Overexpression of kallikrein gene 10 is a biomarker for predicting poor prognosis in gastric cancer

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AIM: To analyze the expression of kallikrein gene 10 (KLK10) in gastric cancer and to determine whether KLK10 has independent prognostic value in gastric cancer.

METHODS: We studied KLK10 expression in 80 histologically confirmed gastric cancer samples using real-time quantitative reverse transcription-PCR and hK10 expression using immunohistochemistry. Correlations with clinicopathological variables (lymph node metastasis, depth of invasion and histology) and with outcomes (disease-free survival and overall survival) during a median follow-up period of 31 mo were assessed. Gastric cancer tissues were then classified as KLK10 positive or negative.

RESULTS: KLK10 was found to be highly expressed in 57/80 (70%) of gastric cancer samples, while its expression was very low in normal gastric tissues. Positive relationships between KLK10 expression and lymph node metastasis (P = 0.048), depth of invasion (P = 0.034) and histology (P = 0.015) were observed. Univariate survival analysis revealed that gastric cancer patients with positive KLK10 expression had an increased risk for relapse/metastasis and death (P = 0.005 and 0.002, respectively). Cox multivariate analysis indicated that KLK10 was an independent prognostic indicator of disease-free survival and overall survival in patients with gastric cancer.

CONCLUSION: KLK10 expression is an independent biomarker of unfavorable prognosis in patients with gastric cancer.
INTRODUCTION

Gastric cancer is the fourth most common cancer, and the second leading cause of cancer death worldwide[1]. Mortality due to gastric cancer has risen in China over the past 20 years, especially in rural areas and in aging populations[2,5]. Although the increased use of screening for early disease diagnosis and the widespread administration of systemic adjuvant therapies have led to a decline in mortality rates, the incidence and mortality of gastric cancer are still second only to lung cancer[4][5].

The kallikrein gene family of secreted serine proteases, consisting of 15 genes, is localized in tandem on chromosome 19q13.4 and shows significant homologies at both the nucleotide and the protein levels[6,7]. Kallikrein-related peptidase 10 is a member of the kallikrein family and has been shown in numerous reports to be upregulated in ovarian cancer[8,9]. The human kallikrein (KLK) gene 10 encodes human kallikrein gene 10 (KLK10) protein. Recent studies have shown that human KLKs are involved in human carcinogenesis and that several KLKs are promising biomarkers of prostate, ovarian, testicular and breast cancer[7][10][11]. For instance, prostate-specific antigen (PSA/hK3) which is encoded by the KLK3 gene is used as a cancer-specific marker for male population screening, early diagnosis and monitoring of prostate cancer[12]. In addition, quantification of KLK5 expression is critical for both the discovery of early cellular and molecular alterations in breast cancer, as well as the identification of novel diagnostic and prognostic biomarkers[13]. Many other KLKs are also expected to act as tumor biomarkers[14][15]. More recent evidence also implicates the KLKs in many cancer-related processes, including cell growth regulation, angiogenesis, invasion and metastasis[15]. Several authors have reported that KLK10 mRNA was highly expressed in ovarian cancer tissue and that hK10 may be a useful serum biomarker for the diagnosis and management of ovarian cancer[15]. However, few studies have focused on KLK10 expression in human gastric cancer.

In the present study, we examined the clinicopathologic and prognostic significance of KLK10 expression in gastric cancer. Based on collective findings, we hypothesize that KLK10 expression in gastric cancer tissues may have prognostic/predictive value in patients with this malignancy.

MATERIALS AND METHODS

Study population

Tumor specimens from 80 consecutive patients undergoing surgical treatment for primary gastric cancer at the Department of General Surgery, Tianjin First Central Hospital (Tianjin, China) were analyzed in this study. Patient age ranged from 35 to 74 years, with a median of 51 years (Table 1). All tumor specimens and matched control samples taken from normal tissues at the incision edge were snap-frozen in liquid nitrogen and stored at -80 °C for subsequent RNA extraction. Investigations were carried out in accordance with the ethical standards of the 1975 Helsinki Declaration, as revised in Tokyo 2004. The patients had not received hormonal therapy or chemotherapy prior to surgery. After surgery, all patients were treated with oxaliplatin-based chemotherapy regimens based on a platinum compound, alone or in combination with other drugs; grade 1 and stage I patients received no further treatment. Follow-up information (median follow-up period of 31 mo) was available for 80 patients (Table 1). Two time-to-event outcomes after surgery were defined as the time interval between the date of primary cancer removal and the date of the first documented evidence of relapse. OS was defined as the time interval between the date of surgery and the date of death, or the date of last follow-up for those who were alive at the end of the study.

Clinical and pathological information documented at the time of surgery included clinical stage, histology, depth of invasion and lymph node metastasis (Table 2). All pathological factors were established as described by the 2010 National Comprehensive Cancer Network Guideline.

Ethics

The study protocol was approved by the Ethics Com-
and synthesized for the target sequence database, gene specific primers were designed based on the mRNA sequences from the NCBI Sequence Database. A KLK10 fragment was amplified using the primers: forward, 5'-CTCTGGCGAGCTGCTG-3' and reverse, 5'-ATAGGCTTCGGGTCCAA-3', whereas the primers for HPRT1 were: forward, 5'-TGGAAAAGGGTGTTATTCCCTCAT-3' and reverse, 5'-ATGTAATCCAGCAGGTCACCAA-3'.

Real-time PCR assays of KLK10 mRNA expression levels were performed using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, United States), and the reagents TaqMan Fast Universal PCR Master Mix (29) (Applied Biosystems, United States) according to the manufacturer’s instructions. With an initial polymerase activation step at 95 °C for 10 min, the amplification conditions of the 40 cycles consisted of denaturation at 95 °C for 15 s, annealing at 59 °C for 30 s, and elongation at 72 °C for 30 s. The products were then subjected to a temperature gradient from 55 °C to 95 °C at 0.1 °C/s with continuous fluorescence monitoring to produce a melting curve of the products. KLK10 mRNA expression was calculated from the standard curve, and quantitative normalization of cDNA in each sample was performed using the expression of GAPDH mRNA as an internal control[19]. We classified the 80 cases into two groups using the mean expression level of KLK10 mRNA in tumor tissues (0.03); i.e., a positive-expression group (≥ 0.03, n = 57) and a negative-expression group (< 0.03, n = 23).

Statistical analysis
Statistical analysis were performed using SPSS for Windows version 19.0 (SPSS, Chicago, IL, United States). Associations between clinicopathological parameters, such as depth of invasion, lymph node metastasis, histology and KLK10 expression were analyzed by the Chi-square test or Fisher’s exact test, where appropriate. Survival analysis were performed by constructing Kaplan-Meier DFS and OS curves and differences between curves were evaluated by the log-rank test (Mantel, 1966), and by estimating the relative risks for relapse and death using the Cox proportional hazards regression model (Cox, 1972). Cox analysis was conducted at both univariate and multivariate levels. Only the patients with known status of KLK10 and all other variables, for which the patients were characterized.

RESULTS

Relationship between KLK10 expression and other parameters
Of the 80 patients included in this study, 57 (70 %) were positive for KLK10 expression in gastric cancer tissues. In normal gastric tissues, the level of KLK10 was undetectable or low. Table 2 shows the distribution of KLK10 expression in tumor tissues and corresponding normal tissues from 80 gastric cancer cases.
The histotype also correlated with these groups \( (P = 0.015) \). In contrast, no significant difference was observed regarding age and sex. The 3-year actuarial OS rates in patients with gastric cancer and positive KLK10 mRNA expression and in patients with negative KLK10 mRNA expression were 20\% and 42\%, respectively (Figure 3). The survival difference between these two groups was statistically significant \( (P = 0.002; \text{log-rank test}) \).

Univariate and multivariate survival analysis

The degree of association between each clinicopathological variable and DFS and OS is shown in Table 3. In univariate analysis, patients with KLK10-positive gastric cancer had a significantly increased risk of relapse (decreased DFS) and death (decreased OS) (hazards ratios of 0.46 and 0.43; \( P = 0.005 \) and 0.002, respectively). Nevertheless, positive KLK10 expression was weakly associated with an increased risk of death (decreased OS) (hazards ratio of 0.55; \( P = 0.03 \)) in multivariate analysis compared with univariate analysis, while no significant association was found between the positive KLK10 expression and relapse (decreased DFS) in patients with gastric cancer in multivariate analysis \( (P = 0.06) \).

Depth of invasion and histotype were the strongest independent indicators of poor prognosis \( (P < 0.05, \text{except for depth of invasion for OS in multivariate analysis}) \). As expected, Kaplan-Meier survival curves (Figure 3) indicated that patients with KLK10-positive gastric cancer had shorter DFS \( (P = 0.003) \) and OS \( (P = 0.002) \) com-

**Immunohistochemistry**

In 55 of the 57 patients who were positive for KLK10 mRNA expression, specific expression of hK10 protein was only found in cancer tissues, but not in the corresponding normal tissues (Figure 2). In 23 cases with negative expression of KLK10 mRNA, 20 exhibited negative or weak expression of hK10 in cancer tissues. In contrast, 2 cases with high KLK10 mRNA expression exhibited negative or poor hK10 protein expression in cancer tissues.

**Clinicopathologic significance of KLK10 mRNA expression in gastric cancer**

The clinicopathologic factors analyzed in relation to KLK10 mRNA expression in tumor tissues are shown in Table 2. The level of lymphatic invasion was significantly higher \( (P = 0.048) \) in the positive-expression group than in the negative-expression group. The depth of gastric wall invasion was greater \( (P = 0.034) \) in the positive-expression group than in the negative-expression group.
pared with KLK10-negative patients.

**DISCUSSION**

Gastric cancer is a common malignant tumor of the gastrointestinal tract. The optimal management of patients with gastric cancer involves a multidisciplinary approach: diagnosis, surgery, and chemotherapy, including the use of biological markers. Several authors have reported that KLK10 mRNA is highly expressed in human cancer tissues. In the current study, we found a significant relationship between KLK10 mRNA expression and lymph node metastasis, depth of invasion and histology in patients with gastric cancer, as shown in Table 1. These findings indicate that the overexpression of KLK10 was significantly associated with both an increased incidence of lymphatic invasion and poor histology in patients with gastric cancer. These results suggest that enhanced expression of KLK10 may play an important role in various pathologic processes of gastric cancer. The results obtained in this study are in agreement with previous studies which examined the association between KLK10 expression status and the clinicopathological features of patients with gastric cancer.

Some members of the KLK family have been identified as potential biological markers of prognosis, including KLK3, KLK14 and KLK7. For example, KLK5 expression is an indicator of poor prognosis in ovarian cancer. Furthermore, stratifying patients based on the presence or absence of such markers may result in a different prognosis in individuals. For example, both KLK8 mRNA and KLK6 mRNA are highly expressed in human breast cancer tissues, however, it is unknown whether a breast cancer patient with high expression of KLK8 has a good/poor prognosis compared to a breast cancer patient with high expression of KLK6.

In this study, we identified KLK10 as a new biomarker of poor prognosis in gastric cancer. Patients with KLK10-positive tumors were more likely to have poor histology and advanced stage disease. Our findings demonstrate that KLK10 expression can reduce DFS in patients with gastric cancer in univariate, but not in multivariate analysis (Table 2). In addition, when assessing KLK10 expression to predict survival outcomes, we found an increased risk of death in patients with KLK10-positive tumors in both univariate and multivariate analysis (Table 2). This indicates that KLK10-positivity may be an independent prognostic factor in patients with gastric cancer. That is, KLK10 may induce gastric cancer cell growth and proliferation. However, the function of the KLK10 signaling pathway is unclear. Some reports indicate that serine proteinases (the KLK gene family of secreted serine proteases includes 15 genes) participate in tumor growth and invasion. However, the function of the KLK10 signaling pathway does not allow us to formulate reasonable hypothesis to explain these observations. More studies with a larger group of patients are necessary to substantiate these findings.

The current study indicates that KLK10 mRNA was significantly overexpressed in gastric cancer tissues and high KLK10 expression levels were associated with lymphatic invasion, tumor invasion and poor patient prog-
nosis. hK3 has been well documented to be an excellent tumor marker for prostate cancer. Moreover, hK10 is a promising serum biomarker for ovarian cancer. Therefore, studies are now underway to investigate whether hK10 may also be a useful biomarker for gastric cancer using serum samples from the patients treated at our hospital.

COMMENTS

Background
Gastric carcinoma is one of the most common tumors worldwide. The expression of kallikreins is involved in cancer cell formation. Abnormal expression of kallikrein gene 10 (KLK10) is associated with carcinogenesis, and it is a promising serum biomarker for cancer.

Research frontiers
Several authors have reported that KLK10 mRNA is highly expressed in ovarian cancer tissue and that hK10 could be a useful serum biomarker for the diagnosis and management of ovarian cancer. However, there is little information on KLK10 expression in human gastric cancer.

Innovations and breakthroughs
This study assessed the clinicopathologic and prognostic significance of KLK10 expression in gastric cancer. Furthermore, based on the collective findings, this study investigated whether KLK10 expression in gastric cancer tissues may have prognostic/predictive value in patients with gastric cancer.

Applications
By exploring the relation between the expression of KLK10 and clinicopathology in gastric cancer, this study may provide a strategy for predicting the prognosis of gastric cancer patients.

Peer review
It is a well written paper. The authors investigated the clinicopathologic and prognostic significance of KLK10 expression in gastric cancer, which helps understand the pathogenesis and predict the prognosis of gastric cancer. The experimental procedure is quite well performed.

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