Original Article

DOI: 10.5582/bst.2018.01293

Daucosterol induces autophagic-dependent apoptosis in prostate cancer *via* JNK activation

Ping Gao[1,§], Xiaopeng Huang[2,§], Tingting Liao[3], Guangsen Li[2], Xujun Yu[4], Yaodong You[2,*], Yuxing Huang[5]

[1] *Department of Urology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China;*
[2] *Department of Andrology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China;*
[3] *Department of Endocrinology, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China;*
[4] *Medicine and Life Sciences College, Chengdu University of Traditional Chinese Medicine, Chengdu, China;*
[5] *Department of Neurosurgery, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China.*

Summary **Plant sterols (phytosterols) have been widely accepted as a natural anti-cancer agent in multiple malignant tumors. This study was designed to investigate the functions of daucosterol in prostate cancer progression and its possible molecular mechanisms. Our results showed that daucosterol inhibited cell proliferation and induced cell cycle arrest. Moreover, daucosterol treatment obviously promoted apoptosis and autophagy. An autophagy inhibitor, 3-methyladenine (3-MA) was proved to counteract daucosterol-triggered autophagy, growth inhibition, and apoptosis, indicating that daucosterol-induced apoptotic response was dependent on autophagy. Additionally, treatment with daucosterol resulted in increased phosphorylation of c-Jun N-terminal kinase (JNK). Furthermore, pre-treatment with a JNK-specific inhibitor SP600125 abated daucosterol-elicited autophagy and apoptotic cell death. Taken together, our findings demonstrated that daucosterol blocked prostate cancer growth at least partly through inducing autophagic-dependent apoptosis *via* activating JNK signaling, providing a promising candidate for the development of antitumor drugs in prostate cancer treatment.**

Keywords: Prostate cancer, daucosterol, cell cycle, apoptosis, autophagy, JNK

1. Introduction

Prostate cancer, the most common non-cutaneous malignancy in male, ranks second in cancer-related death among men in the United States in 2016 (*1*). Chemoprevention has been generally used as a main therapy for prostate cancer patients (*2*). However, high cytotoxicity and drug resistance greatly limited its application (*3*). Hence, it is necessary to identify more effective therapeutic targets for prostate cancer treatment.

Natural compounds, multi-targeted and less toxic, have been found to be involved in the prevention and therapy of multiple cancers (*4,5*). Plant sterols (phytosterols), with similar structural and biological functions to cholesterol, are able to exert anti-cancer effects in different tumors through modulation of cell growth, invasion, metastasis, cell cycle arrest and apoptosis (*6,7*). Daucosterol, a β-sitosterol glycoside, is one of the major phytosterols in higher plants and extracted from an endemic medicinal plant of Iran, Salvia sahendica (*8*). Daucosterol was previously reported to display anti-inflammatory and immunomodulatory activities (*9-11*). Additionally, daucosterol showed neuroprotective action in *in vitro* model of ischemia by activating IGF1 signaling (*12*). Moreover, daucosterol could promote neural stem cells proliferation through regulating multiple genes, especially increasing insulin-like growth factor I (IGF1) expression (*13*).

In recent years, daucosterol is also proved as

Released online in J-STAGE as advance publication April 2, 2019.

[§]These authors contributed equally to this work.

**Address correspondence to:*
Dr. Yaodong You, Hospital of Chengdu University of Traditional Chinese Medicine. No. 39, Shi-er-qiao Road, Chengdu 610072, China.
E-mail: yaodong_youu@163.com

important regulators in the occurrence and progression of cancers. A previous report delineated that daucosterol induced apoptosis in human breast cancer through regulating PTEN/PI3K/Akt pathway (*14*). Zeng *et al*. disclosed that daucosterol repressed proliferation, migration and invasion in hepatocellular carcinoma *via* Wnt/β-catenin signaling pathway (*15*). Zhao *et al*. discovered that daucosterol suppressed cancer cell proliferation by triggering autophagy through reactive oxygen species (ROS)-dependent manner (*16*). However, the exact roles and molecular mechanisms of daucosterol in prostate cancer deserve to be further investigated.

Apoptosis, type-I programmed cell death (PCD), is a common mechanism of cancer cell death for various anticancer drugs (*17*). Autophagy has a controversial role in cancer development. It could relieve tumor cell from nutrient and oxidative stress during the rapid expansion of cancer, while excessive and sustained autophagy may result in cell death and tumor shrinkage (*18*). Many researchers have highlighted the importance of autophagy in tumorigenesis and cancer therapy (*19,20*). Natural compounds and extracts have been documented as potential inducers of autophagy through diverse cellular mechanisms and pathways, thereby leading to cell senescence, apoptosis-independent death, and apoptotic death (*21,22*). Jun N-terminal kinase (JNK), a member of mitogen-activated protein kinase (MAPK) family, plays a vital part in many cellular events, including apoptosis and autophagy (*23*). JNK signaling has been found to be implicated in the process of natural product therapy in a variety of neoplasms (*24,25*).

In this current study, we elucidated that daucosterol suppressed cell proliferation, induced cell cycle arrest and enhanced apoptosis in prostate cancer. Furthermore, the anti-cancer effect of daucosterol was mediated by autophagy *via* JNK signaling.

2. Materials and Methods

2.1. *Cell culture*

Human prostate cancer cell lines (PC3 and LNCap) were purchased from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Cells were maintained in RPMI1640 medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Sigma, St. Louis, MO, USA) at 37°C with an atmosphere of 5% CO_2.

2.2. *Reagents*

Daucosterol was obtained from extractsupplier Biotechnology (Xi'an, China) and dissolved in DMSO at 10 mM as stock. Autophagy inhibitor 3-methyladenine (3-MA) was purchased from Sigma and JNK-specific inhibitor (SP600125) was acquired from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. *Cell Counting Kit-8 (CCK-8) assay*

PC3 and LNCap cells were seeded in 6-well plates (1×10^5 cells/well) and exposed to different concentrations of daucosterol (0, 5, 10, 20, 40, and 80 μM) for 48 h. Then, 10 μl of CCK-8 solution was added and incubated for another 2 h at 37°C. The absorbance at 450 nm was determined, and cell viability was normalized as the percentage of control.

2.4. *Cell cycle and apoptosis assay*

PC3 and LNCap cells were treated with various concentrations of daucosterol (0, 5, 10, 20, 40, and 80 μM) for 48 h. For cell cycle analysis, cells were collected, fixed in 75% ethanol, incubated with 50 μg/mL propidium iodide (PI) containing 40 μg/mL RNase for 30 min. Subsequently, cell cycle distribution was detected by a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA).

After treatment, apoptotic rate was measured using the annexin-V-FITC/PI apoptosis kit (BD Biosciences) by a flow cytometry (FACScan; BD Bioscience).

2.5. *Western blot analysis*

Following daucosterol treatment, cell lysates were separated on 10% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel and then electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes. Then, the membranes were sequentially probed with primary antibodies and peroxidase-conjugated secondary antibody. Finally, the protein bands were detected by ECL Advance Detection System (Amersham Biosciences, Piscataway, NJ, USA). The primary antibodies against cleaved caspase 3, cleaved caspase 9, Bax, Bcl-2, LC3, Beclin 1, p62, were purchased from Cell Signaling Technology (Danvers, MA, USA), and antibodies against p-JNK, JNK and β-actin were obtained from Santa Cruz Biotechnology.

2.6. *Statistical analysis*

All experiments were performed at least 3 times and all data were displayed as mean ± SD. Student's *t* test or one-way ANOVA was used to evaluate statistical difference by GraphPad Prism (GraphPad Software Inc.). $P < 0.05$ is considered as statistically significant.

3. Results

3.1. *Daucosterol suppressed proliferation in prostate cancer cells*

To explore the effects of daucosterol on prostate

cancer, PC3 and LNCap cells were treated with various concentrations of daucosterol for 48 h. CCK-8 assay manifested that daucosterol inhibited PC3 and LNCap cell growth in a dose-dependent manner (Figure 1A and 1B). Moreover, flow cytometry analysis demonstrated that daucosterol treatment resulted in an obvious cell cycle arrest at G1 phase in both PC3 (Figure 1C) and LNCap (Figure 1D) cells. These data suggested that daucosterol impaired cell proliferation in prostate cancer.

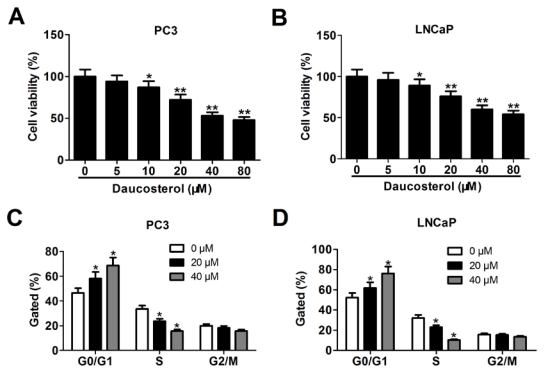

Figure 1. Daucosterol lowers the proliferation potential of prostate cancer cells. (A and **B)** PC3 and LNCap cells were treated with different concentrations of daucosterol (0, 5, 10, 20, 40, and 80 μM) for 48 h, then cell viability was assessed by CCK-8 assay; **(C** and **D)** Flow cytometry analysis was used to measure cell cycle distribution in PC3 and LNCap cells after treatment with various concentrations of daucosterol (0, 20, and 40 μM) for 48 h. *$p < 0.05$, **$p < 0.01$.

3.2. *Daucosterol promoted apoptosis in prostate cancer cells*

To further investigate whether the growth inhibition elicited by daucosterol was associated with apoptosis, we evaluated the apoptotic rate of PC3 and LNCap cells after treatment with different doses of daucosterol for 48 h. Flow cytometry assay displayed that the apoptotic rate was greatly enhanced in both PC3 and LNCap cells following daucosterol treatment (Figure 2A and 2B). Moreover, western blot analysis exhibited a dramatic increase of cleaved caspase 3, cleaved caspase 9 and Bax protein expressions, while a prominent decrease of Bcl-2 expression in PC3 and LNCap cells after treatment with daucosterol (Figure 2C and 2D). Together, these results indicated that daucosterol exerted anticancer activity at least partly by facilitating apoptosis in prostate cancer.

3.3. *Daucosterol induced autophagy in prostate cancer cells*

Autophagy, type-II PCD, is demonstrated to participate

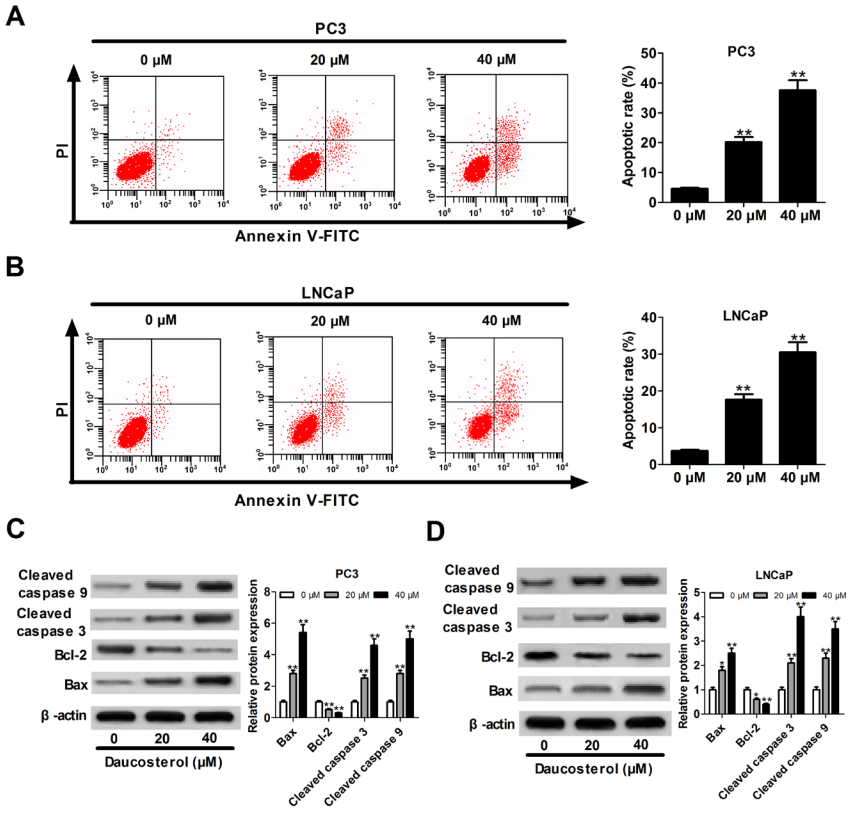

Figure 2. Daucosterol facilitates apoptosis in prostate cancer cells. (A and **B)** The apoptotic rate was detected in PC3 and LNCap cells after treatment with various concentrations of daucosterol (0, 20, and 40 μM) for 48 h; **(C** and **D)** Western blot analysis of cleaved caspase 3, cleaved caspase 3, Bax, and Bcl-1 in PC3 and LNCap cells treated with daucosterol for 48 h. *$p < 0.05$, **$p < 0.01$.

in the regulation of natural products in cancers (26). Thus, we determined the protein levels of autophagy markers (LC3, Beclin 1 and p62) in PC3 and LNCap cells after treatment with different doses of daucosterol for 48 h. Western blot analysis revealed that daucosterol treatment resulted in an increase of LC3II/LC3I ration and Beclin 1 expression, while a decline of p62 expression in PC3 and LNCap cells (Figure 3A and 3B), supporting the induction of autophagy by daucosterol in prostate cancer cells.

3.4. *Suppression of autophagy weakened daucosterol-elicited growth inhibition and apoptosis in prostate cancer cells*

To further analyze whether apoptosis and autophagy were related or independent events in response to daucosterol, we examined the effects of daucosterol on cell proliferation and apoptosis in the presence or absence of autophagy inhibitor 3-MA. As expected, 3-MA pre-treatment effectively attenuated daucosterol-

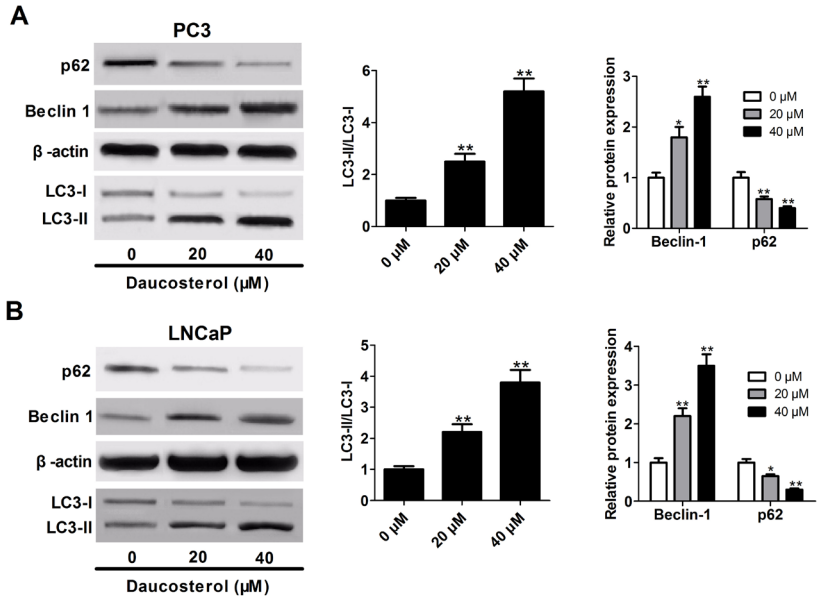

Figure 3. Daucosterol induces autophagy in prostate cancer cells. (A and **B)** The expression levels of LC3II, Beclin 1 and p62 were determined by Western blot in PC3 and LNCap cells following treatment with different concentrations of daucosterol for 48 h. *$p < 0.05$, **$p < 0.01$.

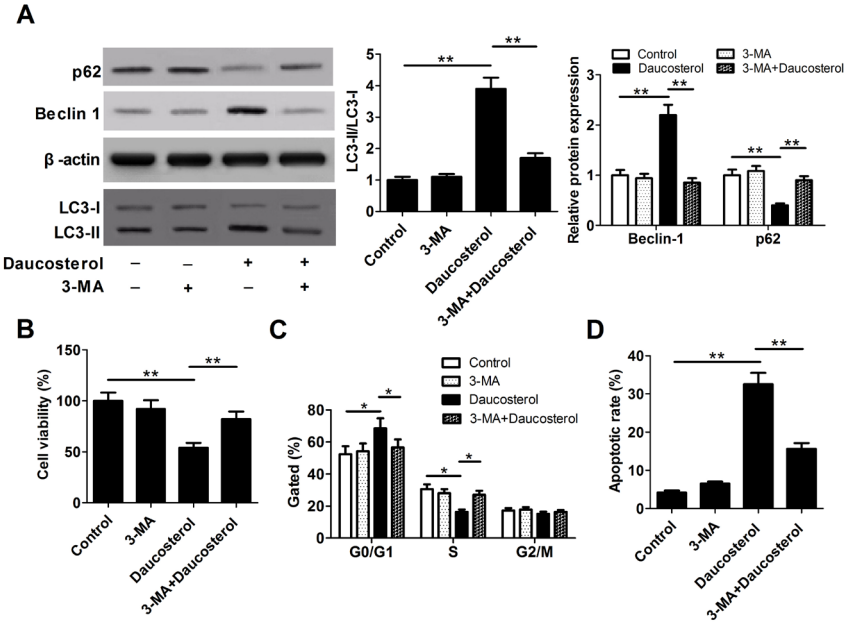

Figure 4. Daucosterol-triggered apoptosis depends on autophagy in prostate cancer cells. PC3 cells were pre-treated with or without 3-MA (5 mM) for 1 h prior to incubation with 40 μM daucosterol for 48 h, then **(A)** western blot was used to assess the protein expressions of LC3II, Beclin 1 and p62, **(B)** cell viability was detected by CCK-8 assay, **(C** and **D)** cell cycle distribution and apoptosis was examined by flow cytometry. *$p < 0.05$, **$p < 0.01$.

induced autophagy in PC3 cells, presented by reduced LC3II and Beclin 1 expressions, while enhanced p62 expression (Figure 4A). Moreover, the growth inhibition triggered by daucosterol was greatly reversed in PC3 cells following pre-treatment with 3-MA (Figure 4B and 4C). Further, treatment of PC3 cells with 3-MA abated daucosterol-induced apoptosis (Figure 4D). Collectively, the pro-apoptotic effect of daucosterol was modulated by autophagy.

3.5. *Daucosterol induced autophagy in prostate cancer cells*

JNK activation has been found to be involved in autophagy and apoptosis induced by natural compound (*27*). In order to explore the potential molecular basis of daucosterol in prostate cancer, we firstly determined the effects of daucosterol on JNK pathway in PC3 cells. As presented in Figure 5A, daucosterol treatment significantly increased the level of phosphorylated JNK. To further confirm whether daucosterol-induced apoptosis and autophagy require JNK activation, PC3 cells were pre-incubated with or without JNK-specific inhibitor (SP600125) prior to daucosterol treatment. The results demonstrated that SP600125 suppressed the conversion of LC3I to LC3II and Beclin 1 expression, while enhanced p62 expression in PC3 cells under daucosterol treatment (Figure 5B), suggesting the inhibitory effect of SP600125 on daucosterol-induced autophagy. Moreover, daucosterol-elicited growth inhibition (Figure 5C), cell cycle arrest (Figure 5D) and apoptosis (Figure 5E) were alleviated following SP600125 pre-treatment. From these data, we draw a conclusion that daucosterol induced autophagic-dependent apoptosis in prostate cancer by activating JNK signaling.

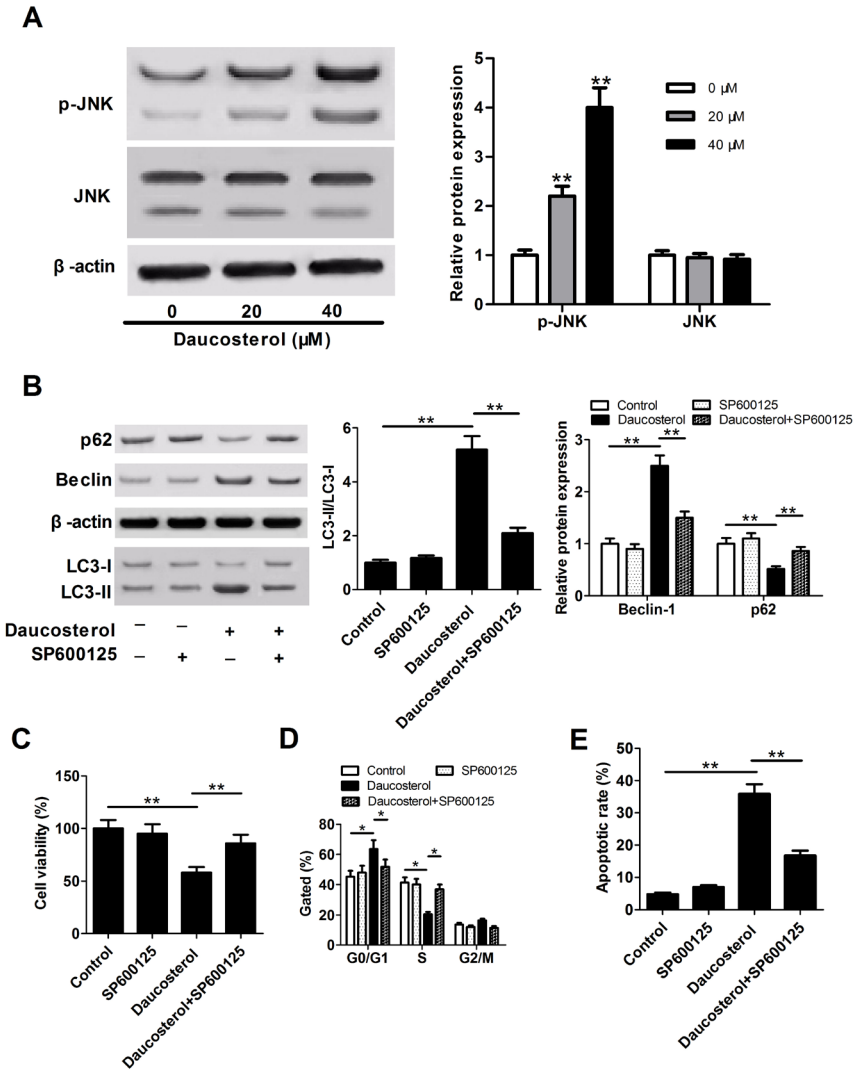

Figure 5. Daucosterol-triggered autophagic-dependent apoptosis by JNK activation in prostate cancer cells. (A) Western blot analysis of JNK and p-JNK expression in PC3 cells treated with daucosterol at indicated concentrations for 48 h; **(B)** PC3 cells were pre-incubated with SP600125 (10 μM) for 1 h prior to treatment with 40 μM daucosterol for 48 h, then (B) western blot was applied to evaluate the protein levels of LC3II, Beclin 1 and p62; (C) CCK-8 assay was performed to monitor cell viability; **(D** and **E)** flow cytometry analysis was conducted to analyze cell cycle distribution and apoptosis. *$p < 0.05$, **$p < 0.01$.

4. Discussion

Natural products have been discovered as a rich source for the discovery and development of cancer preventive and anticancer drugs (*28,29*). Daucosterol, a β-sitosterol glycoside, is one of the major phytosterols in higher plants (*30*). In this study, we found that daucosterol exerted tumor-suppressive function in prostate cancer by inducing autophagic-dependent apoptosis *via* activation of JNK.

Daucosterol are reported as an anti-cancer plant sterol in breast cancer (*14*), hepatic carcinoma (*15*) and gastric cancer (*16*) by various mechanisms and pathways. Rajavel *et al*. also revealed that the presence of phytosteols (β-sitosterol and daucosterol) significantly blocked the growth of lung cancer cells both alone and in combination (*31*). Wang *et al*. disclosed that daucosterol hindered proliferation, enhanced apoptosis, and inhibited migration and invasion in colon cancer *via* regulating caspase signaling (*32*). In this study, we firstly found that daucosterol suppressed prostate cancer cell viability in a dose-dependent manner. Deregulation of cell cycle progression has been regarded to be associated with cancer occurrence and development (*33*). Moreover, controlling or perturbing the cycle of tumor cells is an effective method for natural products to destroying malignant cell proliferation (*34*). Here, our results showed that daucosterol treatment resulted in a dramatic cell cycle arrest.

Apoptosis has been considered as a major mechanism to exert anti-cancer roles for chemotherapeutic agents (*35*). It has been proved that numerous plant-derived chemotherapy drugs kill cancer cells *via* inducting apoptotic cell death (*36,37*). In the present study, we demonstrated that daucosterol repressed cell apoptosis by flow cytometry. Moreover, daucosterol activated the mitochondria-dependent apoptotic signaling pathway, leading to increased expression of pro-apoptotic protein (Cleaved caspase 3, Cleaved caspase 9, Bax) and decrease expression of anti-apoptotic protein Bcl-2. These data suggested that daucosterol was cytotoxic to cells in prostate cancer through facilitating apoptosis.

Autophagy is a homeostatic cellular degradation process for eliminating damaged or unnecessary intracellular organelles and proteins in lysome (*38*). Autophagy is able to suppress early cancer development while contribute to advanced tumor progression (*39*). Numerous natural compounds have been discovered to induce cell apoptosis through modulating autophagy in cancers. For example, natural product peiminine repressed cell growth in colorectal cancer by enhancing autophagic cell death (*40*). Marchantin M promoted apoptosis in prostate cancer through induction of autophagy (*41*). Our results manifested that daucosterol treatment effectively enhanced autophagy in prostate cancer cells, indicated by up-regulated LC3II and Beclin 1 expression, while down-regulated p62 expression. Moreover, autophagy inhibitor 3-MA attenuated daucosterol-triggered growth inhibition and apoptosis in prostate cancer cells. These data confirmed that daucosterol impaired cell growth by contributing to autophagic-dependent cell death.

It is well known that JNKs are master protein kinases that modulate a variety of physiological events, such as inflammatory responses, morphogenesis, cell proliferation, differentiation, survival and death (*42*). Aberrant activation of JNK signaling has been reported to be associated with tumor progression (*43*). Our data exhibited that daucosterol increased the phosphorylation level of JNK in prostate cancer cells. Moreover, JNK-specific inhibitor (SP600125) inhibited daucosterol-induced autophagy. Furthermore, daucosterol-elicited growth inhibition, cell cycle arrest and apoptosis were abated by pre-treatment with SP600125. Together, daucosterol exerted anti-proliferation effect by inducing autophagic-dependent apoptosis *via* activation of JNK signaling. Consistent with our finding, JNK activation has been reported to be participate in apoptosis and autophagy mediated by multiple natural products (*44,45*).

In conclusion, our present study demonstrated that daucosterol inhibited cell proliferation, induced cell cycle arrest, and promoted autophagic-dependent apoptosis in prostate cancer. Moreover, the tumor-suppressive effect of daucosterol was at least partly mediated by JNK activation. Our findings indicated that daucosterol may be developed as a potential anti-cancer agent.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 81503589) and Education Department of Sichuan Provincial of China (No.14ZB0089)

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016; 66:7-30.
2. Halabi S, Lin CY, Kelly WK, Fizazi KS, Moul JW, Kaplan EB, Morris MJ, Small EJ. Updated Prognostic Model for Predicting Overall Survival in First-Line Chemotherapy for Patients With Metastatic Castration-Resistant Prostate Cancer. J Clin Oncol. 2014; 32:671-677.
3. Zhang W, Meng Y, Liu N, Wen XF, Yang T. Insights into Chemoresistance of Prostate Cancer. Int J Biol Sci. 2015; 11:1160-1170.
4. Shanmugam MK, Lee JH, Chai EZ, Kanchi MM, Kar S, Arfuso F, Dharmarajan A, Kumar AP, Ramar PS, Looi CY. Cancer prevention and therapy through the modulation of transcription factors by bioactive natural compounds. Semin Cancer Biol. 2016; 40-41:35-47.
5. Safarzadeh E, Shotorbani SS, Baradaran B. Herbal Medicine as Inducers of Apoptosis in Cancer Treatment.

Adv Pharm Bull. 2014; 4:421-427.
6. Ramprasath VR, Awad AB. Role of Phytosterols in Cancer Prevention and Treatment. J Aoac Int. 2015; 98:735-738.
7. Shahzad N, Khan W, Md S, Ali A, Saluja SS, Sharma S, Al-Allaf FA, Abduljaleel Z, Ibrahim IAA, Abdel-Wahab AF, Afify MA, Al-Ghamdi SS. Phytosterols as a natural anticancer agent: Current status and future perspective. Biomed Pharmacother. 2017; 88:786-794.
8. Mozafarian V. A Dictionary of Iranian Plant Names. Farhang Mosafer Publication, Tehran, Iran. 1996; pp. 90-91.
9. Bouic PJ, Etsebeth S, Liebenberg RW, Albrecht CF, Pegel K, Van Jaarsveld PP. beta-Sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: Implications for their use as an immunomodulatory vitamin combination. Int J Immunopharmacol. 1996; 18:693-700.
10. Choi JN, Choi YH, Lee JM, Noh IC, Park JW, Choi WS, Choi JH. Anti-inflammatory effects of beta-sitosterol-beta-D-glucoside from Trachelospermum jasminoides (Apocynaceae) in lipopolysaccharide-stimulated RAW 264.7 murine macrophages. Nat Prod Res. 2016; 26:2340-2343.
11. Lee JH, Lee JY, Park JH, Jung HS, Kim JS, Kang SS, Kim YS, Han Y. Immunoregulatory activity by daucosterol, a beta-sitosterol glycoside, induces protective Th1 immune response against disseminated Candidiasis in mice. Vaccine. 2007; 25:3834-3840.
12. Jiang LH, Yuan XL, Yang NY, Ren L, Zhao FM, Luo BX, Bian YY, Xu JY, Lu DX, Zheng YY. Daucosterol protects neurons against oxygen-glucose deprivation/reperfusion-mediated injury by activating IGF1 signaling pathway. J Steroid Biochem Mol Biol. 2015; 152:45-52.
13. Jiang LH, Yang NY, Yuan XL, Zou YJ, Zhao FM, Chen JP, Wang MY, Lu DX. Daucosterol promotes the proliferation of neural stem cells. J Steroid Biochem Mol Biol. 2014; 140:90-99.
14. Esmaeili MA, Farimani MM. Inactivation of PI3K/Akt pathway and upregulation of PTEN gene are involved in daucosterol, isolated from Salvia sahendica , induced apoptosis in human breast adenocarcinoma cells. South Afri J Botany. 2014; 93:37-47.
15. Zeng J, Liu X, Li X, Zheng Y, Liu B, Xiao Y. Daucosterol Inhibits the Proliferation, Migration, and Invasion of Hepatocellular Carcinoma Cells *via* Wnt/β-Catenin Signaling. Molecules 2017; 22:E862.
16. Zhao C, She T, Wang L, Su Y, Qu L, Gao Y, Xu S, Cai S, Shou C. Daucosterol inhibits cancer cell proliferation by inducing autophagy through reactive oxygen species-dependent manner. Life Sci. 2015; 137:37-43.
17. Croce CM, Reed JC. Finally, An Apoptosis-Targeting Therapeutic for Cancer. Cancer Res. 2016; 76:5914-5920.
18. Jiang X, Overholtzer M, Thompson CB. Autophagy in cellular metabolism and cancer. J Clin Invest. 2015; 125:47-54.
19. Fulda S, Kögel D. Cell death by autophagy: Emerging molecular mechanisms and implications for cancer therapy. Oncogene. 2015; 34:5105-5113.
20. Rebecca VW, Amaravadi RK. Emerging strategies to effectively target autophagy in cancer. Oncogene. 2016; 35:1-11.
21. Wang N, Feng Y. Elaborating the role of natural products-induced autophagy in cancer treatment: Achievements and artifacts in the state of the art. Biomed Res Int. 2015; 2015:934207.
22. Naponelli V, Modernelli A, Bettuzzi S, Rizzi F. Roles of autophagy induced by natural compounds in prostate cancer. Biomed Res Int. 2015; 2015:121826.
23. Hao H, Zhang D, Shi J, Wang Y, Chen L, Guo Y, Ma J, Jiang X, Jiang H. Sorafenib induces autophagic cell death and apoptosis in hepatic stellate cell through the JNK and Akt signaling pathways. Anticancer Drugs. 2016; 27:192-203.
24. Zhong F, Tong ZT, Fan LL, Zha LX, Wang F, Yao MQ, Gu KS, Cao YX. Guggulsterone-induced apoptosis in cholangiocarcinoma cells through ROS/JNK signaling pathway. Am J Cancer Res. 2015; 6:226-237.
25. Peng F, Wang X, Shu M, Yang M, Wang L, Ouyang Z, Shen C, Hou X, Zhao B, Wang X. Raddeanin a Suppresses Glioblastoma Growth by Inducing ROS Generation and Subsequent JNK Activation to Promote Cell Apoptosis. Cell Physiol Biochem. 2018; 47:1108-1121.
26. Hua F, Shang S, Hu Z. Seeking new anti-cancer agents from autophagy-regulating natural products. J Asian Nat Prod Res. 2017; 19:305-313.
27. Zhao Y, Li ETS, Wang M. Alisol B 23-acetate induces autophagic-dependent apoptosis in human colon cancer cells *via* ROS generation and JNK activation. Oncotarget. 2017; 8:70239-70249.
28. Bishayee A, Sethi G. Bioactive natural products in cancer prevention and therapy: Progress and promise. Semin Cancer Biol. 2016; 40-41:1-3.
29. Cragg G, Pezzuto J. Natural Products as a Vital Source for the Discovery of Cancer Chemotherapeutic and Chemopreventive Agents. Med Princ Pract. 2016; 25:41-59.
30. Pegel K. The importance of sitosterol and sitosterolin in human and animal nutrition. South Afri J Sci. 1997; 93:263-268.
31. Rajavel T, Mohankumar R, Archunan G, Ruckmani K, Devi K. Beta sitosterol and Daucosterol (phytosterols identified in Grewia tiliaefolia) perturbs cell cycle and induces apoptotic cell death in A549 cells. Sci Rep. 2017; 7:3418.
32. Wang GQ, Gu JF, Gao YC, Dai YJ. Daucosterol inhibits colon cancer growth by inducing apoptosis, inhibiting cell migration and invasion and targeting caspase signalling pathway. Bangl J Pharmacol. 2016; 11:395.
33. Schwartz GK, Shah MA. Targeting the Cell Cycle: A New Approach to Cancer Therapy. J Clin Oncol. 2005; 23:9408-9421.
34. Newman DJ, Cragg GM, Holbeck S, Sausville EA. Natural Products and Derivatives as Leads to Cell Cycle Pathway Targets in Cancer Chemotherapy. Curr Cancer Drug Targets. 2002; 2:279-308.
35. Kaufmann SH, Earnshaw WC. Induction of Apoptosis by Cancer Chemotherapy. Exp Cell Res. 2000; 256:42-49.
36. Bosio C, Tomasoni G, Martínez R, Olea AF, Carrasco H, Villena J. Cytotoxic and apoptotic effects of leptocarpin, a plant-derived sesquiterpene lactone, on human cancer cell lines. Chem-Biol Interact. 2015; 242:415-421.
37. Firempong CK, Zhang HY, Wang Y, Chen J, Cao X, Deng W, Zhou J, Wang Q, Tong SS, Yu J. Segetoside I, a plant-derived bisdesmosidic saponin, induces apoptosis in human hepatoma cells *in vitro* and inhibits tumor growth *in vivo*. Pharmacol Res. 2016; 110:101-110.
38. Amaravadi R, Kimmelman AC, White E. Recent insights into the function of autophagy in cancer. Genes Dev.

2016; 30:1913-1930.
39. Kenific CM, Debnath J. Cellular and metabolic functions for autophagy in cancer cells. Trends Cell Biol. 2015; 25:37-45.
40. Lyu Q, Tou F, Su H, Wu X, Chen X, Zheng Z. The natural product peiminine represses colorectal carcinoma tumor growth by inducing autophagic cell death. Biochem Biophys Res Commun. 2015; 462:38-45.
41. Jiang H, Sun J, Xu Q, Liu Y, Wei J, Young C, Yuan H, Lou H. Marchantin M: A novel inhibitor of proteasome induces autophagic cell death in prostate cancer cells. Cell Death Dis. 2013; 4:e761.
42. Bubici C, Papa S. JNK signalling in cancer: In need of new, smarter therapeutic targets. Br J Pharmacol. 2014; 171:24-37.
43. Cicenas J, Zalyte E, Rimkus A, Dapkus D, Noreika R, Urbonavicius S. JNK, p38, ERK, and SGK1 Inhibitors in Cancer. Cancers (Basel). 2017; 10:E1.
44. Li HY, Zhang J, Sun LL, Li BH, Gao HL, Xie T, Zhang N, Ye ZM. Celastrol induces apoptosis and autophagy *via* the ROS/JNK signaling pathway in human osteosarcoma cells: An *in vitro* and *in vivo* study. Cell Death Dis. 2015; 6:e1604.
45. Sun ZL, Dong JL, Wu J. Juglanin induces apoptosis and autophagy in human breast cancer progression *via* ROS/JNK promotion. Biomed Pharmacother. 2017; 85:303-312.

(Received December 7, 2018; Revised March 14, 2019; Accepted March 21, 2019)