysis showed that KPNA2 expression level (P=0.037) and tumor size (P=0.046) were independent prognostic factors of OS. Conclusions: KPNA2 may be a molecular marker and indicator of prognosis in SCCC.

Keywords: Small cell carcinoma of the cervix, karyopherin 2, expression, prognosis

Introduction

Neuroendocrine or small cell carcinoma of the cervix (SCCC) is a rare and aggressive cervical cancer [1]. Its characteristic is a high occurrence of nodal and distant early metastasis. Compared to other cervical cancer subtypes, SCCC has a poorer prognosis [2, 3], and identifying molecular markers and prognostic factors for SCCC and improving treatment strategy is important. However, because it is rare, most SCCC studies have consisted only of case reports or small series. Randomized controlled clinical trials of therapy and disease management are difficult to perform.

Cellular transport machinery deregulation is common in tumors. Accordingly, the expression of karyopherin α2 (KPNA2), a nucleocytoplasmic transport-related protein, is increased in several cancers, including cervical [4], esophageal [5], lung [6], prostate [7], brain [8], liver [9], and bladder [10] cancers, and melanoma [11]. As different cancers frequently feature KPNA2 overexpression, KPNA2 levels might also be upregulated in SCCC.

Human epithelial ovarian carcinoma (EOC) cell lines and tissues overexpress KPNA2 [12]. Moreover, KPNA2 overexpression has been correlated with poor prognosis in both EOC [12] and malignant tumors of ovarian germ cells [13]. Nevertheless, there has been no investigation of the KPNA2 expression level in patients with SCCC and its correlation with prognosis. Accordingly, we investigated KPNA2 expression in such patients and correlated it with their prognosis and the SCCC clinicopathologic features.

Materials and methods

Samples and cases

Tissues obtained from 62 patients from the Sun Yat-Sen University Cancer Center were fixed in formalin and embedded in paraffin. Patients were included if they had undergone

Table 1. Univariate analysis of clinicopathologic factors associated with KPNA2 expression

Variable	KPNA2 expression				*P*-value
	Low	%	High	%	
Age (years)					
≤40	13	52.0	12	48.0	
>40	20	54.1	17	45.9	0.539
FIGO stage					
I-IIa	29	61.7	18	38.3	
IIb-IV	4	26.7	11	73.3	**0.035**
Tumor homology					
Pure	22	57.9	16	42.1	
Mixed	11	52.4	10	47.6	0.786
Depth of stromal invasion					
<2/3	13	76.5	4	23.5	
≥2/3	17	47.2	19	52.8	0.074
Tumor mass size					
<4 cm	22	68.8	10	31.2	
≥4 cm	10	35.7	18	64.3	**0.019**
Lymph node metastasis					
Positive	11	47.8	12	52.2	
Negative	20	60.6	13	39.4	0.417
Lymphovascular space invasion					
Positive	8	38.1	13	61.9	
Negative	24	66.7	12	33.3	0.053

Bold indicates significant values.

surgery between January 2005 and September 2013, their samples were from radical hysterectomy prior to radiotherapy or chemotherapy, and comprehensive clinical data were available. We excluded biopsy specimens, patients who had undergone neoadjuvant radiotherapy or chemotherapy, and patients with postoperative pathology of mixed SCCC.

The included patients did not undergo radiotherapy or chemotherapy preoperatively. The SCCC diagnosis was confirmed pathologically. Patient and disease characteristics, including International Federation of Gynecology and Obstetrics (FIGO) stage, tumor homology, stromal invasion depth, tumor size, lymph node metastasis, lymphovascular space invasion, and age at diagnosis, were evaluated. All patients provided written informed consent for participation in this study.

Immunohistochemistry

Immunohistochemical staining for KPNA2 protein was carried out on the formalin-fixed, paraffin-embedded tissue sections. The 4 μm sections were deparaffinized with xylene, then rehydrated using a series of graded ethanol. The sections were treated with routine procedures and incubated at 4°C overnight in rabbit anti-KPNA2 polyclonal antibody (1:100 dilution in blocking solution; 10819-1-AP, Proteintech), then incubated with secondary antibody conjugated to horseradish peroxidase (MaxVision Immunohistochemical Detection Kit, MXB). We washed the sections, stained them with 3, 3'-diaminobenzidine tetrahydrochloride, and counterstained them with hematoxylin. We assessed immunoreactivity according to the percentage of positive (i.e., tumor) cells and the intensity of staining (i.e., weak, moderate, or strong). Two surgical pathologists assessed the staining independently with a semi-quantitative scale of 0-100% with regard to the proportion of KPNA2-positive cancer cells. We used the average replicate sample score in subsequent analyses. Patients were grouped as low and high KPNA2 expression according to the median score.

Statistical analysis

We assessed the relationship between KPNA2 expression and the SCCC clinicopathologic characteristics with Pearson's χ^2 test. We evaluated disease-free survival (DFS) and overall survival (OS) with the Kaplan-Meier method and log-rank testing. We performed multivariate survival analysis for all parameters with the Cox regression model. We performed the statistical analysis using SPSS 13 and considered a two-sided *P*-value <0.05 significant.

Results

Features of SCCC clinicopathology

The 62 patients were aged 24-66 years (median 42 years). Forty-seven patients (76%) had FIGO stage I-IIa disease and 15 patients (24%) had FIGO stage IIb-IV disease; 37% of the patients (21/57) had lymphovascular space infiltration and 41% (23/56) had lymph node metastasis. The respective estimated 5-year OS and DFS rates of the 62-patient cohort were 32.7% and 28.9%. **Table 1** lists the other clinicopathologic characteristics. The last follow-up was in June 2013; the median duration

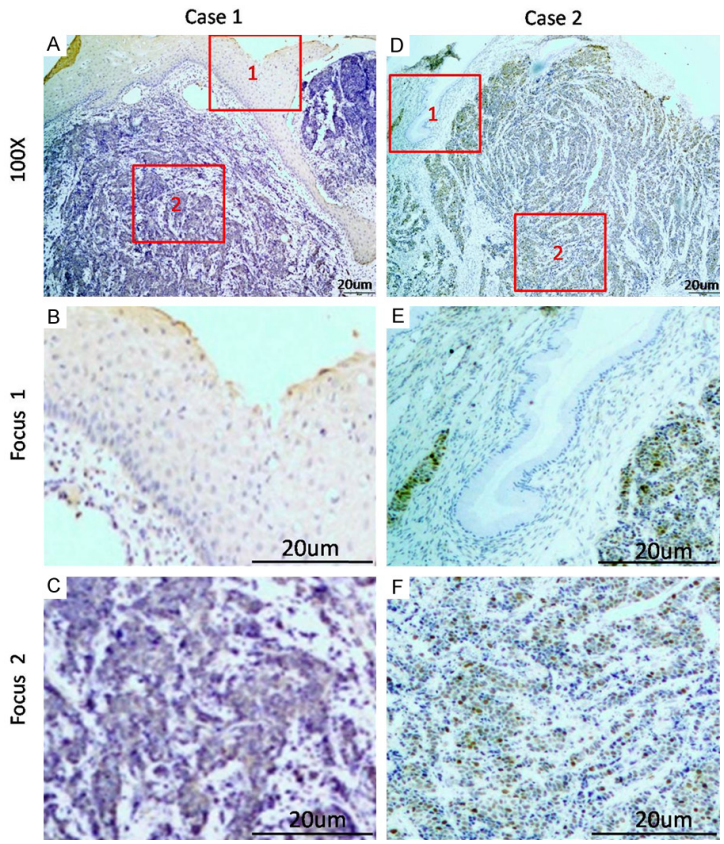

Figure 1. Immunohistochemical analysis of KPNA2 staining in SCCC tissues. KPNA2 protein expression was significantly higher in tumor cells compared with adjacent noncancerous tissues (A-C: case 1; D-F: case 2).

of observation was 20 months (range 2-119 months). There were 28 SCCC-related deaths.

KPNA2 protein was frequently expressed in SCCC

To investigate whether there was high KPNA2 protein expression in SCCC as in other tumors, KPNA2 protein was detected by immunohistochemical staining in the 62 patients. There was KPNA2 protein expression in the SCCC tumor cell nucleus and cytoplasm *in situ*. We detected significantly higher KPNA2 expression in tumor cells than in the adjacent noncancerous tissues (**Figure 1**). KPNA2 expression was detected in 55 patients (89%) (**Figure 2**).

Correlations between KPNA2 expression and SCCC clinicopathologic factors

We examined the correlations between KPNA2 expression and the SCCC clinicopathologic parameters, i.e., age, FIGO stage, tumor histology, tumor size, and lymph node metastasis. The SCCC tissues from the 62 patients were categorized as high KPNA2 (n=29) and low KPNA2 (n=33) according to the median score. In the tumor tissue, there was a significant correlation between high KPNA2 expression and FIGO stage (P=0.035) and tumor size (P=0.019). There was no correlation with the other clinical characteristics (**Table 1**).

Association between KPNA2 expression and patient prognosis

The OS and DFS curves of the high and low KPNA2 expression groups are depicted in **Figure 3**. High-KPNA2 expression patients had significantly inferior OS (P=0.008) and DFS (P=0.004) to those with low KPNA2 levels. The multivariate analysis demonstrated that KPNA2 expression level (hazard ratio [HR] =2.689, 95% confidence interval [CI] =1.060-6.816; P= 0.037) and tumor size (HR=2.736, 95% CI =1.017-7.362; P=0.046) were independent OS prognostic factors (**Table 2**).

Discussion

Uterine cervical small cell carcinoma (SCCC) is rare (0.5-3% of cervical cancers). SCCC is usually aggressive, and management of the disease is difficult. Death usually occurs a few years after diagnosis, although some have reported long-term survival [14-16]. The Gynecologic Oncology Group had endeavored to study SCCC, but could not recruit enough patients. Therefore, SCCC treatment decisions are made according to small-scale studies, and/or the extrapolation of treatment approaches in small cell lung cancer.

Implicated in a variety of malignancies, KPNA2 facilitates signaling molecule import into the nucleus and response molecule export to the cytoplasm [17]. Moreover, KPNA2 was recently identified as a prognostic marker of progression in bladder cancer [10], prostate cancer [7], and lung cancer [6]. We have also demonstrat-

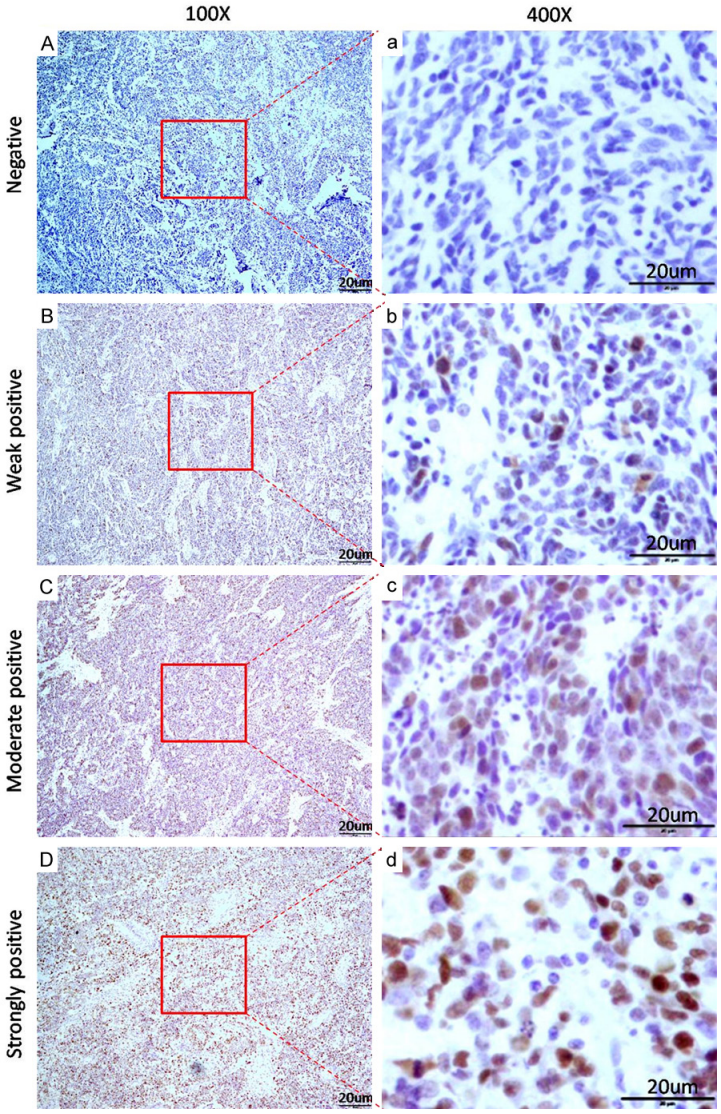

Figure 2. Immunohistochemical analysis of KPNA2 staining in SCCC tissues. KPNA2 protein was expressed in the tumor cell nuclei and cytoplasm *in situ*. Shown are negative (case 1; A: ×100 magnification; a: ×400 magnification), weakly positive (case 2; B: ×100 magnification; b: ×400 magnification), moderate (case 3; C: ×100 magnification; c: ×400 magnification), and strong (case 4; D: ×100 magnification; d: ×400 magnification) KPNA2 staining in the SCCC tissues.

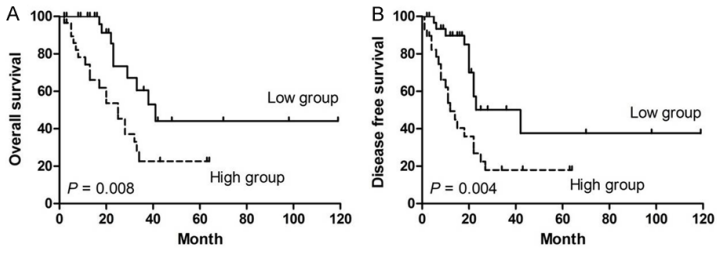

Figure 3. Kaplan-Meier analysis of OS (A) and DFS (B) curves of patients with SCCC with high and low KPNA2 expression. Patients with high KPNA2 expression had significantly poorer overall survival, OS (P=0.008) and disease-free survival, DFS (P=0.004) than patients with low KPNA2 expression.

ed that KPNA2 is overexpressed in human EOC and that it correlates with poor prognosis. In the present study, KPNA2 protein was frequently expressed in SCCC, and high KPNA2 expression in tumor tissue correlated significantly with FIGO stage, tumor size, OS, and DFS. The precise mechanism for the oncogenic functions of KPNA2 is currently unknown. KPNA2 interacts with several cancer-associated proteins, including checkpoint kinase 2 [18], NBS1 [19], and p53 [20]. It is also involved in translocating transcription factors such as E2F transcription factor 1 (E2F1) [21], c-Myc [22], PLAG1 zinc finger (PLAG1) [23], and LOT1 [24]. KPNA2 promotes EOC cell proliferation and tumorigenicity by upregulating c-Myc and downregulating Forkhead box O3 (FOXO3a) [25]. These mechanisms contribute to the transformation of malignant cells and are therapeutic targets.

Our findings indicate that KPNA2 is a molecular and prognostic marker in SCCC. Although the number of cases we included is limited, we hope that our findings contribute to the knowledge of this rare and aggressive tumor.

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Disclosure of conflict of interest

None.

Table 2. Multivariate Cox regression analysis of clinicopathologic factors for OS

Clinicopathologic factor	Subset	HR (95% CI)	*P*-value
Age (years)	>40 vs. ≤40	0.652 (0.242-1.761)	0.399
FIGO stage	IIb-IV vs. I-IIa	1.430 (0.382-5.349)	0.595
Stromal invasion depth	≥2/3 vs. <2/3	3.240 (0.927-11.329)	0.066
Tumor mass size	≥4 cm vs. <4 cm	2.716 (1.017-7.362)	**0.046**
Lymph node metastasis	Negative vs. Positive	0.854 (0.241-3.026)	0.806
Lymphovascular space invasion	Negative vs. Positive	0.655 (0.233-1.843)	0.422
KPNA2 expression	High vs. Low	2.689 (1.060-6.816)	**0.037**

Bold indicates significant values.

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