doi.org/10.36721/PJPS.2026.39.10.274.1

Effect of SJAMP on mTOR/p70S6K signaling pathway and invasion and migration ability of HeLa cells in cervical cancer

Jun Gao#, Yawen Liu# and Guifang Sun*
Department of Obstetrics and Gynecology, Northern Jiangsu People's Hospital affiliated to Yangzhou University, Yangzhou, Jiangsu, 225001, China

Abstract: **Background:** SJAMP, a natural polysaccharide compound, has demonstrated significant inhibitory effects on various tumor models. Whether it plays a role in cervical cancer is unclear. **Objectives**: To explore the effects of SJAMP on the mTOR/p70S6K signaling pathway and the invasion and migration abilities of cervical cancer HeLa cells. **Methods**: The HeLa cells were divided into blank/solvent/polymer control groups and 1.2, 3.2 and 6.4 g/L SJAMP treatment groups. The cell proliferation, migration, invasion abilities, as well as the expression of mTOR/p70S6K protein and mRNA were detected. **Results**: As the concentration of SJAMP increased, the proliferation, migration and invasion abilities of HeLa cells were significantly reduced (all $P < 0.05$) and the protein and mRNA expressions of mTOR and p70S6K also decreased in a concentration-dependent manner (all $P < 0.05$). **Conclusion**: SJAMP can effectively inhibit the proliferation, migration and invasion of cervical cancer HeLa cells and this inhibitory effect is strongly dose-dependent and correlates with the downregulation of the mTOR/p70S6K signaling pathway.

Keywords: HeLa cells in cervical cancer; Invasion ability; mTOR/p70S6K signaling pathway; Migration ability; SJAMP

Submitted on 06-08-2025 – Revised on 23-01-2026 – Accepted on 02-02-2026

INTRODUCTION

Cervical cancer is one of the most common malignancies with the highest incidence and mortality rates among women worldwide, posing a serious threat to public health (Singh *et al.*, 2023, Wu *et al.*, 2024). Statistical data indicate that over 500,000 new cases of cervical cancer are diagnosed each year globally, with more than half of the patients dying from the disease, making its mortality rate the highest among all malignant tumors (Filho *et al.*, 2025). Although early infection with human papillomavirus (HPV) is recognized as the primary cause of cervical cancer, only a small proportion of HPV-infected individuals eventually develop the disease, suggesting that genetic variations and abnormal activation of host molecular signals play critical roles in the progression of cervical cancer (Yuan *et al.*, 2021). From a pathological perspective, the activation and suppression of oncogenes are closely associated with the development and progression of cervical cancer and the process involves the combined effects of multiple factors and genes (Wloszek *et al.*, 2025). The (Yu *et al.*, 2022) signaling pathway has increasingly been recognized as a central hub regulating cell growth, proliferation, metabolism and survival. mTOR integrates various upstream signals, including growth factors, nutrients, energy levels and cellular stress, to coordinate biosynthetic processes by phosphorylating key downstream effector molecules. Among these, P70S6K is the most common and decisive protein downstream of the mTOR signaling pathway. The mTORC1 complex activates p70S6K, which in turn phosphorylates the S6 ribosomal protein, promoting protein translation and cell cycle progression, thereby driving tumor development (Deng *et al.*, 2024). The mTOR/p70S6K signaling pathway is aberrantly activated in various malignant tumors, such as breast cancer and lymphoma and its activation level is closely associated with poor prognosis. However, the specific role of this pathway in cervical cancer and its potential as a therapeutic target remains to be further explored.

In recent years, screening antitumor drugs from natural products has become an important direction in new drug development. SJAMP, a natural polysaccharide compound extracted from the body wall of the sea cucumber *Apostichopus japonicus*, has attracted widespread attention due to its broad biological activities (Li *et al.*, 2025, Lin *et al.*, 2022). SJAMP has demonstrated significant inhibitory effects on various tumor models, including S180 sarcoma, hepatocellular carcinoma H22 xenografts and breast cancer cells. Its mechanisms may involve inducing apoptosis, arresting the cell cycle and modulating immune responses. Moreover, studies have indicated that SJAMP can effectively inhibit the proliferation of cervical cancer HeLa cells and regulate Bax/Bcl-2 protein expression (Karaboga Arslan *et al.*, 2025, Li *et al.*, 2022). However, its precise molecular targets, particularly its regulatory effects on key signaling pathways, remain unclear. Therefore, this study is the first to link the antitumor effects of SJAMP, a marine natural active substance, with the mTOR/p70S6K signaling pathway. This not only provides a new theoretical perspective for the mechanism of SJAMP against cervical cancer but also offers important experimental evidence for the development of naturally derived anticancer drugs targeting the mTOR pathway.

**Corresponding author:* e-mail: 785564283@qq.com
#These authors contributed equally and are the co-first authors.

MATERIALS AND METHODS

Experimental cells

HeLa cell line of cervical cancer was purchased from the Chinese Academy of Medical Sciences.

Reagents and instruments

Reagents and instruments in this experiment are SJAMP, purchased from Tianjin Institute of Materia Medica; high glucose medium and fetal bovine serum, purchased from Gibco, USA; mTOR, P-mTOR and p70S6K antibodies, purchased from Santa Cruz; paraformaldehyde, purchased from Shanghai Biyuntian biotech company; and low-temperature high-speed centrifuge purchased by Coulter.

Experimental method

Cell culture and grouping

Cultivated HeLa cells in fetal bovine serum(FBS), then placed the cells in high glucose medium with penicillin and streptomycin for further culture; then placed cells in the 37°C incubator with 5% CO_2, the liquid in the incubator should be changed every two days, then selected well-growing cells for the following experiments.

HeLa cells were divided into the following groups: A control group (complete culture medium), a solvent control group (the solvent used to dissolve SJAMP, e.g., PBS), a polysaccharide control group (treated with 6.4 g/L dextran) and SJAMP-treated groups. Based on previously reported effective concentrations for antitumor activity (Song *et al.*, 2013), the SJAMP-treated groups were set at 1.2 g/L (24–60 μmol/L), 3.2 g/L (64–160 μmol/L) and 6.4 g/L (128–320 μmol/L).

To verify the specificity of SJAMP's effects, a solvent control group and a polysaccharide control group were established. Preliminary experiments confirmed that, compared with the control group, the solvent control and polysaccharide control groups showed no significant differences in cell viability, migration, or invasion ability ($P > 0.05$). Therefore, in all subsequent experiments examining cell phenotypes and signaling pathways, only data from the control group, 1.2 g/L SJAMP group, 3.2 g/L SJAMP group and 6.4 g/L SJAMP group are presented to clearly demonstrate the dose-dependent effects of SJAMP.

MTT for detecting cell proliferation ability

HeLa cells in the logarithmic growth phase were seeded into 96-well plates at a density of 5×10^3 cells per well. After incubation for 0, 24, 48 and 72 hours, 20 μL of MTT solution (5 mg/mL, dissolved in PBS) was added to each well, followed by further incubation for 4 hours (Ghasemi *et al.*, 2021). The supernatant was then discarded and 150 μL of DMSO was added to each well. The plates were shaken for 10 minutes to fully dissolve the formazan crystals. The absorbance (OD value) of each well was measured at a wavelength of 570 nm using a microplate reader and the cell proliferation rate was calculated. Each group consisted of six replicate wells and the experiment was independently repeated three times.

The effect of 0.5–10 g/L SJAMP on the proliferation of HeLa cells was assessed using the MTT assay. A dose-response curve was plotted and the IC50 value was calculated using GraphPad Prism software.

Scratch test for detecting cell migration ability

Established a scratch model after 72 hours of culture and scratched cells with a sterile pipette tip (Merino-Casallo *et al.*, 2022). For further inspections, the equal distances of the edges of the scratch should be marked clearly; gently wash the cells 2-3 times with PBS to remove the detached cells and then replace them with a medium free of FBS to inhibit cell proliferation. Observed the degree of healing 24 hours later under microscope and took photos. Measured the distance between the scratches and compared it with the primary distance and represented the distance reduction as a percentage. The results reflect the cells' ability to migrate. The above experiment was repeated three times and the average value was taken.

Transwell for detecting cell invasion ability

The Matrigel basement membrane matrix (approximately 10 mg/mL) was diluted with pre-cooled serum-free medium at a ratio of 1:8 to achieve a working concentration of approximately 1.25 mg/mL (Aslan *et al.*, 2021). Then, 50 μL of the diluted Matrigel solution was evenly spread onto the polycarbonate membrane of the upper chamber of the Transwell insert and any excess liquid was removed. The chamber was placed in a 37°C incubator for 1 hour to allow polymerization. Cells from the four experimental groups (control, 1.2, 3.2 and 6.4 g/L SJAMP) were seeded into the upper chamber at a density of 1×10^4 cells per well, while the lower chamber was filled with medium containing FBS for incubation. After incubation, the culture medium was gently washed away with PBS and allowed to air-dry. The membrane was then rinsed gently with PBS. Five random fields were selected under an inverted microscope and the number of cells that had migrated through the membrane in each field was counted. The average value was calculated and used as the number of invasive cells for each sample.

Western blot for detecting mTOR and p70S6K protein expression

HeLa cells from these four groups were lysed using RIPA lysis buffer containing 1 mmol PMSF and phosphatase inhibitors. The lysates were centrifuged at 12,000 rpm for 15 minutes at 4 °C to collect the total protein supernatant. Protein concentration was determined using a BCA protein assay kit. For subcellular fractionation, cytoplasmic proteins were extracted strictly following the instructions of the Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology, P0027).

Equal amounts of proteins were separated by 8–10% SDS-PAGE and then transferred to PVDF membranes. The

membranes were blocked with 5% non-fat milk at room temperature for 1 hour, followed by incubation overnight at 4 ℃ with the following primary antibodies: anti-mTOR (1:1000), anti-p70S6K (1:1000) and anti-β-actin (1:5000; used as a loading control for total and cytoplasmic proteins). For nuclear fraction controls, anti-Lamin B1 (1:1000) and anti-α-tubulin (1:5000) antibodies were used to assess the purity of cytoplasmic and nuclear fractions, respectively.

After washing, the membranes were incubated with appropriate HRP-conjugated secondary antibodies (1:5000) at room temperature for 1 hour. Protein bands were visualized using an enhanced chemiluminescence (ECL) kit and quantified with ImageJ software. The expression levels of mTOR and p70S6K were normalized to β-actin and data are presented as mean ± standard deviation (n = 3).

Reverse transcription-polymerase chain reaction (RT-PCR) for detecting mTOR and p70S6K mRNA expression

Cells were lysed with TrIzol reagent to extract total RNA. RNA concentration and purity were assessed using a spectrophotometer. Prior to reverse transcription, 1 μg of total RNA was treated with DNase I (RNase-free). qPCR was performed using TB Green Premix Ex Taq II on a QuantStudio 5 Real-Time PCR System. Each 20 μL reaction mixture contained: 10 μL of TB Green Premix, 0.8 μL forward primer, 0.8 μL reverse primer, 2 μL cDNA template and 6.4 μL nuclease-free water. The amplification conditions were as follows: initial denaturation at 94 ℃ for 4 minutes; followed by 40 cycles of denaturation (94 ℃ for 30 seconds), annealing (50 ℃ for 40 seconds) and extension (72 ℃ for 7 minutes); finally, a melting curve analysis was performed (Table 1).

Statistical analysis

Data are presented as mean ± standard deviation. All statistical analyses were performed using SPSS software (version 27.0). Normality of data distribution was assessed using the Shapiro-Wilk test and homogeneity of variance was verified by Levene's test. One-way analysis of variance (ANOVA) was conducted to compare differences among the four groups in terms of cell proliferation, migration, invasion and protein/mRNA expression levels. When ANOVA indicated a statistically significant difference ($P < 0.05$), post-hoc pairwise comparisons were carried out using Tukey's HSD test (in case of homogeneous variances) or the Games-Howell test (in case of heterogeneous variances). A P-value of less than 0.05 was considered statistically significant.

RESULTS

Comparison of cell proliferation ability in four groups

The IC50 value of SJAMP for inhibiting HeLa cell proliferation was 4.8 g/L (95% CI: 4.2–5.5). Based on this result, subsequent mechanistic studies employed concentrations of 1.2 g/L and 3.2 g/L (below the IC50) and 6.4 g/L (above the IC50). After 24, 48 and 72 hours of treatment, the proliferative capacity of HeLa cells in the 1.2, 3.2 and 6.4 g/L SJAMP groups was significantly lower than that of the control group ($P<0.05$), showing a concentration-dependent decrease, (Fig. 1).

Comparison of cell migration ability

The cell migration ability in 1.2g/L group, 3.2g/L group and 6.4g/L group was significantly lower than that of the control group ($P<0.05$) and the cell migration ability decreased gradually; the migration ability in the 6.4g/L group was the lowest ($P<0.05$) (Fig. 2).

Comparison of cell invasion ability

The invasion ability in 1.2g/L group, 3.2g/L group and 6.4g/L group was significantly lower than that of control group and gradually declined among groups. The invasion ability in the 6.4g/L group was the lowest ($P<0.05$), (Fig. 3.

Western blot for detecting mTOR and p70S6K protein expression

In the 1.2 g/L, 3.2 g/L and 6.4 g/L SJAMP treatment groups, the expression levels of both mTOR and p70S6K proteins were significantly reduced compared to the control group ($P<0.05$), as determined by analysis of total protein extracts. This inhibitory effect was dose-dependent, with the 6.4 g/L group showing the lowest expression levels ($P<0.05$). Representative Western blot images (Fig. 4) show specific bands for mTOR, p70S6K and the loading control β-actin. Figure 5 presents the quantitative analysis of band intensities normalized to β-actin.

Comparison of mTOR and p70S6K mRNA expression in cells detected by RT-PCR

mTOR and p70S6K mRNA expression in 1.2g/L group, 3.2g/L group and 6.4g/L group were significantly lower ($P<0.05$) compared with the control group and they showed a gradual decrease among groups, with that in 6.4g/L group being the lowest ($P<0.05$) (Table 2 and Fig. 6).

DISCUSSION

The incidence of cervical cancer keeps climbing each year, with more than 500,000 new cervical cancer patients every year, among whom more than 250,000 patients die from it and its proportion of histological types has also undergone tremendous changes, posing a serious threat to female health (Wu et al., 2024). Nowadays, the common treatments for cervical cancer are radiotherapy and chemotherapy, while their effects haven't been desirable and the side effects are a concern in the treatment. Therefore, finding effective anti-tumor drugs is the clinical focus currently.

Table 1: Primer sequence list

mRNA	gene	Primer sequence	Premer length
mTOR	F	5'-TGCTGAAGCTGATGAGGAAG-3'	108bp
	R	5'-GGCAGGTCTTGTAGTTGGTG-3'	
p70S6K	F	5'-CAGCAGCCTCTACGAGTTCA-3'	119bp
	R	5'-TGCAGGACAGGGTCATTATT-3'	
GAPDH	F	5'-GTCTCCTCTGACTTCAACAGCG-3'	101bp
	R	5'-ACCACCCTGTTGCTGTAGCCAA-3'	

Table 2: Comparison of mTOR mRNA and p70S6K mRNA expression ($\bar{X}$±S)

Group	mTOR mRNA (relative level to internal control)	p70S6K mRNA (relative level to internal control)
Control group	0.63±0.31	0.59±0.25
1.2g/Lgroup	0.58±0.13[a]	0.48±0.21[a]
3.2g/Lgroup	0.31±0.07[ab]	0.34±0.15[ab]
6.4g/Lgroup	0.11±0.05[abc]	0.13±0.06[abc]
F	19.63	11.87
P	<0.0001	<0.0001

Note: a means P<0.05 compared with the control group; b means P<0.05 compared with the 1.2g/L group; c means P<0.05 compared with the 3.2g/L group.

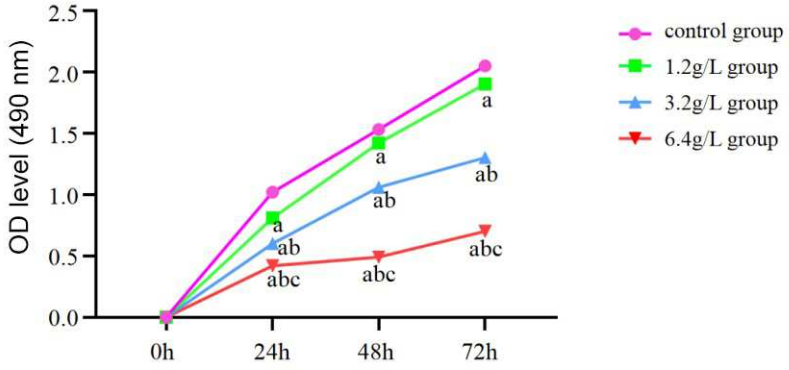

Fig. 1: Comparison of cell proliferation ability (n=3). Note: a means P<0.05 compared with the control group; b means P<0.05 compared with the 1.2g/L group; c means P<0.05 compared with the 3.2g/L group.

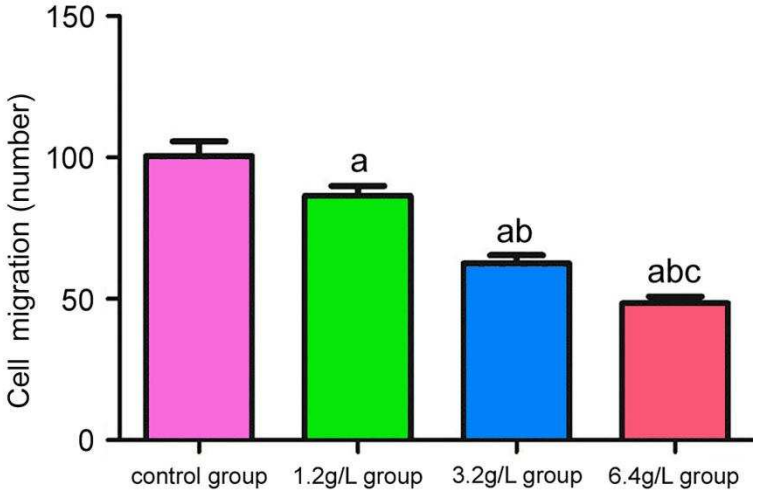

Fig. 2: Comparison of the migration ability (×200, n=3). Note: a means P<0.05 compared with the control group; b means P<0.05 compared with the 1.2g/L group; c means P<0.05 compared with the 3.2g/L group.

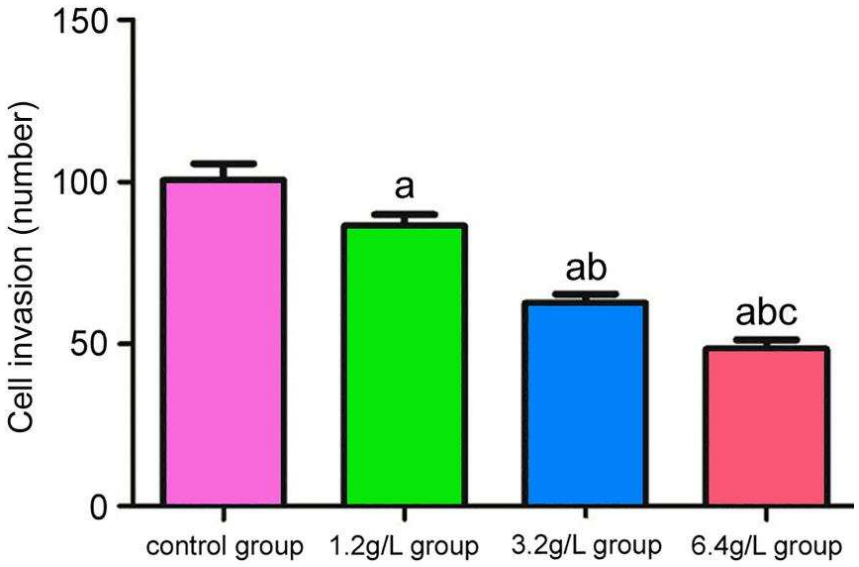

Fig. 3: Comparison of the number of HeLa cell invasion (n=3). Note: a means P<0.05 compared with the control group; b means P<0.05 compared with the 1.2g/L group; c means P<0.05 compared with the 3.2g/L group.

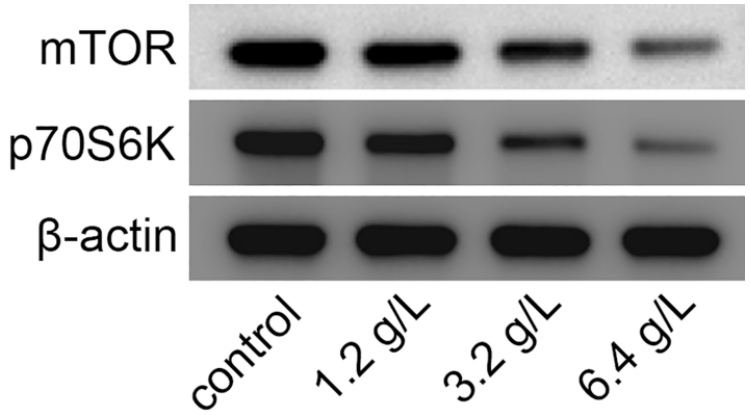

Fig. 4: mTOR and p70S6K protein expression in four groups (The molecular weight markers are displayed on the right side)

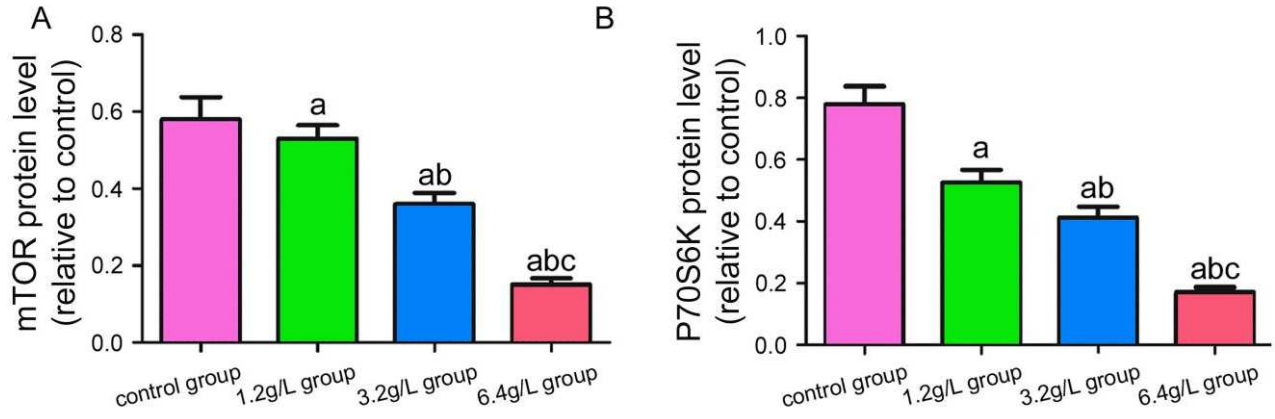

Fig. 5: Comparison of mTOR and p70S6K protein expression in four groups (n=3). Note: (A) is the comparison of mTOR protein in the four groups of cells; (B) is the comparison of p70S6K protein in the four groups of cells; a means P<0.05 compared with the control group; b means P<0.05 compared with the 1.2g/L group; c means P<0.05 compared with the 3.2g/L group.

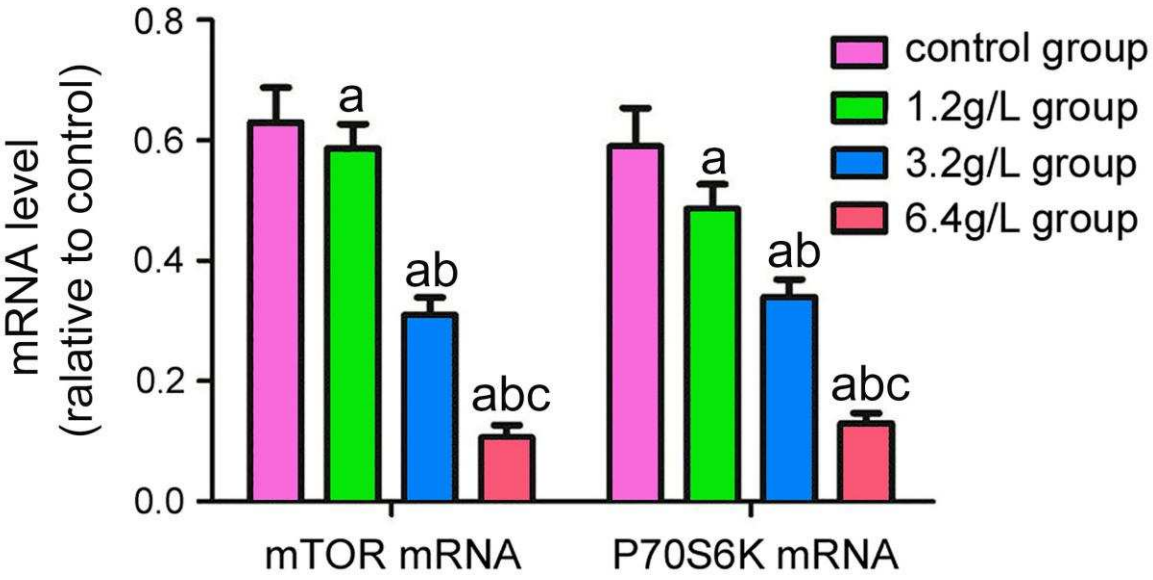

Fig. 6: Comparison of mTOR mRNA and p70S6K mRNA expression (n=3). Note: *a* means P<0.05 compared with the control group; *b* means P<0.05 compared with the 1.2g/L group; *c* means P<0.05 compared with the 3.2g/L group.

Studies have shown that SJAMP has an anti-tumor effect and can inhibit the proliferation and migration of malignant tumor cells while the research on its anti-tumor molecular mechanism still remains in the primary stage (Chen *et al.*, 2021). Furthermore, studies have indicated that SJAMP can inhibit DNA synthesis in rat S180 sarcoma and breast cancer cells, effectively suppressing tumor cell growth without interfering with the DNA synthesis of normal stem cells. This suggests that SJAMP may effectively promote the proliferation of stem cells. A previous study (Zhou *et al.*, 2023) demonstrated in a study on solid tumors in rats that SJAMP could alleviate the immunosuppressive effects induced by prednisone and significantly inhibit the development of solid tumors, lung cancer and gastric fibrosarcoma. Based on these findings, this study investigates the inhibitory effect of the marine bioactive compound SJAMP on the malignant phenotype of cervical cancer HeLa cells through regulation of the mTOR/p70S6K signaling pathway.

HeLa cells in cervical cancer has a strong invasion and metastasis ability and poor prognostic effect. Its mortality rate among all female malignant tumors is relatively high (Amemiya *et al.*, 2022, Guo *et al.*, 2022). It should be noted that a limitation of this initial approach was the use of nuclear protein extracts to detect mTOR and p70S6K, which are primarily localized in the cytoplasm. Subsequently, the study validated our key findings by examining their expression in total protein lysates. No significant differences ($P > 0.05$) were observed in cell viability, migration, or invasion ability among the control, solvent control and polysaccharide control groups, indicating that the observed effects were not attributable to the solvent, osmotic pressure, or general polysaccharide properties. In contrast, SJAMP treatment resulted in significant inhibitory effects compared to these control groups. As the concentration of SJAMP increased, the number of penetrated cervical cancer HeLa cells decreased, demonstrating that SJAMP suppressed both cell invasion and migration capabilities. While this study primarily focused on the effects of SJAMP on invasion, migration and the expression of key pathway molecules, it did not directly assess cell cycle or apoptotic effects. Therefore, the observed inhibition of proliferation at higher concentrations may theoretically include contributions from cytotoxic effects. Future studies employing flow cytometry to examine cell cycle distribution and apoptosis rates will help to more clearly distinguish between SJAMP's specific pathway inhibitory effects and non-specific cytotoxic actions. Although previous studies have confirmed that SJAMP inhibits HeLa cell growth (Le-Trung *et al.*, 2023, Turan *et al.*, 2022), most of its molecular targets remain unknown.

The research on the molecular biology of the occurrence and development of malignant tumors has become increasingly in-depth. The results found that effective regulation of cell growth is quite important in the occurrence of tumors. Therefore, the most important parts in the diagnosis and treatment of tumors are the signal pathways and cytokines, such as VEGFa and mTOR/p70S6K and others which mediate the occurrence of tumors and mTOR/p70S6K is the most important one among all pathways (Wang and Zhong, 2023). When rapamycin is used by different S. cerevisiae mutants to study its resistance, mTOR protein expression is found in many cells, which mediates the cells growth and differentiation. When mTOR signaling pathway is activated, it will inhibit the normal cells apoptosis and greatly promote the proliferation and differentiation of

normal cells. When cancerization occurs, the proliferation of cancer cells is affected by mTOR, thereby influencing cancer occurrence and development. p70S6K and 4EBPI are the two most important signal molecules in the mTOR; and p70S6K is the most direct substrate. The activated mTOR pathway activates p70S6K, which in turn promotes protein synthesis (Zhang *et al.*, 2022). This study determined the IC50 of SJAMP against HeLa cells to be approximately 4.8 g/L through a dose-response curve. The three experimental concentrations selected—1.2, 3.2 and 6.4 g/L—represent low, medium and high levels of inhibition, respectively, ensuring the reliability and physiological relevance of subsequent mechanistic investigations. The results demonstrated that SJAMP significantly inhibited the proliferation, migration and invasion capabilities of cervical cancer HeLa cells, while concurrently downregulating the expression levels of key proteins and genes in the mTOR/p70S6K signaling pathway. Both effects exhibited a significant concentration-dependent manner. These findings strongly suggest that inhibition of the mTOR/p70S6K signaling pathway may be closely associated with the antitumor effects of SJAMP. This aligns with the previous study (Zhou *et al.*, 2025), which indicated that regulation of the mTOR signaling can modulate the PI3K/AKT pathway. This study further precisely links the action of a natural product to this pathway, providing a solid theoretical foundation for the development and application of SJAMP. mTOR promotes apoptosis in cervical cancer cells and is subject to mutations across various tumor tissues, influencing the prognosis of radiotherapy and chemotherapy. However, inhibition of the mTOR/p70S6K pathway may not be the sole mechanism underlying SJAMP's efficacy. Given the extensive crosstalk between this pathway and other signaling networks such as PI3K/Akt and MAPK, the observed effects of SJAMP could result from either direct inhibition of mTOR or indirect mediation through upstream molecules. It is therefore plausible that SJAMP inhibits the mTOR/p70S6K pathway, thereby blocking pro-survival and proliferative signals, which in turn trigger cell cycle arrest and apoptosis. However, based on the current experimental design, this study cannot establish a direct causal relationship between these phenomena. The inhibitory effect of SJAMP on the mTOR/p70S6K pathway could be either its direct molecular target or a downstream consequence. Thus, further studies employing gain-of-function or loss-of-function experiments are necessary to robustly demonstrate the central causal role of the mTOR/p70S6K pathway in SJAMP's anti-cervical cancer effects.

CONCLUSION

In summary, this study demonstrates that SJAMP inhibits the proliferation, migration and invasion capabilities of cervical cancer HeLa cells in a concentration-dependent manner, accompanied by significant downregulation of mTOR/p70S6K signaling pathway activity. These results suggest that the mTOR/p70S6K pathway may represent an important mechanistic target for the antitumor effects of SJAMP. However, the precise causal relationship between SJAMP and pathway inhibition requires further in-depth investigation for validation.

Acknowledgment
This work was supported by the National Natural Science Foundation of China (Grant No. 82072088).

Authors' contributions
Jun Gao: Conceptualized the study, performed cell culture, MTT, wound healing and Transwell assays, collected and analyzed data and wrote the initial draft; Guifang Sun: Oversaw the overall research design, secured funding, supervised the experiments and reviewed and revised the manuscript; Yawen Liu: Contributed to Western blot and RT-PCR experiments, participated in literature research, figure preparation and manuscript proofreading. All the authors have read and approved the final version of the manuscript for submission.

Funding
None.

Data availability statement
All data generated or analysed during this study are included in this published article.

Ethical approval
This study involving human specimens was approved by the Ethics Committee of the Northern Jiangsu People's Hospital Affiliated to Yangzhou University [Approval No.:(2025ky210)].

Conflicts of interest
The authors declare no conflict of interest.

REFERENCES

Amemiya T, Shibata K, Takahashi J, Watanabe M, Nakata S, Nakamura K and Yamaguchi T (2022). Glycolytic oscillations in HeLa cervical cancer cell spheroids. *FEBS J,* **289**(18): 5551-5570.

Aslan M, Hsu EC, Liu SQ and Stoyanova T (2021). Quantifying the invasion and migration ability of cancer cells with a 3D Matrigel drop invasion assay. *Biol Methods Protoc,* **6**(1): bpab014.

Chen L, Wang Z, Du S and Wang G (2021). Antimicrobial activity and functional genes of actinobacteria from Coastal Wetland. *Curr Microbiol,* **78**(8): 3058-3067.

Deng D, Wu Y, Wu K, Zeng N and Li W (2024). Dihydroberberine alleviates Th17/Treg imbalance in premature ovarian insufficiency mice via inhibiting Rheb/mTOR signaling. *Mol Med,* **30**(1): 194.

Filho AM, Laversanne M, Ferlay J, Colombet M, Pineros M, Znaor A, Parkin DM, Soerjomataram I and Bray F (2025). The GLOBOCAN 2022 cancer estimates: Data sources, methods and a snapshot of the cancer burden worldwide. *Int J Cancer,* **156**(7): 1336-1346.

Ghasemi M, Turnbull T, Sebastian S and Kempson I (2021). The MTT assay: Utility, limitations, pitfalls and interpretation in bulk and single-cell analysis. *Int J Mol Sci,* **22**(23): 12827.

Guo F, Liu Y, Cheng Y, Zhang Q, Quan W, Wei Y and Hong L (2022). Transcriptome analysis reveals the potential biological function of FSCN1 in HeLa cervical cancer cells. *PeerJ,* **10**: e12909.

Karaboga Arslan AK, Eminoglu S, Pasayeva L, Bozkurt NM and Tugay O (2025). Centaurea lycaonica extracts induce apoptosis in HeLa human cervical cancer cells via Bax/Bcl-2 modulation and caspase activation: An LC-HRMS-based study. *Food Sci Nutr,* **13**(7): e70528.

Le-Trung N, Duong TM, Dang TTP and Kamei K (2023). Potent anti-cancer activity of *Sphaerocoryne affinis* fruit against cervical cancer HeLa cells via inhibition of cell proliferation and induction of apoptosis. *BMC Complement Med Ther,* **23**(1): 290.

Li C, Zhang L, Liu C, He X, Chen M and Chen J (2022). Lipophilic grape seed proanthocyanidin exerts anti-cervical cancer effects in HeLa cells and a HeLa-Derived xenograft zebrafish model. *Antioxidants (Basel),* **11**(2): 422.

Li S, Xiao Y, Li Q, Su M, Guo Y and Jin X (2025). Recent advances in natural products derived from marine echinoderms and endophytic microbes: Chemical insights and therapeutic potential. *Mar Drugs,* **23**(1): 33.

Lin P, Shen N, Yin F and Guo SD (2022). Sea cucumber-derived compounds for treatment of dyslipidemia: A review. *Front Pharmacol,* **13**:1000315.

Merino-Casallo F, Gomez-Benito MJ, Hervas-Raluy S and Garcia-Aznar JM (2022). Unravelling cell migration: Defining movement from the cell surface. *Cell Adhesion & Migration,* **16**(1): 25-64.

Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B, Arbyn M, Basu P, Bray F and Vaccarella S (2023). Global estimates of incidence and mortality of cervical cancer in 2020: A baseline analysis of the WHO global cervical cancer elimination initiative. *Lancet Glob Health,* **11**(2): e197-e206.

Song Y, Jin SJ, Cui LH, Ji XJ and Yang FG (2013). Immunomodulatory effect of Stichopus japonicus acid mucopolysaccharide on experimental hepatocellular carcinoma in rats. *Molecules,* **18**(6): 7179-7193.

Turan I, Demir S, Yaman SO, Canbolat D, Mentese A and Aliyazicioglu Y (2022). An investigation of the antiproliferative effect of rhododendron luteum extract on cervical cancer (HeLa) cells via Nrf2 signaling pathway. *Nutr Cancer,* **74**(5): 1882-1893.

Wang M and Zhong XG (2023). Detection and significance of AKT/mTOR/P70S6K signaling pathway in gastrointestinal stromal tumors. *Asian J Surg,* **46**(12): 5707-5708.

Wloszek E, Krupa K, Skrok E, Budzik MP, Deptala A and Badowska-Kozakiewicz A (2025). HPV and cervical cancer-biology, prevention and treatment updates. *Curr Oncol,* **32**(3): 122.

Wu S, Jiao J, Yue X and Wang Y (2024). Cervical cancer incidence, mortality and burden in China: A time-trend analysis and comparison with England and India based on the global burden of disease study 2019. *Front Public Health,* **12**: 1358433.

Yu L, Wei J and Liu P (2022). Attacking the PI3K/Akt/mTOR signaling pathway for targeted therapeutic treatment in human cancer. *Semin Cancer Biol,* **85**: 69-94.

Yuan Y, Cai X, Shen F and Ma F (2021). HPV post-infection microenvironment and cervical cancer. *Cancer Lett,* **497**(243-254.

Zhang X, Liu H, Wang H, Zhao R, Lu Q, Liu Y, Han Y, LuluRen, Pan H and Han W (2022). B3galt5 deficiency attenuates hepatocellular carcinoma by suppressing mTOR/p70s6k-mediated glycolysis. *Cell Mol Life Sci,* **80**(1): 8.

Zhou J, Guo Z, Peng X, Wu B, Meng Q, Lu X, Feng L and Guo T (2025). Chrysotoxine regulates ferroptosis and the PI3K/AKT/mTOR pathway to prevent cervical cancer. *J Ethnopharmacol,* **338**(Pt 3): 119126.

Zhou M, Qu R, Yin X, Qiu Y, Peng Y, Liu B, Gao Y, Bi H and Guo D (2023). Prednisone acetate modulates Th1/Th2 and Th17/Treg cell homeostasis in experimental autoimmune uveitis via orchestrating the Notch signaling pathway. *Int Immunopharmacol,* **116**:109809.