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ORIGINAL RESEARCH

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Potential mechanism of Astragalus polysaccharide increasing chemosensitivity of cervical cancer HeLa cells to carboplatin by modulating the PI3K/Akt signaling pathway

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Abstract

The current study investigated the possible mechanisms in which astragalus polysaccharide (APS) elevates the chemosensitivity of cervical cancer (CC) HeLa cells to carboplatin (CBP) by regulating the PI3K/Akt pathway. In this study, HeLa cells were assigned to the control, CBP, APS, and APS + CBP groups, and the proliferation of HeLa cells in each group was detected using the MTT (methyl thiazolyl tetrazolium) assay. The apoptosis and cycle of HeLa cells were examined using flow cytometry, and the expression levels of PI3K (phosphatidylinositol-3-kinases), Akt (Protein Kinase B), p-PI3K (phospho-phosphatidylinositol-3-kinases), and p-Akt (phospho-Akt) proteins in the PI3K/Akt pathway were determined using Western blot. MTT results revealed that the inhibitory effect was more significant within the APS + CBP groups ($p < 0.01$). The flow cytometry results showed that the apoptosis rate was significantly elevated in the APS + CBP groups ($p < 0.01$). Compared with the control group, the most prominent arrest in the G2/M phase and the arrest within the G2/M phase in the APS + CBP groups being the most significant. Western-blot results depicted that the above proteins in the APS + CBP groups revealed more significant expression changes ($p < 0.01$). The above analyses demonstrated that APS could increase the chemosensitivity of CC HeLa cells to CBP by modulating the PI3K/Akt pathway. In conclusions, it is reasonable to believe that the enhancing effect of APS on the sensitivity of chemotherapeutic drugs was associated with blocking the PI3K/Akt pathway, thus, enhancing.

Keywords

Cervical cancer; PI3K/Akt pathway; Chemosensitivity; Astragalus polysaccharide; HeLa cells

1. Introduction

Cervical cancer (CC) is the fourth most prevalent cancer globally among women. Paclitaxel (paclitaxel) + cis-Platinum (cis-Platinum) (TP) is the standard regimen for treating CC, but cis-Platinum has nephrotoxicity and requires hydration treatment [1]. Carboplatin (CBP) is a platinum analog and not as nephrotoxic as cis-Platinum. It has been reported that CBP may be an alternative therapy that is feasible and less toxic in treating advanced or recurrent CC. However, another study demonstrated that paclitaxel + carboplatin (TC) was no less effective than TP. This study compared the efficacy and safety of TC and TP and observed that the overall response, disease control, and survival rates were similar in the TC and TP groups. However, the TC group had better tolerance, a significantly lower incidence of grade III–IV gastrointestinal toxic reactions, and a shorter hospital stay than the TP group. These findings indicate that TC could be a safe and effective alternative to TP for advanced or recurrent CC in clinical practice.

With the increasing number of cancer cases and deaths in the past few decades, chemically synthesized drugs have not significantly improved overall survival. Cancer treatment has a major medical challenge and demands a growing need for therapeutic approaches. The most effective treatment method for cancer is surgery, along with chemotherapy and radiation. Unfortunately, there is an increasing need for more effective anticancer drugs and new strategies to improve the success rate of cancer treatment due to the intrinsic or acquired drug resistance and the side effects of cancer treatment [2]. Therefore, new drugs for cancer prevention and new approaches to improve the anticancer efficiency of the currently used methods can be utilized. Natural compounds derived from plants, known as phytochemicals, are an essential resource for new drugs and anticancer treatments. Some typical examples include vinca alkaloids, including paclitaxel analogs, vinblastine and vincristine, and Podophyllotoxin analogs. These phytochemicals

often regulate molecular pathways related to tumorigenesis and development, including increasing antioxidation, inactivating carcinogens, inhibiting proliferation, inducing cell cycle arrest and apoptosis, and controlling the immune system.

Despite the efficiency of chemotherapeutic drugs, severe side effects and drug resistance affect their application. Natural products with anticancer activity may help to overcome these problems partially. Thus, finding a way to minimize the adverse effects of platinum and reverse drug resistance without affecting the efficacy of chemotherapy could significantly enhance its effectiveness.

The ant-tumor activities of Astragalus polysaccharides (APS) has been achieved through diverse immunomodulatory activities, synergistic effects, toxicity reduction, reversing the resistance of tumor cells to chemotherapeutic drugs, regulating cell autophagy and the expression of autophagy-related proteins, and increases the level of autophagy [3–8]. APS has been applied to the adjuvant treatment of CC and achieved certain results [9]. Based on our previous studies, we have established through network pharmacology using TCGA (The Cancer Genome Atlas) disease and molecular target data that APS could intervene in CC carcinogenesis and progression using the phosphatidylinositol-3-kinases/Akt (PI3K/Akt) signaling pathway. Many PI3K/Akt signaling pathways exist within human cells, and a stable PI3K/Akt signaling pathway regulates normal cellular physiological activities, including cell proliferation, angiogenesis, metabolism, and chemoresistance [10, 11]. This pathway is essential for various physiological and pathological processes and, therefore, is considered a master regulator of cancer. Aberrant expression of proteins associated with the PI3K/Akt pathway can promote carcinogenesis and tumor development [12, 13].

In this experiment, we assessed and elucidated the mechanism of APS in enhancing chemotherapy sensitivity using the effect on CC cell proliferation, cell cycle, and apoptosis using methyl thiazolyl tetrazolium (MTT) assay, PI (Propidium Iodide) staining cell cycle assay, and AnnexinV-FITC (Fluorescein Isothiocyanate) staining apoptosis assay. Moreover, we verified whether it enhances CBP chemotherapy sensitivity through the PI3K/Akt pathway by identifying PI3K (phosphatidylinositol-3-kinases)/AKT (Protein Kinase B) pathway-related protein expression levels.

2. Materials and methods

2.1 Material

2.1.1 Cell strains

The gynecology team provided the CC HeLa cell line from the Experimental Center of the Second Hospital of Nanjing laboratory.

2.1.2 Drugs

Astragalus polysaccharide (APS) lyophilized powder (batch number C54201) was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China), and carboplatin (CBP) (batch number: c54201) was procured from MedChemExpress Co., Ltd (Monmouth Junction, NJ, USA).

2.1.3 Reagents

Dulbecco's modified Eagle's medium (DMEM) was bought from Hyclone (SH30243.01, Logan, UT, USA), the Penicillin mixture (100×) was purchased from Solarbio (P1400–100, Beijing, China), the Trypsin-EDTA (Ethylene Diamine Tetraacetie Acid) digestion solution (0.25%) was obtained from Solarbio (T1300–100, Beijing, China), MTT was procured from Sigma (M5655, St Louis, MO, USA), dimethyl sulfoxide (DMSO) was bought from Amresco (302A0316, Solon, Ohio, USA), RNaseA was purchased from Solarbio (R8020–25, Beijing, China), propidium iodide (PI) was provided by 7Seas Biotechnology Co., Ltd (C001–200, Shanghai, China), anhydrous ethanol was obtained from Sinopharm Chemical Reagent Co., Ltd (100092680, Shanghai, China), fetal bovine serum (FBS) was procured from GIBCO (16000–044, Grand Island, NY, USA), and the Annexin-V-FITC cell apoptosis detection kit was bought from Beyotime Biotechnology Co., Ltd (C1062, Shanghai, China).

2.1.4 Instruments

Centrifugal machines were provided by Lu Xiangyi Centrifuge Instrument Co. Ltd (TDZ4-WS, Shanghai, China; cryogenic freezing centrifuge, TG-16M), pipette guns were obtained from Pipetman®, Gilson, Inc. (P2, P10, P20, P100, P200, P1000, Middleton, Wis, USA), the enzyme label analyzer was purchased from Beijing Perlong New Technology Co., Ltd (DNM-9602, Beijing, China), cell culture consumables were procured from TRUELINE (TR4001, TR4002, TR5000, La Crosse, WI, USA), biological safety cabinets were obtained from Suzhou Jin Purification Equipment Technology Co., Ltd (BHC-1300IIB2, Suzhou, Jiangsu, China), CO_2 constant-temperature incubator was provided by Thermo Fisher Scientific (ThermoForma3111, Waltham, MA, USA), the microscope was purchased from Shanghai Caikon Optical Instrument Co., Ltd (ChinaXDS-600C, Shanghai, China), the flow cytometer was provided by BD Biosciences (AccuriC6, Franklin Lakes, NJ, USA), the real-time PCR (Polymerase Chain Reaction) was procured from (ABI-7300, ABI Inc., Foster City, CA, USA), the water bath kettle was obtained from Leica (HI1210, Solms, Germany), the vortex oscillator was purchased from Shanghai Qingpu-Huxi Instruments Factory (K30, Shanghai, China), the electric homogenizer was purchased from Shanghai FLUKO Equipment Co., Ltd (PRO200, Shanghai, China), the electromotor was bought from Dalian Jingmai Biotechnology Co., Ltd (PS-9, Dalian, Liaoning, China), the microplate reader was provided by Labsystems Ltd. (MK3, OY, Vantaa, Finland), the imaging system was purchased from Shanghai Tanon Technology Co., Ltd (Tanon-5200, Shanghai, China), and the electrophoresis instrument was procured from Bio-Rad Inc (miniprotean 3cell, Hercules, CA, USA).

2.2 Methods

2.2.1 Cell culture

CC HeLa cells were cultured in a complete DMEM medium (containing 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycins) with 5% CO_2 and saturated humidity at 37 °C. During cell passage, the cells were rinsed twice using

phosphate-buffered saline (PBS), digested with 0.25% EDTA-containing trypsin, and centrifuged after the complete medium was terminated. The cells were resuspended and passaged from 1:3 to 1:5.

2.2.2 Drug preparation

APS lyophilized powder was prepared using sterile water into 50 mg/mL of mother liquor, dispensed in 30 μL volumes, and stored inside a refrigerator at 4 ℃.

2.2.3 Selection of drug concentration

To better identify the difference between the effect of APS + CBP on HeLa cells and the drug alone and to select the optimal dosing concentration, relevant pre-experiments were performed, in which the final concentration of APS was 1, 2, 3, 4, 5 and 6 mg/mL, and the final concentration of CBP was 0, 25, 50, 75 and 100 μg/mL. The IC50 (50% inhibitory concentration) of APS became 3.9, 3.8 and 3.6 mg/mL after intervention for 24, 48 and 72 h, respectively. Moreover, the IC50 of CBP was 55.6, 50.4 and 46.5 μg/mL after intervention for 24, 48 and 72 h, respectively. The drug concentrations were based on the pre-experimental results, so the concentration of APS was chosen as 1 mg/mL for low, 3 mg/mL for medium, 6 mg/mL for high concentration, and CBP concentration was selected as 50 μg/mL.

2.2.4 Proliferation inhibition rate of cells measured by MTT assay

HPV (Human Papilloma Virus)-18-positive CC cell line (HeLa) at the logarithmic growth stage was seeded within 96-well culture plates at a cell density of 4×10^3 cells/well. 100 μL cell suspension was cultured per well at 37 ℃ and 5% CO_2 for 24 h. After that, the original culture medium was discarded, and the cells in the blank group were incubated without drugs. The cells were cultured for 24 h and 48 h within the following groups for subsequent experiments: blank, CBP 50 μg/mL, APS low concentration 1 mg/mL, APS medium concentration 3 mg/mL, APS high concentration 6 mg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL groups. The original medium was removed, and 10 μL MTT (mass concentration of 5 mg/mL) was added to the incubator for 4-h incubation. The supernatant was carefully aspirated, and 100 μL DMSO was introduced and shaken lightly for 10 min to terminate the reaction. The OD (optical density) value of each well was detected at 570 nm through an enzyme marker. The effect of APS on cell viability was determined by the cell proliferation rate (%), which was evaluated as follows: cell proliferation rate (%) = $(OD_{APS} - OD_{Blank})/(OD_{Control} - OD_{Blank}) \times 100\%$.

2.2.5 Cell cycle determined by PI single-staining method

The cells were cultured in the following groups for 48 h for subsequent experiments: blank control, APS 1 mg/mL, APS 3 mg/mL, APS 6 mg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL groups. The cells were detached using trypsin, and the cell culture medium was obtained. The cells were washed with PBS, centrifuged at 1000 g, and fixed in 700 μL of −20 ℃ pre-cooled anhydrous ethanol. Then, the cells were washed using PBS solution, and the cell precipitate was slowly resuspended with 100 μL of 1 mg/mL RNase A solution and warmed for 30 min at 37 ℃ to digest the intracellular RNA. Subsequently, the cells were added using 400 μL of PI at a concentration of 50 μg/mL, and the cycle distribution was detected through flow cytometry.

2.2.6 Cell apoptosis examined by Annexin V-FITC staining method

The cells were cultured for 48 h within the following groups for further experiments: blank control, APS 1 mg/mL, APS 3 mg/mL, APS 6 mg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL groups. The cells were detached with trypsin, and the cell culture medium was obtained. The cells were washed using PBS, centrifuged at 1000 g, mixed with 195 μL of Annexin V-FITC conjugate and 5 μL of Annexin V-FITC, incubated for 15 min at 4 ℃, and then mixed with 5 μL of PI. Apoptotic changes were detected using flow cytometry.

Expression levels of associated proteins in the PI3K/Akt signaling pathway determined using Western blot assay.

The cell concentration was adjusted to 2.5×10^6 cells/mL in the complete medium, and HeLa cells were incubated using 6-well plates for 24 h at 37 ℃ and 5% CO_2. The cells were cultured for 48 h at 37 ℃ and 5% CO_2 among the following groups: blank control, CBP 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL groups. HeLa cells were obtained after 48 h of culture, mixed with the lysate, and lysed on ice. The protein supernatant was aspirated for protein concentration through the Bradford method. Protein samples were boiled for 5 min using an equal volume of loading buffer before introducing into the loading wells. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was undergone using 10% separation gel and 5% concentrated gel. The gels were removed, and the protein samples were transferred onto nitrocellulose (NC) membranes through electroelution at 120 V on ice for 20 min. After that, the NC membranes were removed, kept in a sealing solution, and incubated at room temperature for 2 h. Then, the NC membranes were placed in the primary antibody incubation solution overnight at 4 ℃. Finally, the NC membranes were kept in the secondary antibody dilution solution and incubated for 1 h at room temperature. The primary and secondary antibodies were diluted using a blocking solution. PI3K, p-PI3K, Akt, and p-Akt antibodies were diluted at 1:2000, 1:2000, 1:2000, and 1:10,000, respectively. Moreover, the secondary antibody was horseradish peroxidase (HRP)-labeled IgG (Immunoglobulin G) diluted at 1:5000. Lastly, the enhanced chemiluminescence (ECL) reagent was utilized for color development.

3. Results

3.1 Proliferation inhibition of HeLa cells

Compared with the blank control group, the proliferation of HeLa cells within the APS 1 mg/mL, APS 3 mg/mL, APS 6

mg/mL, CBP 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL groups were significantly inhibited ($p < 0.05$). As shown in Fig. 1A, at 24 hours, APS at 1, 3 and 6 mg/mL showed a significant inhibitory effect on cell proliferation, reducing the cell proliferation to lower than 60%, lower than 40% and about 10% respectively. At 48 hours, APS at 1, 3 and 6 mg/mL showed a significant inhibitory effect on cell proliferation, reducing the cell proliferation to about 65%, about 60% and about 8% respectively. The inhibitory effect of APS + CBP groups was significant ($p < 0.01$) and concentration-dependent. Compared with the APS 1 mg/mL and the CBP 50 μg/mL groups alone, the proliferation inhibition rate of cells within the APS + CBP groups was positively related to the elevation in concentration ($p < 0.01$) (Fig. 1B).

3.2 Cell cycle and apoptosis of HeLa cells

Compared with the blank control group, the apoptosis rate of HeLa cells within the APS 1 mg/mL, APS 3 mg/mL, APS 6 mg/mL, CBP 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL groups was significantly increased ($p < 0.05$). As shown in Fig. 2B, APS at 1, 3 and 6 mg/mL showed a significant apoptosis-inducing effect, increasing the apoptotic cell population to 18%, 24% and 30% respectively. Compared with the APS 1 mg/mL and the CBP 50 μg/mL groups alone, the apoptosis rate of cells in the APS + CBP group was significantly increased ($p < 0.01$) (Fig. 2A,B). The apoptosis rate of cells in the administered group was significantly enhanced, and the difference was statistically significant ($p < 0.01$) (Fig. 2A,B). CBP + APS at 1, 3 and 6 mg/mL also showed a significant apoptosis-inducing effect, increasing the apoptotic cell population to 32%, 38% and 42% respectively. Compared with the group administered alone, the number of cells in the G2/M phase increased with drug concentrations. APS at 6 mg/mL was found to be able to increase the proportion of G2 phase by 10% (Fig. 3B). The proportion of cells in the G1 phase decreased with a marked increase in the third peak after administering different concentrations of APS + CBP in HeLa cells for 48 h, indicating that many cells were stalled during the G2/M phase (Fig. 3). By blocking cell cycle progression at G2/M phase, APS could effectively inhibit the mitosis of tumor cells. Moreover, compared with the control group, the most prominent arrest in the G2/M phase and the arrest within the G2/M phase in the APS + CBP groups being the most significant, highlighting the contribution of blockade of mitosis in the synergistic anti-tumor activities of APS + CBP.

3.3 APS promotes the sensitivity of CC cells to CBP by inhibiting the PI3K-Akt signaling pathway

The effect of APS in combination with CBP on the activation of PI3K/Akt signaling was examined using western blotting. As shown in Fig. 4A,B, APS at 1 and 3 mg/mL was able to significantly enhanced the inhibitory effect of CBP on PI3K/Akt signaling pathway. The relative expression levels of p-PI3K and p-Akt proteins in the APS and CBP groups were significantly reduced ($p < 0.05$) compared with the blank control group. In contrast, the above proteins in the APS + CBP groups revealed more significant changes ($p < 0.01$). Meanwhile, the relative expression levels of PI3K and Akt proteins remained the same. Moreover, compared with the APS and CBP groups, there were no significant changes in the relative protein levels within the APS + CBP groups (Fig. 4A–C).

4. Discussion

Previous KEGG (Kyoto Encylopaedia of Genes and Genomes) enrichment analysis of network pharmacology revealed that the differentially expressed genes are primarily enriched within signaling pathways associated with cellular metabolism. The PI3K/Akt signaling pathway is critical for normal cell growth and survival in humans and regulates normal cellular physiological activities [14–16]. It is a significant regulator of cancer [17, 18] and a promising therapeutic target [19, 20] since it is frequently activated among various human cancers. Its possible mechanisms for promoting oncogenic transformation involve stimulation of cell proliferation, metabolic reprogramming, invasion or metastasis, and effective inhibition of autophagy and senescence [21, 22]. In the PI3K/Akt signaling pathway, Akt binds the cell membrane using phosphatidylinositol-dependent kinase. Moreover, threonine and serine phosphorylation contribute to Akt translocation from the cytoplasm to the nucleus and mediates enzymatic biological effects, including those involved during cell proliferation, apoptosis inhibition, cell migration, and carcinogenesis [23, 24]. The modulatory effect of APS on PI3K/Akt signaling has been documented in a number of cells. In Neuroendocrine rat pheochromocytoma PC12 cells, APS has been found to activating PI3K/Akt signaling to increase cellular autophagy level *in vitro*, hence exhibit anti-parkinson activities [8]. Another study by Cao *et al.* [25] showed that APS suppresses doxorubicin-induced cardiotoxicity by activating the PI3K/Akt and p38MAPK pathways. In our studies, we found that APS was able to enhance the inhibitory effect of CBP on the activation of PI3K/Akt signaling, providing experimental evidence that PI3K/Akt signaling might be involved in the anti-cancer mechanisms of APS. These combined results implicated that APS might exert distinct effect on PI3K/Akt signaling in different settings.

APS has the Buzhongyiqi effect (tonifying the middle and benefiting the qi), and it can enhance efficacy and reduce toxicity when combined with cytotoxic drugs [26]. Sun Shuyu *et al.* [27] observed that APS could effectively inhibit the growth of tumors in rats, and its combination with CBP had a synergistic effect. Zhang Mingming [28] identified that chemotherapy could suppress the immune function of gastric cancer patients after surgery, and APS could release this suppression and improve the overall immune status of patients. Yu Dongqing *et al.* [9] found through experiments that APS enhanced the sensitivity of HeLa cells to CBP by increasing the autophagic activity of HeLa cells. Cui Wei *et al.* [29] observed that APS combined with the TP regimen improved the quality of life of cervical cancer patients, reduced bone marrow suppression, alleviated adverse effects, and reduced the ratio of immunosuppressive cells, Treg, and myeloid-derived suppressor cells

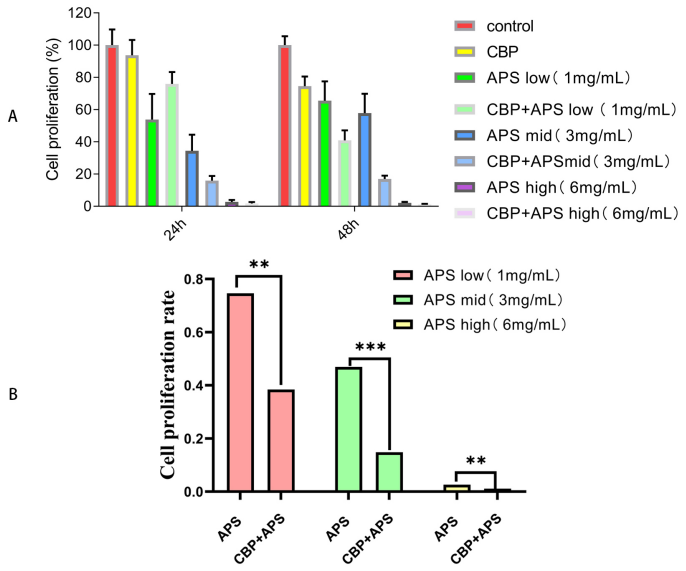

FIGURE 1. MTT analysis of cell proliferation following treatment. HeLa cells were treated with the APS at 1 mg/mL, APS at 3 mg/mL, APS at 6 mg/mL, CBP at 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL. (A, B) The inhibitory effect of APS + CBP groups was significant ($p < 0.01$) and concentration-dependent. $^{**}p < 0.01$ *vs.* APS group; $^{***}p < 0.005$ *vs.* APS group. CBP: cells to carboplatin; APS: Astragalus polysaccharides.

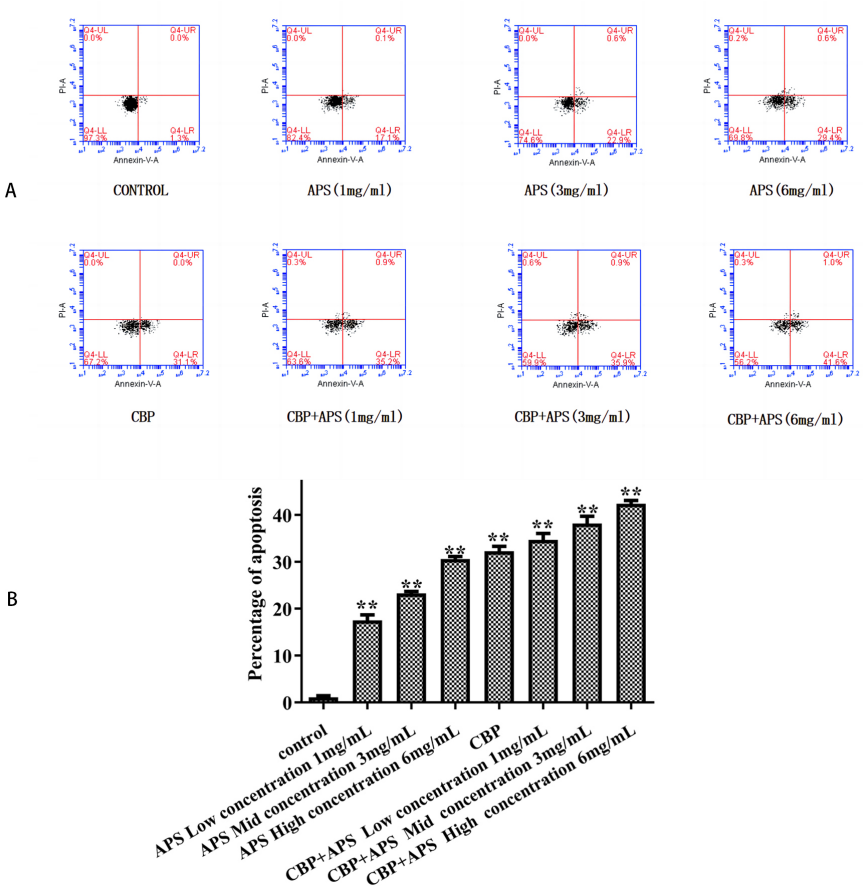

FIGURE 2. Flow cytometry analysis of cell apoptosis following treatment. HeLa cells were treated with the APS at 1 mg/mL, APS at 3 mg/mL, APS at 6 mg/mL, CBP at 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL. (A,B) The apoptosis rate of cells within the APS + CBP groups was significantly increased compared with the APS and CBP groups alone ($p < 0.01$). CBP: cells to carboplatin; APS: Astragalus polysaccharides. $^{**}p < 0.05$ versus control.

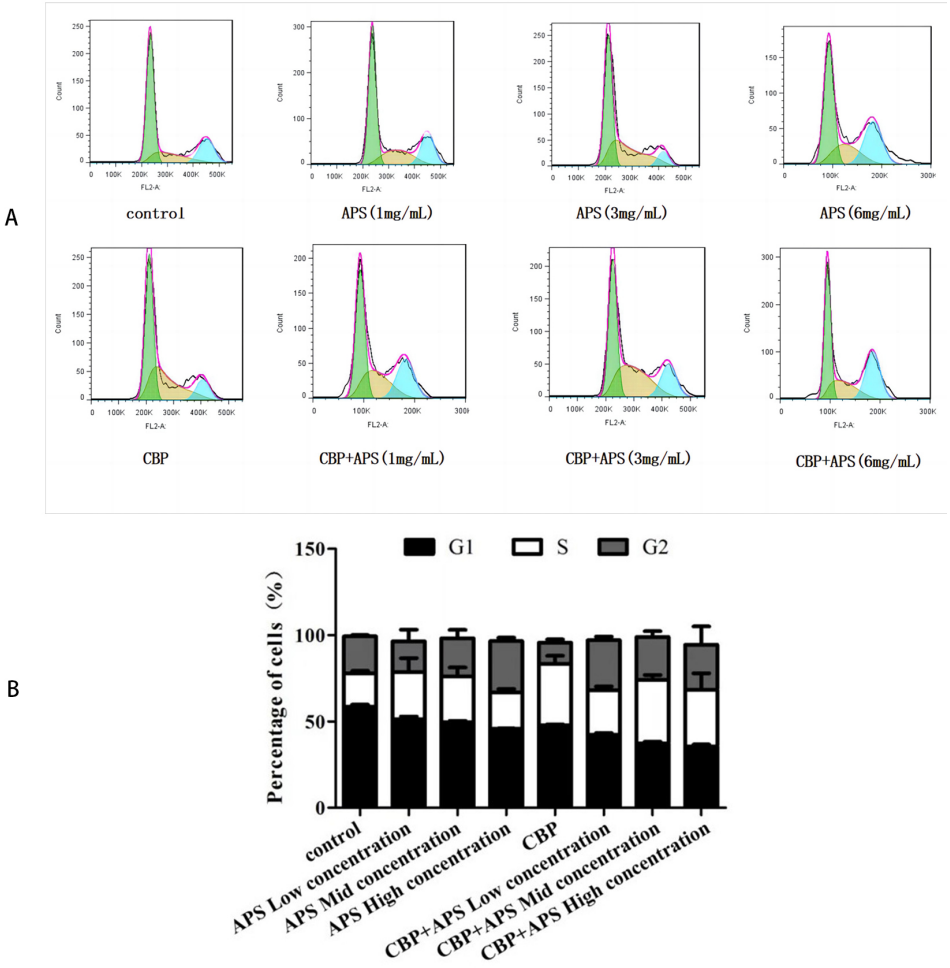

FIGURE 3. Flow cytometry analysis of cell cycle distribution following treatment. HeLa cells were treated with the APS at 1 mg/mL, APS at 3 mg/mL, APS at 6 mg/mL, CBP at 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, APS 3 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL. (A,B) Compared with the control group, the proportion of cells within the G1 phase was reduced, and the third peak was significantly higher, suggesting that many cells were stagnant during the G2/M phase. CBP: cells to carboplatin; APS: Astragalus polysaccharides.

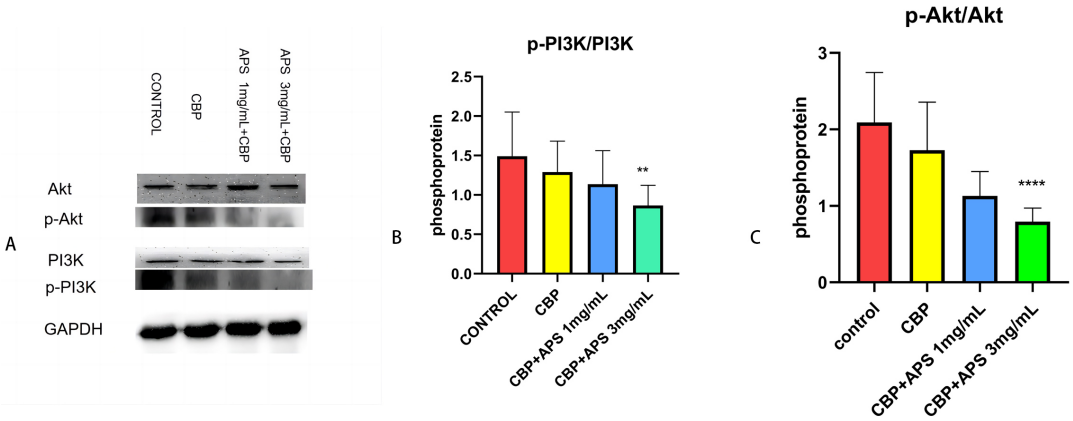

FIGURE 4. Activation of PI3K/Akt pathway following treatment. HeLa cells were treated with CBP at 50 μg/mL, APS 1 mg/mL + CBP 50 μg/mL, and APS 6 mg/mL + CBP 50 μg/mL. (A–C) The relative levels of p-PI3K and p-Akt proteins in the APS and CBP groups were significantly lower ($p < 0.05$) compared with the control group, and the changes in the protein levels in the APS + CBP groups were more significant ($p < 0.01$). In contrast, the relative levels of PI3K and Akt proteins were not significantly altered. CBP: cells to carboplatin; APS: Astragalus polysaccharides; PI3K: Phosphatidylinositol-3-kinases; Akt: Protein Kinase B; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

(MDSCs). In the context of cancer, it has been reported that APS, in combination with cisplatin, had significantly synergistic growth-inhibitory effect on nasopharyngeal carcinoma cell lines, which may be related to cell cycle and migration induction [30]. It has also been reported that APS increased the sensitivity of SKOV3 cells to cisplatin potentially by activating the JNK (c-Jun N-terminal kinase) pathway [7]. The ability of APS to enhance the sensitivity of non-small cell lung cancer and liver cancer cells to chemotherapeutic drug has also been documented in literature [31, 32]. In our study, we found that APS could enhance the anti-cancer activities CBP, which involved promotion of apoptotic cell death and cell cycle blockade. These results indicated that APS has the potential to be employed as an adjuvant chemotherapeutic in a variety of malignancies.

CC patients resist chemotherapeutic drugs in the late stage of treatment, causing poor chemotherapeutic effects and clinical failure. Therefore, it is vital to understand the resistance mechanism of CC cells to platinum drugs and identify drugs that can effectively reverse the resistance to improve the chemotherapeutic effect of CC. The mechanism of multidrug tumor resistance is complex. Under the preliminary network pharmacological analysis, APS acts on CC cells through the PI3K/Akt pathway. Therefore, APS can inhibit the growth of cancer cells, accelerate apoptosis, and elevate the sensitivity of cancer cells to chemotherapeutic drugs when combined with chemotherapeutic drugs. Thus, continuous in-depth study on this pathway could develop a more effective inhibitory therapy or targeted treatment against CC.

ABBREVIATIONS

APS, Astragalus polysaccharides; CBP, Carboplatin; CC, Cervical Cancer; FBS, Fetal bovine serum; HPV, human papillomavirus; IC50, Half maximal inhibitory; MTT, methyl thiazolyl tetrazolium; PBS, Phosphate buffer saline; PI3K/Akt, Phosphatidylinositol-3-kinases/Akt; TCM, traditional Chinese medicine.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

WZL—designed the research study, performed the research, analyzed the data and wrote the manuscript. MMY—provided help and advice on. LZ—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the ethics review board of Mechanism of long non-coding RNA UCA1a regulating the ubiquitination degradation of PKM2 and promoting the proliferation of cervical cancer cells (No. XZR2020070) in accordance with the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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