Upregulated KLK10 inhibits esophageal cancer proliferation and enhances cisplatin sensitivity *in vitro*

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Abstract. The kallikrein-related peptidase 10 (KLK10) gene has tumor-suppressive function in various types of human cancer. However, previous studies showed that KLK10 also acts as an oncogene and is upregulated in gastrointestinal tumors. The role of KLK10 in human esophageal cancer (EC) remains unclear. In the present study, the expression of KLK10 in human esophageal and non-esophageal cancer tissues was investigated by immunohistochemistry. Quantitative RT-PCR and western blot analysis were utilized to detect KLK10 mRNA and protein expression in human esophageal cancer cell lines (TE-1 and Eca-109). Small interference RNA was utilized to specifically knockdown KLK10 expression in Eca-109 and TE-1 cells. Cell proliferation, cell cycle analysis as well as CDDP-dependent apoptosis were determined using a CCK-8 assay and flow cytometry. The results showed that, KLK10 was positive in 67 out of 83 (80.72%) human EC and positive in 3 out of 11 (27.27%) normal tissues (P=0.001). The present study indicated that KLK10 potentially plays a crucial role in Eca-109 cell growth. Additionally, the downregulation of KLK10 induced S-phase arrest and promoted cisplatin-induced apoptosis. The resutls of the present study suggested that KLK10 is a promising novel marker for the diagnostic and therapeutic target of esophageal cancer.

Introduction

Esophageal cancer (EC) ranks eighth in cancer incidence with an estimated 456,000 new cases in 2012 (3.2% of the total patients) and sixth in cancer mortality with an estimated 400,000 deaths (4.9% of the total patients) (1). There are two primary pathological types of EC, esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), which account for 95% of all cases of EC (2). ESCC has a predilection for Asian populations and more than half of all new cases of ESCC worldwide are diagnosed in China (3). The incidence of EAC has increased by 6-fold in the United States during the last few decades (4). Although there have been advances in the field of operative methods, chemotherapy (Cis-platinum CDDP) and radiotherapy (5), the prognosis of EC patients remain to be determined. The development of new biomarkers for the early detection and potential therapeutic targets of EC are important for therapeutic improvement (6).

Serine proteases (plasminogen-plasmin system) is dysregulated in various human cancers, emerging as an important mechanism to regulate cell growth, migration, invasion, metastasis and angiogenesis (7). Kallikrein-related peptidases (KLKs) are the largest cluster among all serine proteases within the human genome (8,9). This cluster consists of a subgroup of 15 homologous secreted trypsin and chymotrypsin-like serine proteases, encoded by a tightly clustered multigene family on chromosome 19q13.4 (8). Kallikreins are often co-expressed in the skin, breast, prostate, pancreas and brain, and secreted by epithelial cells, from which they enter the bodily fluids such as sweat, milk, saliva, seminal plasma and cerebrospinal fluid or pericellular spaces (10). Kallikrein genes/proteins are aberrantly expressed in many types of cancer and their expression is often associated with patient prognosis. Abnormal regulation of KLKs interferes with different stages of cancer growth, including tumor differentiation, angiogenesis and metastasis (11). KLK family members have been reported to be promising diagnostic/prognostic biomarkers for several cancer types, including breast, ovarian, prostate and testicular carcinomas. For example, hK 3 encoded by KLK3, which is well known as prostate-specific antigen (PSA), used as a marker for prostate cancer (8).

The kallikrein-related peptidase 10 (KLK10) gene, also known as normal epithelial cell-specific 1 (NES1), is a member of the human kallikrein-related family of peptidases (8). The KLK10 gene encodes a 31 kDa secreted serine protease with 276 amino acids and is expressed in various tissues and organs such as skin, prostate, fallopian tube, salivary gland, colon and

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testis. KLK10 exerts various physiological functions in tissues and is involved in cancer progression (12). KLK10 has been found to be downregulated in breast cancer, prostate tumor, non-small cell lung cancer and renal cell carcinoma (13-16). Ectopic KLK10 reduces anchorage-independent growth and enhances drug sensitivity in liver and breast cancer. Thus KLK10 probably acts as a tumor suppressor in these cancer types. By contrast, KLK10 is overexpressed in ovarian cancer, uterine papillary serous carcinoma, pancreatic ductal adenocarcinoma, colorectal carcinoma and oral squamous cell carcinoma (11,17-21) and acts as a tumor promoter. Knockdown of KLK10 inhibits proliferation and tumorigenicity in pancreatic cancer. Additionally, the dysregulation of KLK10 expression is useful for diagnosis and associated with prognosis of various types of cancer. KLK10 performs as an unfavourable prognostic role in ovarian cancer, pancreatic ductal adenocarcinoma and gastrointestinal tumors, or has a favourable prognostic role in breast cancer, prostate tumor and non-small cell lung cancer, respectively.

KLK10 expression is affected by several factors at various levels including transcriptional, translational and post-translational modification. In hormone tightly-regulated cancers, KLK10 is mainly upregulated by estrogen, androgen and progestin (22). By contrast, the downregulation of KLK10 is often associated with hypermethylation in CpG rich exon 3 (23), instead of in the promoter region. Additionally, many miRNAs are also involved in the post-transcriptional regulation of KLK10, and let-7f, miR-224 and miR-516a in ovarian carcinoma, as well as miR-21 in prostate cancer, can directly target KLK10 and reduce KLK10 expression. A previous study also found that KLK10 and KLK expression was altered by the transcriptional factor specificity protein 1 (24).

The primary aim of the present study was to examine the expression changes of KLK10 in clinical EC samples in comparison with normal tissues, and experimentally demonstrate the potential biological functions of KLK10 in EC.

Materials and methods

Patients and cell lines. Patients analyzed in this study were diagnosed with EC during the period 1999-2014 at Anshan Cancer Hospital (Anshan, Liaoning, China). None of the patients had been administered chemotherapy or radiotherapy prior to surgery. In total, 83 formalin-fixed, paraffin-embedded specimens were obtained from resected tumors and 11 adjacent normal specimens. The patient age ranged from 31 to 78 years with a mean age of 59.8±10.83 years. Tumors were graded according to the WHO grading system and staged according to the modified Astler-Coller system (MAC).

The human esophageal squamous cancer cell lines (Eca-109 and TE-1) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Eca-109 was cultured in RPMI-1640, supplemented with 10% fetal bovine serum (FBS) (both from Gibco-Life Technologies, Carlsbad, CA, USA) and 100 μg/ml each of penicillin and streptomycin (Invitrogen-Life Technologies, Carlsbad, CA, USA). The TE-1 was cultured in complete medium of high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (FBS) (Gibco-Life Technologies) and 100 μg/ml each of penicillin and streptomycin (Invitrogen-Life Technologies). The cells were maintained in a humidified 5% CO_2 incubator at 37˚C.

Reagents and antibodies. The Cell Counting Kit-8 (CCK-8, cat no. KGA317), Annexin V-FITC Apoptosis Detection kit (KGA108) and the Cell Cycle Detection kit (KGA512) were purchased from KeyGen Biotech (Nanjing, China). cis-Dichlorodiamine platinum (CDDP) was purchased from Haosen Pharmaceutical Inc. (Jiangsu, China). The antibodies used for the western blot analysis were: KLK10 (bs-2531R, diluted 1:500) purchased from Bioss (Beijing, China); β-actin (sc-47778, diluted 1:1,000) from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); and active caspase-3 (AJ1131b, diluted 1:1,000) from Abgent (San Diego, CA, USA). PARP (no. 5625, diluted 1:1,000), CDK2 (no. 2546, diluted 1:1,000) and cyclin A2 (no. 4656, diluted 1:1,000) were purchased from Cell Signaling Technology (Danvers, MA, USA). Goat anti-rabbit, goat anti-mouse IgG, peroxidase-conjugated secondary antibodies (31460 and 31430, both diluted 1:10,000) and goat anti-rabbit IgG (H+L) secondary antibody were purchased from Thermo-Pierce (Rockford, IL, USA).

Immunohistochemistry (IHC). The cytoplasmic expression of KLK10 in the human esophageal squamous cancer cell was evaluated as previously described. The cytoplasmic expression for KLK10 was scored as: i), 0<5% of the squamous cancer cells in the respective lesions; ii), 5-25% of the squamous cancer cells in the respective lesions were rated 1; iii), 26-50% of the squamous cancer cells in the respective lesions were rated 2; iv), 51-75% of the squamous cancer cells in the respective lesions were rated 3; and v), >75% of the squamous cancer cells in the respective lesions were rated 4. The intensity was graded as: i), negative, 0; ii), weak, 1+; iii), moderate, 2+; and iv), strong, 3+. A final score between 0 and 12 was achieved by multiplication of the extent of positivity and intensity. Scores of 9-12 were defined as '(+++)', scores of 5-8 were defined as '(++)', scores of 1-4 were defined as '(+)', and scores of 0 was defined as '(-)'. (-), (+) were considered negative and (++), (+++) were considered positive (25). The slides were blindly labeled and scored by two independent pathologists.

Isolation of total RNA and quantitative RT-PCR. Total RNA was extracted from EC cells (Eca-109 and TE-1) using TRIzol-up reagent (Invitrogen-Life Technologies), according to the manufacturer's instructions. The primers used were: KLK10 forward; 5'-GCCCGGAGAGTGAAGTACAA-3' and reverse, 5'GTAAACACCCCACGAGAGGA-3'; β-actin forward, 5'GCATGGAGTCCTGTGGCAT-3'; and reverse 5'-CTAGAAGCATTTGCGGTGG-3'. mRNA was reverse transcribed to cDNA using a SuperScript™ Two-Step RT-PCR kit (Takara, Dalian, China) by SureCycler 8800 (Agilent Technologies, Santa Clara, CA, USA). The PCR amplification was performed using SYBR-Green PCR Master Mix (Takara) by Stratagene Mx3005P (Agilent Technologies) under the following conditions: 95˚C for 30 sec, 40 cycles of 95˚C for 5 sec and 55˚C for 20 sec. The expression of KLK10 was normalized by using β-actin as an internal control. The relative expression was analyzed using the $2^{-\Delta\Delta Ct}$ method in MxPro-Mx3005P software.

siRNA transfection. Small interfering RNAs (siRNAs) targeting KLK10 and siRNA negative control were chemically synthesized (Genepharma, Shanghai, China). The siRNA sequences used were: KLK10 siRNA 1: 5'-TTGTTGTAC TTCACTCTCCGG-3'; KLK10 siRNA 2: 5'-ATGACTTTA TTGATCCAGGAC-3'; negative control siRNA: 5'-UUC UCCGAACGUGUCACGUTT-3'. The EC cells were seeded in six-well plates and incubated overnight for transfection. The medium was exchanged for serum-free DMEM when the cell confluence reached 60-70%. Complexes of siRNA (final concentration was 20 μM according to the manufacturer) and Lipofectamine 2000 (Invitrogen-Life Technologies) were added into the medium for 6 h. Subsequently, the medium was converted with fresh medium supplemented with 10% cultured FBS. The effectiveness of RNA interference was assessed by quantitative RT-PCR and western blot analysis.

Cell counting kit (CCK-8) assay. Cell proliferation was determined using CCK-8 and KGA317, according to the manufacturer's instructions. Two thousand cells (Eca-109)/wells and 3,000 cells (TE-1)/wells were seeded in a 96-well cell culture plate, and three replicates were prepared for each condition. After the cells were grown at different times (1-6 days) in a 37˚C incubator the medium was replaced with 100 μl of RPMI-1640/DMEM containing 10 μl of CCK-8 reagent, and the cells were incubated for 2 h at 37˚C. The optical density was then measured using an EnSpire™ 2300 Multilabel reader (PerkinElmer, Waltham, MA, USA) at 450 nm, after which the mean values were measured and the growth curves drawn.

Colony formation assay. Eca-109 and TE-1 were used for colony formation analysis. The cells were seeded in 6-well plates at a density of 500 cells (Eca-109)/800 cells (TE-1) of each well in 2 ml of medium (RPMI-1640 and DMEM) containing 10% FBS and cultured for 14 days in a humidified 5% CO_2 incubator at 37˚C. The colonies were fixed in 4% paraformaldehyde for 15 min, and stained with a solution containing 1% crystal violet for 15 min. Subsequently, the plates were washed with PBS and dried at room temperature.

Cell cycle and apoptosis assays. To evaluate the effects on cell cycle arrest and CDDP-induced apoptosis by downregulation of KLK10, the cells were examined using the cell cycle detection kit and the Annexin V-FITC apoptosis detection kit according to the manufacturer's instructions. For cell cycle analysis, Eca-109 cells were seeded in 6-well plates ($1x10^5$ and $2x10^5$ cells/dish for analysis of cell cycle arrest and apoptosis, respectively). After siRNA was transfected for 48 h, a total of $1x10^6$ cells was pelleted by centrifugation and washed twice with PBS. The cell pellets were resuspended in 500 μl of ice-cold 70% ethanol and incubated at -20˚C overnight. The fixed cells were centrifuged and the pellets were washed with PBS. After incubation with 100 μl RNase A (10 μg/ml) for 30 min at 37˚C in the dark, the cells were resuspended in 400 μl PI (50 μg/ml) and placed at 37˚C in the dark for 30 min. The stained cells were analyzed using an Accuri C6 flow cytometer (Accuri Cytometers Inc., Ann Arbor, MI, USA). For the apoptosis analysis, after siRNA were transfected in Eca-109 cells for 48 h followed by treatment with CDDP (5 μg/ml) for 48 h the cells were trypsinized, washed with cold PBS and suspended in PBS. The cells were stained using the Annexin V-FITC reaction reagent [5 μl of Annexin V-FITC and propidium iodide (PI)] at 37˚C for 30 min in the dark. The stained cells were analyzed using an Accuri C6 flow cytometer (Accuri Cytometers Inc.) as previously described (26).

Western blot analysis. After siRNA transfection for 2 days, the cells were harvested and lysed in RIPA buffer (KGP702) and proteins were extracted, and supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF; KGP610 from KeyGen Biotech). The mixture was centrifuged at 12,000 x g for 15 min and the supernatant was collected. The protein concentration was determined using the BCA Assay kit (KGPBCA), and each sample contained 30 μg protein per 10 μl. The protein samples were mixed with loading buffer (KGP101) and the proteins were separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Hercules, CA, USA). After soaking in blocking buffer at room temperature for 2 h, the membranes were incubated at 4˚C overnight with the primary antibody and were subsequently incubated at 37˚C for 2 h with the HRP-conjugated secondary antibody. The bands were visualized by chemiluminescence, imaged using a ChemiDoc XRS and analyzed using Image Lab (both from Bio-Rad). All protein extracts were completed on the ice (26).

Statistical analysis. Data represented at least three independent experiments and were statistically evaluated by Pearson's Chi-square test, correction for continuity analysis or Student's t-test with the statistical analysis software SPSS version 19.0 (IBM). $P<0.05$ was considered to be statistically significant.

Results

Expression of KLK10 in human EC tissue samples and cell lines. To detect the expression of KLK10 in human EC tissue samples, 83 EC specimens were assessed by IHC staining followed by the Chi-square test and correction for continuity analysis. KLK10 showed strong cytoplasmic staining in the tumor samples, while the normal epithelium tissues presented no or weekly cytoplasmic staining (Fig. 1A-C). The data from IHC analysis indicated KLK10 was markedly increased in tumor samples, as the positive staining is 67 out of 83 (80.72%) in human EC and only 3 out of 11 (27.27%) in normal tissues ($P=0.001$, Table I). We analyzed the association between KLK10 expression and other clinical parameters and found that KLK10 expression correlates with tumor diameter ($P=0.046$), although no significant difference was identified in the pathological types, nodal status and tumor grade (Table II). Then, we detected the expression of KLK10 in the Eca-109 and TE-1 EC cell lines. The two cell lines expressed KLK10 protein as shown by western blot analysis with higher KLK10 in Eca-109, than in TE-1 cells (Fig. 1D). However, its expression in mRNA levels was opposite to that of the protein expression (Fig. 1E).

Knockdown of KLK10 inhibits the growth and colony formation of Eca-109 and TE-1 cells. Two small interference RNAs

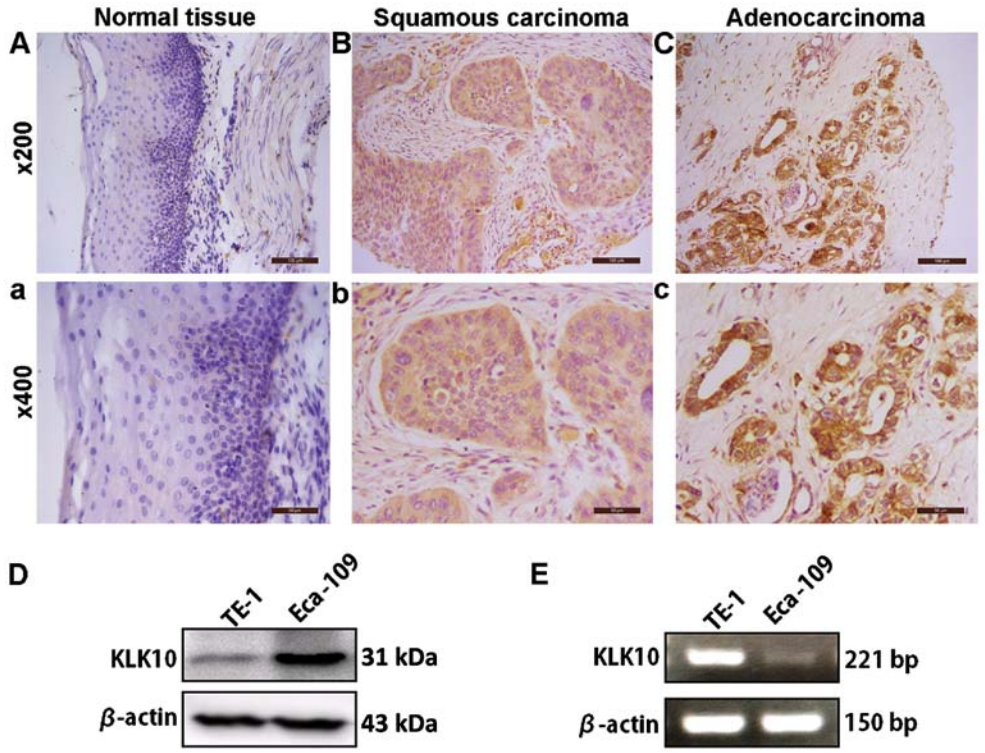

Figure 1. Expression of KLK10 in relative normal esophageal epithelium and EC tissue. (A-C) Immunohistochemical staining showing the KLK10 expression in relative normal tissue (A-a) squamous carcinoma tissue (B-b) and adenocarcinoma (C-c). (D) KLK10 expression in ESCC cell lines (Eca-109 and TE-1) was examined using (E) western blot analysis and RT-qPCR analysis. β-actin was used as a loading control. KLK10, kallikrein-related peptidase 10.

Table I. Associations between KLK10 expression and human esophageal tissue.

Variable		Total patients	Expression of KLK10 protein (-) (+)	Expression of KLK10 protein (++) (+++)	Positive (%)	P-value
General	Normal tissue	11	8	3	27.27	0.001[a]
	Tumor tissue	83	16	67	80.72	

[a]Correction for continuity; (-), no staining; (+), background or faint staining; (++), positive staining; (+++), intense staining. Samples marked (++) or (+++) were considered KLK10 positive, samples marked (-) or (+) were considered KLK10 negative.

were used to downregulate KLK10 expression. As shown by qPCR (Fig. 2A), the mRNA levels of KLK10 were reduced in KLK10 siRNA-1 and -2 treated cells compared with the control siRNA-transfected cells, respectively, which was consistent with KLK10 protein expression (Fig. 2B). In order to investigate the importance of KLK10 in cell proliferation, a CCK-8 assay was used and we observed a significant reduction in cell viability in ESCC cells treated with KLK10 siRNAsiRNA-1 and -2. The cells treated with KLK10 siRNAs exhibited a gradual growth ($P<0.05$) (Fig. 2C) and formed fewer colonies than the Eca-109 control siRNA cells (Fig. 2D, left panel). The bar graph demonstrated that knockdown of KLK10 in Eca-109 cells decreased the number of colonies as much as ~2-fold relative to the control ($P<0.001$ and $P<0.01$) (Fig. 2D, right panel). These results suggested the relationship between KLK10 and the cell proliferation of Eca-109 cells. We also observed a significant reduction of colony formation in KLK10 knockdown TE-1 cells (Fig. 3).

Knockdown of KLK10 leads to S-phase cell cycle arrest and enhancement of cell cycle regulatory proteins in Eca-109 cells. Based on the above results, we determined whether inhibition of cell proliferation after knockdown of KLK10 in Eca-109 cells was associated with cell cycle regulation. For this purpose, the cells were harvested and subjected to cell cycle analysis after transfected for 48 h. We found that the Eca-109 cells were arrested into S phase from 38.3 to 47.1 (siRNA-1) and 47.67% (siRNA-2) in the KLK10 siRNA groups ($P<0.01$) compared to the control (Fig. 4A). Regulatory proteins for S-phase cell cycle were further examined as shown in Fig 4C, and the

Table II. Associations between KLK10 expression and clinical parameters.

Variable	Expression of KLK10 (-)(+)	Expression of KLK10 (++)(+++)	Positive (%)	P-value
Years of age				
<60	9	31	77.5	0.581[a]
≥60	7	36	83.7	
Gender				
Male	13	57	81.4	0.708[a]
Female	3	10	76.9	
Degree of differentiation				
Well	6	21	77.8	0.780[a]
Moderately	8	32	78.0	
Poorly	2	13	86.7	
Tumor diameter (cm)				
<5	2	26	92.9	0.046[a]
≥5	14	41	74.5	
Lymph node metastasis				
Yes	7	34	82.9	0.782[a]
No	9	33	78.6	
Tumor grade				
I-IIa	9	31	79.5	0.776[a]
IIb-III, IV	7	36	83.7	
Pathological types				
Squamous cell carcinoma	15	53	78.3	0.285[b]
Adenocarcinoma	1	14	92.9	

[a]Chi-square test; [b]correction for continuity. KLK10, kallikrein-related peptidase 10.

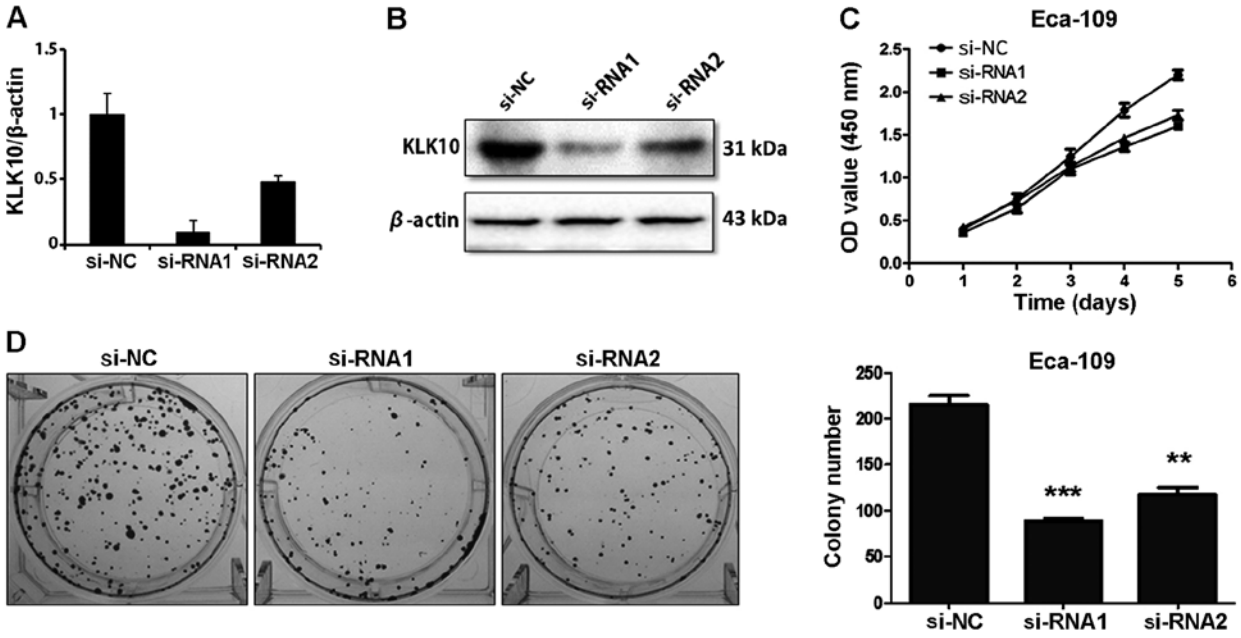

Figure 2. Effect of KLK10 on proliferation and growth in Eca-109 cell lines. (A and B) RT-qPCR and western blot analysis were used to verify the efficiency of KLK10 downregulation. β-actin was used as a loading control. (C) Cell growth was measured using CCK-8 assays after transfection with si-RNAs in Eca-109 cells. (D) The effect of downregulation of KLK10 in the Eca-109 cells was examined by a colony formation assay. The bar graph (right) shows the total colony numbers of siRNA- and si-NC-treated cells. Data are representative of three independent experiments and shown as mean ± SD ([**]P<0.01 and [***]P<0.001). CCK-8, cell count kit; KLK10, kallikrein-related peptidase 10.

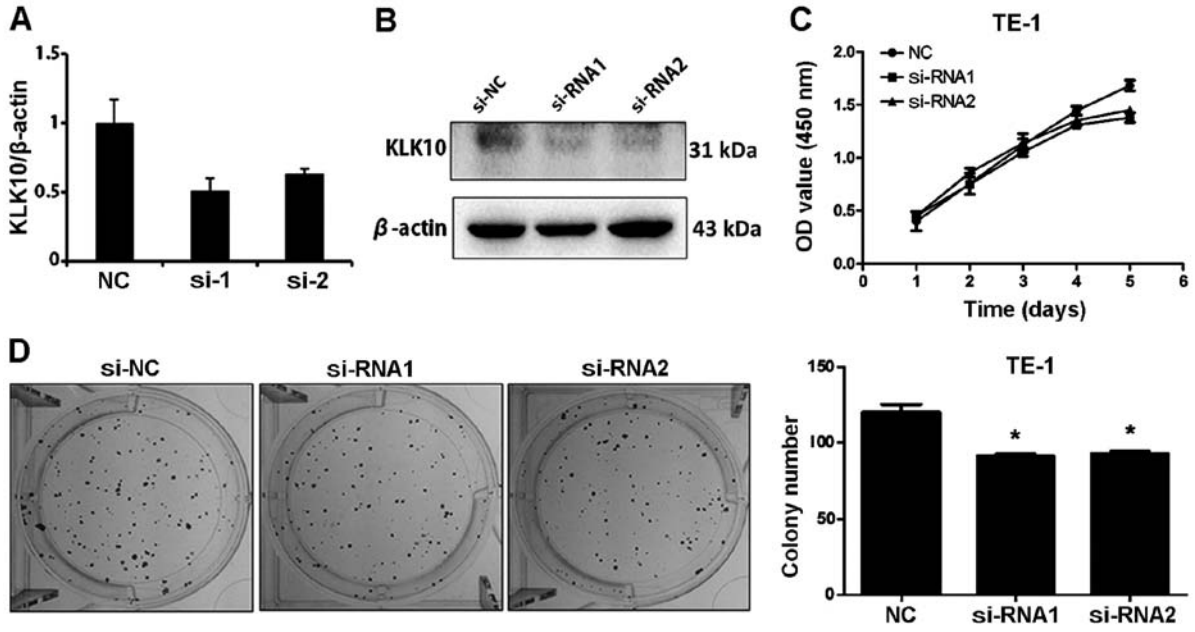

Figure 3. Effect of KLK10 on proliferation and growth of TE-1 cell lines. (A and B) RT-qPCR and western blot analysis were used to verify the efficiency of KLK10 downregulation. β-actin was used as a loading control. (C) Cell growth was measured using CCK-8 assays after transfection in TE-1 cell. (D) The effect of the downregulation of KLK10 in the TE-1 cells was examined by a colony formation assay. The bar graph (right) showing that the total colony numbers of siRNA- and si-NC-treated cells. Data are representative of three independent experiments and are shown as mean ± SD (*P<0.05). CCK-8, cell counting kit; KLK10, kallikrein-related peptidase 10.

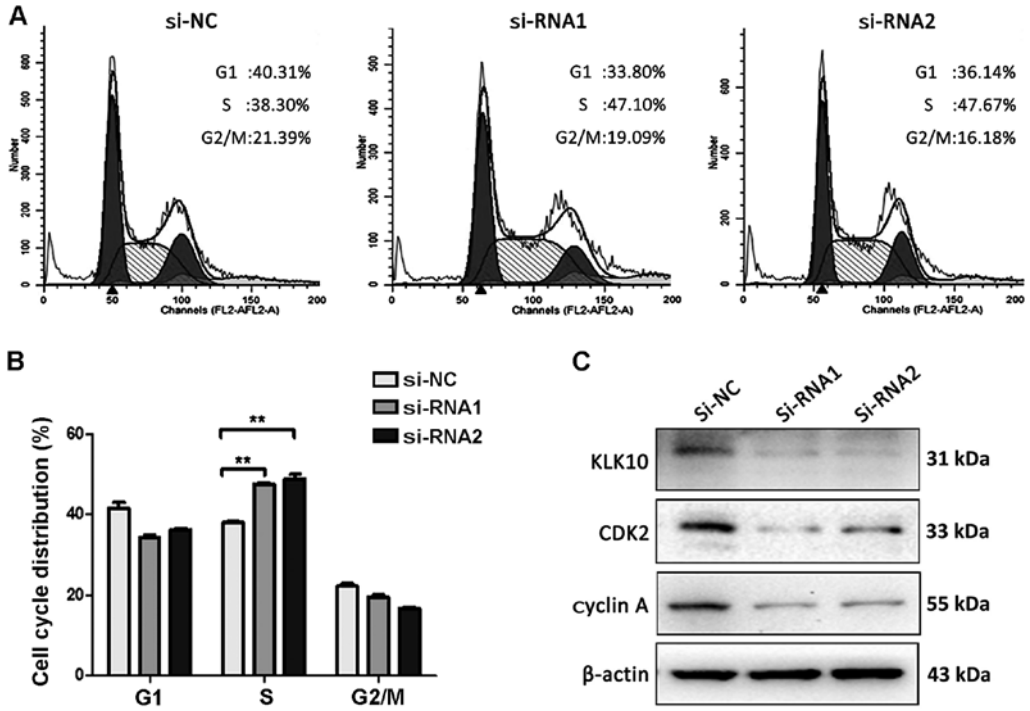

Figure 4. Downregulation of KLK10 in Eca-109 cells blocks S phase of cell cycle progression. (A) Eca-109 cells were stained with PI for FACS analysis after transfection for 48 h. (B) Quantitative data show the cell cycle distribution in the control and si-RNA treated cells. Data are presented as mean ± SD (**P<0.01). (C) Western blot analysis shows KLK10, CDK2 and cyclin A protein expression in Eca-109 after transfection for 48 h. β-actin was used as a loading control. Experiments were performed in triplicate. KLK10, kallikrein-related peptidase 10.

knockdown of KLK10 in Eca-109 cells resulted in the reduction of cyclin A and CDK2 expression.

Knockdown of KLK10 promotes CDDP-induced apoptosis and activation of caspase-3. CDDP is regarded as a first-line chemotherapy for EC patients, but the drug resistance remains a major clinical challenge (4). Therefore, we studied whether knockdown of KLK10 in Eca-109 cells increased the sensitivity to CDDP. siRNA1 was selected as its higher knockdown efficiency. Annexin V/PI staining and FACS analysis showed that apoptosis in KLK10-silenced Eca-109 cells increased to 10.8% as compared to the control (P<0.001) in response to

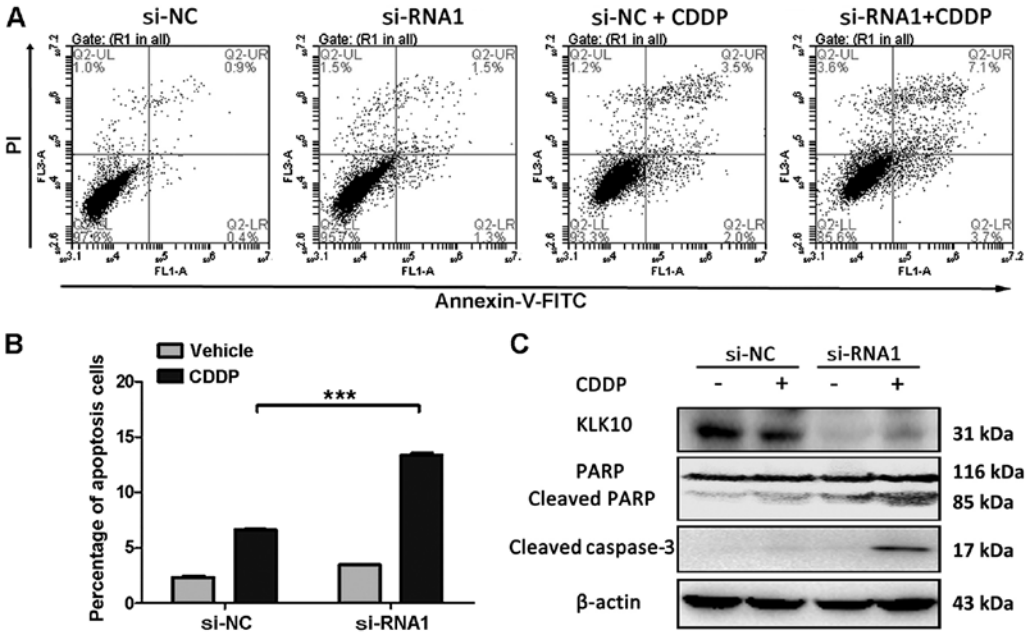

Figure 5. Downregulation of KLK10-enhanced CDDP-induced apoptosis. (A) Apoptosis in Eca-109 cells expressing KLK10 siRNAs or control si-NC with treatment by vehicle or CDDP (5 mmol/l) for 48 h was determined by (FACS) analysis after Annexin-V/PI staining. (B) The bar graph shows quantification summarized from three independent experiments. (C) Western blot analysis shows KLK10, cleaved PARP and cleaved caspase-3 proteins in Eca-109 cells after treatment with vehicle or CDDP. Experiments were performed in triplicate. PI, propidium iodide; KLK10, kallikrein-related peptidase 10.

CDDP. Western blot analysis revealed that CDDP induces apoptosis via knockdown of KLK10, significantly increasing protein levels of active caspase-3 and cleaved form of PARP as compared to the control (Fig. 5).

Discussion

Esophageal cancer is difficult to diagnose, and frequently has a poor prognosis. Therefore, it is of importance to identify new candidate markers to facilitate the early diagnosis and treatment of EC (27).

KLK10 codes a secreted serine protease, whose physiological function remains unknown. Compelling evidence has indicated that the expression of KLK10 is associated with the development of severe malignant tumors including steroid hormone-related cancers and some gastrointestinal tumors (28). KLK10 is considered to be a biomarker of some malignant tumors. In breast cancer, KLK10 is deregulated and acts as a tumor suppressor (14). KLK10 may enhance diagnosis sensitivity and specificity when cooperating with CA125. In NSCLC, ectopic KLK10 reduces anchorage-independent growth, tumorigenicity and enhances drug sensitivity (15), whereas in other tumors, such as prostate cancer, KLK10 acts as a tumor promoter. Knockdown of KLK10 inhibits proliferation and tumorigenicity. In the present study, we aimed to examine the expression and bio-function of KLK10 in esophageal cancer.

Previous findings have shown that a high expression of KLK10 in gastric (29,30) and colorectal cancer (11,31) is associated with poor prognosis. We hypothesized whether the expression of KLK10 is abnormal in esophageal cancer. Our results show an 80.72% positive expression in human EC for KLK10, but only 27.27% in normal tissues. Further analyses revealed that KLK10 expression was inversely correlated with tumor diameter (P=0.046, Chi-square). The diameter of the esophageal cancer is closely associated with the prognosis of patients (32,33). Accordingly, we demonstrated KLK10 is a potential biomarker for the diagnosis of esophageal cancer.

We observed the expression of KLK10 in mRNA and protein levels were different between TE-1 and Eca-109 cell lines used in the present study. TE-1 cells had a higher mRNA expression level of KLK10, but a relatively lower KLK10 protein expression level, which may be caused by the frequent regulation at the transcriptional and post-transcriptional levels by miRNAs (28), as some microRNAs are directly targeted by KLK10. KLK10 expression may be epigenetically influenced according to the results. Therefore, mRNA expression does not represent the protein level of KLK10 in TE-1 cells transfected with small interference RNAs. Furthermore, inhibition in the proliferation and colony formation ability of the KLK10 knockdown groups was not obvious. The results suggest that in certain esophageal carcinoma with a KLK10 high expression, KLK10 is a potential target for therapy.

DNA synthesis and internal checkpoints during S phase are activated when DNA double strands are damaged, such as in fracture (34). ATM/ATR-mediated Cdc25A degradation acting on cyclin E/A-CDK2 is primarily responsible for S-phase progression (34). We found that knockdown of KLK10 induced S-phase arrest by flow cytometry and the cyclin A and CDK2 activity was also decreased in Eca-109 cells. Thus, cell cycle arrest may be caused by cell proliferation inhibition due to knockdown of KLK10.

Cisplatin resistance remains a serious clinical challenge in the post-esophageal cancer (5). The results of the present study suggest that, knockdown of KLK10 can increase the cisplatin sensitivity of esophageal cancer as the apoptotic ratio was significantly higher in the KLK10 knockdown group.

Therefore, KLK10 is a potential target for the treatment of esophageal cancer by reversing chemotherapy resistance in esophageal cancer patients.

In conclusion, to the best of our knowledge, this is the first study to report that KLK10 was highly expressed in human EC and that KLK10 is a potential biomarker for EC diagnosis at an early stage. Knockdown of KLK10 inhibits EC cell proliferation and reverses chemotherapy resistance *in vitro*. Future studies should examine more tissues and *in vivo* assays to gain insight into the detailed mechanisms of KLK10-derived EC diagnosis and therapy.

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