Hindawi
International Journal of Polymer Science
Volume 2019, Article ID 7690764, 7 pages
https://doi.org/10.1155/2019/7690764

Research Article

Effect of Sulfated Polysaccharide from Undaria pinnatifida (SPUP) on Proliferation, Migration, and Apoptosis of Human Prostatic Cancer

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Received 1 August 2019; Accepted 16 September 2019; Published 21 October 2019

Guest Editor: Jianxun Ding

Objective. To observe the effect of sulfated polysaccharide from Undaria pinnatifida (SPUP) on proliferation, migration, and apoptosis of human prostatic cancer. *Methods*. DU145 human prostate cancer cells were cultured in vitro, and the proliferation activity both in the control group and the SPUP treatment groups (25, 50, 100, 200 μg/ml) was measured by CCK-8 assay. The wound healing assay was conducted to detect the cell migration. Cell apoptosis was measured by flow cytometry. The protein and mRNA expressions of matrix metalloproteinase-9 (MMP-9) and apoptosis-related factor Bax were detected by qRT-PCR and Western blot. The expressions of cleaved caspase-3 and cleaved caspase-9 were also determined by Western blot. *Results*. (1) CCK-8 results showed that the proliferative activity of DU145 cells was significantly decreased with the increase of SPUP treatment concentration ($P < 0.05$) in a dose-dependent manner and that the inhibitory effect of SPUP was most significant at 72 h ($P < 0.05$) as compared with the control group; (2) the migration rate of SPUP-treated cells was significantly decreased ($P < 0.05$) as compared with the control group. And the results of qRT-PCR and Western blot assays showed that SPUP inhibited the expression of MMP-9 in DU145 cells; (3) compared with the control group, the SPUP-treated groups had increased apoptosis of the cells. The expressions of apoptosis-related factors cleaved caspase-3, cleaved caspase-9, and Bax were upregulated ($P < 0.05$), and the mRNA expression of Bax was increased ($P < 0.05$). *Conclusion*. SPUP showed an antitumor activity in prostatic cancer, and the underlying mechanism may be pertaining to inhibition of migration, proliferation, and induction of apoptosis of cancer cells.

1. Introduction

Prostate cancer, one of the most common malignancies in men, is the third leading cause of cancer-related deaths in men worldwide [1]. As prostate cancer cells are prone to malignant invasion and metastasis, current treatment for the disease is still facing difficulties [2]. Despite great advances in cancer treatment over the past decades, patients with advanced prostate cancer still suffer from the lack of effective treatments [3]. Early prostate cancer and local lesions can be treated by surgical resection or radiation [4]; however, the mortality rate of prostate cancer with distant metastasis and castration-resistant prostate cancer remains high. Therefore, the discovery of promising and effective treatments for prostate cancer is of great clinical significance.

Recent years have seen increasing attention from cancer experts and scholars to natural antitumor polysaccharides demonstrating significant anticancer activity as well as low toxic and side effects [5]. More and more researchers believe that polysaccharides can inhibit the proliferation of tumor cells; moreover, they can directly induce apoptosis or enhance immune activity in combination with chemotherapy [6]. Undaria pinnatifida, widely distributed in China and the rest of the world, is rich in protein, polysaccharides, and minerals. It has been used as medication and food for centuries [7]. Studies have reported that as Undaria pinnatifida has various biological functions including immunomodulation, anticancer, and antiviral activities [8], it has become one of the most popular food for cancer prevention, for instance, breast cancer [9]. Fucoidan extracted from Undaria

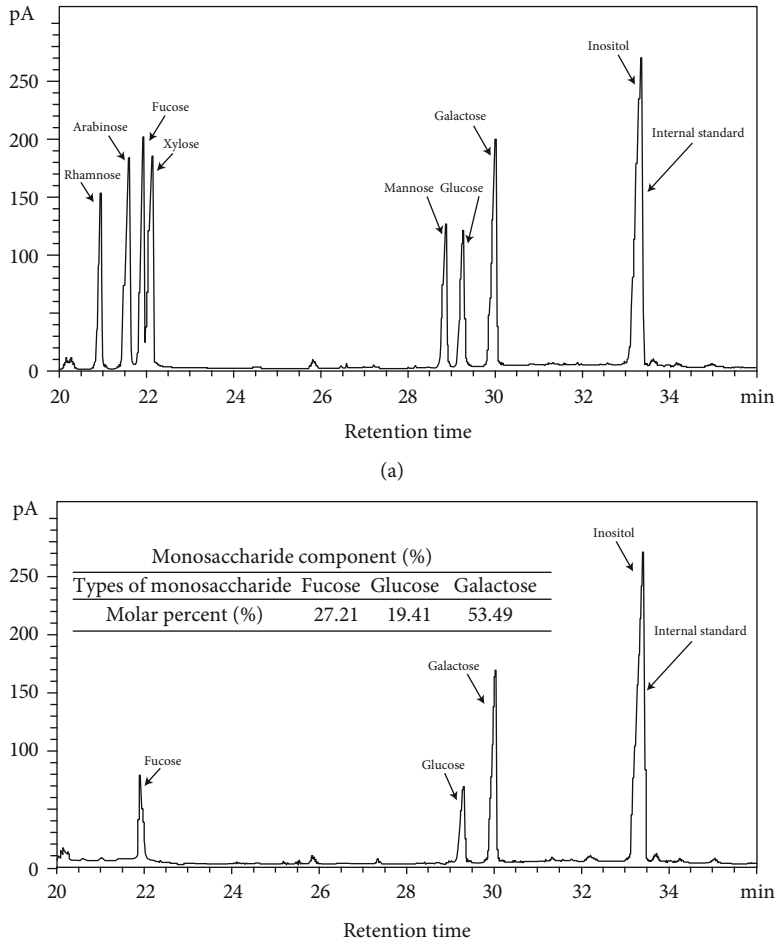

Figure 1: Gas chromatograms of monosaccharide standards (a) and SPUP hydrolysates (b).

pinnatifida has been proven to have antitumor activity in prostate cancer cell PC-3, cervical cancer cell A549, and hepatoma cell HepG2 [10]. In addition, it has been found that sulfated polysaccharide from Undaria pinnatifida (SPUP) inhibits the proliferation and migration activity while promoting apoptosis [11]. However, no study has reported the therapeutic efficacy of SPUP on prostate cancer. Therefore, our study will investigate the effect of SPUP on migration, proliferation, and apoptosis of prostate cancer cell (DU145) to elucidate its anticancer mechanism.

2. Materials and Methods

2.1. Materials and Reagents. The preparation of SPUP was described in the publication by Han et al. [12]. The composition of SPUP was identified by gas chromatography (see Figure 1). The contents of total sugar, ursolic acid, protein, and sulfate in SPUP were 80.32%, 3.19%, 7.07%, and 29.17%, respectively. In addition, SPUP was composed of fucose, glucose, and galactose with a molar ratio of 27.21 : 19.41 : 53.49 and a molecular weight of 97.9 kDa. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies Inc. (Shanghai, China), and Annexin V-FITC/PI Apoptosis Detection Kit was purchased from Sigma-Aldrich Corporation (USA). TRIzol™ LS Reagent, RPMI 1640 medium, and fetal bovine serum were purchased from Thermo Fisher Scientific (USA). Cleaved caspase-3, cleaved caspase-9, Bax, and MMP-9 antibodies were all purchased from Abcam Trading Co., Ltd. (Shanghai, China). PrimeScript RT Master Mix and SYBR-Green Premix were all purchased from Takara Bio Inc. (Japan).

2.2. Cell Culture. DU145 human prostate cancer cells purchased from FuHeng Cell Center, Shanghai, China, were cultured in a RPMI 1640 medium containing 10% fetal bovine serum, 10 mg/ml of streptomycin, and 100 U/ml of penicillin at 37°C, 5% CO_2.

2.3. Cell Viability Detected by CCK-8 Assay. DU145 cells in a logarithmic growth phase were seeded in 96-well plates at a density of 5000/well and divided into the control group and the SPUP experimental groups. When cells adhered to the wells after 24 hours of growth, a RPMI 1640 medium with different concentrations of SPUP (25, 50, 100, and 200 μg/ml) was added into each well, respectively, and the same volume of medium was added into a well as the control group. According to the instruction of the kit, the cell viability of

each well was measured after 0, 12, 24, 36, 48, and 72 h of incubation, respectively.

2.4. Cell Migration Detected by Wound Healing Assay. Cells were seeded in 6-well plates at a density of $1 * 10^5$/well and cultured for 48 h. Two 1 mm wide horizontal lines were drawn across the confluent monolayer of cells in each well using a pipette tip over ruler. The cells washed 3 times with PBS were added with a medium as the control group. And the experimental groups were treated with different concentrations of SPUP (25, 50, 100, and 200 μg/ml). Then, the scratch area was photographed at 0, 48, and 72 h after treatment, respectively, and the scratch width was measured using image analysis software (cell mobility = $(L_{0h} - L_{nh})/L_{0h} * 100\%$; $n = 48, 72$ h).

2.5. Cell Apoptosis Detected by Flow Cytometry. DU145 cells were seeded in 6-well plates at a density of $1 * 10^5$/well and cultured for 24 h. After adherence, the control group was added with SPUP-free medium, while the experimental groups were treated with different concentrations of SPUP (25, 50, 100, and 200 μg/ml) for 72 h. Then, the cells were digested with EDTA-free trypsin digestion solution, collected to the centrifuge tubes, washed with precooled PBS, and centrifuged for three times. The cells were resuspended with 100 μl of binding buffer (1 : 1 ratio). Then, each tube of cells was added with 5 μl of Annexin V-FITC staining solution and 10 μl of PI staining solution and incubated in the dark for 15 min at room temperature. Afterwards, the cells were added with 400 μl of binding solution (1 : 1 ratio), mixed well, and placed on ice. Samples would be tested on the machine within 1 h.

2.6. Protein Expression Detected by Western Blot. DU145 cells were seeded in 6-well plates at a density of $1 * 10^5$/well and cultured for 24 h. After adherence, cells were added into SPUP-free medium as control and treated with different concentrations of SPUP (25, 50, 100, and 200 μg/ml) for 72 h. After that, the whole cell protein was extracted with RIPA whole cell lysate according to the instruction, followed by protein electrophoresis, PVDF membrane transfer, incubation combined with specific primary and secondary antibodies, and ECL fluorescence imaging.

2.7. mRNA Expression Detected by qRT-PCR. The cells were lysed by a TRIzol method to extract total cellular mRNA. Reverse transcription of mRNA into cDNA was then performed according to the instruction of the kit for quantitative fluorescence amplification. β-Actin was used as a housekeeping gene to calculate the relative expression level of the target gene. The primer sequences were as follows: MMP-9 forward primer: 5′-TCCAACCACCACCACCGC-3′, reverse primer: 5′-CAGAGAATCGCCAGTACTT-3′; Bax forward primer: 5′-CCCGAGAGGTCTTTTCCGAG-3′, reverse primer: 5′-CCAGCCCATGATGGTTCTGAT-3′; and β-actin forward primer: 5′-GGCTCCGGCATGTGCAAG-3′, reverse primer: 5′-CCTCGGTCAGCAGCACGG-3′.

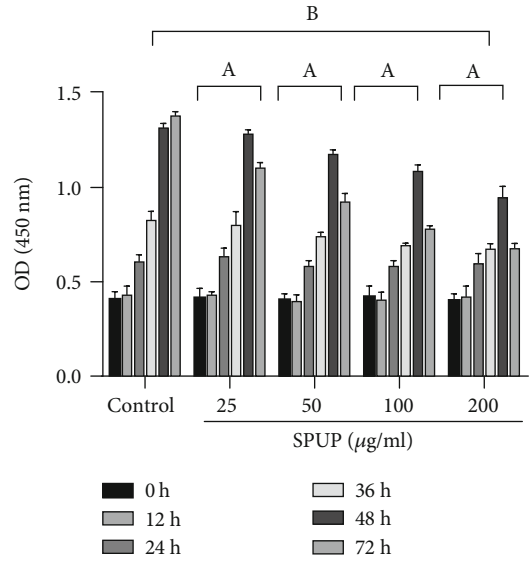

Figure 2: Effect of SPUP on the proliferative activity of DU145 cells. Compared with the control group, A: <0.05; within-group comparison of different time points at the same concentration, B: <0.05.

2.8. Statistical Analysis. All measured data represent as $\bar{x} \pm$ SD, and each experiment was independently repeated at least three times. Data were analyzed using SPSS 20.0 statistical software (USA). Between-group comparison and within-group comparison at different times were performed with one-way analysis of variance, and P values < 0.05 were considered statistically significant.

3. Results

3.1. SPUP Inhibited the Proliferative Activity of DU145 Cells. As shown in Figure 2, with increasing dose, the proliferative activity of cells was significantly decreased ($P < 0.05$), which indicated that the inhibitory effect was dose dependent; the effect was most significant at 72 h ($P < 0.05$). Therefore, we took 48 h and 72 h as treatment time points in subsequent tests.

3.2. SPUP Inhibited the Migratory Activity of DU145 Cells. As shown in Figure 3, we found that SPUP inhibited the closure of DU145 cell scratch wound in a time- and dose-dependent manner. The migration rates of all SPUP-treated cells were significantly smaller than those of the control group ($P < 0.05$). The above results showed that SPUP inhibited the migration of DU145 cells in a dose-dependent manner, which revealed that SPUP can inhibit the migration of prostate cancer cells.

3.3. SPUP Downregulated the mRNA and Protein Expression of MMP-9: A Protein Involved in Cell Migration. As shown in Figure 4, compared with the control group, when the concentration of SPUP was 25 μg/ml, no significant change was observed in MMP-9 protein expression ($P < 0.05$). The expression level of MMP-9 was gradually decreased ($P < 0.05$) with the increase of SPUP concentration (50,

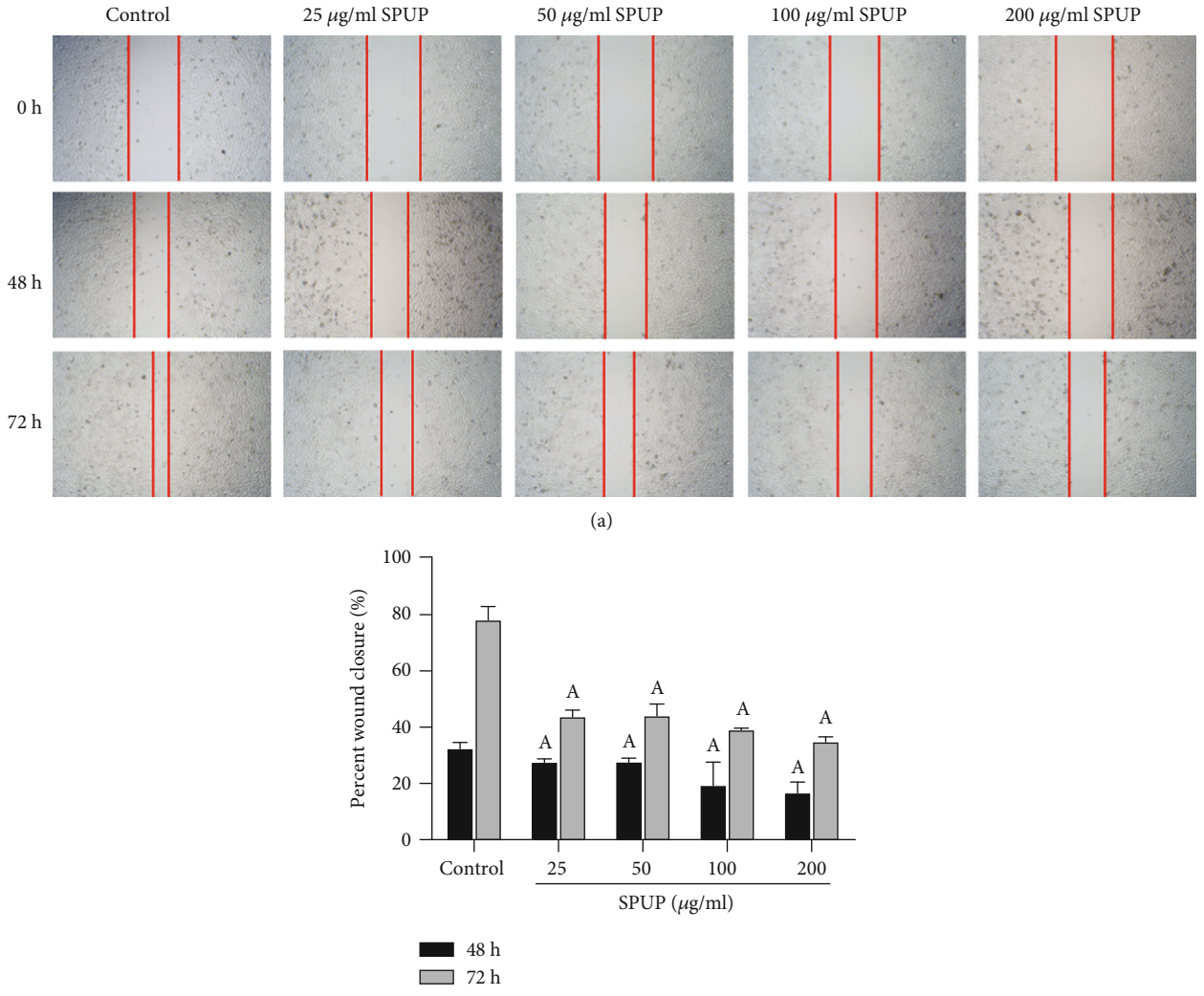

Figure 3: Effect of SPUP on the migration of DU145 cells. Compared with the control group, A: <0.05.

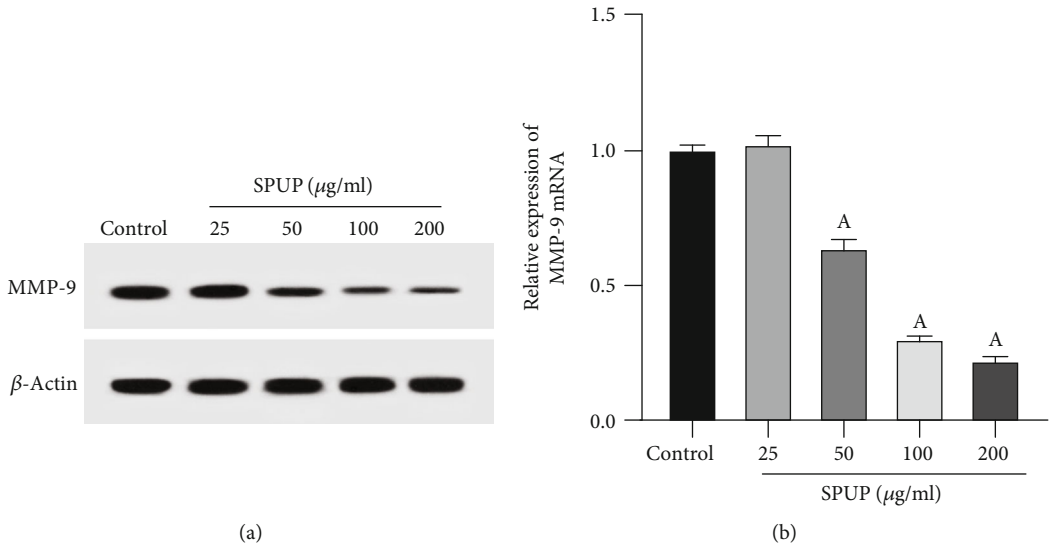

Figure 4: SPUP reduced both the expression of DU145 cells and that of migration-related protein MMP-9. Compared with the control group, A: <0.05.

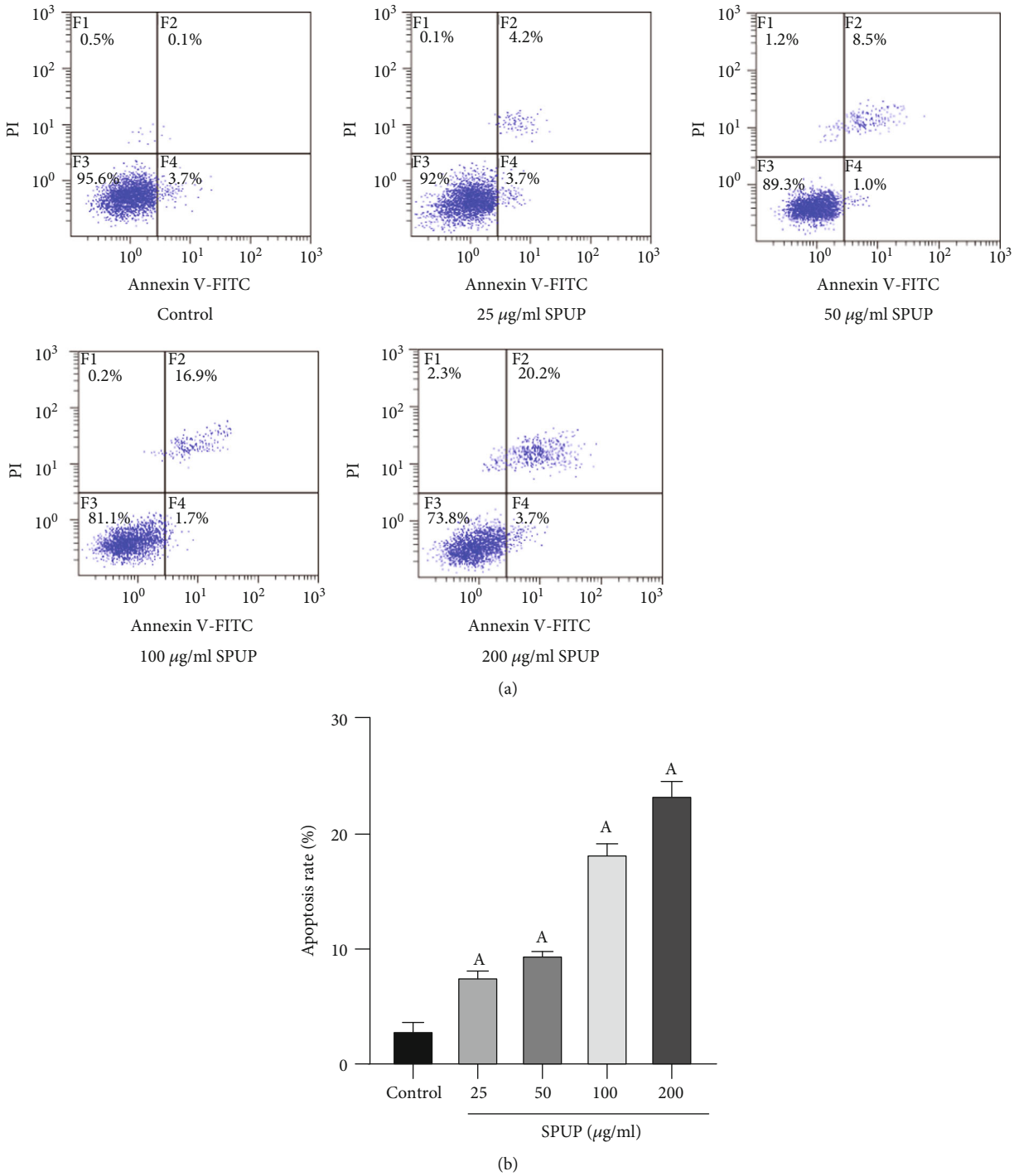

Figure 5: Effect of SPUP on the apoptosis of DU145 cells. Compared with the control group, A: <0.05.

100, and 200 μg/ml), revealing that SPUP inhibited the expression of migration-related protein MMP-9 at a certain concentration and the mRNA expression was consistent with its protein expression.

3.4. SPUP Induced the Apoptosis of DU145 Cells. As shown in Figure 5, after cells were treated with different concentrations of SPUP for 72 h, various degrees of apoptosis were observed in all groups of DU145 cells. Compared with the control group, the apoptosis rates of all SPUP groups were significantly increased ($P < 0.05$).

3.5. SPUP Upregulated the Expressions of Apoptosis-Related Proteins Cleaved Caspase-3, Cleaved Caspase-9, and Bax. As shown in Figure 6, compared with the control group, the expressions of cleaved caspase-3, cleaved caspase-9, and Bax were significantly increased ($P < 0.05$) in high-dose groups after SPUP treatment (25, 50, 100, and 200 μg/ml)

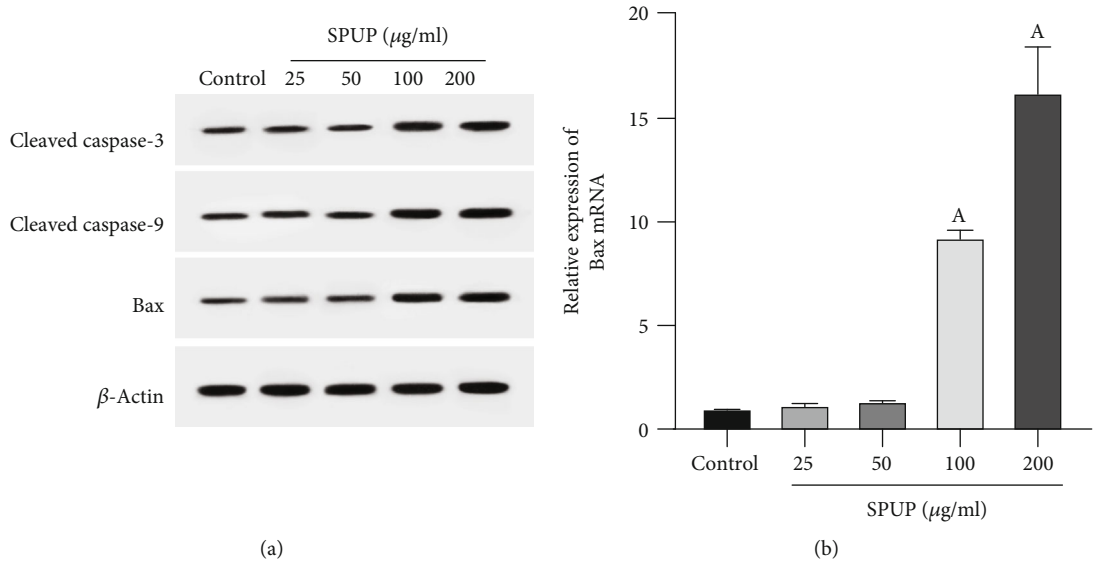

Figure 6: Expression of apoptosis-related protein and mRNA in DU145 cells treated with SPUP. Compared with the control group, A: <0.05.

for 72 h. The mRNA expression of Bax measured by qRT-PCR was consistent with its protein expression ($P < 0.05$).

4. Discussion

In recent years, patients with prostate cancer have suffered from increasingly higher odds of recurrence and limited life expectancy [13]. At present, surgical resection, radiation therapy, and hormone therapy are mainly used for the treatment of prostate cancer. However, for patients terminally ill with metastasis, there is still a lack of effective treatment measures, which can reduce the harm to their body and improve their quality of life. As such, it is urgent to develop new antitumor drugs.

In the study, sulfated polysaccharides isolated from Undaria pinnatifida and purified were studied for inhibitory effects on the growth of prostate cancer cells cultured in vitro. The inhibitory effect of SPUP on the proliferation of DU145 cells was detected by CCK-8 assay. It was found that when DU145 cells were treated with different concentrations of SPUP, the inhibition of proliferation was positively correlated with the SPUP concentration. DU145 cells treated with 200 μg/ml of SPUP for 72 h could show significantly downregulated proliferative activity as compared with the control group. To further understand the role of SPUP in prostate cancer progression, we also performed studies on cell migration and apoptosis, respectively. In the scratch assay, we chose 72 h as treatment time when cell proliferation was significantly inhibited in CCK-8. After treating the cells with different concentrations of SPUP, we found that the degree of closure of the scratches in all SPUP-treated cells was significantly smaller than that in the control group. Furthermore, we found that the effect of SPUP on the scratch closure of DU145 cells was significantly dose dependent, suggesting that SPUP inhibits the migration of prostate cancer cells. Matrix metalloproteinases (MMPs), including MMP2 and MMP9, facilitate the invasiveness and metastasis of a variety of cancer cells by degrading extracellular matrix and other barriers [14]. MMPs have been shown to be important targets for treating metastatic prostate cancer. For example, Larsson et al. found that MMP9 can increase the aggressiveness of metastatic cancer and promote the growth of metastatic tumors by interacting with other factors [15]. Li et al. found that reducing the expression of MMP-9 in prostate cancer cells was accompanied by a decrease in cell invasion and migration [16]. Since we found that SPUP-treated cells inhibit the migration of prostate cancer, we hypothesized that SPUP exerts the effect through cancer cell invasion and metastasis-promoting factor MMP-9 [17] and then found that the higher the concentration of SPUP, the lower the expression of MMP-9, indicating that SPUP may suppress cell migration by inhibiting the expression of MMP-9 protein. In terms of induction of apoptosis, DU145 cells were treated with different SPUP concentrations (25, 50, 100, and 200 μg/ml) for 72 h, and the results indicated that SPUP can induce apoptosis in a dose-dependent manner in DU145 cells. By detecting the expression of apoptosis-related proteins cleaved caspase-3, cleaved caspase-9, and Bax as well as mRNA expression of Bax, we found that the expression level of apoptosis-related proteins significantly increased after treatment of SPUP as compared with the control group, suggesting that SPUP may promote apoptosis in DU145 cells by upregulating the expression of the above induction-related factors; yet, the specific mechanism of pathway needs further study.

The above results showed that SPUP can inhibit the proliferation and migration of DU145 cells and promote apoptotic response, and further study based on the results can underlie necessary data to support the therapeutic effect of SPUP against prostate cancer, making SPUP a promising drug for the treatment of prostate cancer.

5. Conclusion

In the study, sulfated polysaccharide isolated from Undaria pinnatifida and purified was investigated for its mechanism and effect on DU145 human prostate cancer cells cultured

in vitro, expecting to find a newly effective drug with low toxicity against prostate cancer. The experimental results confirmed that SPUP can inhibit DU145 cell proliferation, suppress cell migration by downregulating the expression of MMP-9 protein, and promote apoptotic response by upregulating the expression of apoptosis-related proteins cleaved caspase-3, cleaved caspase-9, and Bax. Thus, it can be seen that SPUP has great potential in treating prostate cancer, while we still need to conduct more in-depth experimental studies on its mechanism and large-scale population-based studies to clarify its therapeutic effect.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Acknowledgments

The present study was supported by the Shanghai Health and Family Planning Commission (grant no. 201840245).

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