Ginsenoside Compound K Inhibits the Proliferation, Migration and Invasion of Eca109 cell via VEGF-A/Pi3k/Akt Pathway

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Research article

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Abstract

Objective: Esophageal cancer, one of the most common cancers of the upper digestive tract and is one of the leading cancer-related mortality worldwide. Accumulating studies found that Ginsenoside compound K (CK) has significantly anti-tumor effects, especially in the suppression of proliferation, migration, as well as invasion of various human cancers. While the effects of Ginsenoside CK have not well been studied in esophageal cancer. In our present study, we aim to explore the functions of Ginsenoside CK in the progression of esophageal cancer cells (Eca109).

Methods: Cell Counting Kit-8 (CCK-8), wound healing, transwell and flow cytometry assays were applied to analyze the effects of Ginsenoside CK in the progression of Eca109 cell, western bolt was used to investigated the potential downstream signal pathway after Ginsenoside CK treatment.

Results: Our study found that Ginsenoside CK can suppressed the cell viability, proliferation, migration and invasion of Eca109 cell. Furthermore, the flow cytometry showed that Ginsenoside CK induced apoptosis rates of Eca109 cell. The western blot results indicated that Ginsenoside CK functions may possibly related to the blockade of the VEGF-A/Pi3k/Akt signaling pathway. Knockdown of VEGF-A gene could suppress poor the progression of Eca109 cells either.

Conclusion: In conclusion, our study suggests that Ginsenoside CK inhibited viability, proliferation, migration, invasion, and induced apoptosis of esophageal cancer cells by VEGF-A/Pi3k/Akt signaling pathway.

Introduction

Esophageal carcinoma (EC) is one of the most malignant tumors worldwide, and present a great threat to the health of society [1, 2]. EC is well known by its high rate of metastasis, aggressive invasion and poor prognosis [3]. Surgery and chemotherapy are effective treatments for EC diagnosed at an early stage, a lot of EC patients comes to recurrence or metastasis, and eventually progress to advanced stages of cancers, which present poor prognosis [4]. Therefore, it is of great urgent to explore new and efficient treatment strategies, so as to improve the poor survival status of EC patients.

Ginsenoside CK is the main metabolic component of ginseng in human body [5]. Previous studies demonstrated that Ginsenoside CK has anti-tumor, anti-inflammatory, anti-oxidation, liver protection, improving immune function and other effects [6]. Furthermore, the therapeutics values of Ginsenoside CK in tumors have well been studied in bladder cancer, colon cancer, liver cancer which can significantly inhibited the proliferation ability of these tumor cells [7-9]. Vascular endothelial growth factor-A (VEGF-A), a highly specific pro-vascular endothelial cell growth factor, which can promote extracellular matrix degeneration, vascular permeability, proliferation, migration and angiogenesis of vascular endothelial cells [10, 11]. Study suggested that ginsenoside CK suppress tumor angiogenesis by suppressing the proliferation and migration of vascular endothelial cells, inhibiting the activity of VEGF-A and it's signaling pathway, and the degradation of vascular extracellular matrix in neuroblastoma

cells [12]. However, there are few reports about the effects of Ginsenoside CK on esophageal cancer cells and its molecular mechanism. As a targeted drug with high efficiency and low toxicity, ginsenoside CK has further development potential in esophageal cancer.

In our present study, we aim to further explore the effects of Ginsenoside CK on the cell proliferation, migration, invasion and related mechanisms on Eca109 cell.

Materials And Methods

Cell lines and culture

Human esophageal cancer cell (Eca109) was purchased from the American Type Culture Collection (Manassas, USA). Eca109 cell maintained in RPMI 1640 medium (Gibco, USA) containing with 10% feta bovine serum (Gibco, USA) and 1% penicillin streptomycin (Gbico, USA) in an atmosphere of 5% CO_2 at 37 °C.

Cell viability assay

The changes of cell viability were evaluated by employing the Cell Counting Kit-8 (CCK-8) assay. Cells (2.5×10^3) per well were cultured in 96-well plate overnight. To investigate the proliferation effect of Ginsenoside CK on Eca109 cell, cells were maintained with ginsenoside CK for 72 h. Similarly, lentivirus transfection cells were maintained in 96-well plate for 24 h, 48 h, 72 h or 96 h, then added with 10ul CCK-8 reagent (New Cell & Molecular Biotech, China) to each well, and then incubated for 2h at 37 ◦C. The absorbance value (OD450) was detected by using the microplate reader (Bio-Tek, USA).

VEGF-A knockdown cell line

Eca109 cells were transfected with knockdown lentivirus sh-VEGF-A, and the corresponding negative control lentivirus sh-NCwhich were purchased from Hanbio (Shanghai, China). Puromycin was used to screen the stably transfected cells. Western blot analysis was applied to evaluated the efficiency of lentivirus transfection.

Wound healing assay

Eca109 cell (5×10^5) were seeded in 6-well plates for 24h and scraped by a sterile pipette tip. Cells were cultured with DMSO or ginsenoside CK in FBS-free medium. sh-NC and sh-VEGF-A cells were maintained with FBS-free medium. The Zen Imaging software (Carl Zeiss, Germany) was applied to observe images at 0h, 24h and 48h. The Image J software (USA) was employed to calculate the scratch area.

Cell migration and invasion assays

The migration and invasion assays were employed by transwell chamber (BD, USA) with the presence of Matrigel (BD, USA) for invasion, and absence of Matrigel for migration. Cells ($5x10^4$) per well pretreat with

Ginsenoside CK or lentivirus were planted into the upper chamber with 100 ml serum-free medium, and the lower chamber with 600 ml complete medium. After incubation at 37°C for 24 h, the lower chamber cells were fixed with 70% methanol and then stained by crystal violent (Beyotime, China). The migrated or invaded cells were counted.

Flow Cytometry

Ec109 cells (1.5×10^5) were maintained in 6-well plates at overnight and treated with ginsenoside CK or DMSO for 48 h. sh-NC and sh-VEGF-A cells (1.5×10^5) were planted in 6-well plates for 48 h. The rates of apoptosis cells were assessed by applying the Annexin V-FITC Apoptosis Detection Kit (Beyotime). The results were detected by the CytoFLEX flow cytometer (Beckman).

Western blot analysis

Membrane and Cytosol Protein Extraction Kit (Beyotime, China) and Bicinchoninic Acid Protein Assay Kit (Beyotime) were used for protein extracted and quantified. Proteins were separated on 10% SDS polyacrylamide gel (Beyotime) and then blotted onto PVDF membranes (Millipore, USA). The PVDF membranes were incubated in 5% skim milk for 2h. After that, all membranes were cultured with the primary antibodies of anti-Tubulin (Affinity, USA), anti-VEGF-A (Affinity), anti-Pi3k (Affinity), anti-P-Pi3k (Affinity), anti-Akt (Affinity) and anti-P-Akt (Affinity) overnight at 4 °C. The membranes were then incubated with corresponding secondary antibody (Affinity) for 1h. The protein bands were finally detected by chemiluminescence detection system (ProteinSimple, USA).

Statistical analysis

SPSS 24.0 software (SPSS Inc., Chicago, USA) was applied to data analysis, presented as mean ±SD. The difference between two groups were analyzed by Student's t-test. P value less than 0.05 was defined as statistically significant.

Results

Ginsenoside CK suppress cell viability, proliferation, migration and invasion of Eca109

After the incubation with Ginsenoside CK for 72h in Eca109 cell, the cell viability was decreased with the increased concentration of Ginsenoside CK, as shown in Figure 1. Moreover, wound healing and transwell assay indicated that both the migration and invasion abilities were reduced notably in Eca109 cell after the treatment of Ginsenoside CK (Fig. 2A, B).

Knockdown of VEGF-A gene suppress cell viability, proliferation, migration and invasion of Eca109

Eca109 cell were transfected with VEGF-A knockdown lentivirus (sh-VEGF-A) or the corresponding negative control lentivirus (sh-NC) (Fig. 3A). The western blot analysis confirmed that Eca109 cell were stably transfected with lentivirus (Fig. 3B). The CCK-8 assay showed that cellviability is remarkably

reduced after sh-VEGF-A transfection (Fig. 3C). Wound healing and transwell assay further confirmed that VEGF-A gene knockdown suppress the migration and invasion of Eca109 cell (Figs. 4A, B).

Ginsenoside CK inhibition and knockdown of VEGF-A gene promote apoptosis in Eca109 cell

To investigate the function of Ginsenoside CK inhibition and VEGF-A gene knockdown in cell apoptosis, Annexin V-FITC and PI staining was applied. The flow cytometry analysis of apoptosis showed that Ginsenoside CK inhibition and VEGF-A gene knockdown promoted the apoptosis rate of Eca109 cell (Fig. 5).

Ginsenoside CK influences Eca109 cell progression via VEGF-A/Pi3k/Akt pathway

It is confirmed that the VEGF-A/ Phosphoinositide 3-kinase (Pi3k)/protein kinase B (Akt) signaling pathway play important roles for the tumor progression on proliferation, migration, and invasion behaviors [13]. The present study detected the expression of VEGF-A/Pi3k/Akt pathway related proteins in Eca109 cell by Ginsenoside CK inhibition and VEGF-A knockdown. The results found that the expressions of VEGF-A, P-Pi3k, and P-Akt proteins were obviously reduced in both the inhibition and knockdown groups, while the total proteins of Pi3k and Akt were not changed (Fig. 6), which suggested that the VEGF-A/Pi3k/Akt pathway may present as downstream of Ginsenoside CK inhibition in Eca109 cell.

Discussion

Esophageal cancer is a highly malignant digestive tract tumor, ranking the 5th and 8th among the causes of cancer-related death in worldwide [14]. Although the diagnostic techniques and treatment methods for esophageal cancer have been continuously improved recently, the overall survival status of esophageal cancer patients is still not ideal due to the characteristics of early invasion and distant metastasis [15]. Ginseng, as a treasure of human pharmaceutical culture, contains a variety of active components, among which ginsenoside is the main bioactive compound in ginseng and has broad implications in human disease [16]. Ginsenoside CK is a natural diol-type ginsenoside with medicinal activity in vivo [17]. Studies found that Ginsenoside CK has inhibiting effects on various tumor cells, containing lung cancer, colon cancer, and liver cancer, as well as bone tumor [18-20]. The anti-tumor effects of Ginsenoside CK are mainly reflected in its ability to reduce the proliferation, migration, and invasion in tumor cells [21]. Chen et al. [22] Study found that Ginsenoside CK can induce cell apoptosis and inhibit the biological activities of human osteosarcoma cells via blocking the Pi3k signaling pathway. Oh et al. [23] revealed that Ginsenoside CK promote cell autophagic and apoptosis which can inhibit human neuroblastoma cells viability both in vitro and vivo. In the present study, we investigated the anti-tumor effect of ginsenoside CK and its related mechanism in Eca109 cell. Our study found that Ginsenoside CK can suppress Eca109 cell viability, proliferation, migration, invasion and induce apoptosis via inhibition of VEGF-A signal pathway. Moreover, knockdown of VEGF-A gene by lentivirus transfection confirmed the suppression of proliferation, migration, invasion ability, as well as promote cell apoptosis of esophageal cancer cells. Furthermore, the western blot results revealed the decrease expression of VEGF-A, P-Pi3k, and P-Akt proteins after Ginsenoside CK intervention and VEGF-A knockdown.

VEGF-A playing as an important regulator of angiogenesis and presenting as the mediator of endothelial cells proliferation [24]. Pi3k/Akt signaling pathway can be activated by angiogenesis inducers and growth factors, like angiopoietins and VEGF-A [25]. Some studies found that Pi3k/Akt presented as the main downstream signaling pathway mediating the biological effects of VEGF-A, and the VEGF-A/Pi3k/Akt signaling pathway playing significant roles in proliferation, migration and invasion of various cellular processes [26]. In our study, the results of western blot demonstrated the Ginsenoside CK inhibition decrease the expression of VEGF-A, P-Pi3k, and P-Akt proteins. Therefore, we speculated that inhibition of the VEGF-A/Pi3k/Akt signaling pathway may be the main way that ginsenoside CK inhibits cell viability, proliferation, migration, invasion, and induce apoptosis in Eca109 cell. Furthermore, the VEGF-A gene knockdown investigation supported the hypothesis that the therapeutic effect of ginsenoside CK may mainly be via affecting Pi3k/Akt signal activation.

Conclusions

In conclusion, our present study found that Ginsenoside CK can suppress the cell proliferation, migration, invasion, and induce apoptosis of Eca109 cell via VEGF-A/Pi3k/Akt signaling pathway, which suggests that Ginsenoside CK may serve as an effective treatment in EC.

Abbreviations

EC: Esophageal carcinoma; CCK-8: Cell Counting Kit-8; Ginsenoside CK: Ginsenoside compound K; VEGF-A: Vascular endothelial growth factor-A.

Declarations

Authors' contributions

LZ and WX responsible for the study design. HH performed the experiments and draft the manuscript. PD, LF, HY, HG and HX participated in the data analysis. All authors have read and approved the final manuscript.

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Availability of data and materials

The data the support the findings of this study are available on request from corresponding author.

Consent for publication

Not applicable.

Competing interests

The authors declare none conflict of interest.

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Figures

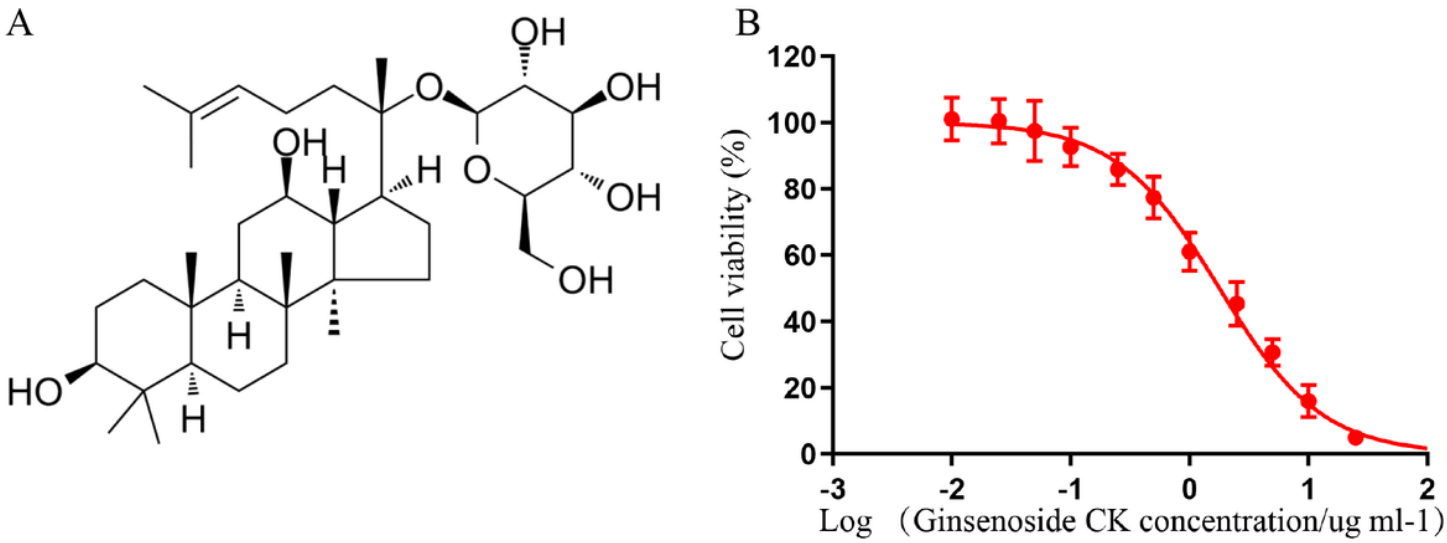

Figure 1

The drug formula of Ginsenoside CK (A) and cell viability changes with increased concentration of Ginsenoside CK cultured for 72h in Eca109 cell (B).

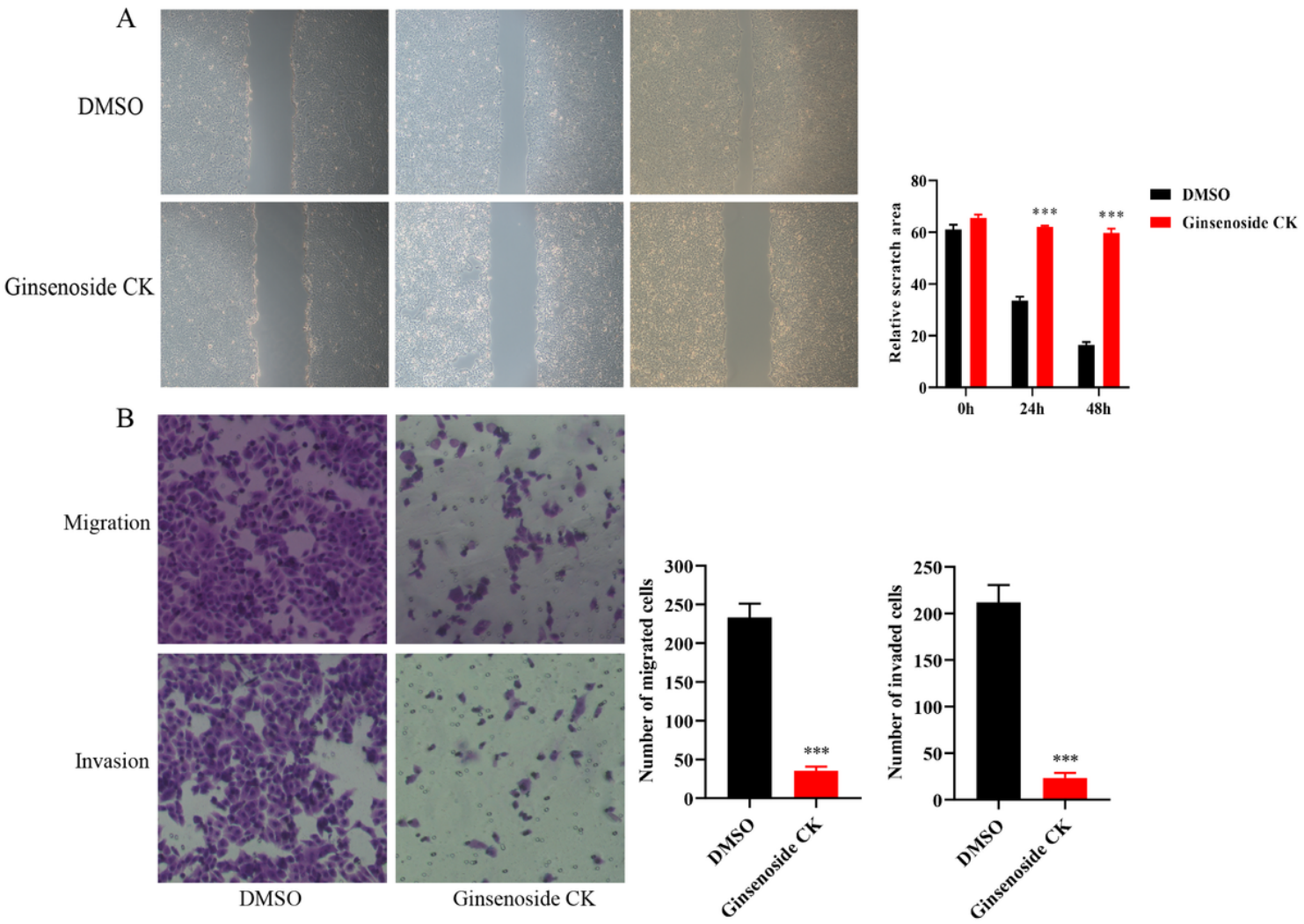

Figure 2

Ginsenoside CK suppressed the migration and invasion of Eca109 cell. (A) Wound healing assay showed that the migration ability was reduced after Ginsenoside CK treatment. (B) Transwell assay demonstrated that cell migration and invasion ability decreased in Ginsenoside CK intervention. ***P□0.001

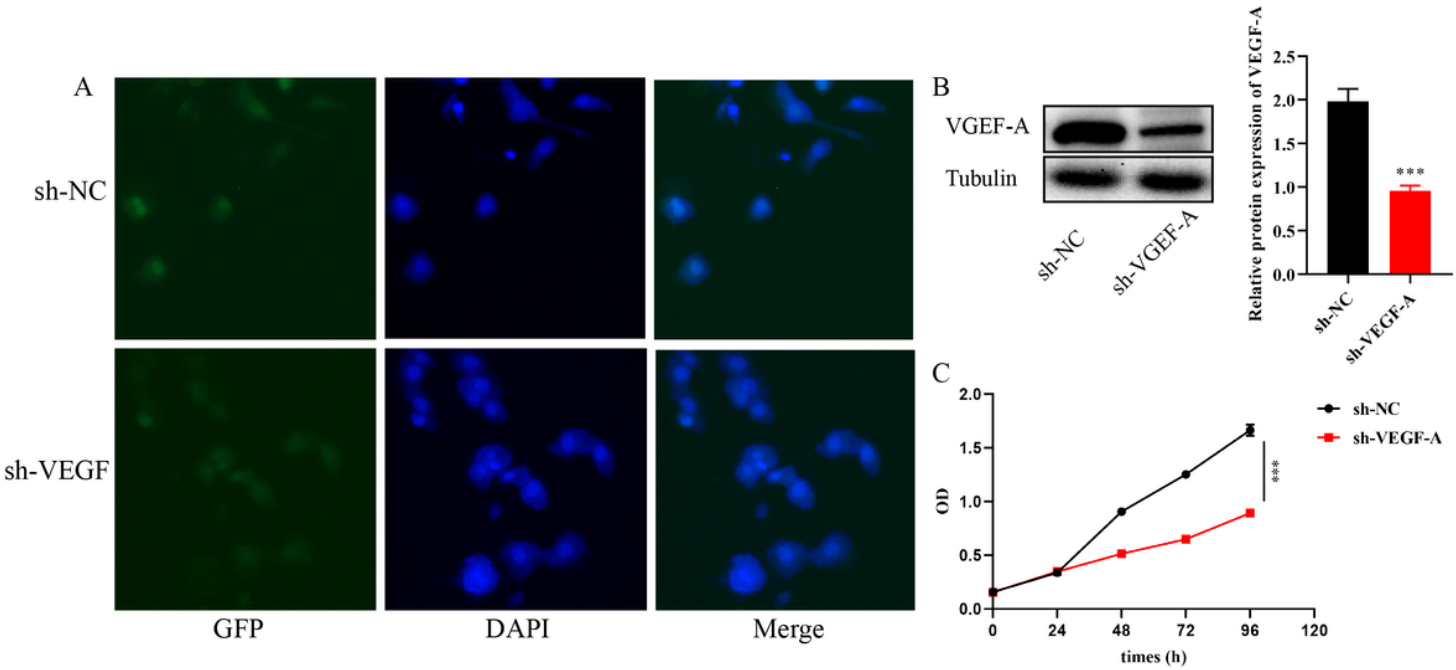

Figure 3

The knockdown of VEGF-A in Eca109 cell and cell viability changes. (A) Green fluorescent and western bolt analysis showed that Eca109 cell were stably transfected with VEGF-A knockdown gene. (B) Cell viability changes by CCK-8 after incubation for 4 days. ***P□0.001

Figure 4

VEGF-A gene knockdown suppressed the migration and invasion of Eca109 cell. (A) Wound healing assay found that cell migration ability was inhibited after VEGF-A gene knockdown. (B) The migration and invasion ability changes in VEGF-A gene knockdown group. ***P□0.001

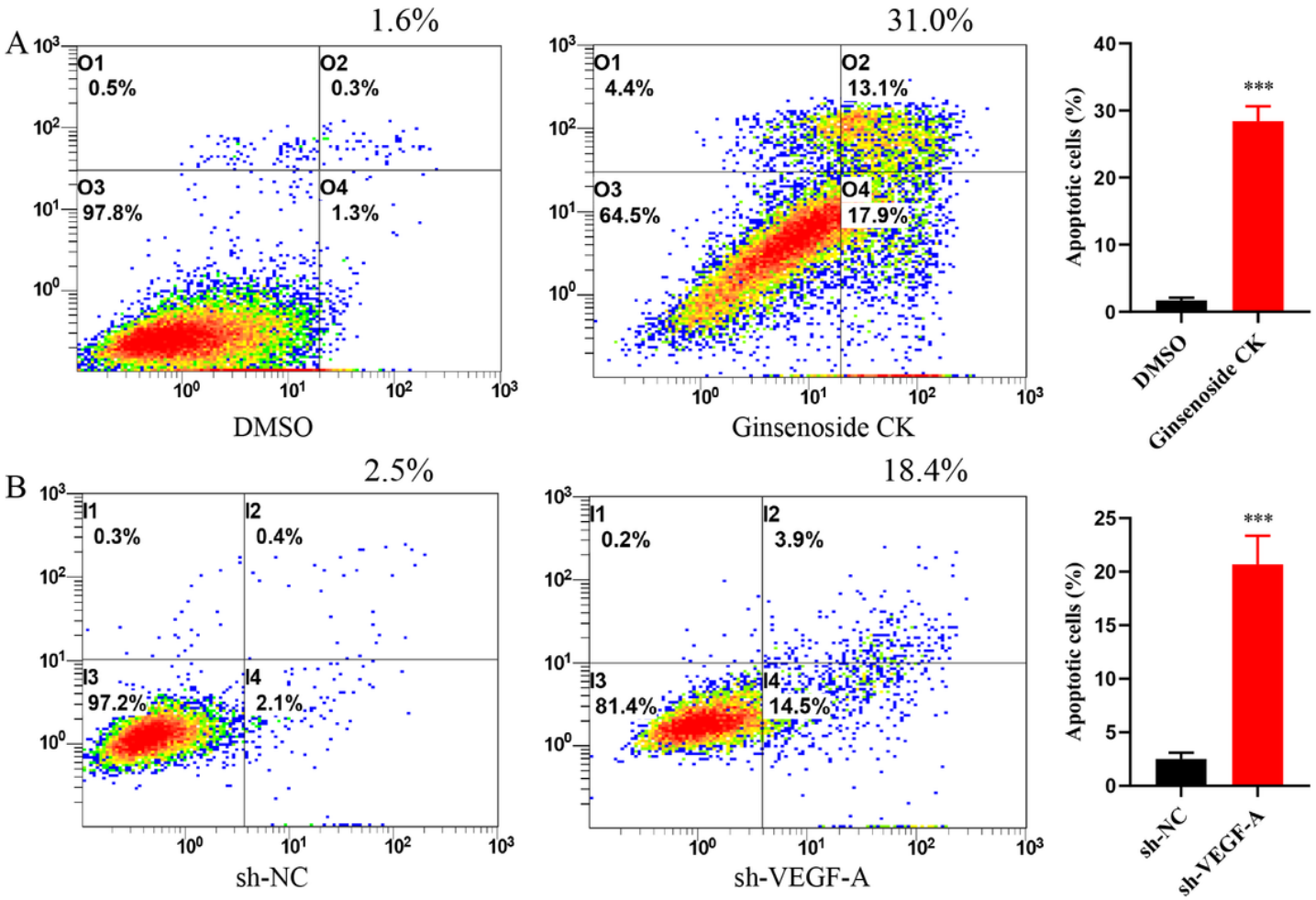

Figure 5

The involvement of Ginsenoside CK and VEGF-A in the pro-apoptotic in Eca109 cell. (A) The cell apoptosis rates of Eca109 cell were detected by flow cytometry with DMSO and Ginsenoside CK treatments. (B) The cell apoptosis rates of Eca109 cell in sh-NC and sh-VEGF-A. ***P□0.001

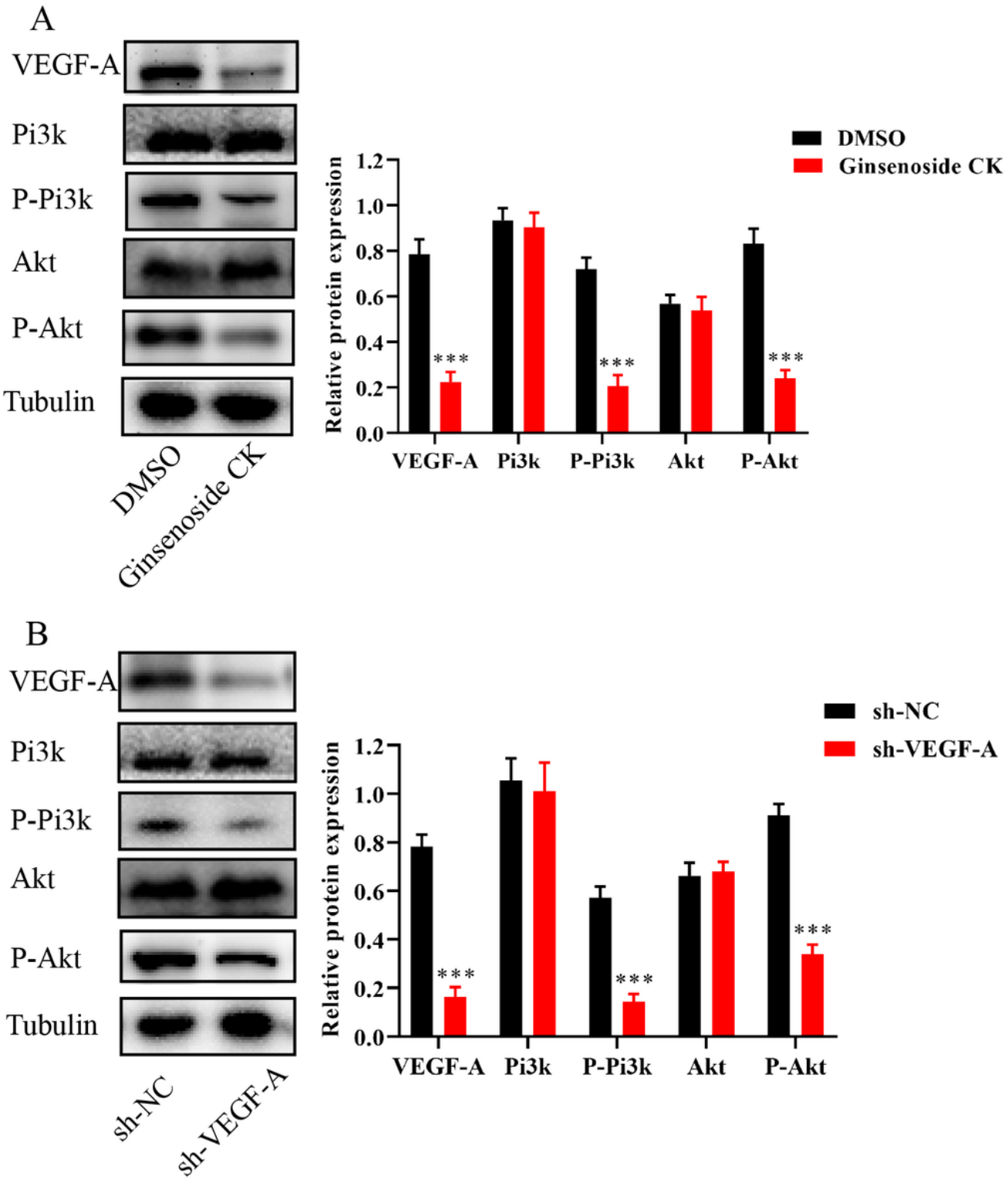

Figure 6

The involvement of VEGF-A/Pi3k/Akt pathway in Ginsenoside CK inhibition and VEGF-A gene knockdown of Eca109 cell. Expressions of VEGF-A, Pi3k, P-Pi3k, Akt, P-Akt in Ginsenoside CK inhibition (A) and VEGF-A gene knockdown (B) groups. ***P□0.001