Int J Clin Exp Med 2016;9(3):6316-6322
www.ijcem.com /ISSN:1940-5901/IJCEM0019923

Original Article

Overexpression of KPNA2 is associated with unfavorable prognosis in cervical carcinoma

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Received November 17, 2015; Accepted February 11, 2016; Epub March 15, 2016; Published March 30, 2016

Abstract: Aims: The present study is to investigate the clinical significance of KPNA2 in the development of cervical cancer. Methods: KPNA2 mRNA levels in 8 paired cervical cancer tissues and the adjacent non-tumor tissues were examined using real-time PCR. The expression and prognostic value of KPNA2 were examined in 94 cervical cancer patients after surgical resection. The overall and recurrent-free survival rates were estimated using Kaplan-Meier method and compared with the log-rank test. The prognostic analysis was carried out with univariate and multivariate Cox regressions models. Results: Compared to non-tumor tissues, KPNA2 mRNA was clearly increased in cervical cancer tissues. High expression of KPNA2 was significantly associated with tumor stage (P = 0.034) and tumor size (P = 0.025) of the disease. Moreover, high expression of KPNA2 was significantly associated with poorer overall (OS) and recurrent free (RFS) survival (P = 0.002 and = 0.004, respectively) of cervical cancer patients. Multivariate analysis suggested that reduced expression of KPNA2 was an independent prognostic marker of cervical cancer (P = 0.038). Conclusion: KPNA2 may serve as oncogene in the development of cervical cancer, and may serve as clinicopathologic biomarkers for prognosis of cervical cancer patients.

Keywords: KPNA2, karyopherin, cervical cancer, prognosis

Introduction

Cervical carcinoma is one of the most common malignancies in female reproductive system, especially in developing countries. There are estimated of 529,800 new cases and 275,100 deaths annually [1]. Despite advances in surgical and medical care, patients with metastatic or recurrent cervical cancer were not curable with standard treatment [2]. Local invasion and the spread of a cancer are directly linked to patient's clinical survival and are closely relevant to the processes involved in cervical carcinogenesis [3], but their molecular mechanisms are not fully understood. Therefore, the discovery of novel biomarkers involved in the development and progression of cervical cancer is of great value in finding effective therapeutic strategies and novel therapeutic targets.

Karyopherin alpha 2 (KPNA2), a member of the karyopherin family, is a 58-kDa protein with 529 amino acids [4]. The KPNA2 protein comprises a central hydrophobic region containing of 10 armadillo repeats, which binds to the NLS site of the cargo protein; an N-terminal hydrophilic import in β-binding domain; and a short acidic C terminus, which has no reported function [5]. KPNA2 interacts with the NLSs of DNA helicase Q1 and SV40 T antigen and may be involved in the energy-independent docking of proteins to the nuclear envelope. KPNA2 also may play a role in cytoplasmic transport [6]. Elevated levels of KPNA2 have been reported in many cancers, including esophageal squamous cell carcinoma [7], non-small cell lung cancer [8], hepatocellular carcinoma [9], bladder cancer [10], and prostatic cancer [11]. Moreover, KPNA2 has been reported to be an important factor in the tumorigenesis and progression of human cancers [12]. However, currently it is lack of relevant studies concerning the roles of KPNA2 in cervical cancer.

In the present study, we aimed to investigate the expression of KPNA2 and further explore the clinical significance and biological functions of KPNA2 in cervical cancer. We examined the expression levels of KPNA2 in cervical cancer tissues by using qRT-PCR and immunohisto-

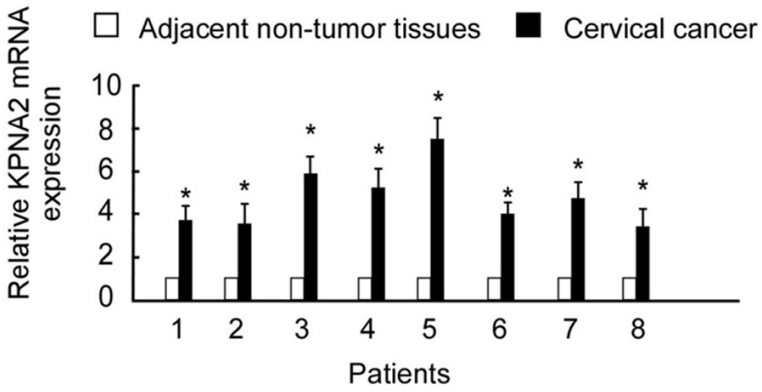

Figure 1. Real time-PCR analysis of KPNA2 expression in 8 pairs of cervical cancer and adjacent non-tumor tissues. asterisks, $P < 0.05$.

chemical assay. In addition, we analyzed the correlation of KPNA2 expression with clinicopathological characters and prognosis in order to determine the clinical and prognostic significance of KPNA2 in cervical cancer. Our research revealed a novel molecule involved in the development and progression of cervical cancer.

Materials and methods

Patients and specimens

We collected tumors from 94 cervical cancer patients who had undergone surgery at The First Affiliated Hospital of Henan University of Traditional Chinese Medicine from January 2007 to December 2009. For the use of these clinical materials for research purposes, prior patient's consent and approval from the Institute Research Ethics Committee was obtained. None of the patients had undergone either chemotherapy or radiotherapy before the collection of the samples. Histopathological diagnoses were made according to the pathological classification system of the International Federation of Gynecology and Obstetrics (FIGO). The patients' clinicopathological features included patients' age, tumor stage, tumor size, histological type, tumor grade and clinical survival.

Extraction of RNA and real-time reverse transcription-polymerase chain reaction

Total RNA was extracted from 8 pairs of frozen cervical cancer samples. Total RNA in the frozen tissues was extracted using Trizol (Invitrogen) following the manufacturer's recommendations. The RNA was treated with DNase, and 2 ug of total RNA was used for cDNA synthesis using random hexamers. Real-time reverse transcription polymerase chain reaction (qRT-PCR) was carried out using SYBER green kit in a Light Cycler system (Roche Applied Science). For the evaluation of the relationship between KPNA2 and GAPDH (internal control), the primer selected were as follows: KPNA2, forward, 5'-ATTGCAGGTGATGGCTCAGT-3' and reverse, 5'-CTGCTCAACAGCATCTATCG-3; GAPDH, forward, 5'-TGCACCACCAACTGCTTAGC-3', andreverse, 5'-GGCATGGACTGTGGTCATGAG-3'.

Immunohistochemistry staining and analysis

All paraffin-embedded archival specimens were cut in 4-μm-thick sections, and mounted on glass slides. Each slide was dewaxed in xylene and rehydrated in grade alcohol, followed by boiling in 10 mmol/L of citrate buffer (pH 6.0) for antigen retrieval. After inhibition of endogenous peroxidase activities by 3% hydrogen peroxide in methanol, slides were treated with 1% bovine serum albumin to block non-specific binding. The sections were then incubated overnight at 4°C with anti-KPNA2 antibody (Santa Cruz Biotechnology, 1:100). After washing, the tissue sections were then incubated with the biotinylated secondary antibody followed by further incubation with streptavidin-horseradish peroxidase complex. Finally, the sections were developed with diaminobenzidine tetrahydrochloride (DAB) and counterstained with hematoxylin.

The degree of immunostaining of formalin-fixed, paraffin-embedded sections was reviewed and scored independently by two observers, based on both the proportion of positively stained tumor cells and the intensity of staining. The intensity of the staining was scored using the following scale: 0: no staining; 1: weak staining; 2: positive staining; and 3: strong staining. The area of staining was evaluated and recorded as a percentage: 0: no staining; 1: positive staining in < 10% of tumor cells; 2: positive staining in 10% to 50% of tumor cells; 3: positive staining in > 50% of tumor cells. The staining index was calculated as by

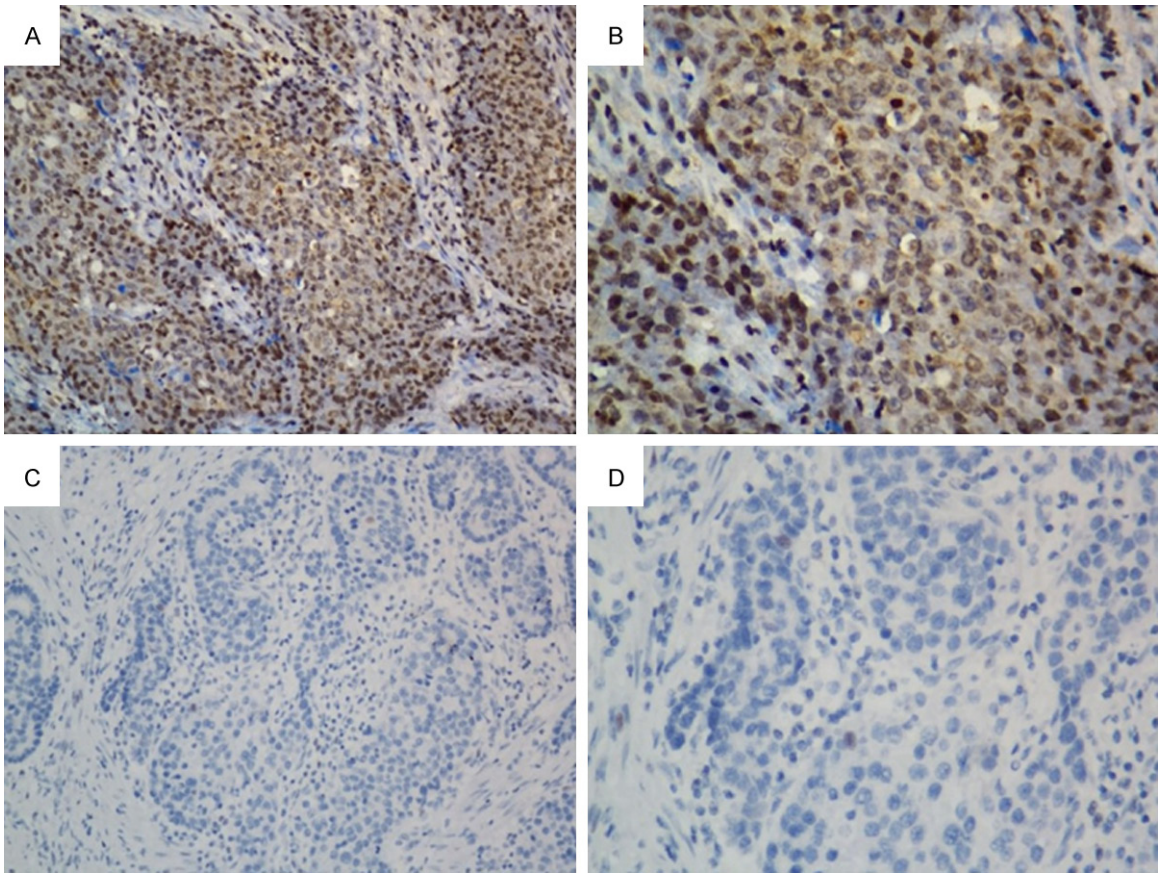

Figure 2. Representative images of KPNA2 from immunohistochemistry assays in cervical cancer specimens (high expression for A and B; low expression for C and D) (200× for A and C, 400× for B and D).

Table 1. Relationship between KPNA2 expression and clinicopathological characteristics in cervical cancer

Parameters	Group	Total	KPNA2		*P*
			Low	High	
Age	≤ 50 y	57	24	33	0.139
	> 50 y	37	22	15	
Tumor stage	IB	73	40	33	0.034
	> IB	21	6	15	
Tumor size	≤ 4 cm	70	39	31	0.025
	> 4 cm	24	7	17	
Tumor grade	1/2	77	37	40	0.715
	3	17	9	8	
histological type	SCC	65	31	34	0.824
	AC	29	15	14	
LN Metastasis	No	85	42	43	0.777
	Yes	9	4	5	

SCC: squamous cell cancer; AC: Adenocarcinoma.

multiplying the positive area and the staining intensity.

Statistical analysis

All statistical analyses were carried out using the SPSS 16.0 statistical software package. The χ^2 test for proportion was used to analyze the relationship between KPNA2 expression and clinicopathological features. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. The significance of various variables for survival was analyzed by the Cox proportional hazards model in the multivariate analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression of KPNA2 in cervical cancer tissues

The expression of KPNA2 was examined by qRT-PCR in 8 pairs of frozen cervical samples (cervical cancer and adjacent non-tumor tissues). As shown in **Figure 1**, KPNA2 mRNA lev-

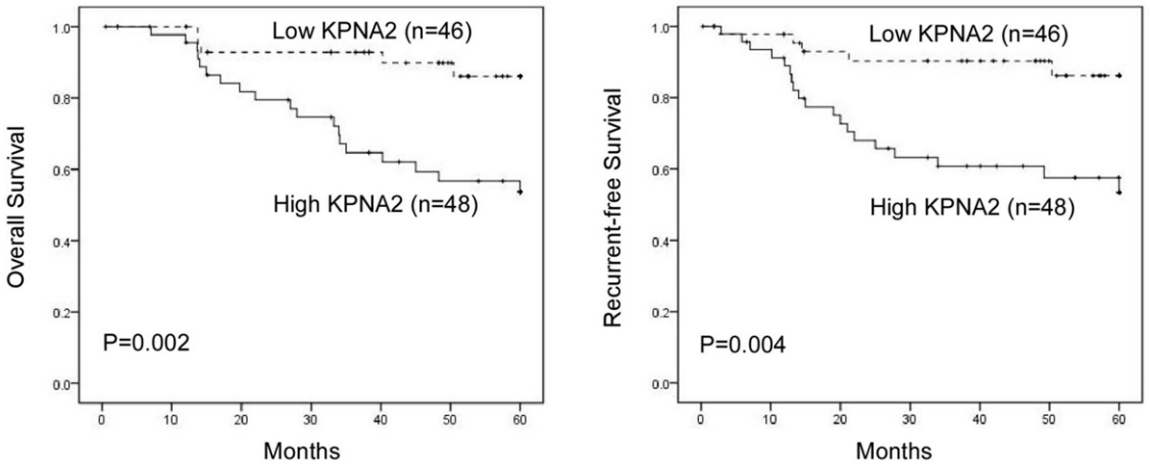

Figure 3. Kaplan-Meier curves of overall survival and recurrence-free survival in relation to KPNA2 expression in 94 cervical cancer patients.

Table 2. Cox regression analyses for predictors of outcome

Prognostic variables	OS		RFS	
	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*
Age (> 50 y vs ≤ 50 y)	1.440 (0.273-4.276)	0.464	1.384 (0.131-4.726)	0.856
Tumor Stage (> IB vs IB)	2.455 (0.122-5.375)	0.027	2.354 (0.426-6.276)	0.065
Tumor size (> 4 cm vs ≤ 4 cm)	1.829 (0.622-5.276)	0.067	1.406 (0.643-5.644)	0.079
Tumor grade (Grade 3 vs 1/2)	1.308 (0.254-4.673)	0.301	1.209 (0.226-4.736)	0.483
Histological type (SCC vs AC)	1.722 (0.375-7.649)	0.708	1.615 (0.039-5.774)	0.673
LN Metastasis (yes vs no)	3.569 (1.471-9.262)	0.045	3.026 (1.373-10.611)	0.078
KPNA2 expression (high vs low)	2.526 (1.110-8.772)	0.038	2.247 (1.149-8.305)	0.095

els were elevated in cervical cancer tissues compared to that of adjacent non-tumor tissues. Based on Student's t-test, statistically significant differences were found between the KPNA2 mRNA levels in tumor and non-tumor tissues.

Immunohistochemistry was performed to further investigate the expression of KPNA2 in cervical cancer. KPNA2 protein expression was displayed in the nucleus, but not in the cytoplasm. The representative immunostaining of KPNA2 in cervical cancer was shown in **Figure 2**. Forty-eight cases (51.0%) exhibited high expression of KPNA2 (**Table 1**).

Correlations between KPNA2 expression and clinical features of cervical cancer

The correlation of KPNA2 expression and clinicopathological features were analyzed according to the IHC assay results (**Table 1**). Statistical comparison revealed a significant positive association between KPNA2 expression and tumor stage (P = 0.034) and tumor size (P = 0.025). No significant differences were found between KPNA2 expression and other clinicopathological findings such as age, histological type, tumor grade, or lymph node metastasis.

Correlations between KPNA2 expression and clinical prognosis of cervical cancer

We further investigated the relationship of KPNA2 expression and the clinical outcomes of the 94 patients with cervical cancer. The median observation period was 41.7 months (range, 2.4 to 60 months). Kaplan-Meier analysis was used to compare the survival rates of cervical cancer patients with tumors expressing low or high levels of KPNA2. The results showed that the expression of KPNA2 was significantly associated with overall survival (OS) and recurrent-free survival (RFS) in cervical cancer

patients (P = 0.002 and = 0.004, respectively). Thus, patients with tumors having a high expression of KPNA2 had a poorer prognosis than those with tumors of low KPNA2 expression (**Figure 3**). Multivariate Cox proportional hazards model analysis revealed that KPNA2 expression was an independent prognostic factor for OS (P = 0.038, **Table 2**).

Discussion

In this study, we present the first evidence that KPNA2 is upregulated in cervical carcinoma tissues. KPNA2 protein was observed in 92.7% of cervical carcinoma specimens, and the expression level of KPNA2 protein was found to be significantly associated with clinical staging and tumor size of cervical cancer and the prognosis of patients with cervical carcinoma.

KPNA2 is a karyopherin α protein that play crucial role in nucleocytoplasmic transport. Nuclear retention of KPNA2 results in cytoplasmic retention of NLS-containing cargo proteins, as the KPNA2 not recycled back to the cytoplasm leads to a lack of free KPNA2 to bind its cargo in the cytoplasm [13]. Misregulation of KPNA2 during pluripotent embryonic stem cell differentiation significantly impairs clock development [14]. In cancer cells, nuclear accumulation of KPNA2 in response to cellular stress suppresses the nuclear import [15], which may contribute to the aberrant cell proliferation. Teng et al. hypothesized that KPNA2-mediated nuclear transport of proteins necessary for maintaining cell proliferation such as transcription factors, promote tumor cell growth [16]. Alshareeda et al. demonstrated that KPNA2 expression was associated with the cytoplasmic localisation and low/negative nuclear expression of key DDR proteins and cell cycle associated proteins, which could possibly be related to the induction of proliferation [17]. Huang et al. found that KPNA2 promotes tumor cell proliferation and tumorigenicity in epithelial ovarian carcinoma through upregulation of c-Myc and downregulation of FOXO3a [5]. Tan et al. reported that targeting KPNA2 by miR-26 could mediate breast cancer cell proliferation [18]. Our results revealed that KPNA2 expression was significantly correlated with tumor stage and tumor size of cervical cancer, suggesting that KPNA2 may be an important factor in modulating cell proliferation in cervical cancer.

Recent studies have indicated that the upregulation of KPNA2 was associated with the clinical prognosis of human cancers. Shi et al. proposed that high expression of KPNA2 defines poor prognosis in patients with upper tract urothelial carcinoma after radical nephroureterectomy [19]. Li et al. found that KPNA2 or Oct4 positive expression was significantly correlated with lower overall survival, while KPNA2 or Oct4 negative expression was associated with higher overall survival in patients with non-small-cell lung cancer [20]. Li et al. demonstrated that overexpression of KPNA2 is closely related to progression and poor prognosis of gastric adenocarcinoma patients [21]. Our study was in line with these studies in that upregulated expression of KPNA2 was associated with the poor prognosis in cervical cancer patients. Collectively, these studies described the prognostic roles of KPNA2 in human tumors.

It is noteworthy that KPNA2 expression was, in our study, upregulated in cervical cancer tissues compared with normal cervical tissues. Matching this result, van der Watt et al. found that KPNA2 has been found to be overexpressed in cervical cancer cells compared to normal cells, but inhibiting the expression of KPNA2 had no effect to cell death [22]. The results may be due to different levels of KPNA2 between in vivo and in vitro assays. Further studies are still needed to investigate the roles of KPNA2 in both cervical cancer tissues and cell lines.

In conclusion, our findings suggest that the downregulation of KPNA2 may be useful as a prognostic marker of cervical cancer development and progression. Further study of the detailed molecular mechanism of KPNA2 involvement in the tumorigenesis and progression of cervical cancer is warranted. Nevertheless, our study has provided a basis for the development of a novel potent marker and therapeutic target for cervical cancer.

Disclosure of conflict of interest

None.

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