

ORIGINAL RESEARCH

Polygonatum sibiricum polysaccharide inhibits the proliferation of endometrial cancer cells by promoting cell death

Xianfeng Ma¹, Wan Liu^{2,*}

¹Department of Splenogastric Oncology, Daqing Hospital of Traditional Chinese Medicine, 163000 Daqing, Heilongjiang, China

²Department of Obstetrics and Gynecology, The First Hospital of Yulin, 718000 Yulin, Shaanxi, China

***Correspondence**

liuwan1770@163.com
(Wan Liu)

Abstract

Endometrial cancer (EC) is a type of malignant tumor with increasing global incidence and mortality rates, especially in regions with high rates of obesity. Polygonatum sibiricum polysaccharide (PSP) has shown promise in addressing deficiencies associated with EC. While PSP has demonstrated anti-cancer properties across various cancer types, its efficacy in EC remains underexplored. This study investigates the inhibitory effects of PSP on EC cell proliferation and autophagy. Our data demonstrate that PSP effectively inhibits the growth of EC cells and induces apoptosis. Furthermore, PSP triggers autophagy in EC cells. Mechanistically, PSP suppresses the Toll-like receptors (TLR4)/Signal Transducer and Activator of Transcription 3 (STAT3) pathway, thereby inhibiting EC progression. In conclusion, our findings highlight the significant therapeutic potential of PSP in EC treatment by inhibiting cell growth, inducing apoptosis, and triggering autophagy *via* the TLR4/STAT3 pathway.

Keywords

Endometrial cancer (EC); Polysaccharide (PSP); Autophagy; Apoptosis; TLR4/STAT3

1. Introduction

Endometrial cancer (EC) is a malignant tumor of the endometrial epithelium with a continuously increasing global incidence [1]. In 2020, statistical reports indicated that approximately 417,367 women worldwide were diagnosed with EC, with North America and Western Europe having the highest disease burden [2], and is believed to be largely attributed to lifestyle factors, particularly obesity, which accounts for about 50% of EC cases. The prognosis of patients with EC significantly depends on the stage of diagnosis. Patients with tumors confined to the uterus have an overall five-year survival (OFS) rate of $\geq 95\%$. However, this rate sharply decreases with disease progression, reaching 69% for those with local metastasis and 17% for individuals with distant metastasis [3].

According to Traditional Chinese Medicine (TCM) theory, deficiencies in the five organs and the balance of qi (life energy) and blood (the vital substance circulating through the body for nourishment and energy) are fundamental factors contributing to depression onset. Polygonatum sibiricum polysaccharide (PSP), a component of TCM, is well-known for its ability to nourish blood and yin (the passive, cooling energy), tonify the liver and spleen, moisten the lungs, generate fluids, and benefit the kidneys and qi. It serves to supplement deficiencies in the five organs, as well as in qi, blood, yin and yang (the active, warming energy) [4]. PSP has received considerable attention due to its diverse biological activities, such as immunomodulatory effects, antioxidant properties, anti-

inflammatory actions and potential as an anti-cancer agent. It has demonstrated potential in regulating blood sugar and lipids, enhancing hematopoiesis and protecting against depression-like behaviors through the reduction of oxidative stress and inflammation [5, 6]. In addition, PSP is recognized for its potential as a cancer chemopreventive agent. For instance, PSP derived from Siberian Solomon's Seal has been found to inhibit liver cancer within a simulated tumor microenvironment *via* the Toll-like Receptor 4 (TLR4)/Signal Transducer and Activator of Transcription 3 (STAT3) axis [7]. Moreover, PSP extracted from Polygonatum sibiricum has demonstrated anti-cancer potential in HepG2 cells by inducing cell cycle arrest and apoptosis. Furthermore, it has been shown to stimulate autophagy in prostate cancer-associated fibroblasts, thereby inhibiting their growth [8].

While the anti-cancer effects of PSP on different types of cancer have been extensively investigated, there is a lack of reports and ambiguity regarding its role in EC. In this study, we hypothesized that PSP holds potential as a promising therapeutic agent against EC. Thus, we investigated the inhibitory effects of PSP on the proliferation of EC cells by stimulating autophagy through modulation of the TLR4/STAT3 pathway.

2. Materials and methods

2.1 Cell culture

The human EC cell lines HEC-1A and Ishikawa were purchased from American Type Culture Collection (ATCC) (Man-

assas, VA, USA). Both cell lines were cultured using Roswell Park Memorial Institute (RPMI)-1640 complete medium and treated with PSP (Sigma, catalog No. P9275, St. Louis, MO, USA) for 24 hours at concentrations of 0, 20, 50 and 100 $\mu\text{g}/\text{mL}$.

2.2 Immunoblot

The proteins were initially separated *via* Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto Polyvinylidene Fluoride (PVDF) membranes and blocked with 5% milk for 1 hour. Following this, the membranes were incubated with primary antibodies, including BCL-2-associated X protein (Bax, 1:500, ab32503), B-cell lymphoma 2 (BCL-2, 1:1000, ab182858), Cleaved caspase-3 (1:1000, ab32042), Cleaved poly ADP-ribose polymerase (PARP) (1:1000, ab32064), Microtubule-associated proteins 1A/1B light chain 3B (LC3) (1:500, ab192890), Sequestosome 1 (P62, 1:1000, ab109012), STAT3 (1:500, ab68153), p-STAT3 (phosphorylation Y705, ab267373; 1:1000), TLR4 (1:1000, ab22048) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:3000, ab8245, Abcam, Cambridge, UK). Then, they were incubated with secondary antibodies for 1 hour, and the membranes were photographed following chemiluminescence (ECL).

2.3 Cell counting kit-8 (CCK-8) assay

A total of 1000 cells per well were seeded into 96-well plates and cultured for 48 hours, following which they were treated with CCK-8 (C0038, Beyotime, Beijing, China) for 4 hours, and their optical density was measured at 450 nm (OD450).

2.4 5-Ethynyl-2'-deoxyuridine (Edu) assay

EC cells were incubated with an Edu agent (Abcam, ab219801, Cambridge, UK) for 2 hours, then the agent was removed, and the cells were photographed.

2.5 Colony formation assay

The cells were initially seeded at a density of 400 cells per well in a 6-well plate and cultured for approximately two weeks. Then, they were fixed and stained with crystal violet, and the resulting cell colonies were manually counted.

2.6 Cell apoptosis assay

HEC-1A and Ishikawa cells were initially washed, fixed using 70% ethanol at $-20\text{ }^{\circ}\text{C}$ for 2 hours and stained with Propidium Iodide (PI) and Fluorescein Isothiocyanate (FITC) Annexin V at $4\text{ }^{\circ}\text{C}$, following which apoptosis levels were measured.

2.7 Statistics

GraphPad 5.0 software (GraphPad company, San Diego, CA, USA) was used for statistical analysis, and the data are represented as mean \pm Standard deviation (SD). Values with $p < 0.05$ were considered significantly significant.

3. Results

3.1 PSP inhibits the growth of EC cells

To evaluate the effects of PSP on the EC growth in cells, we first examined its effects on HEC-1A and Ishikawa cells at concentrations of 0, 20, 50 and 100 $\mu\text{g}/\text{mL}$ for 24 hours. CCK-8 assays revealed a decrease in the OD450 value with PSP treatment (Fig. 1A), Edu assays demonstrated a reduction in the percentage of Edu-positive cells following PSP treatment, indicating suppressed cell growth (Fig. 1B), and colony formation assays confirmed that PSP inhibited the growth of both HEC-1A and Ishikawa cells (Fig. 1C). These results collectively suggest that PSP treatment could impede EC cell growth.

3.2 PSP promotes apoptosis in EC cells

The results showed that PSP treatment promoted EC cell apoptosis, as evidenced by the increased apoptosis percentage in Fig. 2A. Immunoblot assays further confirmed these observations, demonstrating elevated expression levels of Bax, cleaved caspase-3, and cleaved PARP, along with decreased expression of BCL-2 upon PSP treatment (Fig. 2B,C). These results strongly indicate that PSP treatment could be used to increase the apoptosis of EC cells.

3.3 PSP induces autophagy in EC cells

Next, we explored the effects of PSP on the autophagy of EC cells by conducting immunoblot assays. Following treatment with PSP for 24 hours, HEC-1A and Ishikawa cells were examined and the results indicated that PSP treatment suppressed the expression of P62 in EC cells, indicative of autophagy activation (Fig. 3). Similarly, PSP treatment increased the ratio of LC3II/LC3I in EC cells, also suggesting enhanced autophagy (Fig. 3). Thus, these findings support the potential induction of autophagy by PSP in EC cells.

3.4 PSP suppresses the TLR4/STAT3 pathway in EC cells

Lastly, we detected the effects of PSP on the TLR4/STAT3 axis in EC cells by conducting immunoblot assays. The results revealed that PSP treatment led to decreased phosphorylation levels of STAT3 in EC cells. Additionally, the expression of TLR4 was suppressed following PSP treatment, suggesting inhibition of the TLR4/STAT3 pathway (Fig. 4). Taken together, our findings indicate that PSP could suppress the TLR4/STAT3 axis in EC cells.

4. Discussion

EC is a complex disease with increasing global incidence and mortality rates, particularly in North America and Western Europe [9]. Herein, we investigated the potential of PSP in inhibiting the proliferation of EC cells *via* autophagy through the TLR4/STAT3 signaling pathway, and the results demonstrated that PSP could be a therapeutic agent for EC.

PSP has demonstrated promising anti-tumor effects across various cancer types due to its anti-proliferative, pro-apoptotic, and anti-metastatic properties in liver cancer, prostate cancer,

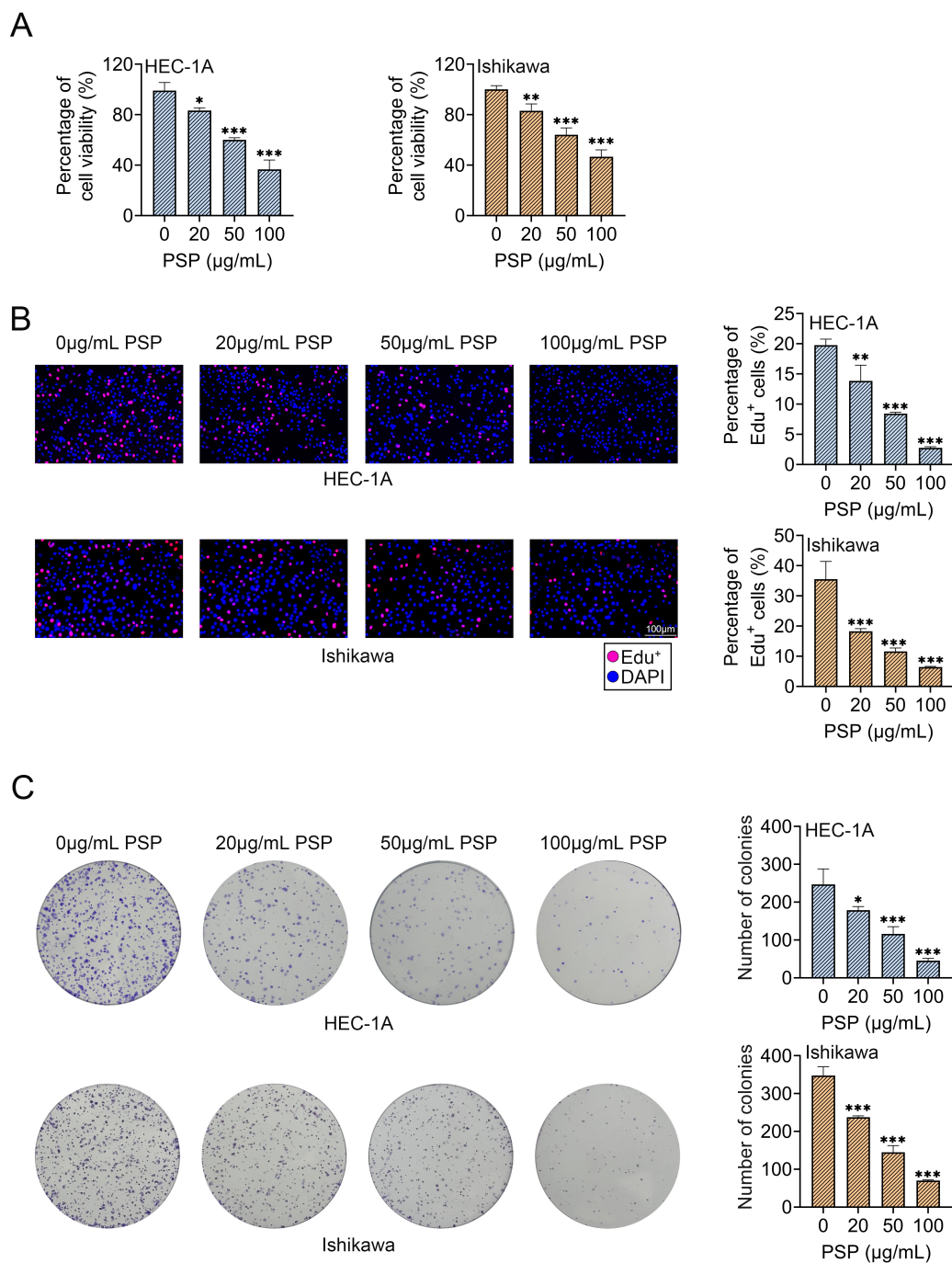


FIGURE 1. PSP inhibits the growth of endometrial cancer cells. (A) CCK-8 assays illustrating the impact of PSP on Ishikawa and HEC-1A cell growth at concentrations of 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours, and the corresponding OD450 values. (B) Edu assays depicting the effects of PSP on Ishikawa and HEC-1A cell growth at concentrations of 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. The percentage of Edu-positive cells was quantified. (C) Colony formation assays demonstrating the effects of PSP on Ishikawa and HEC-1A cell growth at concentrations of 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. Colony numbers were counted. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, PSP vs. control. PSP: Polygonatum sibiricum polysaccharide; DAPI: 4,6-diamidino-2-phenylindole; Edu: 5-ethynyl-2'-deoxyuridine.

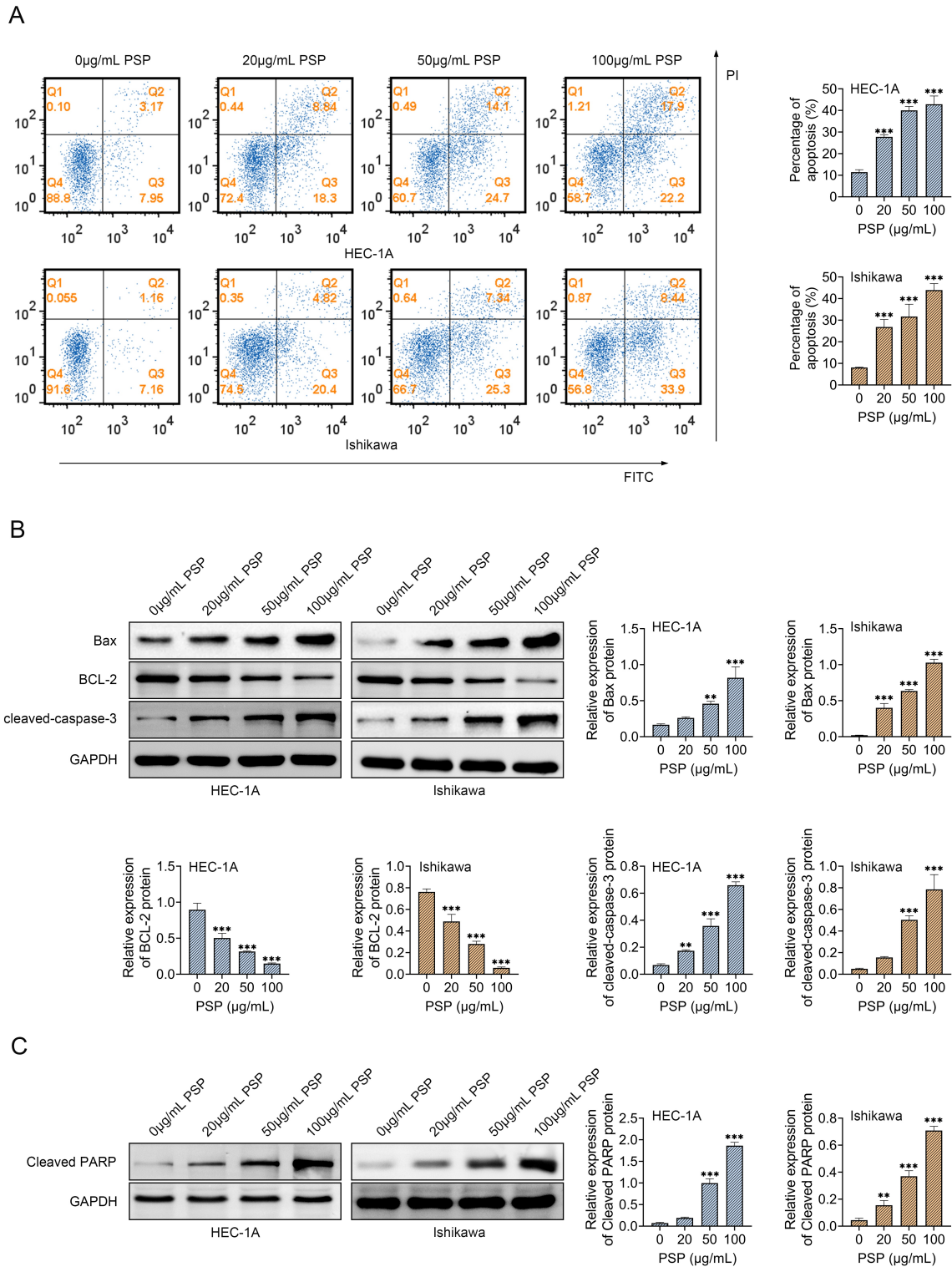


FIGURE 2. PSP promotes apoptosis in endometrial cancer cells. (A) Flow Cytometry (FCM) assays showing the effects of PSP on apoptosis in Ishikawa and HEC-1A cells at 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. The percentage of apoptotic cells was quantified. (B) Immunoblot assays illustrating the expression levels of Bax, BCL-2 and cleaved caspase-3 in Ishikawa and HEC-1A cells following PSP treatment at 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. The relative expression of these proteins was quantified, with GAPDH used as the loading control. (C) Immunoblot assays demonstrating the expression of cleaved PARP in Ishikawa and HEC-1A cells upon PSP treatment at concentrations of 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. $**p < 0.01$, $***p < 0.001$, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; PSP vs. control. PSP: Polygonatum sibiricum polysaccharide; PI: Propidium Iodide; FITC: Fluorescein Isothiocyanate; PARP: poly ADP-ribose polymerase.

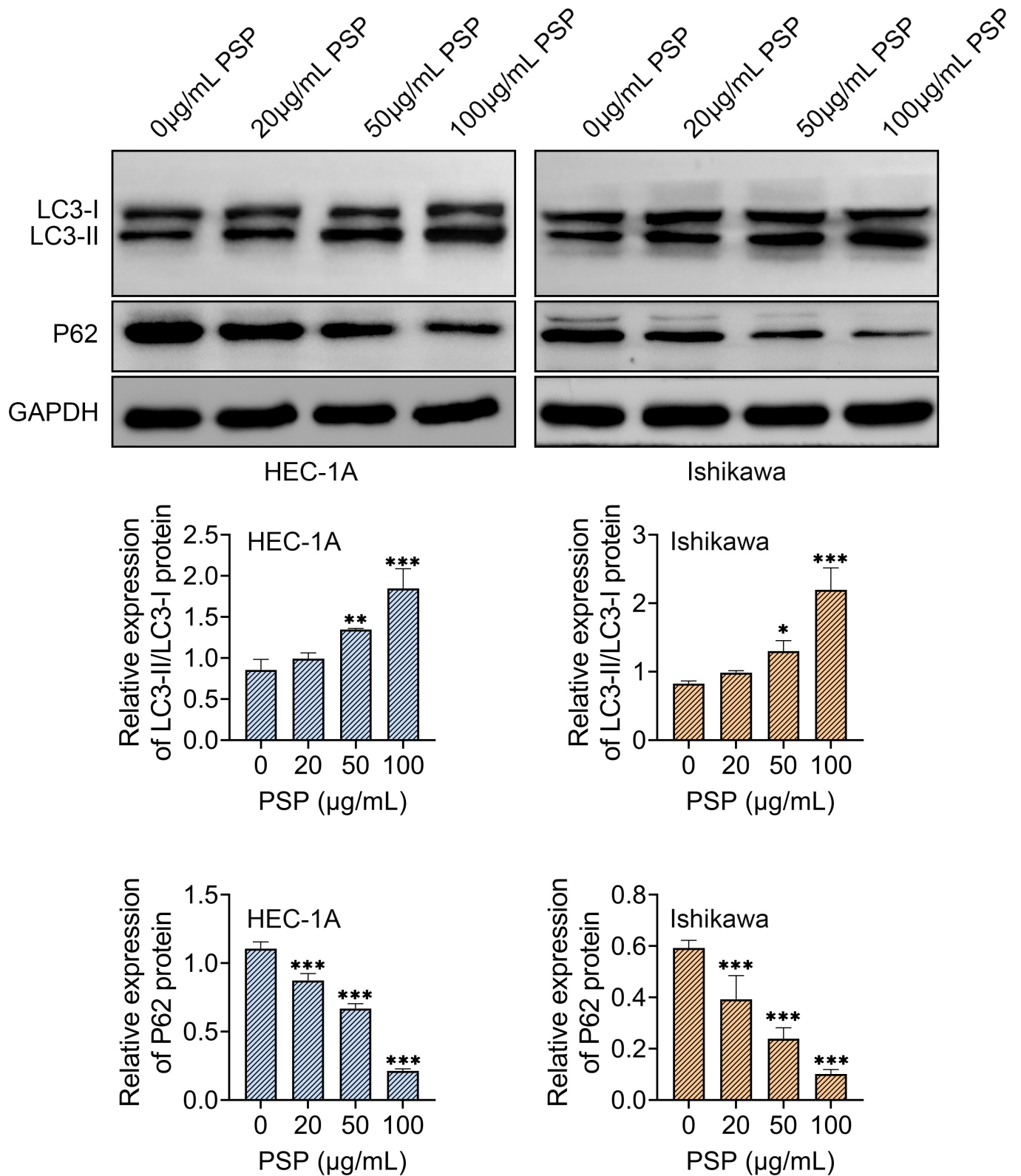


FIGURE 3. PSP induces autophagy in endometrial cancer cells. Immunoblot assays showing the expression levels of LC3 and P62 in Ishikawa and HEC-1A cells following PSP treatment at 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. The relative expression of P62 and the ratio of LC3II/LC3I were quantified, with GAPDH as the loading control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, PSP vs. control. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; PSP: Polygonatum sibiricum polysaccharide; LC3: light chain 3.

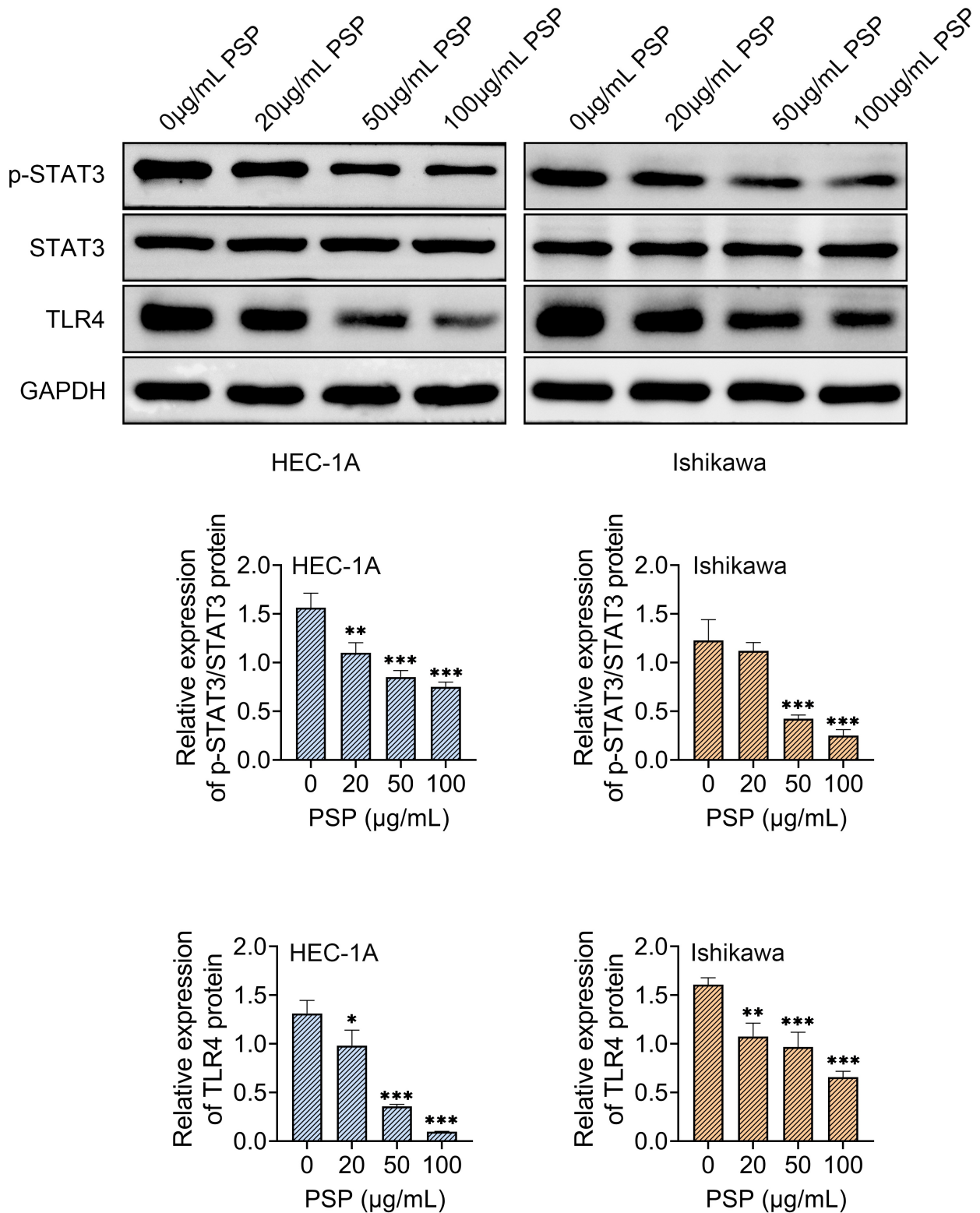


FIGURE 4. PSP suppresses the TLR4/STAT3 pathway in endometrial cancer cells. Immunoblot assays illustrating the expression levels of TLR4 and STAT3, as well as the phosphorylation status of STAT3 in Ishikawa and HEC-1A cells upon PSP treatment at 20, 50 and 100 $\mu\text{g/mL}$ for 24 hours. The relative expression or phosphorylation was quantified, with GAPDH used as the loading control. * $p < 0.015$, ** $p < 0.01$, *** $p < 0.001$, PSP vs. control. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; PSP: Polygonatum sibiricum polysaccharide; STAT3: Signal Transducer and Activator of Transcription 3; TLR4: Toll-like Receptor 4.

breast cancer and others [4, 10]. Studies have indicated that PSP can inhibit cancer cell growth, induce apoptosis, and reduce cancer cell metastasis [7, 11, 12]. Furthermore, PSP has been observed to modulate diverse signaling pathways implicated in cancer progression, including the TLR4/STAT3 pathway, hinting at its potential as a novel therapeutic anti-tumor agent. In this study, PSP was found to effectively restrain the growth of Ishikawa and HEC-1A EC cells, aligning with prior research demonstrating PSP's anti-proliferative effects across various cancer types. The inhibition of cell proliferation by PSP highlights its potential as a promising anti-cancer agent for EC.

Moreover, PSP was observed to enhance apoptosis in EC cells, demonstrated by an elevation in apoptotic markers such as Bax and cleaved caspase-3 and a reduction in the anti-apoptotic marker BCL-2. These findings suggest that PSP induces apoptosis in EC cells, in line with its demonstrated anti-cancer effects across other cancer types, such as liver cancer, prostate cancer, and breast cancer [10–12]. Increasing apoptosis is an essential strategy for impeding cancer cell growth and is regarded as a hallmark of effective anti-cancer agents [13].

Furthermore, PSP was observed to stimulate autophagy in EC cells, as evidenced by the increased LC3II/I ratio and decreased levels of P62. Autophagy is a cellular process that exerts a dual role in cancer, functioning as both a tumor suppressor mechanism and a pro-survival mechanism [13, 14]. In the context of this study, the induction of autophagy by PSP suggests that it may trigger a protective response in EC cells, ultimately leading to cell death.

The TLR4/STAT3 axis has been implicated in various cancers, including EC [15–17]. In this study, PSP was observed to inhibit the TLR4/STAT3 pathway in EC cells, as indicated by the downregulation of TLR4, STAT3 and p-STAT3, suggesting that the anti-cancer effects of PSP may be mediated through the inhibition of the TLR4/STAT3 axis.

Currently, the standard treatment for EC comprises surgery, chemotherapy, and radiation therapy [18]. However, these treatments are often accompanied by various side effects. In this regard, the use of natural compounds like PSP as adjuvant therapy for EC could present a safer and potentially more effective alternative [18].

Moreover, combining PSP with other anti-cancer agents could enhance its therapeutic efficacy and mitigate the risk of drug resistance [9]. Future investigations should focus on elucidating the underlying mechanisms of PSP-induced autophagy in EC cells and exploring its potential synergistic effects with other anti-cancer agents.

Despite the promising findings, this study had several limitations. Firstly, the *in vitro* nature of the study restricts its ability to fully replicate the complex interactions that occur *in vivo*, and further studies employing animal models are necessary to validate the findings. Secondly, we only focused on the TLR4/STAT3 axis, while other potential mechanisms underlying the anti-cancer effects of PSP in EC cells remain unexplored. Additionally, the study utilized a limited number of EC cell lines, necessitating further investigations with a broader range of cell lines to validate the results. Lastly, the study did not assess the potential side effects of PSP, which is

essential for evaluating its safety and feasibility as a therapeutic agent for EC.

5. Conclusions

In conclusion, our study highlights that PSP effectively inhibits EC cell growth by promoting autophagy through TLR4/STAT3 axis regulation. These findings suggest that PSP holds promise against EC and highlight the need for further investigation into its clinical utility as an anti-cancer agent.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

XFM—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript. XFM and WL—supervised the data collection, analyzed the data, interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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