Knockdown of serpin peptidase inhibitor clade C member 1 inhibits the growth of nasopharyngeal carcinoma cells

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Abstract. Nasopharyngeal carcinoma (NPC) is a type of cancer originating in the nasopharynx. There are no NPC-specific treatments available at present. Serpin peptidase inhibitor clade C member 1 (SERPINC1) serves roles in anticoagulation and anti-inflammation. The aim of the present study was to investigate the role of SERPINC1 in the proliferation and apoptosis of NPC cells. Tumor and adjacent healthy tissue samples were collected from patients with NPC. Additionally, the SERPINC1 gene was silenced in the HNE3 cell line using short interfering RNA targeted against SERPINC1 (SERPINC1-siRNA). Cell viability was determined via a Cell Counting Kit-8 assay; furthermore, proliferation and apoptosis were investigated via flow cytometry. Western blotting and reverse transcription-quantitative polymerase chain reaction analysis were performed to determine the expression levels of protein and mRNA. It was revealed that the expression levels of SERPINC1 mRNA and protein were increased in NPC tumor tissues compared with in adjacent healthy tissues. The expression of SERPINC1 mRNA and protein in HNE3 cells decreased following SERPINC1-siRNA transfection. Furthermore, knockdown of SERPINC1 promoted apoptosis and inhibited proliferation. It was also demonstrated that silencing SERPINC1 upregulated the expression of B-cell lymphoma-2 (Bcl-2)-associated X protein and p53 mRNA and protein, and downregulated that of Bcl-2, survivin and cyclin D1. Downregulation of SERPINC1 reduced the phosphorylation of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt) and mammalian target of rapamycin (mTOR). Thus, SERPINC1 knockdown may promote the apoptosis of HNE3 cells and inhibit proliferation via the suppression of the PI3K/Akt/mTOR signaling pathway.

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignancy of the head and neck that exhibits a unique geographical distribution (1,2). NPC usually develops in the nasopharyngeal mucosa; the majority of NPCs are squamous cell carcinomas (3). NPC is more common in males than in females (4); however, the incidence of nasopharyngeal cancer has increased in previous decades (5).

Chemotherapy, radiotherapy and surgery are three main methods for treating NPC (6). As NPC is more sensitive to chemotherapy, a combined therapy, including induction, concurrent and adjuvant chemotherapy is commonly used to treat late-stage NPC (7-9). Clinical studies reported that combined chemotherapy demonstrated beneficial short-term effects on NPC (10,11); however, the incidences of acute recurrence and toxicity following chemotherapy are high (12,13). A novel anticancer approach, targeted therapy, has effectively treated lung cancer, melanoma, leukemia and other tumors (14-17). Therefore, it is important to investigate the mechanisms underlying the pathogenesis of NPC to aid the identification of potential novel targets in the treatment of NPC.

The gene encoding serpin peptidase inhibitor clade C member 1 (SERPINC1), also known as antithrombin III (ATIII), is located on chromosome 1q23-25.1 (18). ATIII regulates coagulation via the inhibition of coagulation factors, and exhibits anti-inflammatory effects on epithelial cells (19-21). Studies have previously reported that the occurrence and development of certain cancers was also closely associated with SERPINC1; Zietek *et al* (22,23) reported increased levels of ATIII in the serum of patients with kidney cancer and the tumor tissues of patients with bladder cancer. Other studies observed that SERPINC1-encoded proteins exhibited inhibitory effects on angiogenesis and suppressed proliferation (24,25). To the best of our knowledge, the role of SERPINC1 in the occurrence and development of NPC has not been investigated.

RNA interference is commonly used to study the role of genes by targeting the mRNA, and it allows the selective silencing of one or several genes (26). The present study investigated the role of SERPINC1 in the proliferation of NPC cells via determining the expression of the SERPINC1 gene in tissues from patients with NPC and silencing the gene in NPC cells. The results may provide novel insight and targets for the treatment of NPC.

Materials and methods

NPC samples. From September 2016 to September 2017, 41 NPC tumor and adjacent healthy tissues were collected from 19 males and 22 females (aged 49-80 years, with a mean of 58.53±6.36 years) at the Department of Pathology, Tongde Hospital of Zhejiang Province (Hangzhou, China). The samples were stored at -80˚C prior to subsequent experimentation. Patients included in the present study exhibited NPC that was histologically confirmed by biopsy, and no history of previous head and neck cancer. Patients with incomplete clinical data were excluded. In addition, NPC patients were staged according to the criteria of the 8th edition of The Union for International Cancer Control/American Joint Committee on Cancer staging system (27). Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed to determine the expression levels of SERPINC1 mRNA in each tissue sample. The patients were divided into low- and high-expression groups according to the median value of SERPINC1 mRNA expression. A total of 6 cases were randomly selected from the 41 patients the analysis of SERPINC1 protein via western blotting. All patients provided signed informed consent, and the experiment was approved by the Ethics Committee of Tongde Hospital of Zhejiang Province.

Cell culture. The poorly differentiated squamous cell carcinoma NPC cell line HNE3 was purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin at 37˚C in an incubator with 5% CO_2. All culture reagents were purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Transfection of short interfering RNA (siRNA). In total, $2x10^5$ cells/well were seeded in 24-well culture plates, and 0.5 ml medium without antibiotic was added in each well. Cells at 90% confluence were transfected. siRNA targeted against SERPINC1 (SERPINC1-siRNA) was purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). siRNA (50 nM) transfection was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) to generate the SERPINC1-siRNA group. Negative-siRNA (Thermo Fisher Scientific, Inc.) was inserted into an empty vector (pcDNA3.1; Thermo Fisher Scientific, Inc.), which served as the empty vector control group; a non-transfected control group was also established. The sequences of the siRNAs used in the present study were as follows: SERPINC1-siRNA forward, AUC ACAUUGGAAUACAUGGCC and reverse, CCAUGUAUU CCAAUGUGAUAG; negative-siRNA forward, CAUGUG GUCUGUCGCAUAAUA and reverse, CGGUACACCAGA CAGCGUAUU. Following transfection for 48 h, cells were harvested, and the efficiency of transfection was determined via RT-qPCR and western blot analysis.

Cell viability. A Cell Counting Kit-8 (CCK-8) assay was used to determine cell viability. Transfected cells at a density of $3x10^3$ cells/well were inoculated in a 96-well plate and incubated at 37˚C with 5% CO_2 for 12, 24 and 48 h following transfection. CCK-8 reagent (10 ml; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was then added to each well and cultured together at 37˚C with 5% CO_2 for 4 h. The absorbance of each well at 450 nm was measured using a microplate reader (ELx800; BioTek Instruments Inc., Winooski, VT, USA), and cell viability was calculated according to the standard curve.

Flow cytometry for cell apoptosis and proliferation. Flow cytometry was used to evaluate cell apoptosis. Cells ($1x10^6$) were washed with PBS at 4˚C and resuspended to a concentration of $4x10^5$ cells/ml. Phycoerythrin-Annexin V Apoptosis Detection kit (5 μl; BD Pharmingen; BD Biosciences, San Jose, CA, USA) was added to cell culture (200 μl), and then 10 μl 7-aminoactinomycin D (20 μg/ml; BD Biosciences) was added. The samples were incubated at room temperature in the dark for 10 min. The BD FACSCanto flow cytometer (BD Biosciences) was used to analyze apoptosis at 488 nm, analysis of data was performed using the FSC Express software (version 3; De Novo Software, Glendale, CA, USA). Living cells are presented in the lower left quadrant, necrotic cells in the upper left quadrant, advanced apoptotic cells in the upper right quadrant, and early apoptotic cells in the lower right quadrant. The proliferation of HNE3 cells was also analyzed via flow cytometry. Click-iT® Plus EdU Pacific Blue flow cytometry kit (Thermo Fisher Scientific, Inc.) was applied to detected the cell proliferation.

RT-qPCR. RT-qPCR was performed to investigate the expression levels of SERPINC1, B-cell lymphoma-2 (Bcl-2)-associated X protein (Bax), Bcl-2, survivin, cyclin D1 and p53 mRNA; GADPH was used as a reference gene. RNA was extracted from NPC tissues and cells using TRIzol® (Thermo Fisher Scientific, Inc.) at 0˚C for 5 min, isolated with $CHCl_3$ (Aladdin Shanghai Biochemical Technology Co., Ltd., Shanghai, China) and then dissolved in diethyl pyrocarbonate-treated water (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). RNA concentration was determined using a NanoDrop One Microvolume UV-Vis Spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc., Wilmington, DE, USA). RT was performed on RNA samples using the PrimeScript first Strand cDNA synthesis kit (Takara Bio, Inc., Otsu, Japan) to synthesize cDNA. The RT reaction was performed at 37˚C for 15 min, followed by reverse transcriptase inactivation at 85˚C for 15 sec. RT-qPCR reactions were performed using an ABI 7500 real-time PCR system (Thermo Fisher Scientific, Inc.), using the SYBR Prellix Ex Taq™ Real-Time PCR Kit (Takara Bio, Inc.). qPCR was performed by activating the DNA polymerase at 95˚C for 5 min, followed by 40 cycles of two-step PCR (at 95˚C for 10 sec and at 60˚C for 30 sec) and a final extension at 75˚C for 10 min prior to holding at 4˚C. DNase- and RNase-free water were used as negative control templates. All primers used were purchased from Genewiz, Inc. (Suzhou, China) and are presented in Table I. The $2^{-\Delta\Delta Cq}$ method (28) was applied to analyze relative expression levels of target genes normalized to GADPH. Each experiment was performed in triplicate.

Western blotting. The expression of SERPINC1, apoptosis-, cell cycle-proteins, and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway-associated proteins were determined via

Table I. Sequences of the primers used for reverse transcription-quantitative polymerase chain reaction.

Primer name	Sequence (5'-3')	Product size (bp)
SERPINC1, forward	GCCTGAAGGTAGCAGCTTGT	
SERPINC1, reverse	CCCACACTCCCTCACTCTTC	313
Bax, forward	TCCACCAAGAAGCTGAGCGAG	
Bax, reverse	TTCTTTGAGTTCGGTGGGGTC	345
Bcl-2, forward	CTGGTGGACAACATCGC	
Bcl-2, reverse	GGAGAAATCAAACAGAGGC	317
Survivin, forward	CCCTTTCTCAAGGACCACCGCATC	
Survivin, reverse	GCCAAGTCTGGCTCGTTCTCAGT	133
Cyclin D1, forward	CTGGCCATGAACTACCTGGA	
Cyclin D1, reverse	GTCACACTTGATCACTCTGG	245
p53, forward	CTGAGGTCGGCTCCGACTATACCACTATCC	
p53, reverse	CTGATTCAGCTCTCGGAACATCTCGAAGCG	360
GAPDH, forward	CCATCTTCCAGGAGCGAGAT	
GAPDH, reverse	TGCTGATGATCTTGAGGCTG	222

Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; SERPINC1, serpin peptidase inhibitor clade C member 1.

western blot analysis; phosphorylation levels were also determined. Tissues and cells were lysed using liquid nitrogen and radioimmunoprecipitation assay buffer (Abmole Bioscience, Inc., Houston, TX, USA), and subsequently subjected to cleavage and lysis using 1% phenylmethane sulfonyl fluoride protease and phosphatase inhibitors (Abmole Bioscience, Inc.), for 30 min at 4˚C. The supernatant was collected by centrifugation at 12,000 x g at 4˚C for 15 min. Total protein concentration was determined using the bicinchoninic acid method. In total, 10 μg protein was loaded in each lane. Proteins were separated by 10% SDS-PAGE. The separated proteins were transferred onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using a Trans-Blot Transfer Slot (Bio-Rad Laboratories, Inc.) and blocked with 5% skimmed milk for 2 h at room temperature. The membranes were incubated with the following primary antibodies obtained from Abcam (Cambridge, UK): Anti-SERPINC1 (1:800; ab126598); anti-Bax (1:700; ab32503); anti-Bcl-2 (1:900; ab32124); anti-survivin (1:700; ab76424); anti-cyclin D1 (1:800; ab134175); anti-p53 (1:600; ab26); anti-PI3K (1:700; ab125633); anti-phosphorylated (p)-PI3K (1:600; ab138364); anti-Akt (1:800; ab8805); anti-p-Akt (1:800; ab38449); anti-mTOR (1:800; ab2732); anti-p-mTOR (1:600; ab109268) and anti-GADPH (1:800; ab8245). Following the application of primary antibodies, membranes were agitated at room temperature for 2 h, and then incubated at 4˚C for 12 h. Subsequently, membranes were incubated at room temperature for 1.5 h with the following secondary antibodies: Fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1:8,000; ab6785; Abcam); horseradish peroxidase (HRP)-conjugated mouse anti-rabbit IgG (1:9,000; ab99697; Abcam); mouse anti-rabbit IgG (1:7,000; BA1034; Invitrogen; Thermo Fisher Scientific, Inc.); NL557-conjugated donkey anti-rabbit IgG (1:5,000; NL004; R&D Systems, Inc., Minneapolis, MN, USA); HRP-conjugated rabbit anti-human IgG (1:10,000, ab6759; Abcam). Protein bands were visualized using an enhanced chemiluminescence reagent (Thermo Fisher Scientific, Inc.). The optical density was quantified using ImageJ software (version 1.46; National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. Experimental data were presented as the mean ± standard deviation. Data were analyzed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). All experiments were performed three times. Differences between two groups were analyzed using paired Student's t-tests. One-way analyses of variance were performed to analyze differences between experimental groups, with Tukey's multiple comparison test used as a post hoc test. Associations between clinicopathological data and SERPINC1 expression were analyzed using χ^2 tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

SERPINC1 is upregulated in NPC tissue. RT-qPCR and western blotting were performed to analyze the levels of SERPINC1 expression in tumor and adjacent healthy tissues from patients with NPC. It was revealed that SERPINC1 protein and mRNA expression levels were significantly increased in NPC tissues compared with in adjacent tissues ($P<0.05$; Fig. 1A-C). As presented in Table II, patients were separated into low- and high-expression groups according to the median value of SERPINC1 mRNA expression, and associations with clinicopathological features were investigated. It was demonstrated that increased SERPINC1 expression was significantly associated with the metastasis of NPC. The results suggested that the SERPINC1 gene was overexpressed in NPC tissues.

Transfection with SERPINC1-siRNA reduces SERPINC1 expression and the proliferation of HNE3 cells. The human poorly-differentiated squamous cell carcinoma NPC cell

Table II. Associations between clinicopathological data of patients with nasopharyngeal carcinoma and SERPINC1 expression.

		SERPINC1 expression		
Clinicopathological factor	N	Low	High	P-value
Gender				0.756
Female	23	11	12	
Male	18	10	8	
Age				0.756
<50	18	10	8	
>50	23	11	12	
TNM stage				0.536
I-II	19	11	8	
III-IV	22	10	12	
Metastasis				0.015[a]
Yes	11	2	9	
No	30	19	11	

[a]P<0.05. SERPINC1, serpin peptidase inhibitor clade C member 1; TNM, tumor, node and metastasis.

line HNE3 was used to study the role of SERPINC1 in NPC; si-SERPINC1 was transfected into HNE3 cells. The efficiency of SERPINC1-siRNA transfection was determined via RT-qPCR and western blot analysis. It was demonstrated that the expression levels of SERPINC1 mRNA and protein were significantly decreased in HNE3 cells following SERPINC1 knockdown compared with the negative siRNA-transfected (empty vector) control group (P<0.01; Fig. 2A-C). The proliferation of HNE3 cells was determined via a CCK-8 assay; cell proliferation was significantly reduced at 12, 24 and 48 h following transfection with SERPINC1-siRNA compared with the empty vector control (P<0.05, P<0.05 and P<0.01; Fig. 2D). The results indicated that transfection of the SERPINC1-siRNA were successfully conducted, and that downregulation of SERPINC1 expression induced a decrease in the proliferation of NPC cells.

SERPINC1 gene silencing inhibits cell proliferation and promotes apoptosis. Flow cytometry was performed to investigate the apoptosis and proliferation of HNE3 cells following SERPINC1-siRNA transfection. No significant differences in apoptosis or proliferation were reported between the empty vector and non-transfected control groups. Conversely, the apoptotic rate of the SERPINC1-siRNA group was significantly increased compared with the empty vector group (P<0.01; Fig. 3A and B), whereas proliferation was reduced following SERPINC1 knockdown compared with the empty vector group (P<0.01; Fig. 3C and D). The results indicated that SERPINC1 gene silencing inhibited the proliferation and promoted the apoptosis of NPC cells.

Downregulation of SERPINC1 gene affects cell cycle- and apoptosis-associated proteins. To further investigate the effects of SERPINC1 knockdown on the proliferation and apoptosis of HNE3 cells, RT-qPCR and western blot analyses were conducted to determine the expression of apoptosis-associated (Bax, Bcl-2 and survivin) and cell cycle-associated (cyclin D1 and p53) genes and proteins, respectively. It was revealed that the expression levels of Bax mRNA were significantly upregulated in SERPINC1-siRNA-transfected cells compared with the empty vector group, whereas those of Bcl-2 and survivin mRNA were downregulated (P<0.01; Fig. 4A-C). Additionally, the expression levels of cyclin D1 mRNA were significantly decreased and those of p53 increased following silencing of the SERPINC1 gene compared with the empty vector group (P<0.01; Fig. 4D-G). Similar alterations in the expression levels of the genes were also reported at the protein level (P<0.01; Fig. 4F and G), indicating that the suppression of SERPINC1 expression promoted the expression of proapoptotic factors, inhibited the expression of antiapoptotic factors and suppressed proliferation via regulation of cell cycle-associated genes in NPC cells.

Knockdown of SERPINC1 inhibits the phosphorylation of proteins involved in the PI3K/Akt/mTOR signaling pathway. The mechanisms underlying the effects of SERPINC1 on the proliferation and apoptosis of NPC cells were investigated by evaluating the expression and phosphorylation levels of PI3K/Akt/mTOR-associated proteins. It was revealed that the phosphorylation levels of PI3K, Akt and mTOR were significantly decreased in HNE3 cells following transfection with SERPINC1-siRNA compared with the empty vector control group (P<0.01; Fig. 5A-D), suggesting that SERPINC1 knockdown inhibited the activity of the PI3K/Akt/mTOR pathway.

Discussion

NPC is a malignant tumor prevalent in Southeast Asia that occurs at the top of the nasopharyngeal cavity, and seriously affects the survival and quality of life of patients (29). In addition, >50% of patients with NPC are diagnosed at an advanced stage as the initial clinical symptoms of NPC and the site of lesions are difficult to identify (30). NPC is sensitive to chemotherapy and radiotherapy, but surgery is an effect method for treating NPC (31); however, treatment outcomes are usually poor due to late diagnosis. It was reported that the recurrence rate of NPC was 10-15% and the 5-year survival rate was ~60% (30,31). NPC exhibits high sensitivity to radiotherapy; however, patients commonly experience strong adverse reactions and side effects following treatment. Radiation brain injury was reported in 28.5% of patients treated with radiotherapy, whereas 38.1% of patients exhibited severe hearing loss, 40.6% patients possessed nasal pharyngeal mucosal injuries and >50% patients succumbed to mortality following severe radiation damage (32-34). Therefore, identifying a safer and more effective treatment of NPC is of great importance.

The SERPINC1 gene encodes ATIII, a serine protease inhibitor involved in coagulation cascades (18). The main roles of ATIII have been associated with the regulation of coagulation and hemostasis, and the induction of anti-inflammatory processes (35). A previous study reported that the activity of ATIII in the serum of patients with renal and bladder cancer was significantly increased compared with in healthy

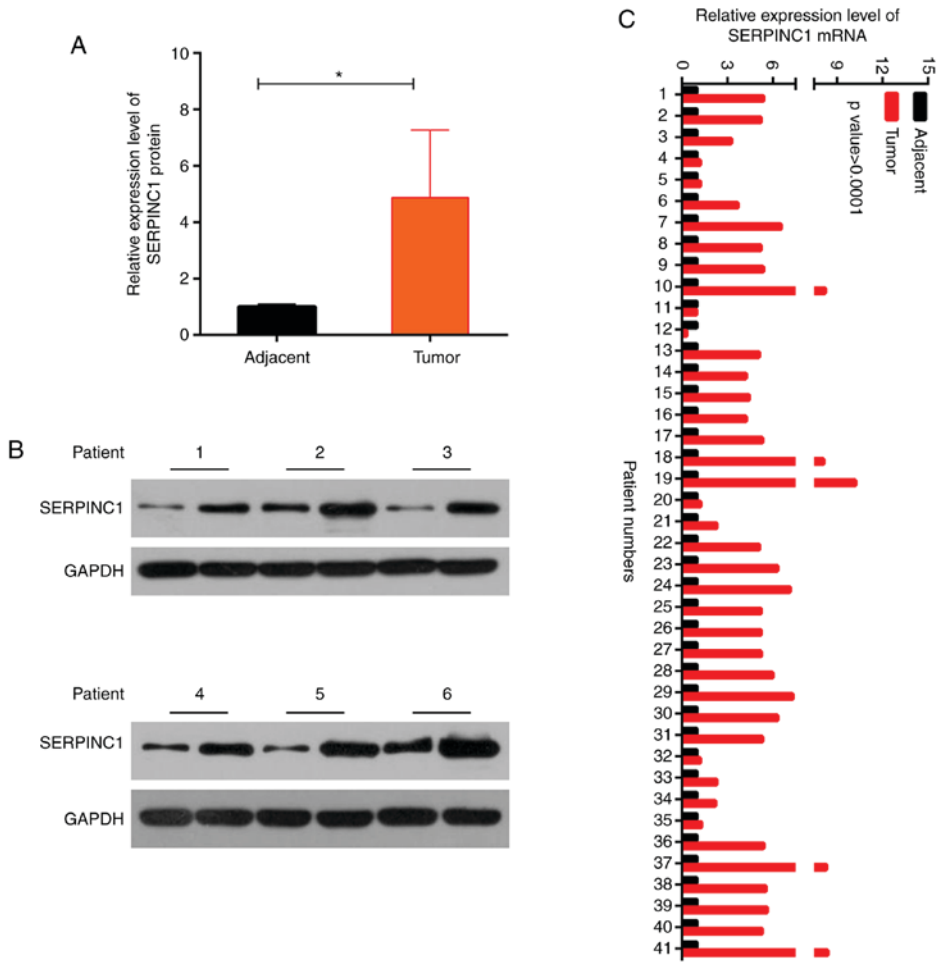

Figure 1. Expression of SERPINC1 in NPC tissues. (A and B) Expression levels of SERPINC1 protein were determined in six paired tumor and healthy adjacent tissue samples collected from patients with NPC by performing western blot analysis. (C) Expression of SERPINC1 mRNA was analyzed in 41 patients by reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean ± standard deviation. *P<0.05. SERPINC1, serpin peptidase inhibitor clade C member 1.

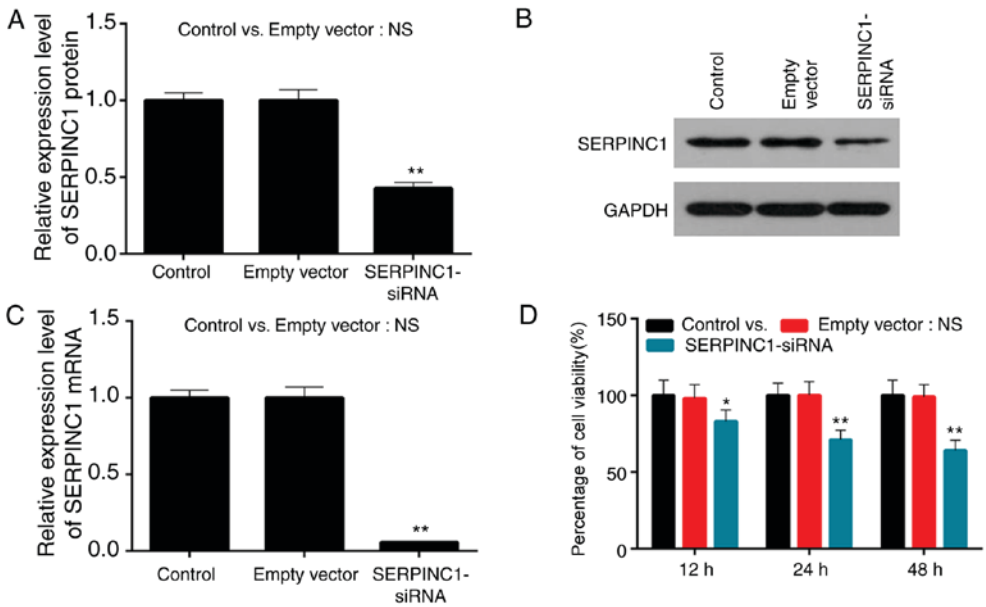

Figure 2. Effects of SERPINC1-siRNA transfection on the expression of SERPINC1 and the viability of HNE3 cells. HNE3 cells were treated with PBS, transfected with empty vector or SERPINC1-siRNA. (A and B) Western blot analysis was performed to determine the expression levels of SERPINC1 protein in each group. (C) Reverse transcription-quantitative polymerase chain reaction was performed to determine the mRNA expression of SERPINC1 in each group. (D) Cell viability was analyzed using a Cell Counting Kit-8 assay. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.01 vs. the empty vector group. Empty vector, negative control siRNA; NS, not significant; SERPINC1, serpin peptidase inhibitor clade C member 1; siRNA, small interfering RNA.

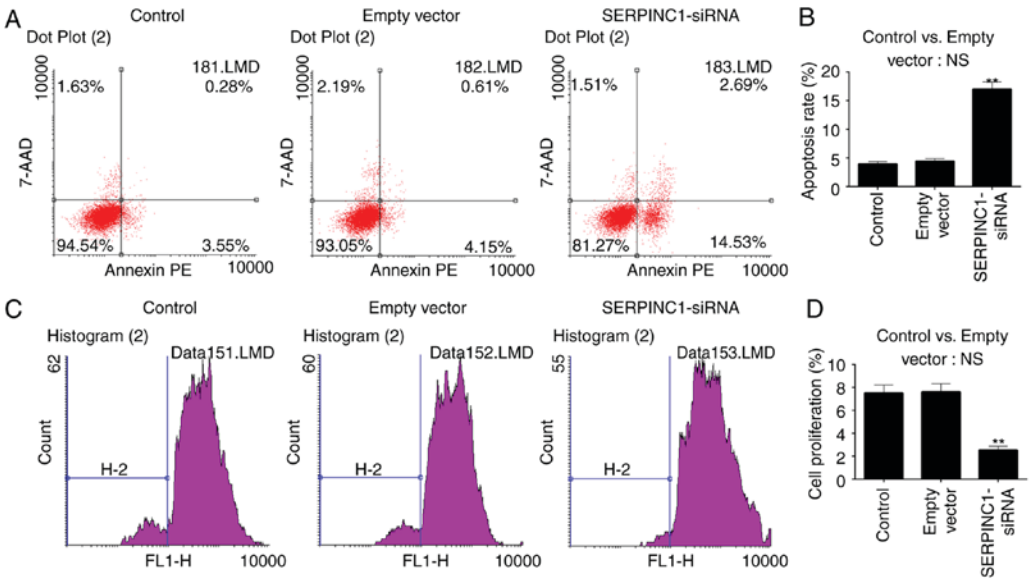

Figure 3. Effects of SERPINC1-siRNA transfection on apoptosis and proliferation of HNE3 cells. (A and B) Flow cytometry was performed to evaluate apoptosis in non-transfected, empty vector-transfected and SERPINC1-siRNA-transfected cells. (C and D) Flow cytometry was performed to analyze the proliferation of each group following transfection. Data are presented as the mean ± standard deviation. **P<0.01 vs. the empty vector group. 7-AAD, 7-amino-actinomycin D; empty vector, negative control siRNA; NS, not significant; PE, phycoerythrin; SERPINC1, serpin peptidase inhibitor clade C member 1; siRNA, small interfering RNA.

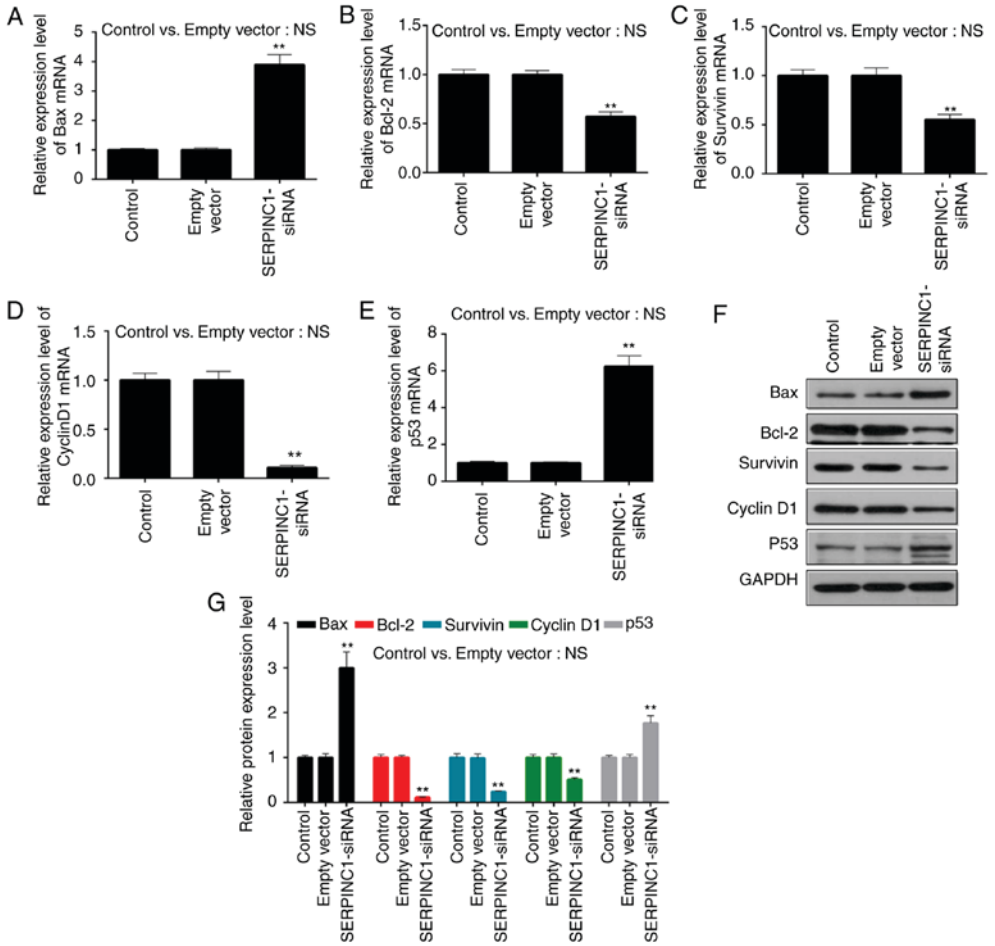

Figure 4. Effects of SERPINC1-siRNA transfection on the expression of apoptosis and cell cycle-associated genes in HNE3 cells. Reverse transcription-quantitative polymerase chain reaction was performed to determine the expression levels of (A) Bax, (B) Bcl-2, (C) survivin, (D) cyclin D1 and (E) p53 mRNA in non-transfected, empty vector-transfected and SERPINC1-siRNA-transfected cells. (F and G) Expression levels of Bax, Bcl-2, survivin, cyclin D1 and p53 proteins were determined via western blot analysis. Data are presented as the mean ± standard deviation. **P<0.01 vs. the empty vector group. Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; empty vector, negative control siRNA; NS, not significant; SERPINC1, serpin peptidase inhibitor clade C member 1; siRNA, small interfering RNA.

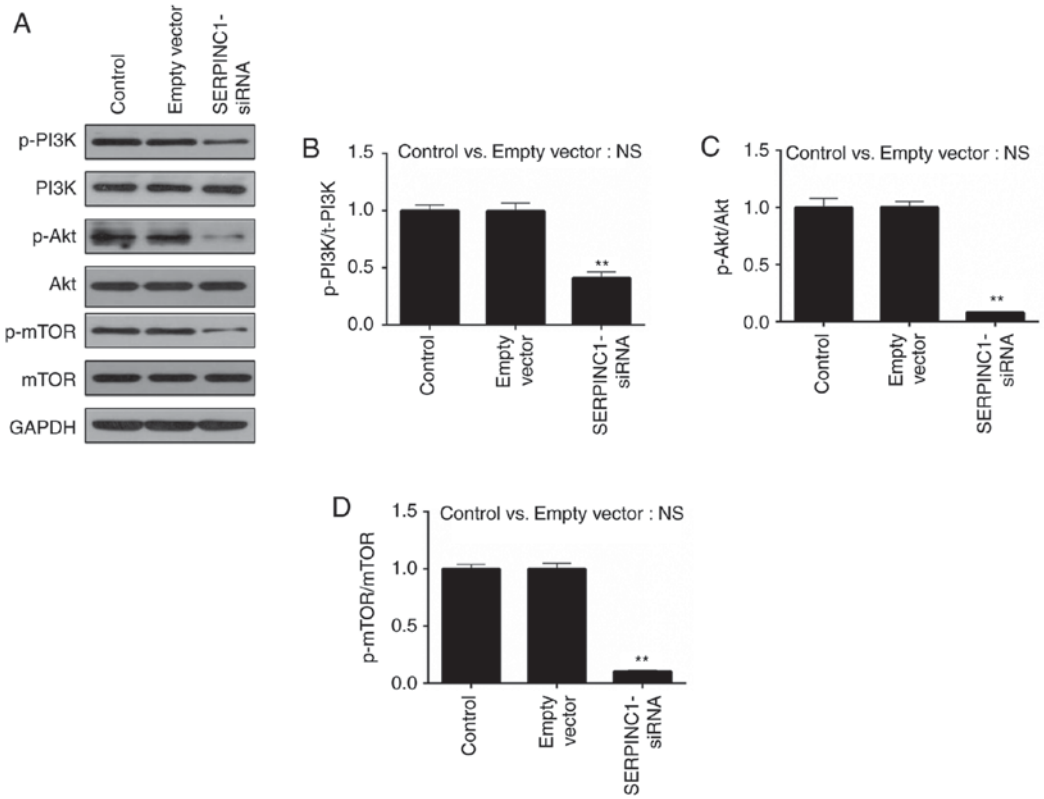

Figure 5. Effects of SERPINC1-siRNA transfection on PI3K/Akt/mTOR pathway. (A) Western blot analysis was performed to determine the expression and phosphorylation of PI3K, Akt and mTOR proteins. (B-D) p-PI3K/PI3K, p-Akt/Akt and p-mTOR/mTOR ratios were calculated for non-transfected, empty vector-transfected and SERPINC1-siRNA-transfected cells. Data are presented as the mean ± standard deviation. **P<0.01 vs. the empty vector group. Akt, protein kinase B; empty vector, negative control siRNA; mTOR, mammalian target of rapamycin; NS, not significant; p, phosphorylated; PI3K, phosphatidylinositol 3-kinase; SERPINC1, serpin peptidase inhibitor clade C member 1; siRNA, small interfering RNA.

individuals (23). ATIII promotes the inhibition of proteases via interactions with heparin-like substances on the surface of endothelial cells (36). As NPC is a tumor that derives from a malignant lesion of epithelial cells (37), the expression of SERPINC1 in NPC was investigated. The results of the present study revealed that SERPINC1 was significantly upregulated in NPC tissues and the metastasis of NPC was highly associated with elevated expression of SERPINC1.

The present study proposed that knockdown of SERPINC1 may protect against the progression of NPC. The data revealed that the proliferation of the NPC cell line HNE3 was suppressed by SERPINC1-siRNA. To further investigate the effects of SERPINC1 on NPC, flow cytometry analysis was performed on cells following transfection with SERPINC1-siRNA. The results demonstrated that silencing SERPINC1 promoted the apoptosis and inhibited the proliferation of HNE3 cells. Therefore, the expression of apoptosis- and cell cycle-associated genes following SERPINC1-siRNA transfection was investigated. Cyclin D1 binds to and activates cyclin-dependent kinase 4 during G1 to regulate G1/S cell cycle transition, thereby promoting cell proliferation (38). Conversely, the tumor suppressor gene p53 inhibits cell proliferation (39). Bax directly regulates the activity of proapoptotic target proteins to induce apoptosis (40), whereas Bcl-2 exhibits antiapoptotic effects; overexpression of Bcl-2 is common in NPC (41). Survivin is expressed only in tumors and embryonic tissues, and is associated with cellular immortality (42). It was observed that the downregulation of SERPINC1 increased the expression of Bax and p53, but decreased that of Bcl-2, survivin and cyclin D1. Collectively, SERPINC1 silencing promoted the apoptosis and inhibited the proliferation of NPC cells by regulating the expression of apoptosis-associated proteins and cyclin D1, suggesting that SERPINC1 may contribute to the pathogenesis of NPC.

The PI3K/Akt/mTOR signaling pathway contributes to the pathogenesis of numerous tumors (43,44). Similar to SERPINC1, the PI3K/Akt pathway serves roles in coagulation and anti-inflammatory processes (45,46). Therefore, the activity of the PI3K/Akt/mTOR signaling pathway was investigated following silencing of SERPINC1 in the present study. The results revealed that the expression levels of PI3K, Akt and mTOR proteins were markedly unaltered by transfection with SERPINC1-siRNA; however, the levels of phosphorylation of each protein were significantly downregulated. It was recently reported that the PI3K-dependent activation of Akt induced the phosphorylation of the Ser136/Ser112 residues of Bcl-2-associated death promoter (Bad), promoting apoptosis via the separation of Bad/Bcl-2 heterodimers (47). Furthermore, activation of the PI3K-Akt pathway promotes the phosphorylation of the Ser184 residue of Bax, inhibiting the antiapoptotic effects of the protein (48). Additionally, PI3K/Akt/mTOR promotes cell proliferation via regulation of cell cycle-associated proteins (49-52). Collectively, it was proposed that downregulation of SERPINC1 in NPC cells may suppress proliferation and induce apoptosis by inhibiting the activation of the PI3K/Akt/mTOR signaling pathway;

however, the association between this signaling pathway, and the proliferation and apoptosis of cells require further investigation. Furthermore, due to the crosstalk that occurs between signaling pathways, the possibility that other pathways may also be involved in the effects of SERPINC1 downregulation cannot be excluded. Additionally, the findings of the present study were obtained from a single cell line *in vitro*; thus, performing similar experiments in additional NPC cell lines or animals models may provide insight into the roles of SERPINC1 in the pathogenesis of NPC. Finally, the use of specific inhibitors of the PI3K/Akt/mTOR signaling pathway is required to further demonstrate the role of this pathway in the effects of SERPINC1 on NPC cells.

In conclusion, SERPINC1 was upregulated in tumor tissues from patients with NPC. Knockdown of SERPINC1 suppressed the proliferation and promoted the apoptosis of HNE3 cells by regulating the expression levels of cell cycle- and apoptosis-associated proteins. The activation of the PI3K/Akt/mTOR signaling pathway was suppressed by silencing of the SERPINC1 gene. Collectively, these findings suggested that SERPINC1 may be a potential target for the treatment of patients with NPC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JX and YYi conceived and designed the present study. GX, LL, QW, YYa acquired, analyzed and interpreted data. YYi, JX, YYa drafted the article or critically revised it for important intellectual content. All authors read and approved the final manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

All patients provided signed informed consent. The present study was approved by The Ethics Committee of Tongde Hospital of Zhejiang Province (Hangzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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