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Inhibition of constructed SEC3-ES lentiviral vector to proliferation, migration of Hela cells

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ABSTRACT

Aim: To construct a lentiviral vector with endostatin (ES) and staphylococcal enterotoxin C_3(SEC3) gene, and investigate its capacities of inhibition on proliferation and migration of Hela cells.
Methods: By inserting ES and SEC3 gene into the plasmid and then transfect 293 T cell, the co-expressed (SEC3-ES) vector were constructed. A series of experiments in vitro were carried out to detect its anti-tumor capacity.
Results: SEC3 expression of the vector is about 3 times of GV365-SEC3 vector, and ES expression is over 22.5-fold compared with GV365-ES vector. Moreover, OD490 value of CO group (1.212 ± 0.003) was notably lower than NC (negative control) group (1.124 ± 0.01) ($P < 0.05$) in MTT assay. Cell cycle analysis showed it could block Hela cells in S phase. Meanwhile, in wound healing assay, cells of CO group migrated at a slower rate (0.59 ± 0.02) compared with NC group (0.65 ± 0.02)($P < 0.01$).
Conclusion: The successful construction of co-expressed vector lays the foundation for further studies in vivo. These promising results suggest a new strategy to treating cervical cancer.

1. Introduction

There are an estimated 14.1 million new cancer cases and 8.2 million cancer deaths occurred in 2012 [1], based on GLOBALCAN. Cancer is a leading cause of death in the world, responsible for about 14.6% of all human deaths [2]. Gene therapy as a modern therapy to cancer, except for the conventional treatments including chemotherapy, radiotherapy and surgery, has been around for about a decade. Currently 64.4% of all gene therapy trials has been aimed at treatment of cancer [3]. Because of its potentiality to treat a disease at its genetic roots, gene therapy has become a good alternative and held a great promise for the treatment of cancer, as evidenced by a significant number of clinical trials [4–6].

One of the major challenges in gene therapy is the gene delivery system. Gene transferring systems are divided into two groups: non-viral methods and viral methods. Viral gene delivery vehicles remain the most popular so far, having been used in nearly two-thirds of trials performed to date [3]. Different viruses are used as gene delivery vehicle: retroviruses, adenoviruses, lentiviruses and so on. Currently, lentiviral vectors are derived from multiple species. Because of the most extensive and thorough studies of HIV-1 and the most understanding of its biological characteristics, lentiviral vector derived from HIV-1 has become representative [7]. In order to avoid the generation of replication-competent virus (RCV),the HIV-1 genome typically is separated into several plasmid vectors, including transfer plasmid, helper plasmid and envelop expression plasmid. Thus, the vector has only once infectious ability with no replicative capacity [8]. Compared with other viral vectors, lentivirus can also efficiently affect non-dividing cells, including nerve cells, endothelial cells and suspension cells [9,10].

In cancer gene therapy, there are several major strategies, including enhancement of the immune response against tumor cells [11], anti-angiogenic gene therapy [12], suicide gene therapy [13] and RNA interference [14]. Antiangiogenic therapy for cancer has been suggested as a potent therapeutic method, because angiogenesis have a critical role in the occurrence, development, invasion and metabolism of tumor [15]. Endostatin, a 20 kDa C-terminal fragment derived from a1 chain of type XVIII collagen and firstly isolated from cultured hemangio endothelioma (EOMA) cells [16], is a promising substance for treating tumors. However, endostatin has a short half-life in vivo, complicated protein-purification process and low resultant yield rate [17,18]. To circumvent this obstacle, delivery of the vector encoding endostatin has been attempted [19]. Preclinical studies indicated that the effect of

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endostatin was minimizing the size of tumor, instead of removing tumor [20]. Thus, we constructed the co-expressed lentiviral vector expressing two gene products, ES and SEC3, in order to enhance the anti-tumor capacity. SEC3 is a kind of Staphylococcus enterotoxins (SEs),known as the strongest and most potent cytokine inducer of human lymphocyte [21]. Meanwhile, SEs have the capacity of improving immune dysfunction induced by tumor [22] to enhance the ability of body to kill cancer cells. Therefore, it is of high possibility that SEC3 also have a powerful anti-tumor capacity

The aim of this study was to construct co-expressed vector carrying ES gene and SEC3 gene, and to detect the capacity of its inhibition effect on the proliferation and migration of Hela cells, which were transduced with the co-expressed vector, by a series of assays in vitro.

2. Materials and methods

2.1. Cell and culture

Human embryonic kidney (HEK) 293 T and Hela cells were obtained from Jikai gene company and grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 10% FBS (Shanghai weike biotechnology, China), 100 μg/ml streptomycin and 100 U/ml penicillin. The cells were plated in 25 cm^2 flasks and cultured in DMEM at 37℃ in a humidified atmosphere of 95% air/5% CO2 with medium exchanges every 2 days. Cells were harvested from the medium after 2 weeks of incubation.

2.2. Lentiviral vectors construction

The target gene SEC3 was derived from PUC57-SEC3 with forward primer - gene(15950-1)-P1 and reverse primer - gene(15950-1)-P2 (Table 1) by polymerase chain reaction(PCR). Then the vector GV365 was linearized with AgeI / AgeI. To generate the inducible vector GV365-SEC3, target gene fragment containing the SEC3 gene, inducible promoter and enhancer were ligated into the AgeI / AgeI site of GV365 by homologous recombination with competent cell. Target gene SEC3 1 u L, linearized plasmid vector 2.5 u L, ddH_2O 3.5 u L, 5 × CE II Buffer 2 u L and Exnase TMII 1 u L were mixed on ice. After reaction at 37℃ for 30 min, the production was immediately cooled on ice for 5 min and transfected. 10 u L of production was added to 100 u L competent cells and reacted at following condition: 30 min on ice, followed by 90 s at 42℃, and a final incubation on ice for 2 min. The production was further shaking cultured in 500 u L of culture-medium for 1 h at 37℃. Appropriate fluid volume was applied evenly to plate containing antibiotics and invert-cultured in the incubator for 12–16 h. Similarly, according to PUC57-ES, the GV365-ES was constructed with forward primer - gene(16093-1)-P1 and reverse primer - gene(16093-1)-P2 (Table 1).

The co-expression plasmid was constructed by cloning the HpaI/EcoRI fragment containing ES gene, inducible promoter and enhancer into the HpaI/EcoRI site of GV365-SEC3 vector. The target ES gene was derived from PUC57-ES with forward primer - gene(16139-1)-P1 and reverse primer - gene(16139-1)-P2 (Table 1).Specific steps were as above.

The inducible combined expression lentiviral vector was generated by transient co-transfection of HEK 293 T cells with a three-plasmid combination. Briefly, 1 mL transfection agent (Jikai Gene, China), mixed with 10ug envelope plasmid pHelper 1.0(Jikai Gene, China), 15ug packaging plasmid pHelper 2.0 (Jikai Gene, China) and 20ug of the co-expression lentiviral vector plasmid, were incubated at room temperature (RT) for 15 min. HEK 293 T cells were added to the mixture. 48 h post-transfection, lentivirus containing supernatant was harvested and used for next experiments.

2.3. Western blot

HEK 293 T cells (4×10^5 per dish) were incubated on 24-well plate at 37℃ in a humidified atmosphere of 95% air/5% CO_2 to cover 80%. Then medium was exchanged to opti-MEM (Invitrogen, US). 2 u L of Lipofectamine (Invitrogen, US) was added to 1 ug of plasmid DNA diluted in opti-MEM and incubated for 20 min before added to cells. Cells were incubated for 5 h at 37℃ and the medium was replaced with DMEM supplemented with 10% FBS. After incubated for 24 h, the fluorescence from the GFP was examined under the fluorescent microscope (Olympus, Japan). After transduction of 36 h, the cells were harvested to perform Western blot. Extracts of HEK 293 T cells were prepared in cooled Lysis Buffer. Equal amounts of total protein, as assayed by BCA assay (HyClone-Pierce, US), were separated in 12.5% SDS-polyacrylamide gel and subsequently transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking in TBST solution with 5% defatted milk, the PVDF membrane was incubated with the primary antibodies. After several washing steps, the membrane was incubated with the secondary antibodies. The results were visualized by chemiluminescence (ECL-PLUS/Kit, Amersham, US).

2.4. Titration of lentiviral vector

Vector particles were harvested from the supernatant at 48 h post-transfection by centrifuge(4000 g,10 min). Subsequently, the harvested supernatant was filtered through 0.45-micron filter and centrifuged for 2 h with 25,000 rpm. The supernatant was discarded, and resuspended with the virus preservation solution. At last, virus concentrate can be obtained by being centrifuged for 5 min with 10,000 rpm.

The concentrated purified virus solution was diluted to 10^5-10^7 times. The standards were diluted to five dilution,125 pg/mL,31.3 pg/mL,15.6 pg/mL,7.8 pg/mL,0 pg/mL. 200 u L of samples were respectively added to 24 well plate. The plate was sealed with the sealing plate membrane at 37℃ for 1.5 h and washed for four times with wash buffer. 100 u L HIV-1 P24 Detector Antibody (Dakewe, China) were then added to each hole except the control hole following the sealing process as above. After washing away HIV-1 P24 Detector Antibody (Dakewe, China), 100 u L substrate were added to each hole and

Table 1
Sequences of primer.

Primer	Sequence
gene(15950-1)-P1	GAGGATCCCCGGGTACCGGTCGCCACCATGGAGAGTCAACCAGACCCTATGCCAGATG
gene(15950-1)-P2	TCCTTGTAGTCCATACCTCCATTCTTTGTTGTAAGGTG
gene(16093-1)-P1	GAGGATCCCCGGGTACCGGTCGCCACCATGCACAGCCACCGCGACTTC
gene(16093-1)-P2	TCCTTGTAGTCCATACCCTTGGAGGCAGTCATGAAGCTG
gene(16139-1)-P1	ACCGTCAGATCCGTTAACGCTACCGGACGCCACCATGCACAGCCACCGCGACTTC
gene(16139-1)-P2	ATAAGCTTGATATCGAATTCCTACTTGGAGGCAGTCATGAAG
Ubi-F	GGGTCAATATGTAATTTTCAGTG
FLAG-R-2	CCTTATAGTCCTTATCATCGTC
gene(16139-1)-P3	CCGTGCCCATCGTCAACCTC
pGC-E1-SEQR	AGCGTAAAAGGAGCAACATAG

incubated at room temperature for 30 min without sealing and light. 100 u L Stop solution (Dakewe, China) were finally added to each hole to stop the reaction and measured at A450 by Microplate reader within 15 min.

2.5. Cell transduction

A total of 3–5 × 10^3 Hela cells/well were prepared in a 96-wells plate so that they were growing exponentially and no more than 30–50% confluent before tranduction. On the following day, the cells in each well were transduced with packaged co-expressed lentivirus at an MOI of 5 in DMEM medium containing 10% FBS with 5 ug/ml polybrene and Eni.S (Jikai gene, China). After 16 h, transduction media was replaced with fresh DMEM with 10% FBS and incubated for 72 h at 37℃ and 5% CO2.

2.6. RT qPCR

After transduction of Hela cells, total RNA was extracted with Trizol (pufei company, shanghai), according to the manufacturer's instruction. Reverse transcription was performed with M-MLV Reverse Transcription reagent Kit (promega, US) following the instructions. The yielded cDNA was used for PCR reaction, including 1 u L of cDNA dilution and 8 u L of RNase-Free H_2O in a total of 20 u L solution containing 10 u L of SYBR premix ex taq and 2.5 umol/L of sense and antisense primers. The GAPDH gene was used for normalization.

2.7. MTT assay

Hela cells transduced with lentiviral vectors to be tested were harvested by trypsinization, resuspended in complete medium and plated in the wells of 96-well microtiter plates. 6 replicate wells were used for each group. About 2000 cells were plated in each well and cultured for 5 days. At the end of incubation, 20 u L of a 5 mg/mL solution of MTT (Genview, US) was added. After 4 h of incubation with MTT, the supernatant was carefully removed, and 100 u L DMSO was added to each well. Plates were placed in microplate shaker for 5 min, and the absorbance at 490/570 nm was read by an automatic microplate reader (Tecan infinite, Switzerland).

2.8. Cell cycle analysis by flow cytometry

Cell-cycle progression was determined by PI (propidium iodide) staining using a flow cytometer (Millipore, USA). Transduced Hela cells were incubated at 37℃ until 80% confluence. After trypsinization, the cells were harvested and washed with ice-cold PBS and fixed with 75% cold alcohol for 1 h. After centrifugation, cells were resuspended in PI/RNase/D-Hanks solution and incubated in dark for 30 min at room temperature and analyzed by fluorescence activated cell sorting on flow cytometer. Each experiment was performed in triplicate and repeated three times.

2.9. Wound healing assay

3 × 10^4 cells after transduction, were incubated at 37℃ until cells reach 90% confluence to form a monolayer. A 96 Wounding Replicator (VP scientific, US) was used to create a scratch of the cell monolayer. The plate was washed twice and incubated with the serum-free medium at 37℃ and 5% CO2. The wounds were observed and photographed at 0 h, 8 h and 24 h. Each experiment was repeated at least 3 times.

3. Results

3.1. Successful construction and stable expression of co-expressed vector

Firstly, the plasmid was linearized. Purified plasmid GV365 vector were mixed with ddH_2O 42 u L, 10 × CutSmart Buffer 25 u L and AgeI 1 u L, then incubated at 37℃ for 3 h. The identification of linear vector was determined by Southern blot analyses. The molecular size of plasmid is 10.7 kb. Essentially all the plasmids were linearized, while the nonlinearized plasmids, including spire, open-loop and lineared, had different migration rate and showed different bands.

The SEC3 target gene, fused with the linear plasmid, was obtained as described in Methods. Identification of acquired target gene was also determined by gel electrophoresis (Fig. 1A). Virtually, the acquired gene was SEC3 target gene as its molecular size is 765bp (Fig. 1A, lanes 2).

After the construction of GV365-SEC3 plasmid, colonies were picked out on the plate to carry out PCR with forward primer - Ubi-F-P2 (Table 1) and reverse primer - FLAG-R-2 (Table 1). The molecular size of empty vector is 185bp, so the negative control (empty vector) showed the band as the picture below (Fig. 1B, lanes 3). Furthermore,

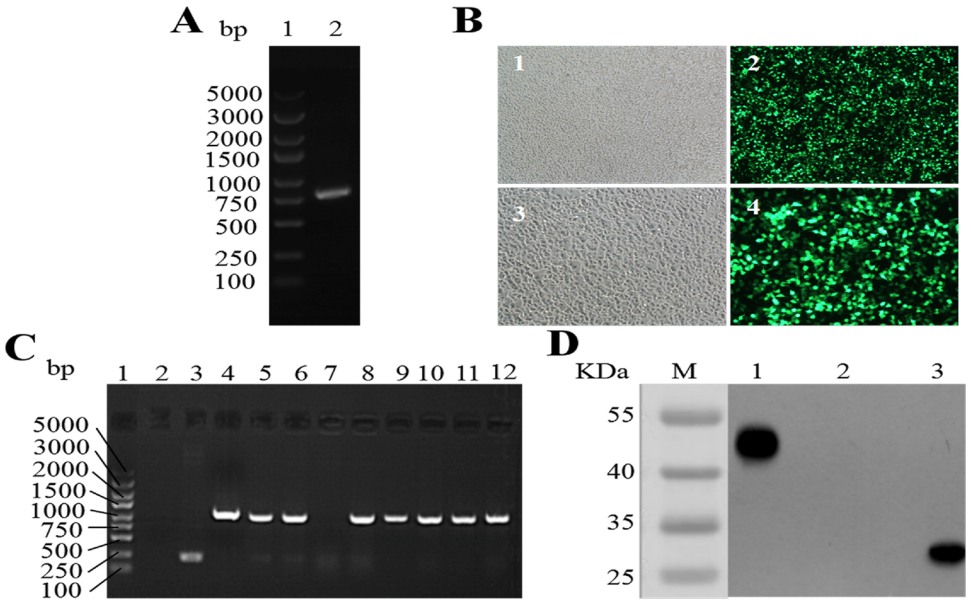

Fig. 1. (A)Southern blot analysis of the target gene.The target gene was 765bp fragment(lanes 2).(B)Southern blot analysis of the positive clone of GV365-SEC3 plasmid.The negtive control of ddH2O(lanes 2),the negtive control of empty vector(lanes 3), the positive control of GAPDH (lanes 4) and the samples(lanes 5–12).(C)Green fluorescence picture (2 and 4) and white light picture (1 and 3) of transfected 293 T cell by GV365-ES plasmid.The magnification of 1 and 2 is 100 ×,The magnification of 3 and 4 is 200 × . (D)Electrophoretic profile of SEC3 purified protein.The positive control (lanes 1) of WB standard material,SURVIVIN-3FLAG-GFP,which is 48KD,the negtive control (lanes 2) of 293 T cells which were not transduced with plasmids,and 293 T cells which were transduced with GV365-SEC3 plasmids (lane 3) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

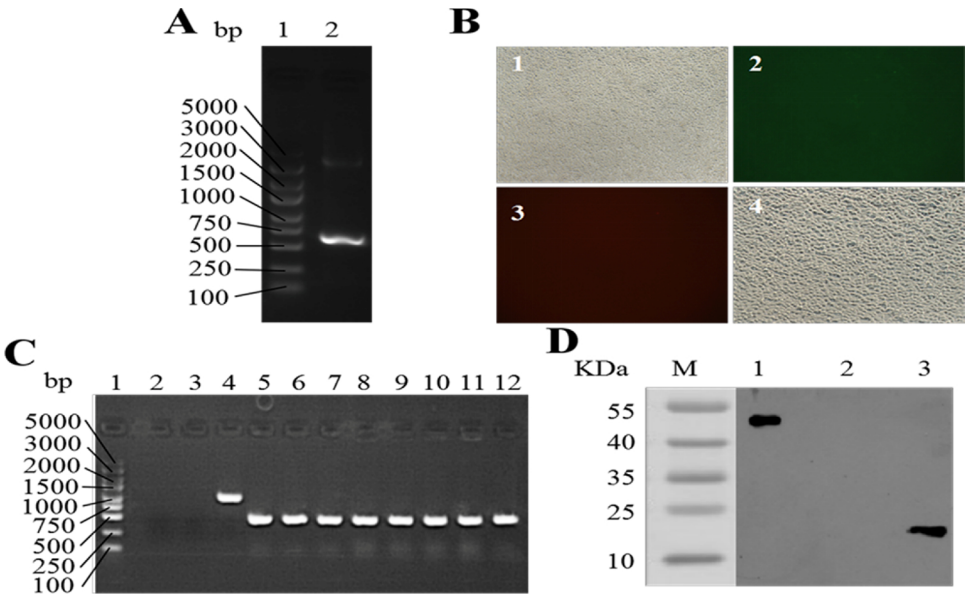

Fig. 2. (A)Southern blot analysis of the target gene.The target gene was 609 bp fragment (lanes 2).(B)Southern blot analysis of the positive clone of co-expressed plasmid.The negtive control of ddH2O(lanes 2),the negtive control of empty vector(lanes 3), the positive control of GAPDH(lanes 4) and the samples (lanes 5–12).(C)Green fluorescence picture (2),Green fluorescence picture (3)and white light picture (1 and 4) of transfected 293 T cell by co-expressed plasmid.The magnification of 1,2 and 3 is 100×,The magnification of 4 is 200 × .(D)Electrophoretic profile of ES purified protein.The positive control (lanes 1) of WB standard material,SURVIVIN-3FLAG-GFP,which is 48KD,the negtive control (lanes 2) of 293 T cells which were not transduced with plasmids,and 293 T cells which were transduced with co-expressed plasmids (lane 3) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the molecular size of SEC3 is 765bp, thus showed the band as the picture below (Fig. 1B, lanes 4). In addition, the migrate band of sample 1–8 was lanes 5–12 (Fig. 1B). As is shown, the plasmid of sample 3 was not constructed successfully (Fig. 1B, lanes 7). According to the result of sequencing and blasting, the plasmids of samples were constructed successfully.

To explore whether transduction of GV365-SEC3 plasmid was successful, HEK 293 T cells were subjected to fluorescent microscope. GFP displayed (Fig. 1C) a distribution at location of the cells, thus, we verified GFP expression after transient transfection of transfer plasmids in 293 T cells. Furthermore, Western blot analysis (Fig. 1D) confirmed the stable expression of transgene, the molecular weight of which is 29KD. Owing to transduced gene containing the flag gene, which is 3KD, thus, the molecular weight of protein separated by Western blot was more than the SEC3 gene.

In order to attain the co-expressed vector, the ES target gene should be segregated from PUC57-ES, described in Methods. Identification of acquired target gene was determined by Southern blot analyses as well (Fig. 2A). The molecular size of target gene is 609 bp, so, the acquired gene was ES target gene (Fig. 2A, lanes 2).

More importantly, the ES gene was insert into the location of GFP removed by endonuclease HpaI/EcoRI. After the construction of co-expressed plasmid, we picked out colonies on the plate to carry out PCR with forward primer - gene (16139-1)-P3 (Table 1) and reverse primer - pGC-E1-SEQR (Table 1).The molecular size of GAPDH is 960bp, so, it showed the band as the picture below (Fig 2B, lanes 4).In addition, the migrate band of sample 1–8 was lanes 5–12(Fig 2B). As shown above, all the plasmid of samples was constructed successfully. According to the results of sequencing and blasting, the plasmids of samples were constructed successfully.

Because the GFP gene was replaced by ES gene, we cannot see any fluorescent light (Fig. 2C). Western blot analysis (Fig. 2D) showed the molecular weight of the protein is 23KD, which is consistent with the sum of that of ES and flag gene. Therefore, it suggested the co-expressed vector was transduced successfully and expressed stably.

After Hela cells were transduced with co-expressed vector, initially, we performed analysis of the expression in Hela cells at the level of mRNA. As shown (Fig. 3A), the proliferation rate of Hela cells with SEC3-ES vector was significantly lower than NC group, and apparently, the morphology of cells has changed significantly. Real-time quantitative PCR was carried out (Fig. 3B) to further detect the expression of two transgenes in Hela cells. Total RNA was extracted from Hela cells transduced with GV365-SEC3 vector, GV365-ES vector, co-expressed vector and empty vector, which were amplified respectively by classical RT-PCR, using the same primers as for real-time quantitative PCR. At the mRNA level, co-expressed vector expression was the highest among four vectors. Moreover, the SEC3 expression of co-expressed vector is about 3 times more than GV365-SEC3 vector, and the ES expression of co-expressed vector is increased more than 22.5-fold when compared with GV365-ES vector. As the picture reveals (Fig. 3B), mRNA level of two transgenes of the co-expressed vector is significantly higher than others.

3.2. Detection of the titer of the lentivirus vectors

Titration of the lentivirus was determined by ELISA. The standard curve $Y = 0.01105X + 0.01165$ was obtained. According to the standard curve equation, the resultant virus titer was calculated to be 1.64×10^8TU/mL by ELISA kit conversion relationship between concentration and titer, which were about 5TU / mL = 1 pg / mL.

3.3. Transfection of Hela cells with co-expressed vector inhibited cell proliferation by infecting cell cycle progression

After transduction, the proliferation and shape of cells were impacted significantly. Thus, MTT assay (Fig. 4A) was performed to detect cell viability. As shown, the OD490 value of CO (co-expression vector) group (1.212 ± 0.003) was notably lower than NC group (1.124 ± 0.01). Moreover, the mortality of CO group was significantly higher than the NC group.

To find out the underlying mechanism of inhibition of cell proliferation, we analyzed phases of cell cycle of each group by flow cytometry with PI staining. As is shown (Fig. 4B and C),$49.13 \pm 1.03\%$ cells of CO group were at S phase, which was higher than NC group ($40.92 \pm 0.63\%$) with statistical significance ($P < 0.001$). Similarly, less cells of CO group were at G2/M phase. This means that the cell cycle was impaired by co-expressed vector. Moreover, this result demonstrated that co-expressed vector inhibited Hela cell growth possibly by inducing cell-cycle to arrest in the S phase.

3.4. Transfection of Hela cells with co-expressed vector inhibited the migration and invasion ability of Hela cells

Fig. 5 showed that wound healing assay was used to track migration of Hela cells. Interestingly, after being cultured for 8 h, the difference between two groups was not notable. However, after 24 h, cells of CO

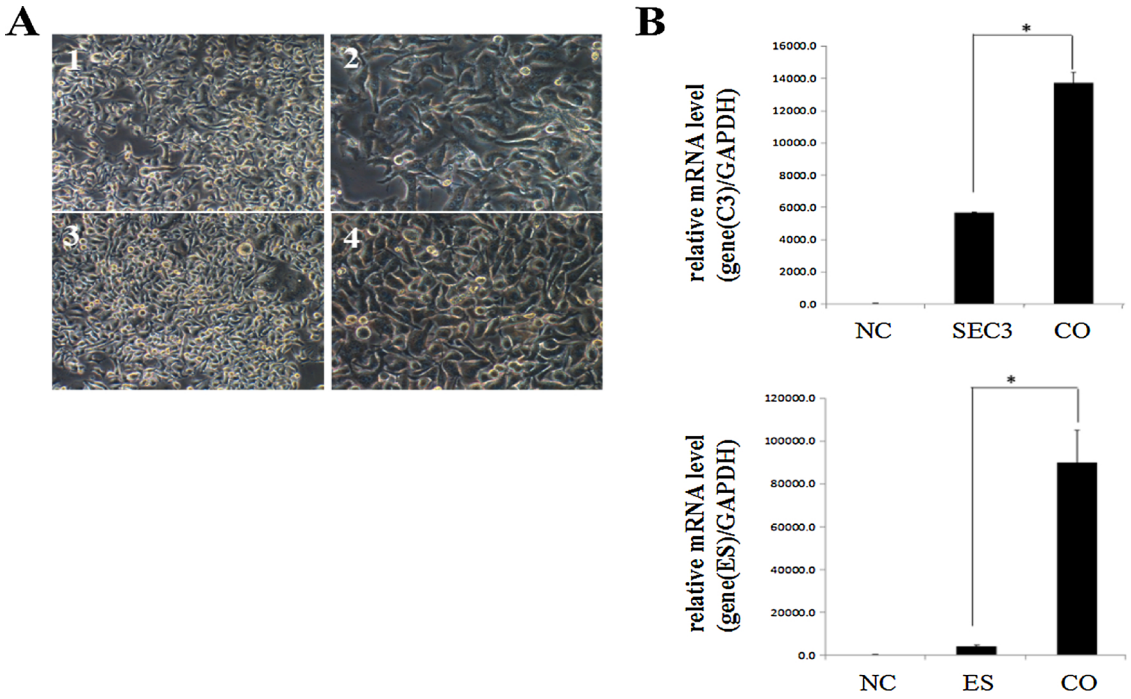

Fig. 3. (A)Representative pictures of Hela cells following transduction.Test group,Hela cells were cultured with lentiviral vector (1,magnification,100×;2,magnification,400×),NC group,Hela cells were cultured with empty vector (3,magnification,10×;4,magnification,400×),and CON group,Hela cells were cultured with nothing(5,magnification,100×;6,magnification,400×).(B).mRNA levels of the ES and SEC3 gene of each group. *:P < 0.05.

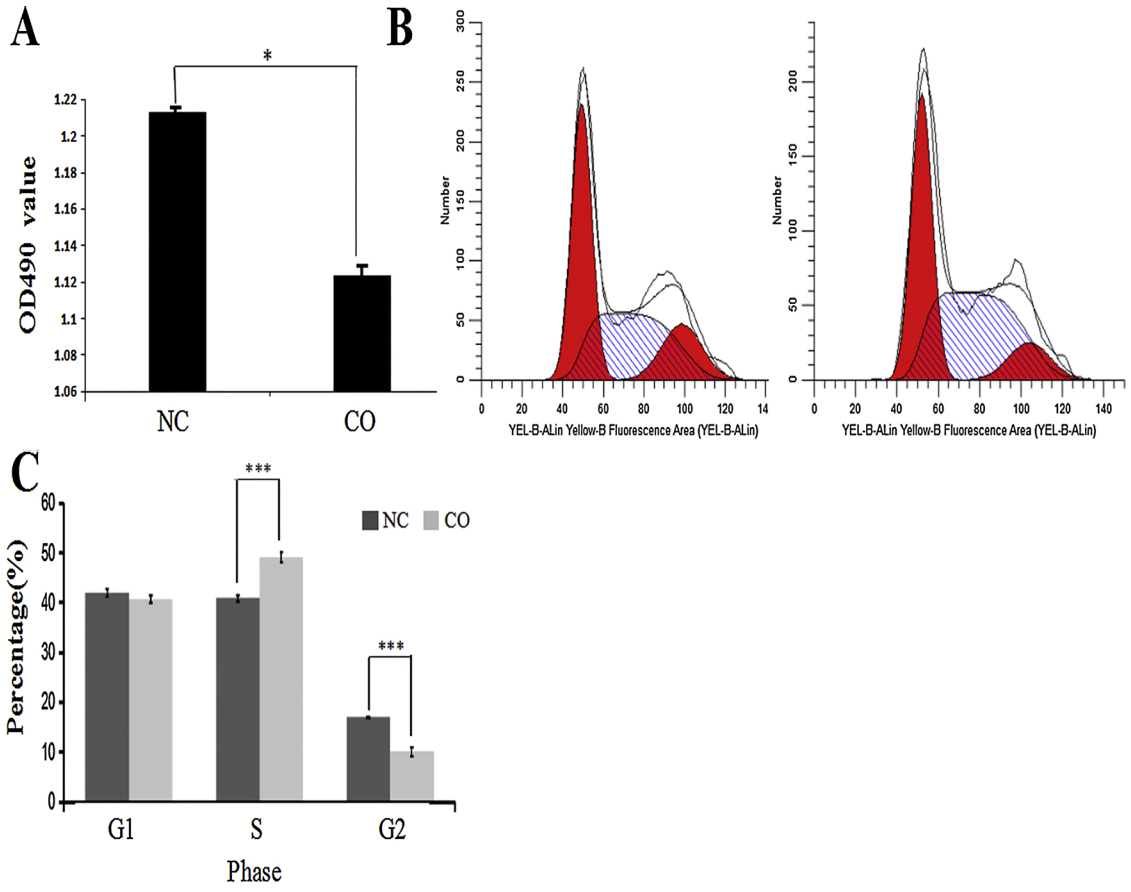

Fig. 4. (A)The result of MTT assay,the difference of OD490 values between two groups is of statistic significance. (B)Representative graphs of flow cytometry analysis of Hela cell cycle using PI staining. (C)Statistic analysis of percentages of Hela cells at different cell cycle stages (G1, S and G2 phase).*:P < 0.05; ***, p < 0.001.

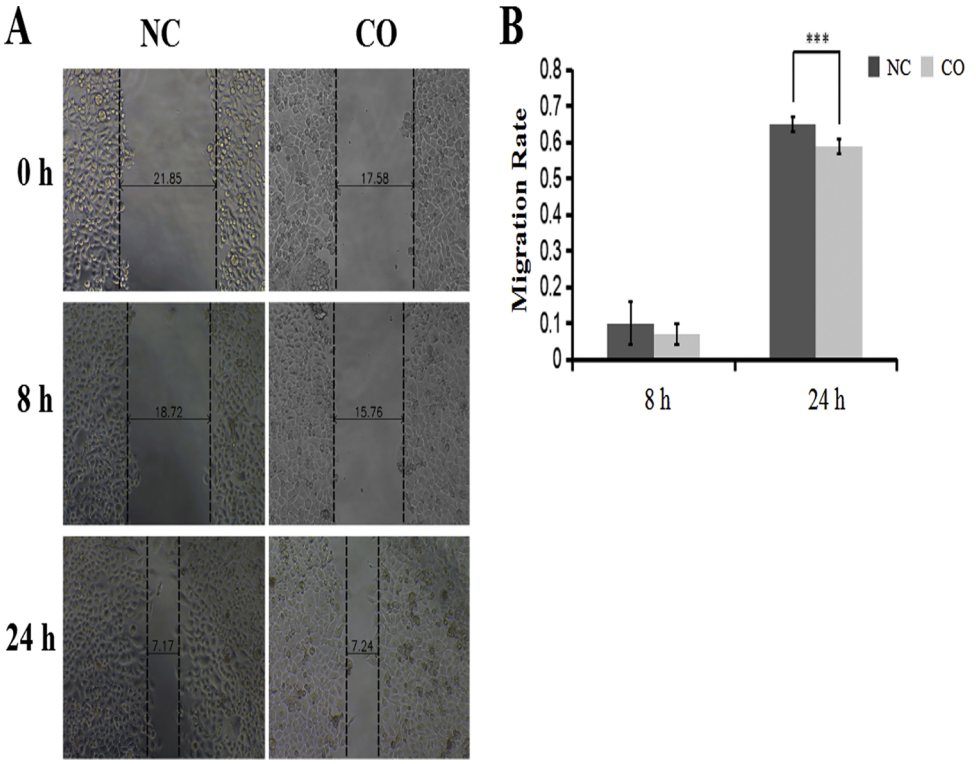

Fig. 5. Wound healing assay of Hela cells.(A) Hela cells of two groups were subjected to in vitro scratch assay with images captured at 0,8 and 24 h after incubation using inverted microscope.(B) Statistic analysis of migration rate comparing NC group and CO group.***, $p < 0.001$.

group migrated at a significantly slower rate (0.59 ± 0.02) compared with cells of NC group (0.65 ± 0.02) ($P < 0.01$). It suggested that co-expressed vector eventually weakened the migration and invasion capacity of Hela cells.

4. Discussion

Although a dramatic progress in cancer treatment took place in the last century, the effects of therapies are still not satisfactory. Recently, much attention has been focused on the cancer gene therapy [3]. Inspiringly, a dynamic development of several approaches of cancer gene therapy over the last two decades seems to herald a future breakthrough. In the past, cancer therapy focused more on inhibiting and killing tumor cells. However, because of the instability, high mutation and heterogeneity of cancer genes, the high resistance has been a difficult problem to the treatment of cancer [23].In view of the genetical stability of endothelial cells [24] and potent immune enhancement of superantigen [22], we thus constructed lentiviral vectors carrying ES and SEC3 gene for having a prolonged and sustained capacity to inhibit and kill tumor cells.

In view of the infectivity of HIV-1 virus, we used 3-plasmid system to obtain the safety and efficiency relying on the segregation of cis- and trans-acting sequences. Moreover, the envelope protein was replaced by the heterologous envelope of G glycoprotein of the vesicular stomatitis virus (VSV-G), resulting in rendering the vector pantropic and conferring high stability on it [8]. As shown, the mRNA and protein level of two transgene was significantly higher than NC group, which means the vector highly expressed dual transgenes. Furthermore, Leonardo O et al suggest the SEB can upregulate the expression of ES [25], likewise, in our study, the mRNA level of ES in Hela cells transduced with co-expressed vector was notably higher than SEC3. This indicated the expression of SEC3 and ES gene may be influenced by each other.

Our MTT result suggests that co-expressed vector can inhibit the proliferation of Hela cells. Analogously, previous compelling evidence suggest SEs can formed the TCR-SAg-MHC II ternary complex to attack tumor cells through activation of massive T cells and the secretion of cytokines via a cascade of biochemical events including many kinds of kinases such as protein tyrosine kinases, phospholipase C, protein kinase C, protein kinase A, and p38MAPK (mitogen-activated protein kinase) [26–28]. It means SEC3 of co-expressed vector may also inhibit Hela cells by such signal pathways. H.Y. Kang et al suggest that ES inhibited the signal pathway of anti-apoptosis by increasing the phosphorylation of PKB via activation of PI3-kinase/PKB pathway to break the balance of growth and apoptosis [29]. Moreover, there are studies determined that ES affects cell cycle by decreasing the p53 and p21 gene product and down-regulated cyclin D1 mRNA and protein [30]. However, there are many studies verified that superantigen influences cell cycle via leukomonocyte but not itself. Thus, SEC3 expressed by co-expressed vector used in vivo, as a kind of superantigen, can exert this capacity. In our study, the results of Flow Cytometry indicated Hela cell was arrested in S phase. Thus, we realized one underlying mechanism of inhibition for cell proliferation of co-expressed vector in vitro is the influence on cell cycle.

The other important result of this study was the impact of co-expressed vector on the migration capacity of Hela cells. As shown in Fig. 5, co-expressed vector significantly inhibited the migration capacity of Hela cells after 24 h. Previously, ES was demonstrated to inhibit tumor cellular invasion by blocking the activation and catalytic activity of matrix metalloproteinase-2(MMP-2) [31]. And although the influence of SEC3 on cell migration has not been completely revealed, a study found that Staphylococcal superantigen-like 10 inhibited CXCL12-induced calcium mobilization and cell migration by binding CXCR4 expressed on human T acute lymphoblastic leukemia, lymphoma, and cervical carcinoma cell lines [32], it means SEC3 as a superantigen, may interfere with this process by regulating related genes and molecules or cross-linking receptors to affect cell migration.

In conclusion, constructed SEC3-ES vector could efficiently express transgenes in Hela cells. Furthermore, the results of the assays in vitro demonstrated that constructed co-expressed vector can inhibit Hela cells proliferation by affecting cell cycle progression and impairing the

migration and invasion ability of Hela cells. However, the effect of co-expressed vector in vivo is not yet clear. Thus, the constructed co-expressed vector will provide a useful tool in future studies to demonstrate its antitumor effect in vivo. Moreover, we believe that this co-expressed lentiviral vector would be used as an ideal strategy to treat cervical cancer in clinical.

Acknowledgements

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