Mechanism and clinical study of inhibition of invasion and metastasis of ovarian cancer by pinogenin targeting THBS1

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Introduction. Pinicin inhibits the migration and invasion of ovarian cancer by promoting THBS1 expression and reducing THBS1 expression. THBS1 promotes the migration and cleaning of ovarian cancer cells, and expresses higher than normal ovarian tissue pine in ovarian cancer, inhibiting the proliferation, migration and invasion of ovarian cancer cells. THBS1 can be directly bound to promote its ubiquitination, and it can also be deubiquitinated by binding with upS 7. mia formation of THBS1 was significantly inhibited at a concentration of 40 microm, but at a concentration of 80. Mra levels have actually risen. The direct binding energy of scisquirrel to uusp7 verified by CETsa test could not be judged by whether there was an interaction between THBS1 and UPS. Through various inducible factors and protein synthesis, scisquirrel can be directly blocked by cx to observe protein synthesis in ovarian cancer cells. The co-expression of THBS1 and ubiquitin was determined by the influence of THBS1 degradation, and the co-expression of THBS1 and usp 7 was determined by the influence of scisquirrel on THBS1 vulgification. THBS1 promotes the migration of ovarian cancer cells, and the higher expression of THBS1 in ovarian tissue is positively correlated with the poor prognosis of ovarian cancer. In this paper, we explored the expression and clinical significance of THBS1 in ovarian cancer to clarify the specific role of THBS1 in ovarian cancer cell migration. Pinicin inhibits the cell activity, proliferation, migration and invasion of ovarian cancer cells, which is one of its core targets. Pinicin down-regulates THBS1 expression in a dose-dependent manner, which is most closely related to migration and invasion. Pinicin is an evaluation of potential target genes. It can inhibit the expression of many proteins. When ecdherin expression is up-regulated, pinoid inhibits the migration and cleaning ability of ovarian cancer cells and the proliferation process of ovarian cancer cells.

Keywords: Ovary; Pinicin; THBS1; Ubiquitin

INTRODUCTION

The clinical application of pinicin in the treatment of ovarian cancer studied in this paper provides a theoretical basis for the research and development of new drugs for ovarian cancer, especially the development of natural drugs derived from animals and plants. The expression significance of THBS1 in ovarian cancer was explored, and the mechanism of scisquirrel in reducing THBS1 level in ovarian cancer cells was explored in vitro experiments. Normal ovarian tissue samples from a hospital were collected, and biological information was used to screen the effect of pinesin. Multi-molecular biological experiments proved that THBS1 is an important target for pinesin to inhibit ovarian cancer migration. Unfortunately, the effect, target and mechanism of pinesin on ovarian cancer are still unclear. Although the antitumor effects of scisquirrel in vitro and the experimental results are rich, pinogenin has been shown to inhibit the proliferation of melanocytes in a dose-dependent manner, low concentrations of pinogenin also significantly inhibit the migration and invasion ability of breast cancer cells, induce cell cycle arrest and promote cell apoptosis. In vitro tests have shown inhibition of breast cancer proliferation, among the various factors of breast cancer, pinogenin has been found to inhibit[1].

The autophagy function of cancer cells inhibits and prevents their proliferation. Pinactin can also inhibit the activation of various THBS1 induced skin fibroblasts, showing excellent anti-inflammatory and anti-fiber activities. In vivo, pinactin can significantly reduce gastric ulcers. Induced oxidative and inflammatory responses in rat models, thereby treating gastric ulcers. In recent years, it has anti-tumor, anti-inflammatory, antibacterial and other biological activities, so it has quickly become a hot spot in the research and development of natural drugs, and pinogenin is one of the high natural flavonoids. Among them, the content of Chinese propolis is THBS1, a typical representative of pinus, which is a protein in the violation of platelet

agglutinin[2]. At present, the mechanism of action of THBS1 in ovarian cancer is not very clear, so this paper analyzes whether there is a broader prospect of inhibiting and treating ovarian cancer by targeting THBS1 therapy. In the emt process of ovarian cancer cells, the expression of matrix metalloproteinases is up-regulated, especially mmp2 and mmp9[3]. The metastasis process of ovarian cancer depends on the tumor microenvironment. THBS1 is a dynamic network composed of various cells in the extracellular matrix and infiltrating stroma of tumor cells. Tumor cells themselves and fibroblast tumor-related stromal cells in THBS1 can secrete various cytokines to inhibit tumor. Ovarian cancer is one of the most common gynecological malignant tumors in modern society, and we must explore as much as possible to curb the harm caused by this disease[4].

1. Function and Target Screening of Pinogenin in Ovarian Cancer Cells

1.1 Experimental materials

The cell lines used in this experiment are es rod type II and human ovarian cell sKOv3, both from a biotechnology company, and have been identified by qualified tests. Other materials required for the experiment. There are mould-free suction heads in boxes, and all models have ordinary suction heads centrifuge tube orifice plate Culture dish Cell culture dish Cell counter plate sealing film plate anhydrous ethanol-trichloromethane-isopropyl alcohol-methanol sealing film supersensitive chemiluminescence detection reagent Enhanced chemiluminescence kit Antibody[5]. They are also available in various models, which fully meet the requirements of the experiment, as well as buffers, pre-dyed protein needles, filters and other skimmed milk powder buffers, amino methane, glycine, sodium 12 alkyl sulfate. Antidiluent solution, iodized probutin inhibitor cell lysate and histopathic cell fixation solution. Crystal violet dye[6].

Digestive fluid dimethylidene. Green streptomycin mixture liquid petri dish, etc. The equipment used in the experiment includes a super-clean table carbon dioxide

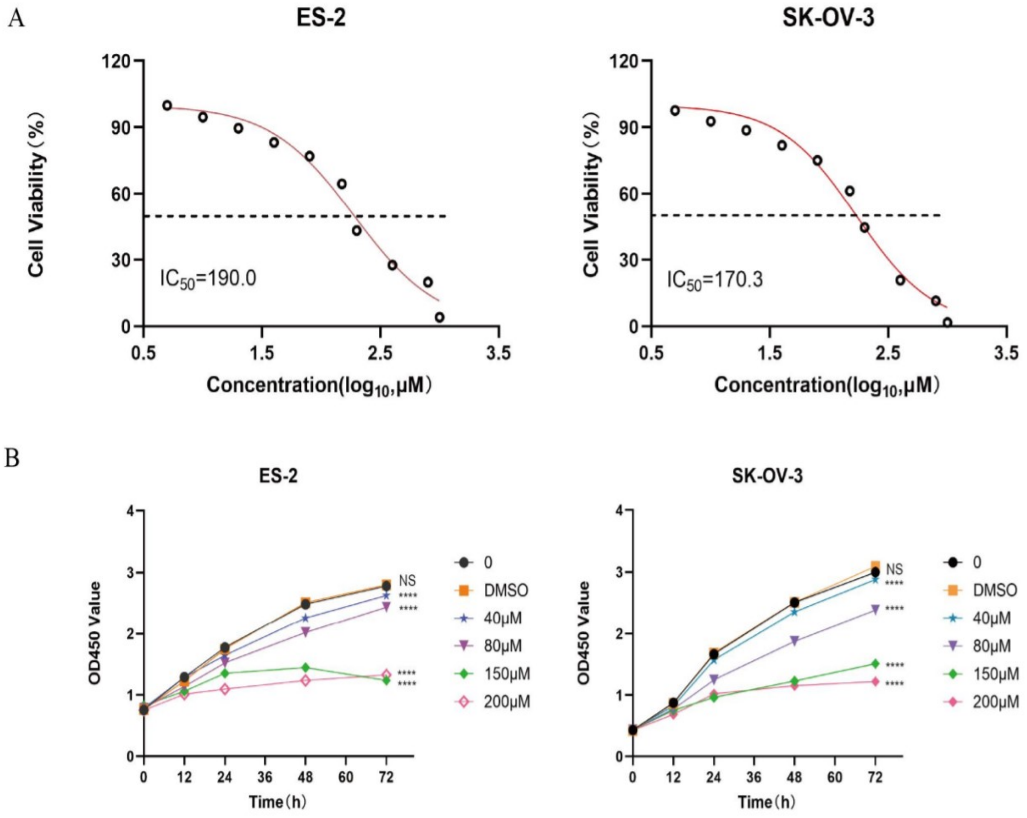
incubator with water isolation electric thermostatic incubator with thermostatic water bath biosafety cabinet with high speed refrigerated centrifuge table top centrifuge pocket centrifuge general refrigerated frozen water tank Ultrasonic cell breaker Adjustable speed whirlpool mixer Low temperature preservation box pipette gun inverted fluorescence microscope high pressure steam sterilizer ice machine electronic balance fine Close weighing balance Enzyme label Instrument Flow Cytometry Luminescence image Analysis System Automatic cell counter Liquid nitrogen container tank programmed cooling freeze-storage box ultra-pure water inverted fluorescence microscope decolorization table Multi-purpose rotating table microplate centrifuge, constant temperature metal bath protein vertical electrophoresis system Electric temperature constant temperature blast drying box ultraviolet-spectrophotometer Multi-function microplate detector. In terms of bioinformatics tools, there are many databases that cannot be described here[7].

1.2 Experimental Methods

This test method should go through the following procedures: First, the preparation of the solution, the experiment is divided into main solution and other solutions, the main solution needs to use the reserve and working solution to complete, the others include complete culture medium cells, complete culture medium and pbs buffer, ammonium persulfate sds solution and electrophoresis buffer transmembrane buffer. Closed buffer and so on. After that, to complete the cell culture, first of all, the cell passage process, then the cell freezing stage, then the cell recovery stage, the cell counting stage, and then the CCK-8 experiment, and then the plate cloning, formation experiment, so that after the cell scratch experiment is completed, we can carry out the cell migration experiment, after the cell invasion experiment. Protein sample collection and gel electrophoresis process. The stage of mold rotation was carried out, and then the immune reaction was performed, and the double-sandwich antibody Elisa assay was performed. Bioinformatics prediction, target of action, experiment and then statistical analysis[8].

1.3 Experimental Results

We can obtain a series of experimental results on the role of pinogenin in ovarian cancer. First, data recording is completed, data screening and integration are carried out, and then data curve fitting analysis is performed by summarizing and comparing, so that there is no significant statistical difference between the blank group and the control group. Although the results show that pine can inhibit the proliferation and active response of ovarian cancer cells in a dose-dependent and time-dependent manner[9].



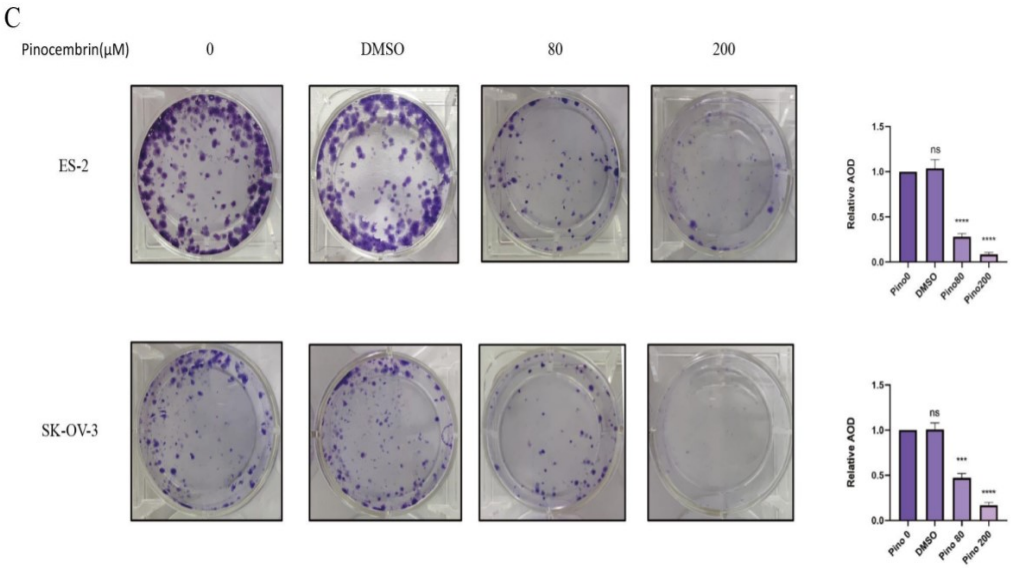


Figure 1. The inhibitory effect of pinesin on the proliferation of ovarian cancer cells

Picture caption：(A) Cell viability curves and IC50 values of ES-2 and SK-OV-3 cells treated with different concentrations of pinesin for 24 hours; (B) The OD450 values of ES-2 and SK-OV-3 cells were changed after 0, 12, 24, 48 and 72 hours of treatment with different concentrations of pinophylline, and only 0.01%DMSO was used in DMSO group; (C) The effect of different concentrations of pinophyllin on the flat clones of ES-2 and SK-OV-3 cells was observed in 0μM group as negative control and only 0.01% DMSO-treated cells in DMSO group. The Relative AOD value of cells in each group after staining compared with negative control was used as the evaluation index. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

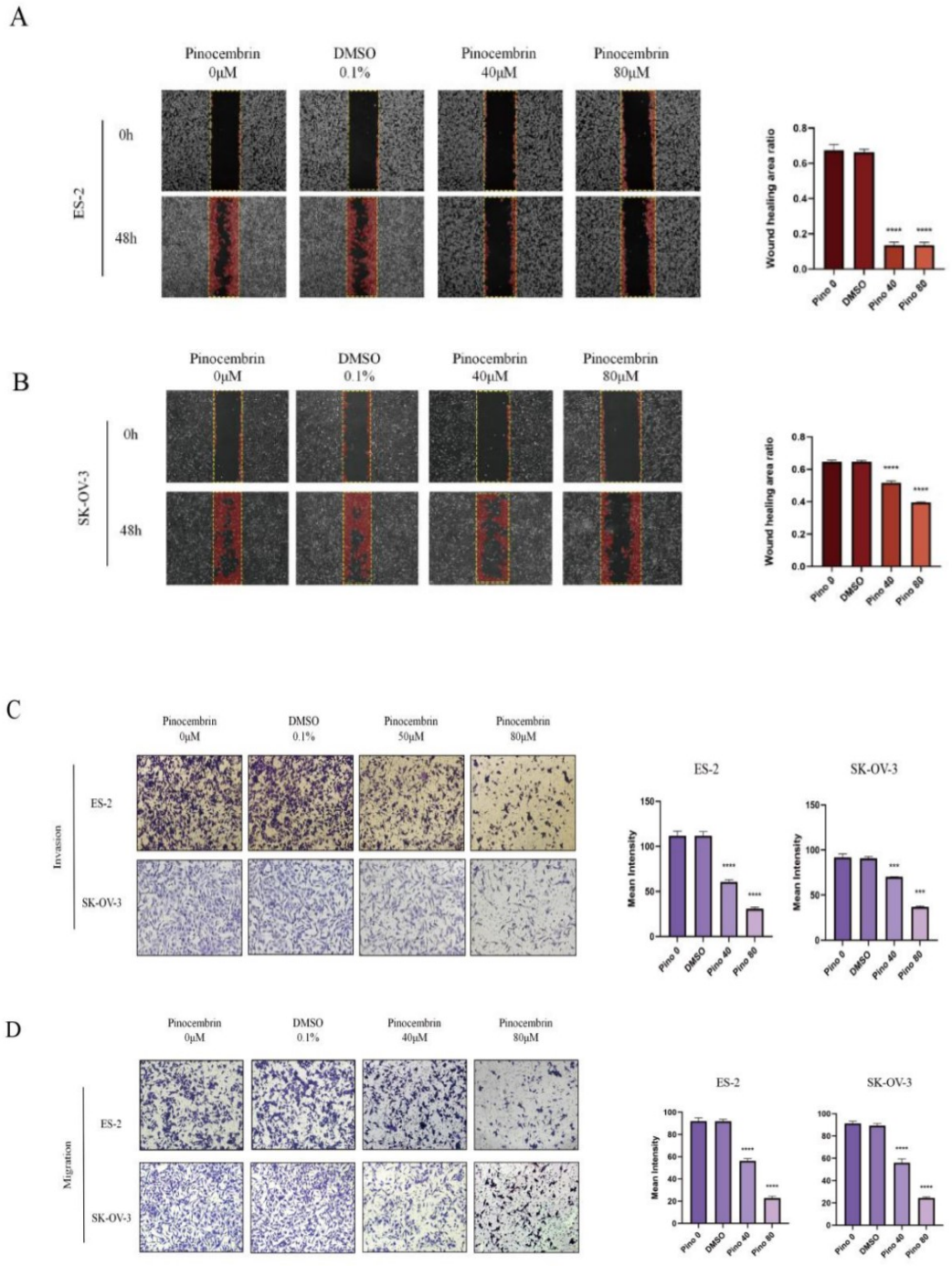
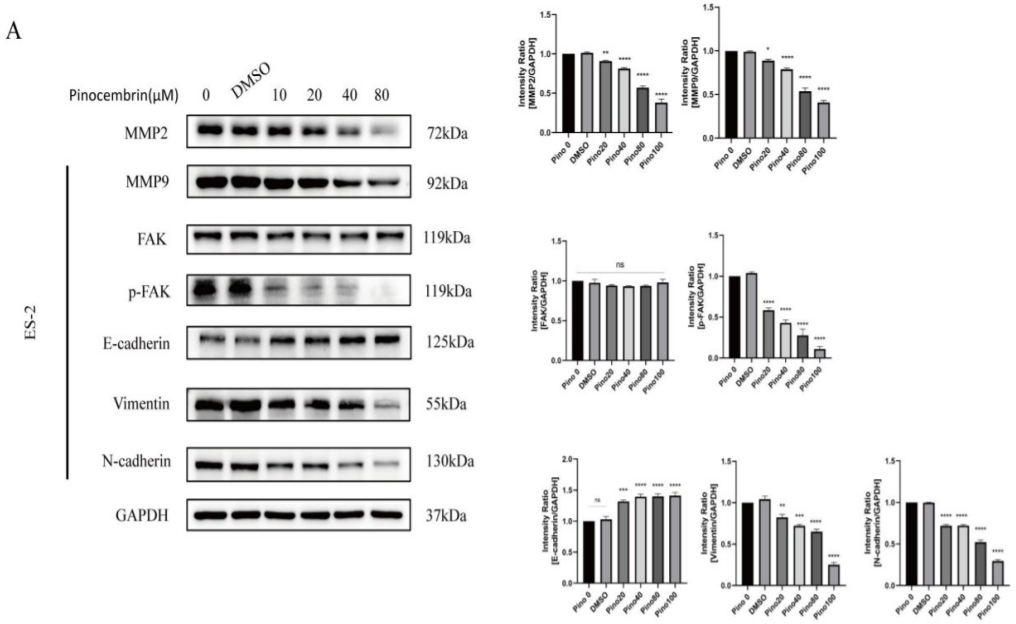


Figure 2. Pinogenin inhibits the invasion and migration of ES-2 and SK-OV-3 ovarian cancer cells

Picture caption：(A) Different concentrations of pinicin in scratch tests.The effect of ES-2 cell migration ability after 48 hours; (B) The migration ability of SK-OV-3

cells after 48 h treatment with different concentrations of pinogenin in scratch test; (C) In Transwell cell migration experiment, different concentrations of pinicin were finer to ES-2 and SK-OV-3The effect of cell invasion ability; (D) The migration ability of ES-2 and SK-OV-3 cells with different concentrations of pinicin in Transwell experiment Influence; The migration ability was evaluated by the Wound healing ratio in the scratch test and Transwell test.Mean Intensity of transplanted cells was used as evaluation index, *p < 0.05, **p < 0.01, ***p < 0.001, *** P < 0.001, * P < 0.05, *** P < 0.001****p < 0.0001.



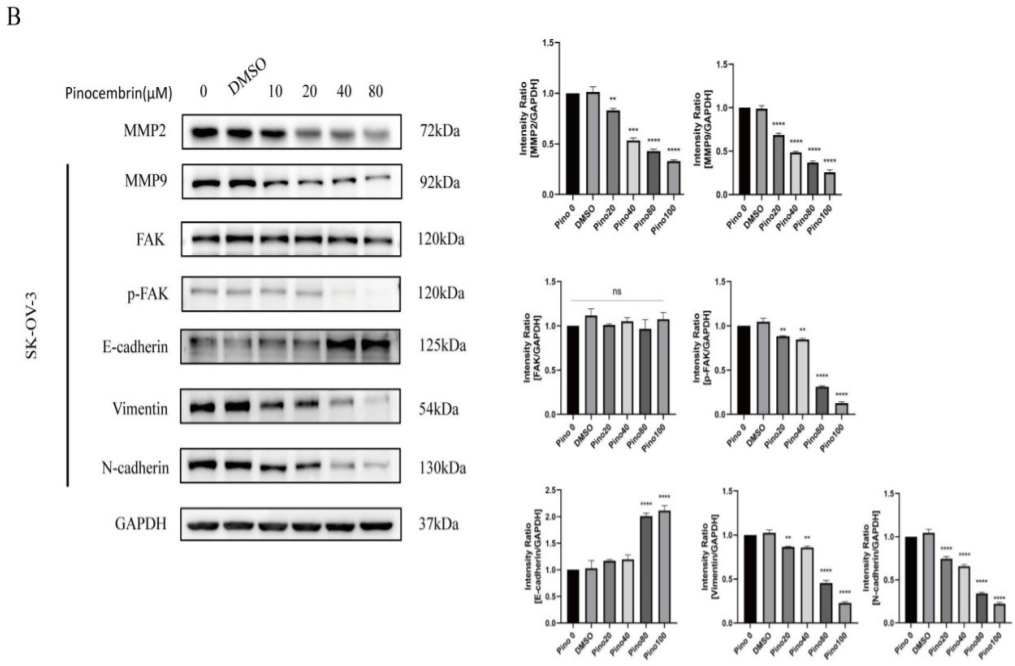
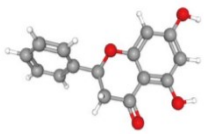


Figure 3. Effect of pinicin on key proteins during invasion and metastasis of ovarian cancer

Picture caption : (A)Western Blot test to verify the effect of pinesin on key proteins of metastasis and invasion in ES2 cells; (B)Western Blot test to verify the effect of pinicin on key proteins of metastasis and invasion in SK-OV-3 cells; The Intensity ratio of each experimental group to the negative control group (pino 0μM) was used as the evaluation index, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. We retrieved a lot of data and chemical structures from the database, and studied them separately as shown in the figure below.

A



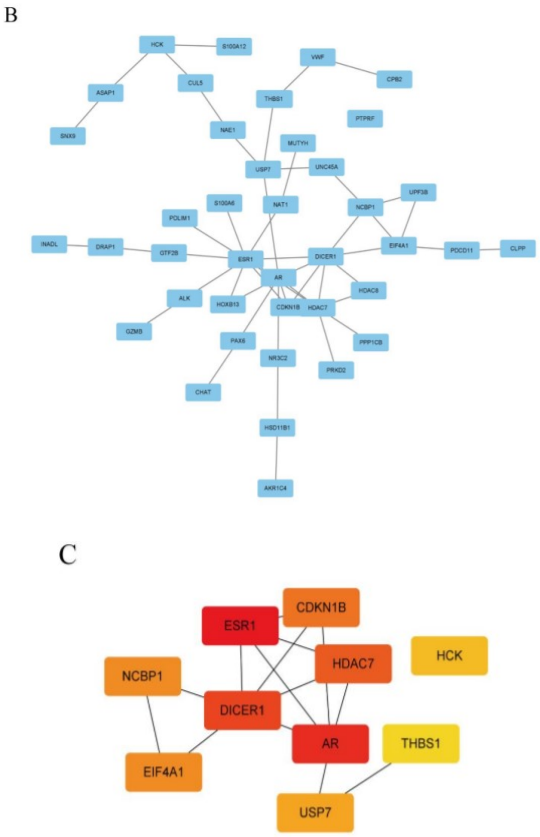


Figure 4. Screening of pinoid target genes

(A) 3D model of the chemical structure of pinicin; (B) PPI network of pinogenin targets Part I Function and target screening of pinogenin in ovarian cancer cells (C) 10 core target gene sets of pinocybin after Cytohubba calculation

Gene	Score	Gene	Score	Gene	Score
UNC45A	0.6776	TLR1	0.4666	HDAC7	0.3797
UPF3B	0.5884	DICER1	0.4645	ALK	0.3789
HOXB13	0.5788	EIF4A1	0.4626	ESR1	0.3753
PDLIM1	0.5767	PRKD2	0.4502	LSS	0.3747
RARG	0.5711	AR	0.4413	NCBP1	0.3737
GRB14	0.5693	PPP1CB	0.4385	HIBCH	0.372
CAPN9	0.565	GZMB	0.4291	PTPRF	0.3715
IGHG1	0.5599	HSD11B1	0.4262	NT5C3A	0.3714
GALM	0.5555	AKR1C4	0.42	ME2	0.3687
TRAPPC6B	0.5425	VWF	0.4178	BCL9	0.3679
THBS1	0.527	HDAC8	0.4165	CLPP	0.3662
DRAP1	0.5247	NR3C2	0.412	VEGFB	0.3658
PAX6	0.5	GSTM2	0.4085	PDCD11	0.3653
SNX9	0.4909	GTF2B	0.4048	NAE1	0.3653
GAN	0.4892	ASAP1	0.4042	CHAT	0.3652
RNF8	0.4872	NMRK1	0.4035	MUTYH	0.3623
CUL5	0.485	HCK	0.4035	CPB2	0.3613
USP7	0.4843	LHX9	0.4	S100A6	0.3602
CELF4	0.4838	ZFP36L2	0.3867	IQUB	0.36
SLC30A9	0.4833	S100A12	0.3855	ZNF462	0.3594
ARHGEF12	0.4814	VPS4B	0.3845	CDKN1B	0.3565
NAT1	0.4773	DHRS1	0.38	INADL	0.3561

Table 1. Target fraction of pinicin

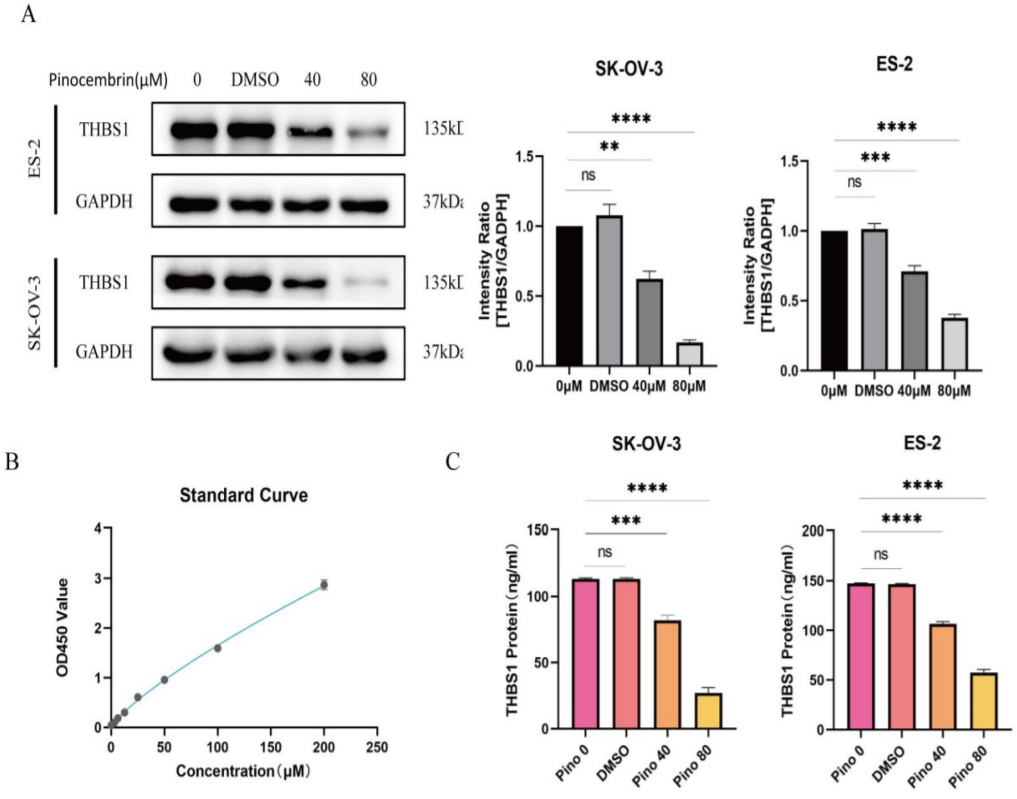


Figure 5. Pinicin inhibits the expression and secretion of ovarian cancer cells

2. Clinical significance of THBS1 in ovarian cancer

To clarify the correlation between THBS1 expression and prognosis in ovarian cancer, collect clinical information of ovarian cancer patients and their tumor specimens, and explore its influence on the migration and invasion of ovarian cancer. In experiments, we altered THBS1 expression in ovarian cancer, but there is no direct evidence of THBS1's role and expression in ovarian cancer. Current research can look at a variety of ways THBS1 appears to have a catalytic effect. From the perspective of multiple data mining and molecular biological experiments, THBS1 was verified as a potential target of squirrelin inhibiting ovarian cancer migration and cleaning[10].

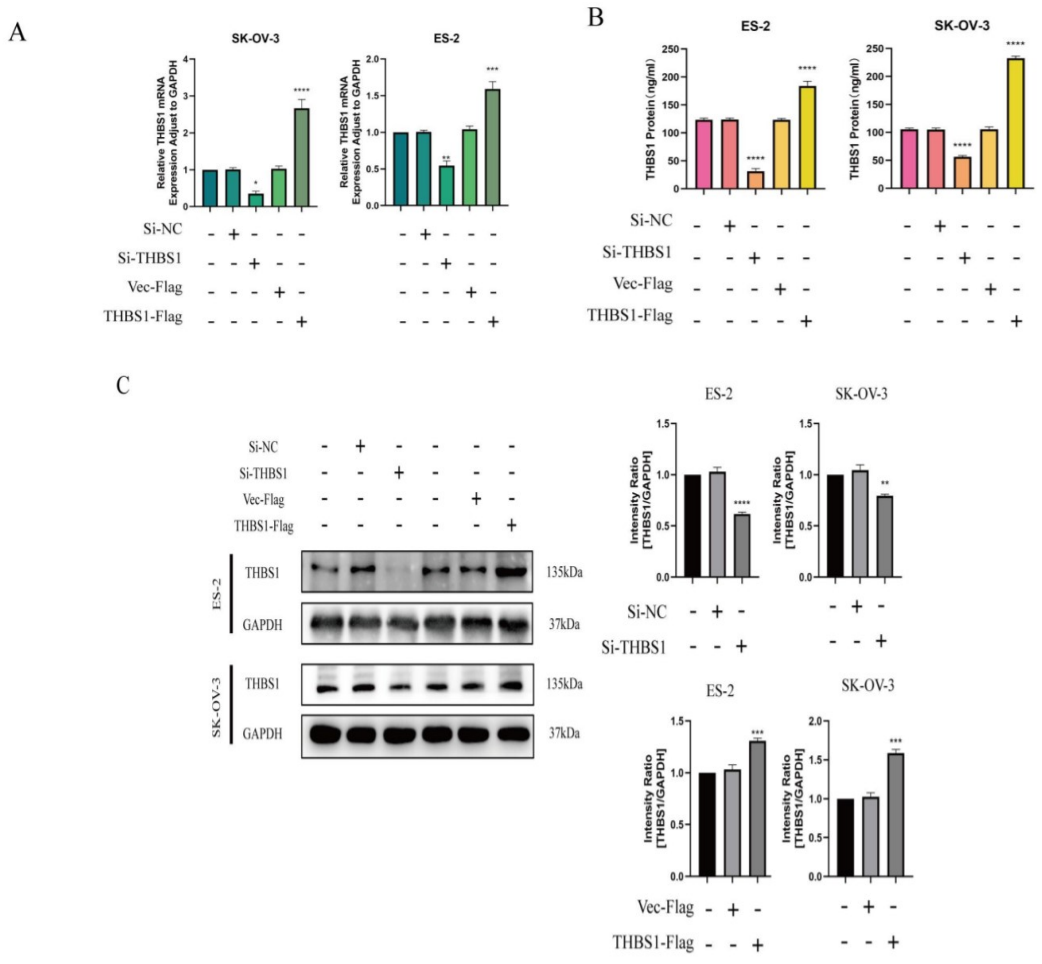


Figure 6. Overexpression of THBS1 in ES-2 and SK-OV-3 cells

(A)RT-qPCR was used to detect the change of THBS1 mRNA after knockdown and overexpression of THBS1. Ovarian cancer cells without any treatment were used as blank control, and the Relative change of THBS1 mRNA between each experimental group and the blank control group was used as the evaluation index.(B) Double antibody sandwich Elisa was used to detect the THBS1 content in the cell culture supernatant after THBS1 knockdown and overexpression.(C)Western Blot was used to detect the change of THBS1 expression in the whole protein of ovarian cancer cells after knockdown and overexpression of THBS1. Ovarian cancer cells without any treatment were used as blank control, and the density ratio of each experimental group compared with the blank control group was used as the evaluation index. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

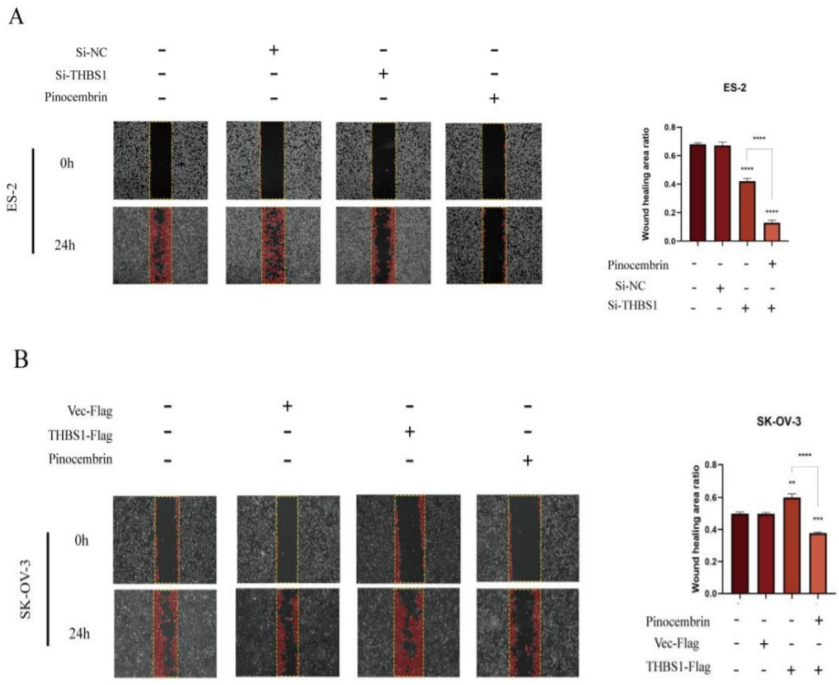


Figure 7.Changes in migration ability of ovarian cancer cells in scratch assay after knockdown and overexpression of THBS1

(A) changes in the migration ability of ovarian cancer cells after THBS1 knockdown;(B) after expressing THBS1 changes of ovarian cancer cell migration ability.The area rate of scratch healing was used to evaluate migration ability. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

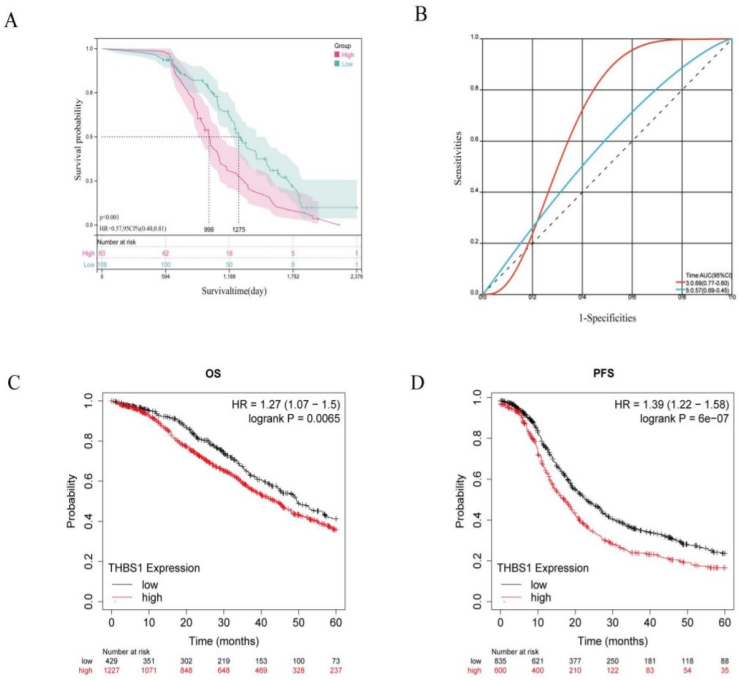


Figure 8.THBS1 relationship with the prognosis of ovarian cancer

(A)this study collected THBS1 expression of ovarian cancer patients with the relationship between the OS;(B) to THBS1 expression in risk factors for evaluating the prognosis of ovarian cancer model of ROC curve.(C) The relationship between THBS1 in the database and OS in ovarian cancer patients;(D) KM - plot database THBS1 the relationship between the patients with ovarian cancer PFS.

Through the above experiments, it is not difficult to find that the inhibitory effect of THBS1 in ovarian cancer is relatively obvious. Although some data and pictures do not fully show its advantages in preventing ovarian cancer. But in most pictures it's already. THBS1 can promote the migration and invasion of ovarian cancer cells.

Meanwhile, high expression of THBS1 was positively correlated with poor prognosis of ovarian cancer patients[11-14].

3. DISCUSSION

Experiments have proved that THBS1 is one of the targets of pinesin, and the results show that pinesin can indeed down-regulate the expression and secretion of THBS1 cells. Therefore, in the process of exploring the influence of pinesin on the expression and secretion of ovarian cells by experiments and double antibody sandwich method, it is particularly close to the organisms with tumor migration and invasion. Indeed, this is a multi-structure. Multifunctional calcium-binding glycoproteins, which are synthesized and secreted by tumor cells and various mechanisms, are associated with the biological behavior of matrix degradation in the core target genes of extracellular matrix degradation in ovarian cancer. The potential targets and bioinformatics tools of pinogenin were calculated by open source database mining. Pinogenin sites are also less complex than those for tumor inhibition, so it can be further demonstrated that pinogenin has an inhibitory effect on ovarian cancer migratory cells, and inhibiting the emt prohibition of ovarian cancer cells marks the outcome of these proteins to promote the development of aggressive phenotypes in cancer metastasis[15-18].

The recombinant cells share a loss of adhesion between adjacent epithelial cells of the top cells, resulting in upregulation of mesenchymal cell markers in tumor cells. In the progression of emt in epithelial tumors represented by ovarian cancer, epithelial cell markers are often missing and can downregulate the interstitium. Cell marker proteinophosphorin treats attractive targets and subsequently signals critical steps in adhesion behavior. In contrast, self-phosphide acid is the main catalytic activity. It is a non-receptor tyrosine that regulates multiple biological functions of tumor migration and invasion. Stimulating extracellular matrix degradation can also promote the invasion and expansion of cell endothelium, allowing cancer cells to pass through the

basal membrane and enter the vascular system, promoting the migration and invasion of cancer cells[19]. These proteins should be studied and paid attention to. In the ovarian cancer cell migration effect, the results make the proliferation effect of these pinoids not particularly obvious, but also can be inhibited. Metastatic cleaning of ovarian cancer cells[20].

4. CONCLUSION

These processes can be studied in this experiment. Pinicin may affect the expression and secretion of THBS1 to affect ovarian metastasis and invasion, which must be the target of condoning surgery, and can significantly inhibit the invasion and metastasis of ovarian cancer at a low concentration. Gonogenin has a fairly obvious inhibitory effect on ovarian cancer cells. There is a positive correlation between THBS1 and poor prognosis in ovarian cancer patients with high mrna or protein expression. THBS1 promotes the migration, invasion and expression of ovarian cancer cells. Through comparative analysis of experimental studies and data charts, we can draw a conclusion. In the following experimental conclusions, pinicin can inhibit the transformation of THBS1, and can also bind and promote the development of ubiquitination. In the follow-up experimental studies, it is necessary to well study the detailed process of inhibiting the migration and proliferation of ovarian cancer cells. Pinicin reduces the ability of inhibiting ovarian cell migration by promoting THBS1 ubiquitination expression.

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