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Diagnostic and prognostic values of KLK11 in nasopharyngeal carcinoma

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Abstract. – OBJECTIVE: This study aims to clarify potential diagnostic and prognostic values of KLK11 in nasopharyngeal carcinoma (NPC).

PATIENTS AND METHODS: KLK11 levels in 81 primary NPC tissues, 24 recurrent NPC tissues, and 60 nasopharyngeal tissues with chronic mucosal inflammation were determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Then, receiver operating characteristic (ROC) curves were depicted for assessing the diagnostic value of KLK11 in primary and recurrent NPC. Next, correlation between KLK11 level and pathological indexes of NPC patients was analyzed by Chi-square test. Enrolled NPC patients were followed up for 5 years, and the follow-up data were recorded to determine the potential influence of KLK11 on overall survival by Kaplan-Meier method. In addition, Cox regression model was applied for assessing factors that could affect prognosis of NPC patients.

RESULTS: It was found that KLK11 level was higher in primary NPC tissues than that in nasopharyngeal tissues with chronic mucosal inflammation. In recurrent NPC tissues, KLK11 was upregulated relative to primary ones. In addition, ROC curves revealed a certain diagnostic value of KLK11 in NPC. Overall survival was worse in primary and recurrent NPC patients expressing a high level of KLK11. By analyzing the pathological indexes of NPC patients, KLK11 level was found to be correlated with age, T stage, and clinical stage of NPC patients. Furthermore, KLK11 level was found to be the risk factor influencing the survival of NPC patients.

CONCLUSIONS: KLK11 is upregulated in NPC tissues, and unfavorable to the prognosis of NPC. Besides, it can be utilized as a potential hallmark for diagnosing NPC.

Key Words:
Nasopharyngeal carcinoma, KLK11, Prognosis, Diagnosis, Biological hallmark.

Introduction

Nasopharyngeal carcinoma (NPC) is one of the common malignant tumors, with a high degree of malignancy and early lymph node metastasis[1,2]. Radiotherapy is a preferred therapeutic approach for NPC. With the progression in radiography and radiotherapy, control rate of local NPC has been largely improved[3]. Nevertheless, distant metastasis of NPC is difficult to be monitored and controlled. It is necessary to develop effective and sensitive targets for NPC, thus providing a theoretical basis for tumor precision treatment and prognosis prediction.

Novel tumor hallmarks for NPC have been highlighted in the clinical diagnosis of NPC. It is reported that Cks1 (cyclin-dependent protein kinase regulatory subunit 1), P27, and CENP-F (centromere protein-F) are involved in cell cycle progression, which may be promising targets for NPC[4-6]. Some carcinogenic proteins and tumor-suppressor genes are critical during the proliferative progression of tumor cells, including RAP2A (RAS-related protein 2A), PTP4A2 (protein tyrosine phosphatase 4A2), and ECRG4 (esophageal cancer-related gene 4)[7,8]. Abnormally expressed genes in NPC are closely linked to prognosis of NPC patients, presenting prognostic potentials.

Kallikreins (KLKs) are a proteolytic enzyme subgroup of the serine protease family. Genes encoded by KLKs are located on human chromatin19q13.3-q13.4, containing about 300 kb continuous gene sequences. A total of 15 encoded proteins, KLK1-15, widely exist in human body fluids and various tissues[9,10]. KLKs are differentially expressed in normal tissues and involved in various biological functions, i.e., blood pressure regulation, tissue remodeling,

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hormone regulation, and skin desquamation[11]. In tumor tissues, KLKs are associated with tumor progression, invasion, and metastasis[12,13]. Abnormally expressed KLK11 has been discovered in many types of tumors[14-17]. This paper aims to clarify potential diagnostic and prognostic values of KLK11 in NPC, thus providing novel strategies for diagnosis and individualized therapy of NPC.

Patients and Methods

Baseline Characteristics

A total of 81 primary NPC tissues, 24 recurrent NPC tissues and 60 nasopharyngeal tissues with chronic mucosal inflammation confirmed by biopsy were collected from Weifang People's Hospital from May 2016 to December 2018.

Patient inclusion criteria: (1) patients newly diagnosed with type 2 or 3 NPC of WHO; (2) readjusted to III-IVb (T1-2N2-3M0 and T3-4N0-3M0, 8th edition based on AJCC/UICC staging system) radiography or computed tomography (CT), abdominal ultrasound or CT, whole-body bone scan or [^{18}F]-fluorodeoxyglucose positron emission Tomography combined with computed tomography (PET/CT); (3) IMRT plus concurrent chemotherapy alone. Patients were excluded if they were under the following conditions: (1) they had received anti-cancer treatment before diagnosed in our hospital; (2) they were pregnant or breastfeeding; (3) they were diagnosed with synchronous/synchronous cancer lesions during or before treatment or follow-up. Clinical data and follow-up information of each subject were complete. Tissues were immediately frozen in liquid nitrogen and stored at -80°C. Patients and their families in this study have been fully informed. This study was approved by Ethics Committee of Weifang People's Hospital.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Tissues were lysed for isolating total RNA using TRIzol method (Invitrogen, Carlsbad, CA, USA). Then, the RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT Reagent (TaKaRa, Otsu, Shiga, Japan), and applied for qRT-PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the internal reference. Primer sequences are as follows: KLK11: forward: 5'-TGGCAACAGGGCTTG-TAGGG-3' and reverse: 5'-GTAGCCGCGTCT-TCTCGAAC-3', and GAPDH: forward: 5'-GAGGCTGGGAACCTTAAGGT-3' and reverse: 5'-AGGGCCGCTGGTCAGAAGTT-3'.

Postoperative Follow-Up

Each subject was followed up through outpatient review, telephone or e-mail once within the first month, followed by once every three months in the first year, once every six months in the second year, and once a year since after. Follow-up data were recorded for 5 years.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was used for data analyses. Measurement data were expressed as mean ± SD ($\bar{x} \pm s$). The differences between two groups were compared by the *t*-test. Enumeration data were compared by χ^2-test. Diagnostic value of KLK11 in NPC was assessed by receiver operating characteristic (ROC) curves, and its prognostic value was evaluated by Kaplan-Meier method, followed by log-rank test for comparing differences between two curves. Cox regression model was applied for assessing influences of KLK11 on prognosis of primary NPC patients. $p<0.05$ suggested that the difference was statistically significant.

Results

KLK11 Was Upregulated In NPC Tissues

Compared with that in nasopharyngeal tissues with chronic mucosal inflammation, KLK11 was upregulated in primary and recurrent NPC tissues, and KLK11 level remained the highest in recurrent NPC tissues (Figure 1). It is suggested that KLK11 may be related to the progression of NPC.

Influence of KLK11 on the Survival of NPC Patients

Enrolled NPC patients were followed up for at least 5 years. Survival analysis uncovered worse prognosis in primary NPC patients expressing a high level of KLK11 (HR=4.032, p=0.0446) (Figure 2A). Similarly, recurrent NPC patients expressing a high level of KLK11 suffered worse prognosis (HR=4.611, p=0.0318) (Figure 2B). As a result, KLK11 is an unfavorable factor for the survival of NPC patients.

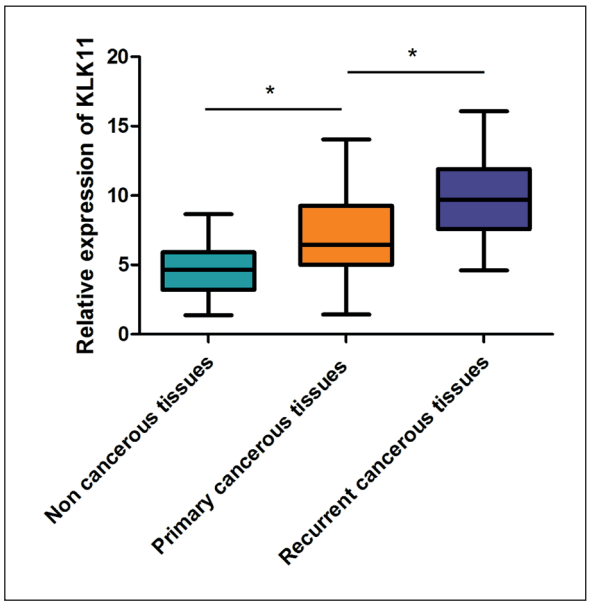

Figure 1. KLK11 is upregulated in NPC tissues. QRT-PCR data reveal a higher level of KLK11 in primary and recurrent NPC tissues (especially recurrent NPC) than those in controls.

Correlation Between KLK11 Level and Pathological Indexes of NPC

To further uncover the clinical significance of KLK11 in NPC, the enrolled 81 primary NPC patients were classified into high-level group (n=48) and low-level group (n=33) based on the median level of KLK11 (5.46±2.46). Chi-square analysis showed that KLK11 level was unrelated to sex, pathological grade, and N stage of NPC patients (p>0.05) but remarkably related to age, T stage, and clinical stage of NPC patients (p<0.05) (Table I).

Univariate and Multivariate Analyses on Potential Factors That May Influence the Prognosis of NPC

Prognostic potential of KLK11 on NPC was analyzed by Cox regression model. After adjustment of age, T stage and clinical stage of primary NPC patients, KLK11 was identified to be the risk factor for survival of NPC (HR=1.788, 95% CI=1.398-2.994) (Table II). It is believed that KLK11 is unfavorable for the prognosis of NPC.

ROC Curves Introduced for Analyzing the Significance of KLK11 in NPC

Subsequently, ROC curves were depicted to clarify the significance of KLK11 in primary and recurrent NPC. By analyzing primary NPC patients, AUC was 0.757 (p=0.040) (Figure 3A). In recurrent NPC patients, AUC was 0.917 (p=0.034) (Figure 3B). Therefore, the diagnostic possibility of KLK11 in NPC was proved.

Discussion

The incidence of NPC has evident regional characteristics, which is relatively high in South China with 33/100,000, especially in Guangxi, Guangdong, and Fujian Province. As a result, NPC is also named as "Guangdong cancer"[18,19]. In recent years, therapeutic strategies for NPC have been improved. Nevertheless, the 5-year survival of NPC is unsatisfied due to local recurrence and/or distant metastasis. Bone, liver and lungs are mainly affected by metastatic NPC[20]. Thus, it is necessary to search for effective hallmarks so as to improve the prognosis of NPC.

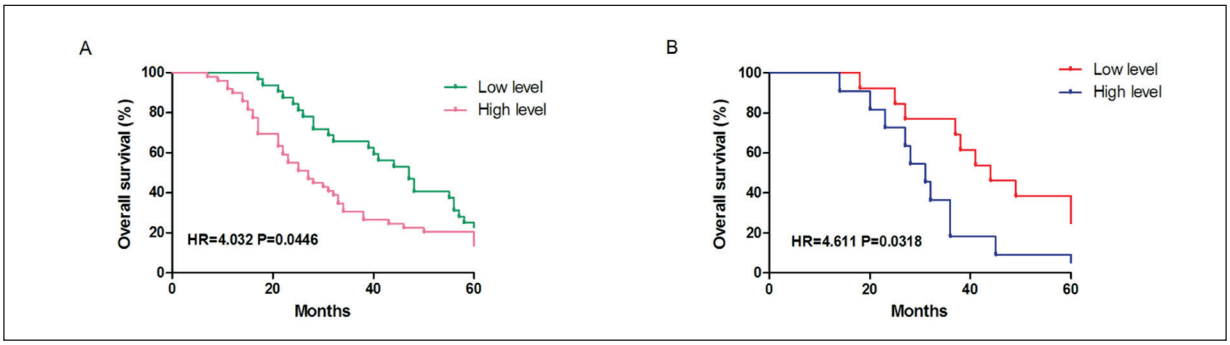

Figure 2. Influence of KLK11 on the survival of NPC patients. **A,** Worse prognosis in primary NPC patients expressing a high level of KLK11 than those expressing a low level. **B,** Worse prognosis in recurrent NPC patients expressing a high level of KLK11 than those expressing a low level.

Table I. Correlation between KLK11 level and pathological indexes of NPC patients.

Variable	No.	KLK11		p
		High level (n = 48)	Low level (n = 33)	
Sex				
Male	57	19	16	0.497
Female	24	29	17	
Age				
< 65	30	11	19	0.002*
≥ 65	51	37	14	
Pathological grade				
Low	29	18	11	0.815
Medium/High	52	30	22	
T stage				
T1-T2	35	26	31	< 0.001*
T3-T4	46	22	2	
N stage				
N0-N1	32	18	14	0.817
N2-N3	49	30	19	
Clinical stage				
I-II	26	10	16	0.015*
III-IV	55	38	17	/

*p<0.05.

Table II. Univariate and multivariate analyses on potential factors that may influence the prognosis of NPC.

Variable	HR[a]	95% CI[b]	p
Univariate analysis		1.558-4.821	0.027
KLK11-Low level	1.000		
KLK11-High level	1.942		
Multivariate analysis[c]		1.398-2.994	0.018
KLK11-Low level	1.000		
KLK11-High level	1.788		

a: Hazard ratio (HR) estimated from Cox proportional hazard regression model. b: Confidence interval of the estimated HR. c: Multivariate models were adjusted for patients' age, T stage, and clinical stage.

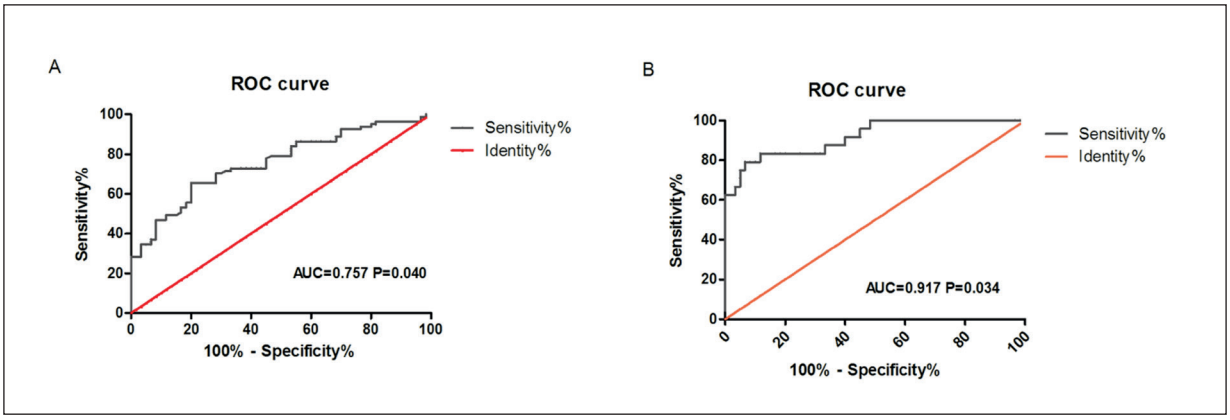

Figure 3. Diagnostic values of KLK11 in primary NPC (**A**) and recurrent NPC (**B**).

KLK11 was originally isolated from human hippocampus, but later found to be expressed in multiple human tissues, including skin, salivary glands, stomach, prostate, and intestines. It is reported that KLK11 is upregulated in 70% cases of ovarian cancer and 60% cases of prostate cancer, suggesting the potential of KLK11 as a tumor hallmark[21]. Alexopoulou et al[22] detected KLK11 levels in 120 colorectal cancer tissues and paracancerous ones. They demonstrated that KLK11 is upregulated in tumor tissues, which is correlated with invasive depth (p=0.013) and pathological grade (p=0.004). Moreover, it is also an independent factor for OS (p=0.026) and DFS (p=0.048) in colorectal cancer patients. Hence, it is believed that KLK11 is of significance to evaluate prognosis of tumor patients, especially those with high invasiveness and lymphatic metastasis.

In this analysis, KLK11 was upregulated in primary and recurrent NPC tissues than those of controls, especially in the recurrent tumor tissues. Through a series of analyses, KLK11 was confirmed to be an unfavorable factor for the survival and prognosis of NPC patients. Moreover, the diagnostic potential of KLK11 in NPC has been identified. Collectively, the findings of this study provide a new hallmark, KLK11, in diagnosing and predicting the prognosis of NPC patients.

Conclusions

Shortly, KLK11 is upregulated in NPC tissues, and unfavorable to the prognosis of NPC. Besides, it can be utilized as a potential hallmark for diagnosing NPC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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