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Original Article

Expression of cystatin C and its effect on EC9706 cells in esophageal carcinoma

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Abstract: Objective: To investigate the expression of cystatin C gene and its effect on the proliferation, apoptosis and invasiveness of EC9706 cells in esophageal carcinoma. Methods: 56 cases of esophageal carcinoma were randomly collected from our hospital. Samples of human esophageal carcinomas and matched normal esophageal mucosal epithelium were selected by resection operation from these patients. Expression of cathepsin B and cystatin C in these specimens were determined by immunohistochemistry and qRT-PCR. Next, lentiviral vectors of over-expression and interference for cystatin C gene were constructed, and both were transfected into EC9706 cells, and then the levels of cystatin C mRNA and protein were detected by qRT-PCR and Western blot. The effect of over and low-expressed cystatin C on the proliferation, apoptosis and invasiveness of esophageal carcinoma cells were detected by MTT assay, flow cytometry and Transwell assay. Results: Compared with normal esophageal epithelial tissues, mRNA and protein levels of cathepsin B and cystatin C in esophageal carcinoma tissues were significantly increased ($P<0.05$). Lentiviral vectors of over-expression and interference for cystatin C gene were successfully transfected into EC9706 cells. Over or low-expression cystatin C had no effect on EC9706 cells proliferation but had a reverse relationship with the apoptosis. However, cystatin C over-expression significantly decreased tumor invasiveness ($P<0.05$) while the invasiveness of EC9706 cells was significantly enhanced by RNAi-mediated abrogation of cystatin C gene expression ($P<0.05$). Conclusion: Over-expressed cystatin C could inhibit the invasiveness of esophageal carcinoma cells.

Keywords: Cathepsin B, cystatin C, esophageal carcinoma, proliferation, apoptosis, invasiveness

Introduction

Esophageal carcinoma is not only the most common malignant tumor in the world with the top ten ranking, but also one of the most common malignant tumors of digestive system. Esophageal carcinoma possesses the highest morbidity in Linzhou, Henan province, China. Its mortality comes first in the world and second to gastric carcinoma in China. The invasiveness and metastasis of malignant tumors are often the leading causes of death in cancer patients. Cathepsin B is a lysosomal cysteine protease, which plays a key role in the development and progression of cancer by involving in the degradation of extracellular matrix proteins and promoting tumor invasiveness and metastasis as well as tumor angiogenesis [1-3]. Cysteine proteases are regulated mainly by their endogenous inhibitor such as cystatin superfamily (cystatins, stefins, and kininogens) and thyropin. With the deepening of the knowledge concerning the mechanisms of tumorigenesis, more and more findings have suggested that cystatins which have been identified as the main endogenous inhibitors of cysteine protease are closely related to the progression, invasion and metastasis of cancer cells. Cystatin C is a cysteine protease inhibitor, which also plays an important role in the development and progression of cancer by interacting with cathepsin as a cathepsin inhibitor [4-6]. An inverse correlation between cystatin C levels and tumor grade has been noted in a variety of tumors. Thus cystatin C could be a good diagnostic biological marker for cancers.

This study explored the expression of cathepsin B and cystatin C in human esophageal carcinoma and its relationship with clinical features.

Then lentivirus transfection technique was used for over and low-expression cystatin C in EC9706 cells, and the levels of cystatin C mRNA and protein were detected by qRT-PCR and Western blot. After successful expression, the effect of over and low-expression cystatin C on the proliferation, apoptosis and invasiveness of esophageal carcinoma cells were detected by MTT assay, flow cytometry and Transwell test, and then the possibility of cystatin C for the treatment of esophageal carcinoma was further evaluated.

Subjects and methods

Patients and specimens

Surgical specimens (esophageal carcinoma tissues and matched normal esophageal mucosal epithelium) and preoperative serum samples were selected from 56 cases with esophageal carcinoma randomly collected from the First Affiliated Hospital of Zhengzhou University from March 2009 to May 2011. All patients were diagnosed with esophageal carcinoma by histopathological examination. Normal esophageal mucosal epithelium was selected from the excised esophageal stump, about 5 cm away from the tumor margin, and was confirmed as normal tissue by pathology. All patients were not received preoperative treatments for cancer, such as chemotherapy, radiotherapy or Chinese medicine therapy. All fresh tissues after surgical separation were immediately placed in liquid nitrogen, and part of them were embedded in paraffin. In this study, 56 patients were composed of 31 males and 25 females, and the age was 36-77 years old and the average age was 59.5 years old. Clinico-pathological diagnosis of all patients was evaluated by our pathologists based on WHO classification standard. All esophageal carcinoma tissues were divided into esophageal squamous cell carcinoma and adenocarcinoma according to pathological types; into well-differentiated group G1, moderately-differentiated group G2 and poorly-differentiated group G3 according to the degree of differentiation; into T1 (cancer cells invasion of the submucosa or lamina propria), T2 (cancer cells invasion of myometrium), T3 (cancer cells invasion of fiber membrane), T4 (cancer cells invasion of adjacent structures) according to the invasive depth of primary cancer and into lymph node metastasis and no lymph node metastasis according to different metastasis situations. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

Immunohistochemical assay

The tissue samples were paraffin-embedded and cut at 5 μm thickness. After deparaffinized and rehydrated, the paraffin sections were immersed in 0.01 mol/L citrate buffer. The mixture was heated to 95°C for 15 min; after 3% hydrogen peroxide solution was added, it was incubated at room temperature in the dark. Along with cystatin C (Proteintech Group Inc, Wuhan, China) (catalog no. 12245-1-AP; dilution, 1:100) and cathepsin B antibody (Proteintech Group Inc, Wuhan, China) (catalog no. 12216-1-AP; dilution, 1:100) were added, the mixture was incubated in the wet box overnight at 4°C. Next day, after Peroxidase-conjugated Affinipure Goat Anti-Rabbit IgG (H+L) (Proteintech Group Inc, Wuhan, China) (catalog no. SA00001-2; dilution, 1:5000) was added, the mixture was incubated at room temperature. Then the sections were washed for three times to remove excess antibodies, and diaminobenzidine (DAB) was used as the chromogen; after thoroughly rinsed with distilled water, they were counterstained with hematoxylin and differentiated with 1% hydrochloric acid alcohol, then gradient dehydrated in ethanol with xylene as clearing medium and mounted by neutral resin. Above sections was observed under the microscope. The matched normal mucosal epithelium was made paraffin sections using PBS instead of primary antibody as negative control. Photos for positive control were provided by the company from which the antibody was bought. Immunohistochemical results were scored by two experienced pathologists observing the sections independently without learning any clinico-pathological data. The scores were adopted under the condition of their consensus. Otherwise the results would be renegotiated. The dots that took on pale yellow, brown and tan were cystatin C- and cathepsin B-positive cells. That cell color being darker than background color, or background being not colored while cytoplasm being colored was regarded as the positive. Mattern semi-quanti-

tative integration method was used for semi-quantitative assessment of classification in both genes expression according to the coloration degree of the cells and the percentage of the coloration-positive epithelial cells (%).

qRT-PCR

RNA in tissues was extracted with Trizol reagent (TaKaRa, Dalian, China) with reverse transcription to cDNA by reverse transcription kit (TaKaRa, Dalian, China). qRT-PCR primers were designed according to cDNA sequences of cystatin C and cathepsin B genes. CysC-QF: CCGTCGGCGAGTACAACAAAG, CysC-QR: CCAGCTCCACGTCCAAGAAGTAGT; CathB-QF: GCTACAGCCCGACCTACAAACAG, CathB-QR: GAGCAGGAAGTCCGAATACACAGA; GADPH-QF: AGAAGGCTGGGGCTCATTTG, GADPH-QR: AGGGGCCATCCACAGTCTTC.

ABI 7500 Fast Real Time PCR System *(Applied Biosystems,* Foster City, CA, *USA)* was used for PCR reaction and $2^{-\Delta\Delta Ct}$ was adopted for calculation.

Construction of over-expression Lenti-CysC vector

Based on the template of cystatin C cDNA cloning vector (available from Beijing Sino Biological Technology Co., Ltd., Item No. HG10439M), the primers were designed for PCR amplification according to the cDNA sequence. Cystatin C-F: TTCTAGAGCTAGCGAATTCGCCACCATGGCCGGGCCCCTGCGC; Cystatin C-R: GCAGATCCTTGCGGCCGCGGATCCCTAGGCGTCCTGACAGGT. PCR products were performed for electrophoresis identification with gel extraction. Lentiviral expression vector was pCDH-CMV-MC-EF1-copGFP and it was linearized with EcoR I and BamH I enzymes. Then over-expression Lenti-CysC vector was constructed using Seamless Cloning. After successfully constructed, it was sent to Shanghai Biological Engineering Co., Ltd. for sequencing.

Construction of interference Lenti-CysC-shRNA vector

3 target sequences were designed for synthesis of shRNA according to cystatin C gene (GenBank, Item NO. NM_000099.2). The target sequence was CCTTGTCGAAATCCACCTG. shRNA3: 5'- --CcggCCTTGTCGAAATCCACCTGTTCAAGAGACAGGTGGATTTCGACAAGGTTTTTTg-3'. And then interference Lenti-CysC-shRNA vector was constructed. After successfully constructed, it was also sent to Shanghai Biological Engineering Co., Ltd. for sequencing.

Cystatin C mRNA level assay

EC9706 cells were seeded into 6-well plates with 3×10^5 in each well and transfected with Lenti (-), Lenti-CysC and Lenti-CysC-shRNA (recombinant lentiviruses with MOI=100) for 96 h. Three replicates for each group. The spent medium was discarded, and the residue was washed twice with PBS, and then total RNA was extracted in 1 ml Trizol. cDNA was synthesized through reverse transcription as described above and the level of cystatin C mRNA was detected by qRT-PCR. Fluorescence quantitative detection was performed with ABI Real time PCR 7500 Fast system. All data was analyzed by $2^{-\Delta\Delta Ct}$ method and expressed as Mean ± SD. LSD variance was used for statistical analysis.

Cystatin C protein level assay

EC9706 cells were seeded into 6-well plates with 3×10^5 in each well and transfected with Lenti (-), Lenti-CysC and Lenti-CysC-shRNA (recombinant lentiviruses with MOI=100) for 96 h. Three replicates for each group. The spent medium was discarded, and the residue was washed twice with PBS. The transfected cells was performed for lysis on ice in 200 μl RIPA lysis buffer with 10 mM PMSF for 1 h, and centrifuged at 12000× g for 15 min. The supernatant was performed for SDS-PAGE electrophoresis with 5%-12% polyacrylamide gel, grafted on PVDF membrane, then sealed in TBST with 5% skim milk at room temperature for 1 h, and incubated overnight at 4°C with Rabbit anti-Cystatin C polyclonal antibody (Proteintech Group Inc, Wuhan, China) (catalog no. 12245-1-AP; dilution, 1:100). After washed with TBST for three times, the mixture was incubated in Peroxidase-conjugated Affinipure Goat Anti-Rabbit IgG (H+L) (Proteintech Group Inc, Wuhan, China) (catalog no. SA00001-2; dilution, 1:5000) at room temperature for 1 h and washed with TBST for three times again. After chemiluminescent reagents (Beyotime Biotech, Nantong, China) was added, the remainder was photographed with GE chemical shine imager A600.

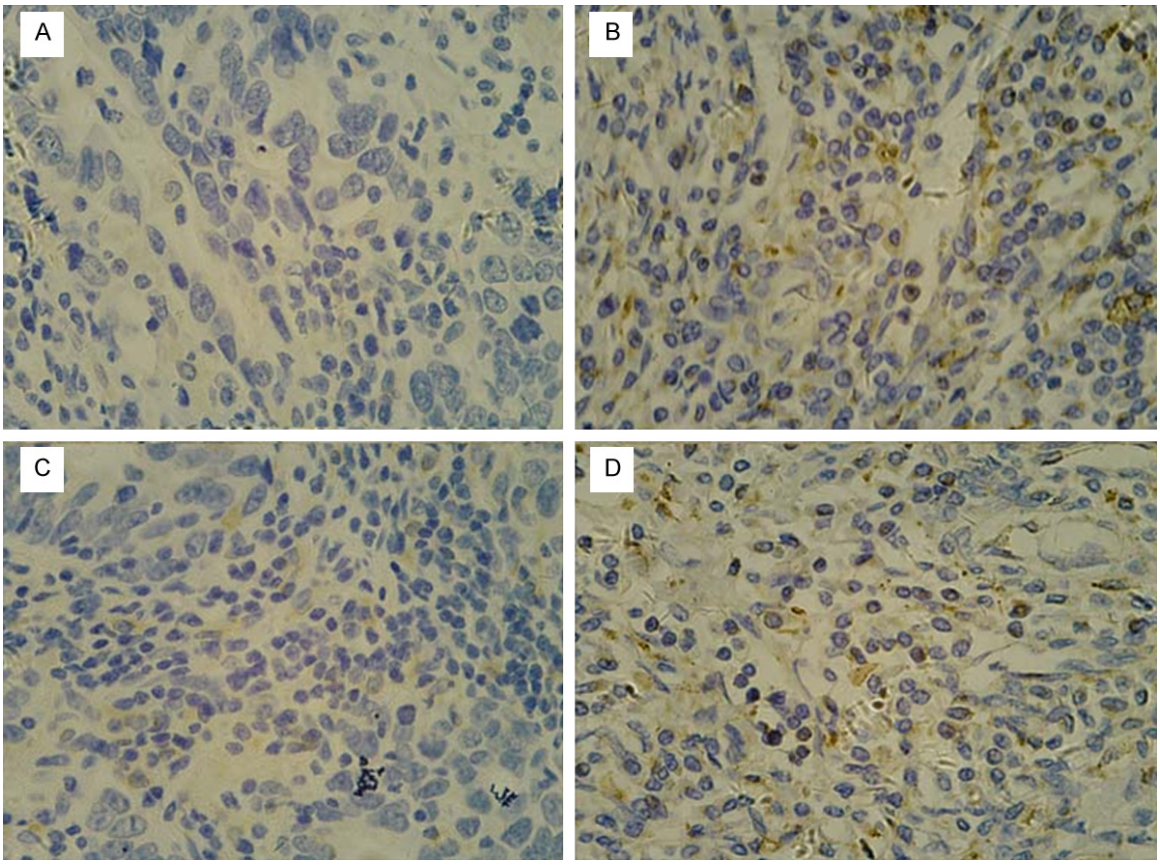

Figure 1. Expression of cystatin C and cathepsin B in normal and cancer tissue (Magnification ×400). A. Cystatin C gene (normal tissue); B. Cystatin C gene (cancer tissue); C. Cathepsin B gene (normal tissue); D: Cathepsin B gene (cancer tissue).

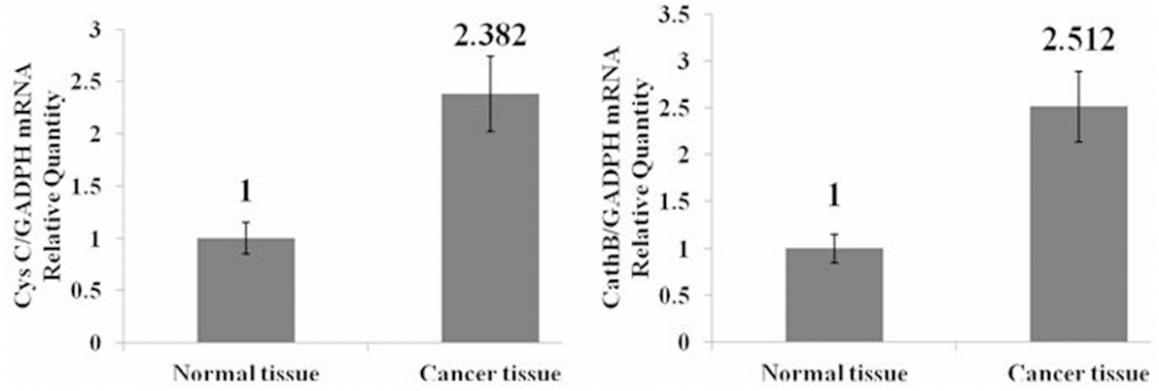

Figure 2. Level of cystatin C and cathepsin B mRNA in normal and cancer tissue.

EC9706 cell proliferation assay

EC9706 cells were seeded into 96-well plates with blank wells (no cell) and control wells (containing 2×10^4 cells in each well and medium) and transfected with Lenti (-), Lenti-CysC and Lenti-CysC-shRNA (recombinant lentiviruses with MOI=100). Five replicates for each group. MTT assay was performed at 24, 48, 72, 96 h. 20 μl 0.5% MTT solution was added in each well and the plates were incubated for 4 h. The medium was discarded after centrifugation, and each well was added with 150 μl DMSO. And then the plates were placed on a shaker for

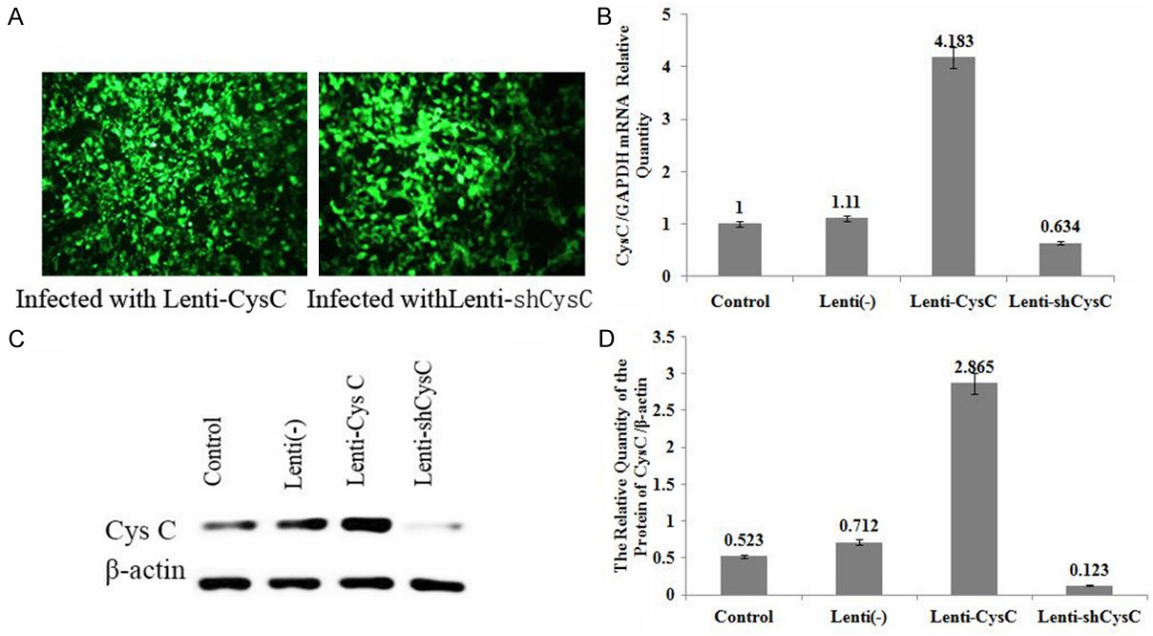

Figure 3. Expression of cystatin C regulated by recombination lentivirus. A: 9706 cells were transfected with Lenti-CysC and Lenti-shCysC; B: The relative quantity of cystatin C mRNA; C: The expression of cysC protein; D: The relative quantity of cystatin C protein.

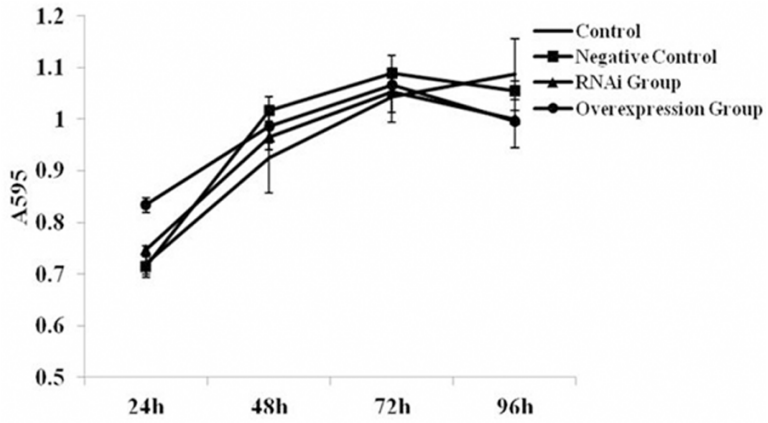

Figure 4. Effect of cystatin C on the proliferation of 9706 cells.

oscillation at slow speed for 10 min until crystal was fully dissolved. The absorbance of each well was measured by enzyme-linked immunosorbent assay at OD490 nm.

EC9706 cell apoptosis assay

EC9706 cells were seeded into 6-well plates with 3×10^5 in each well and transfected with Lenti (-), Lenti-CysC and Lenti-CysC-shRNA (recombinant lentiviruses with MOI=100) for 96 h. Three replicates for each group. The cells were digested with trypsin-free EDTA and washed with PBS for 2 times. 1×10^6/ml cells were collected and resuspended with 500 ul labeled solution according to cell apoptosis kit's instructions (7-AAD/PE Apoptosis Detection Kit; BD Biosciences, New Jersey, USA) (Catalog no. 559763). After 5 ul 7-AAD dye was added with uniform mixing, 1 ul Annexin-PE dye was added, and then the mixture was incubated in the dark at room temperature for 10 min. Excitation wavelength from flow cytometry was detected at 488 nm.

EC9706 cell invasion assay

EC9706 cells were seeded into 6-well plates with 3×10^5 in each well and transfected with Lenti (-), Lenti-CysC and Lenti-CysC-shRNA (recombinant lentiviruses with MOI=100). The plates were added the final concentration of 8 μg/ml Polybrene during transfection. After 12 h, the spent medium was replaced with the fresh. Transwell text was performed after 24 h. Lentivirus-infected cells were plated into 24-well plates with basement membrane matrix of 1:4 diluted Matrigel (BD Biosciences, New

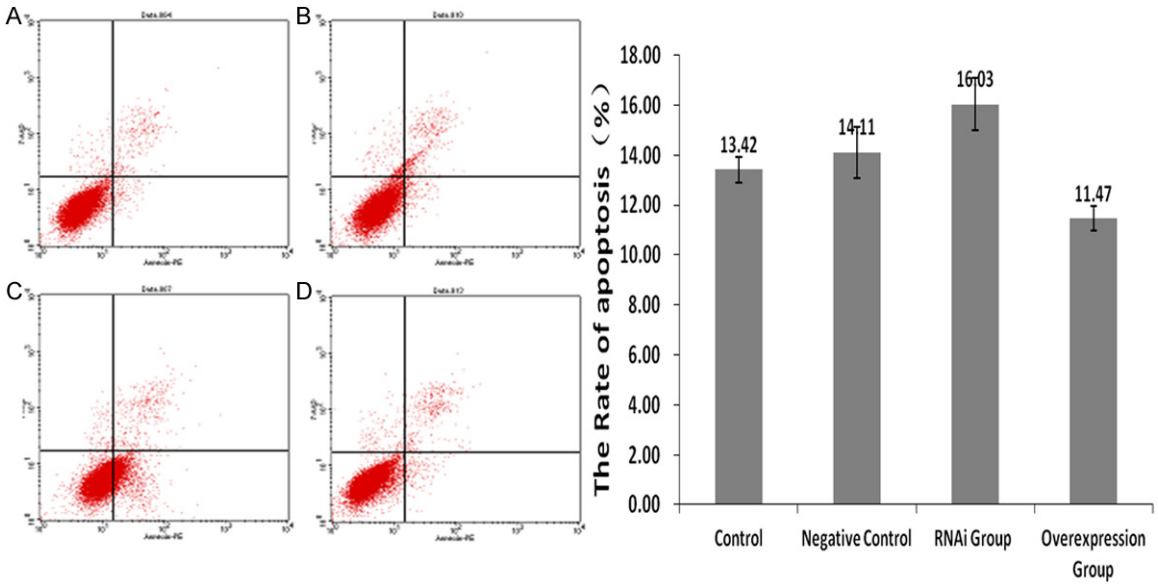

Figure 5. Effect of cystatin C on the apoptosis rate of EC9706 cells. A: Control group; B: Negative control group; C: RNAi group; D: Overexpression group.

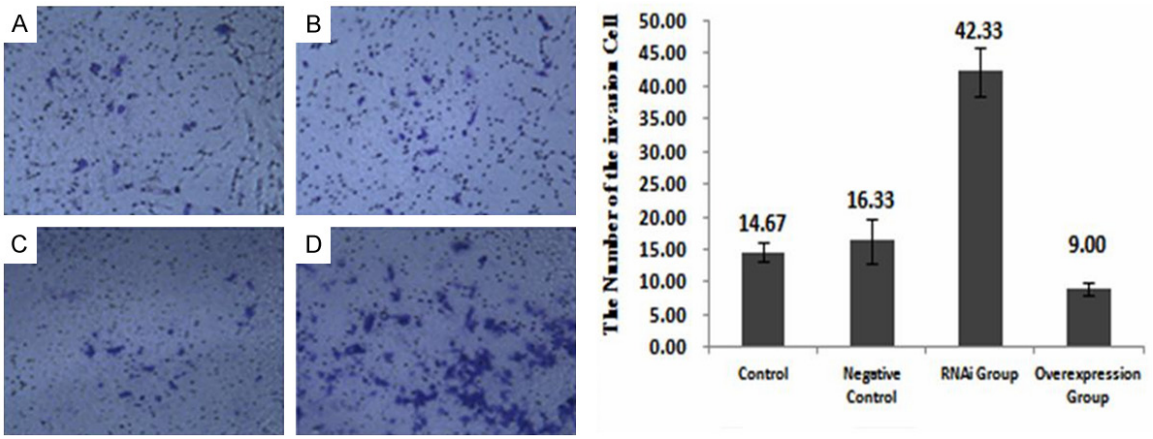

Figure 6. Effect of cystatin C on the invasion of EC9706 cells. A: Control group; B: Negative control group; C: RNAi group; D: Overexpression group.

Jersey, USA) (Catalog no. 356234) with 50 ul in each chamber. Above cells (1×10^5) were plated in the top chamber with the medium of serum-free. The bottom chamber was added with 10% serum. After 24 h incubation, Matrigel was removed, and remaining cells were removed from the top layer by scrubbing with a sterile cotton swab. Invading cells from the bottom surface were stained with 0.1% crystal violet prior to being examined. They were counted and photographed using digital microscopy. Cell numbers were calculated in five random fields for each chamber, and the average value was calculated.

Statistical analysis

All data were performed using SPSS version 14.0 software (SPSS Inc, Chicago, IL, USA). Comparison of averages was analyzed using t-test between the two groups, whereas multiple data was analyzed by the chi-square test. LSD method was applied for pairwise comparison of multiple samples. Comparison of ranked data was analyzed according to nonparametric Mann-Whitney test. The data are shown as the mean ± SD from at least three independent experiments. $P<0.05$ was considered statistically significant.

Results

Comparison of the level of cathepsin B and cystatin C protein in normal and cancer tissues

Immunohistochemical assay was performed to detect the level of cathepsin B and cystatin C protein in esophageal carcinoma tissues and corresponding adjacent normal mucosal epithelium. As shown in **Figure 1**, in all samples, cystatin C and cathepsin B proteins were expressed at different levels, while cathepsin B protein was mainly expressed in the cytoplasm and the adjacent cytomembrane, and the positive rate of protein expression was 60.7% (34/56) and 19.6% (11/56) respectively with significant difference (P=0.000<0.001). Whereas cystatin C protein was mainly expressed in the cytoplasm and took on pale yellow, brown or tan granules while a few also were expressed in the cytomembrane; it also had a weak expression in some adjacent normal epithelium, and the positive rate of protein expression was 94.6% (53/56) and 12.5% (7/56) respectively with significant difference (P=0.000< 0.001; **Figure 1**). Non-parametric Mann-Whitney test was used in this detection.

Comparison of the level of cathepsin B and cystatin C mRNA in normal and cancer tissues

qRT-PCR was performed to detect the level of cathepsin B and cystatin C mRNA in cancer tissues and corresponding adjacent normal epithelium. As shown in **Figure 2**, the relative quantity of cathepsin B and cystatin C mRNA in normal tissues was set to 1 as control, so their expression in cancer tissue were significantly increased (P=0.0002<0.001; **Figure 2**).

Expression of cystatin C in EC9706 cells regulated by recombination lentivirus

As shown in **Figure 3**, the relative quantity of cystatin C mRNA in EC9706 cells transfected with over-expression Lenti-CysC was significantly higher than that in control cells (P<0.01). Whereas the relative quantity of cystatin C mRNA in EC9706 cells transfected with interference Lenti-shCysC was significantly lower than that in control cells (P<0.01). Furthermore, the relative quantity of cystatin C protein in EC9706 cells transfected with over-expression Lenti-CysC was significantly higher than that in control cells (P<0.01). Whereas the relative quantity of cystatin C protein in EC9706 cells transfected with interference Lenti-shCysC was significantly lower than that in control cells (P<0.01; **Figure 3**).

Effect of cystatin C on the proliferation of EC9706 cells

MTT assay was performed to detect the effect of cystatin C on the proliferation of EC9706 cells with transfection. As shown in **Figure 4**, the results showed no statistical differences among over-expression group, RNAi group, control group and negative control group at 24, 48, 72 and 96 h (P>0.05; **Figure 4**), which indicated that the expression of cystatin C had little effect on EC9706 cell proliferation.

Effect of cystatin C on the apoptosis rate of EC9706 cells

Flow cytometry was adopted to detect the effect of cystatin C on EC9706 cell apoptosis in all groups. As shown in **Figure 5**, the apoptosis rate of EC9706 in over-expression group was 11.466 ± 1.361, which was lower than that in control group and negative control group (P=0.019<0.05), while its apoptosis rate in RANi group was 16.03 ± 1.045, which was significantly higher than that in control group and negative control group (P=0.004<0.05; **Figure 5**). This suggested that the expression of cystatin C gene had a reverse relation with the apoptosis rate of EC9706 cells.

Effect of cystatin C on the invasion of EC9706 cells

Transwell test was used to detect the effect of cystyatin C on the invasive ability of EC9706 cells. As shown in **Figure 6**, the invasive ability of over-expression group was 9.00 ± 1.001, which was lower than that in control group and negative control group (P=0.000<0.001), while the invasive ability of RANi group was 42.333 ± 3.786, which was significantly higher than that in control group and negative control group (P=0.000<0.001) (**Figure 6**). This showed that cystatin C expression affected the invasive ability of EC9706 cells.

Discussion

Malignant tumor cells with characteristics of invasiveness and metastasis, can produce a variety of hydrolytic enzymes, such as cysteine protease, which can degrade extracellular ma-

trix, damage mucosal barrier and induce tumor cell invasiveness of the basement membrane to the deeper tissues [7]. Cathepsin B is a kind of cysteine protease and exists in the cytoplasm lysosomes, which participate in the degradation of many kinds of extracellular matrixes, such as multi-type collagen and fibronectin, and involves in the invasion and metastasis of malignant tumor cells. Previous studies have demonstrated a close correlation between cathepsin B levels and the invasive potential of malignant tumours. It has been found that the expression of cathepsin B in tissues of prostate cancer, head and neck cancer, lung cancer, colorectal cancer were increased significantly [8-11]. Cystatin C is a major inhibitor of cathepsin B, which can combine with cysteine proteases to form an enzyme-inhibitor complex, and this complex can prevent cathepsin B from damaging the interstitial tissues and basement membrane, thereby inhibiting the invasion of tumor cells [12, 13]. Many studies have indicated that the expression of cystatin C was increased in some tumor tissues and cells, such as prostate cancer 11, non-small cell lung cancer [14, 15]. On the contrary, Yano reported that cystatin C mRNA expression is lower in breast cancer tissue than that in noncancerous tissue, and the expression of cystatin C is significantly lower relative to cathepsin B in cancerous tissue [7]. Kothapalli showed that cystatin C gene was down regulated in large granular lymphocyte leukaemia [16]. However, the relationship between cystatin C and esophageal carcinoma was poorly understood. In this study, we found that the protein and mRNA levels of cystatin C and cathepsin B protein and mRNA in cancer tissues were significantly higher than that in normal tissues, which indicated that both genes were closely related with the development and progression of esophageal carcinoma. In addition, increased cystatin C expression in cancer tissues could be due to the body's feedback regulation, because increased cathepsin B expression caused an increase in the expression of cystatin C with feedback, and high expression of cystatin C inhibited the activity of cathepsin B, thereby reducing the invasion of cancer cells.

In normal cells and tissues, cathepsin B and its endogenous inhibitor (stefin A, stefin B, cystatin C, etc.) are in equilibrium; once it is broken, the invasive ability will be affected. Cystatin C is a member of cysteine protease inhibitor family, which not only has a strong affinity for cathepsin B, but inhibits cathepsin K, L and H. Cystatin C can combine with cysteine proteases to form an enzyme-inhibitor complex, which can prevent cathepsin B from damaging the interstitial tissues and basement membrane, thereby inhibiting the invasion of tumor cells. Konduri showed that sense-transfected cells were markedly less invasive than control cells after SNB19 glioblastoma cells stably was transfected with cDNA construct of the sense orientation or empty vectors for human cystatin C [17]. Corticchiato found that cysteine proteinase inhibitors could weaken matrix degradation induced by pepsin-activated conditioned medium [18]. Coulibaly showed that SCC-VII cells transfected with human cystatin C encoded by cDNA could result in a remarkable reduction in Matrigel invasion [19]. In our study, over-expression Lenti-CysC vector and interference Lenti-shCysC vector were constructed successfully in vitro on the basis of histological level, and then were transfected into EC9706 cells. The results showed that the level of cystatin C mRNA and protein were higher and lower respectively than that in control cells, which indicated that the construction of both vectors was a success. Next MTT assay, flow cytometry and Transwell test were adopted to detect the effect of over-expression and low-expression cystatin C on the proliferation, apoptosis and invasion of esophageal carcinoma cells and the results showed that over-expression cystatin C reduced cell invasion significantly whereas low-expression cystatin C increased cell invasion markedly, which was consistent with previous reports on other carcinomas.

Furthermore, our results also showed that over-expression Lenti-CysC and interference Lenti-shCysC had little effect on EC9706 cell proliferation and had a reverse relation with EC9706 cell apoptosis rate. Nishikawa showed that cystatin C could inhibit the activity of cathepsin B, which could significantly inhibit the invasion of cancer cells, but did not affect the growth rate of cancer cells [5]. In the study on malignant melanoma, Cox constructed the eukaryotic-expression vector for cystatin C, which was transfected into melanoma cell line B16 [20]. The results showed that the metastatic ability of tumor cells was significantly inhibited but the growth rate was not affected compared with

control cells. Pietras showed that increased cathepsin B secretion had a parallel relationship with the proliferation of urinary bladder epithelial cells and endometrial cells [21]. Tummalapalli showed that tumor growth was slowed down by RNAi-mediated abrogation of expression of cathepsin B and MMP-9 genes in human malignant meningioma [22]. All these studies demonstrated that cathepsin B could facilitate cell proliferation, which could contribute much to tumor formation and development. However, related researches showed that cathepsins B was regarded as effector protease in the TNF cascade of cell death [23, 24], and the protease can lead to cell death by stimulating mitochondrial permeabilization mitochondrial permeabilization [25]. Nagaraj showed that cathepsin B played an important role in cell apoptosis induced by TRAIL (TNF-related apoptosis-induced ligand) and it could induce cell apoptosis [26]. Therefore, cathepsins B could promote not only tumor cell proliferation but also the apoptosis of tumor cells. As an inhibitor of cathepsin B, over-expression cystatin C could inhibit cell proliferation as well as apoptosis, therefore, it may have no significantly affect the growth rate. Sokol showed that cystatin C could be identified as a novel antagonist of tumor growth factor-b (TGF-b) receptor which is considered to possess both tumor-promoting and tumor-suppressing function, which could provide an explanation for the dual effect of cystatin C [27]. Sun indicated that mouse 3T3 fibroblasts encoded with cystatin C gene had an enhanced rate of cell proliferation [28]. Therefore, it still needed further study on the effect of cystatin C on the proliferation of cancer cells and its mechanism in the future.

Overexpression of cathepsin B and cystatin C in esophageal carcinoma tissues can be used as one of diagnostic indicators for esophageal carcinoma. Cystatin C overexpression can inhibit the invasion of cancer cells, and the specific inhibition mechanism involved need to be explored in the future, which could provide new clues for the diagnosis and molecular targeted therapy of esophageal carcinoma.

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Disclosure of conflict of interest

None.

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