School of Life Sciences[1], Liaoning Provincial Key Laboratory of Biotechnology and Drug Discovery, Liaoning Normal University, Dalian; Central Research Laboratory[2], Jilin University Bethune Second Hospital, Changchun, P. R. China; Department of Zoology[3], University of the Punjab, Lahore, Pakistan

Vitamin K4 induces tumor cytotoxicity in human prostate carcinoma PC-3 cells via the mitochondria-related apoptotic pathway

Yu Jiang[1], Jingbo Yang[2], Chunhui Yang[2], Fanmin Meng[1], Yongfeng Zhou[1], Bo Yu[1], Muhammad Khan[3], Hong Yang[1]

Received November 11, 2012, accepted December 7, 2012

Hong Yang, School of Life Sciences, Liaoning Provincial Key Laboratory of Biotechnology and Drug Discovery, Liaoning Normal University, Dalian 116029, P.R. China
hyanglnnu@gmail.com

Pharmazie 68: 442–448 (2013) *doi: 10.1691/ph.2013.2872*

Vitamin K4 (VK4) is a synthetic hydrophilic menadione compound, which is clinically used as hemostasis medicine. It has been reported that several vitamin Ks had inhibitory effects on various cancer cells. However, there is no report about VK4 s anticancer activity. The goal of this study was to investigate the inhibitory effect of VK4 on human prostate PC-3 cells and the mechanisms involved. We found that VK4 dose-dependently inhibited cell proliferation in PC-3 cells with an IC_{50} value of about 20.94 μM. Hoechst 33258 Staining results showed that VK4 caused DNA fragmentation in PC-3 cells. PI staining results indicated that VK4-induced PC-3 cell cycle arrest at the S phase. Further mechanistic studies revealed that VK4-mediated induction of apoptosis in PC-3 cell is associated with disruption of mitochondrial membrane potential, down-regulation of Bcl-2, and up-regulation of Bax, release of cytochrome c from mitochondria, and activation of caspase-3 and PARP. Thus, VK4 might be useful in prostate cancer chemotherapy.

1. Introduction

Prostate cancer is the second most frequently diagnosed cancer worldwide, and is the second most common diagnosed cancer of American males. In 2012, there are about approximately 240,000 new cases and 28,000 people die of this malignancy as estimated by a survey report (American Cancer Society 2012). Despite recent progress in diagnostic and multi-modal therapies of initial prostate cancer, there is no effective therapy in treating androgen-insensitive or hormone-refractory recurrent cases and the mortality rate associated with recurrent prostate cancer is still very high (American Cancer Society 2012). So, there is an urgent need to identify new therapeutic agents to improve the overall survival of patients with prostate cancer.

Vitamin K (VK) is a generic name for a class of organic compounds that share a methylated naphthoquinone ring substituted with variable side chains. VKs exist in both natural and synthetic forms (Shearer 1995). VK1 (phylloquinone) and VK2 (menaquinones) are naturally occurring VKs that are synthesized by plants and bacteria, respectively. VK3 and VK4 (menadione) are synthetic derivatives of VK1 and VK2 (Vermeer 2012). VKs play important roles in the normal blood coagulation system, in which they act as cofactors for the synthesis of gammacarboxyglutamate (Gla) protein family such as prothrombin and factors VII, IX, and X (Berkner 2005; Cranenburg et al. 2007). Actually it has long been regarded that hemostasis is the only physiological process VKs involved. Recent studies demonstrated that VK1, VK2, and VK3 had anticancer activities against various human carcinomas including hepatic, leukemia, colonic, lung, oral, breast, and bladder cancers *in vitro* (Mamede et al. 2011; Kitano et al. 2012; Ishibashi et al. 2012; Wu et al. 2012; Baran et al. 2010; Li et al. 2010; Wei et al. 2010; Showalter et al. 2010; Akiyoshi et al. 2009; Kawakita et al. 2009). VK3 (2-methyl-1,4-naphthoquinone) and VK4 (2-methyl-1,4-naphthalenediyldiacetate) are chemically synthesized VKs all belong to menadione compounds. VK4 is the diacetate form of VK3. Both compounds have short side-chains and are hydrophilic. Previous reports showed that VK3 had high potent cytotoxicity against several cancer cell lines (Kitano et al. 2012; Wu et al. 2012; Akiyoshi et al. 2009). As an anticoagulation medicine, VK3 induced severe haemolytic anaemia *in vivo*, so it was left out of mainstay cancer treatment regimens (Shearer 1995). Though VK4 is structurally similar to that of VK3, it shows positive effects on blood coagulation, we hypothesized that VK4 might confer both advantages of the growth inhibitory effect against cancerous cells and less toxicity to normal cells of VK3, thus VK4 might function as potent anti-tumour agents.

In the present study, we investigated cytotoxic effects of VK4 on human prostate carcinoma cells. We found that VK4 induces apoptosis involving DNA fragmentation in PC-3 cells. This apoptosis was initiated via mitochondrial dysfunction and then followed by activation of the caspase-3-related apoptosis pathway.

2. Investigations and results

2.1. Cytotoxicity of VK4 on human prostate carcinoma PC-3 cells

The inhibitory effect of VK4 (chemical structure shown in Fig. 1A) on human prostate PC-3 cells was measured using the MTT method. The cells were incubated with different

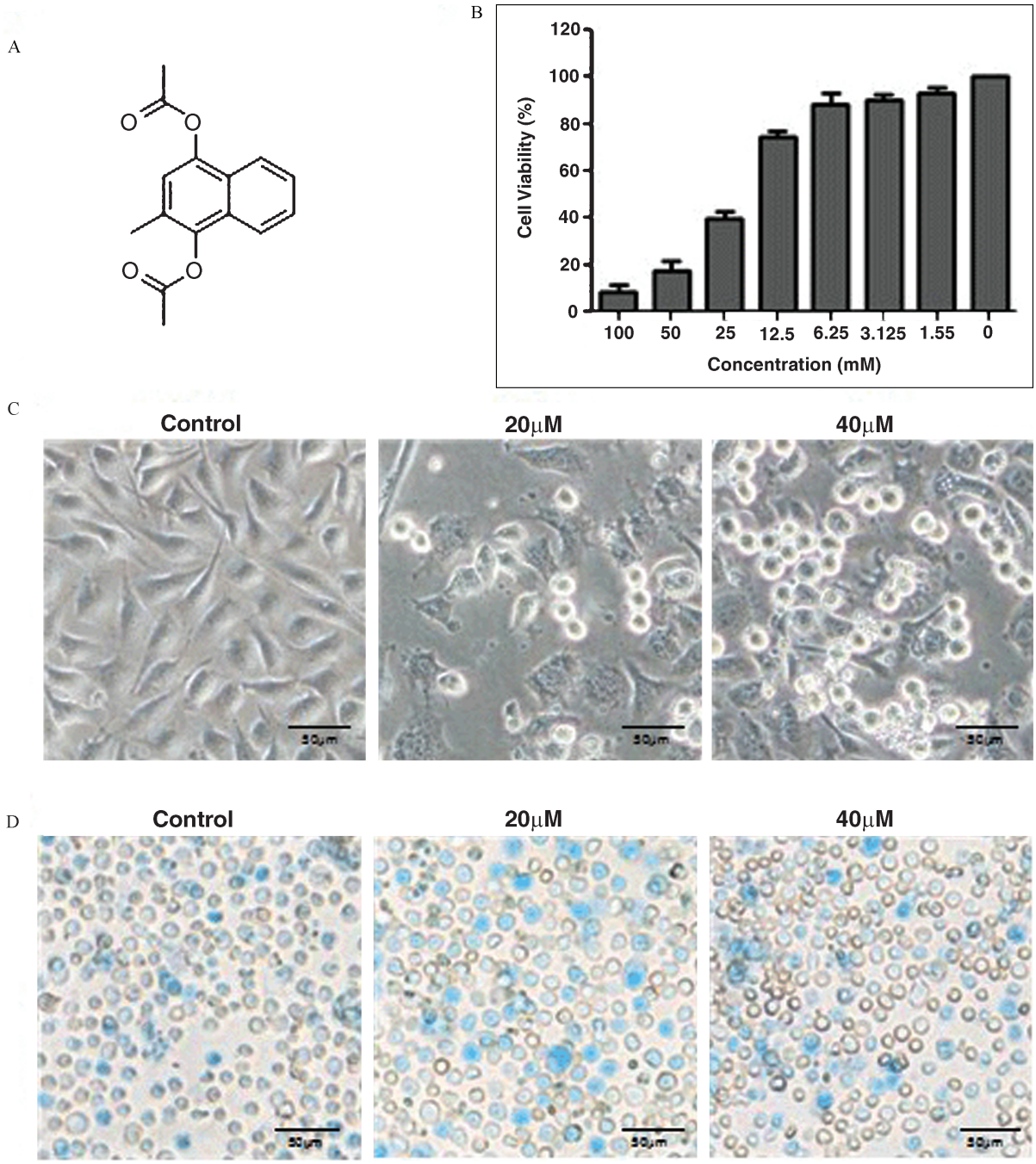

Fig. 1: Effects of VK4 on viability and morphological characteristics of PC-3 cells. (A) Chemical structure of VK4. (B) PC-3 cells were treated with various concentrations of VK4 for 24 h. Cell survival rate was measured using MTT method. Data are expressed as Mean ± SE of three independent experiments with similar results. Morphological changes of PC-3 cells (C) and mouse splenocytes (D) observed under a phase-contrast microscopy after the cells treated with DMSO (negative control) or indicated concentrations of VK4. Scale bar = 50 μm

concentrations of VK4 for 24 h. As shown in Fig. 1B, VK4 concentration-dependently decreased the viability of the PC-3. IC_{50} value of the inhibition was around 20.94 μM. Cell morphology was checked under a phase contrast microscope after treatment with VK4, which resulted in rounded and shrunken cells and a decreased number of cells as compared to the control group (Fig. 1C). We also examined the effect of VK4 on mouse splenocytes, trypan blue method revealed VK4 had little toxic effect on the cells.

2.2. VK4 Induces phase arrest in human prostate carcinoma PC-3 cells

Cell cycle arrest is a possible mechanism involved in the induction of cell proliferation inhibition. It has been fully established that checkpoints play key roles in normal cell cycle progression. Loss of checkpoint control results in uncontrolled cell proliferation. Many factors such as DNA damage, exogenous stress signals, and defects during the DNA replication or failure of chromosomes to attach with the mitotic spindle may disturb the functions of various checkpoints. Various cell checkpoints arrest may lead to DNA content change, so they can be detected by measuring DNA content changes (Bartek and Lukas 2007; Houtgraaf et al. 2006). To investigate whether inhibition of PC-3 is involved in cell cycle arrest, we treated the cells with different concentrations of VK4 for 24 h. VK4 arrested the cell cycle at S phase in a dose-dependent way. The percentage of accumulation of cells in the S phase was increased from 23.7% ± 0.0054 in the control group to 33.3% ± 0.0087, 36% ± 0.0070 and 48.5 % ± 0.0149 in the cells treated with 20, 30, and 40 μM VK4,

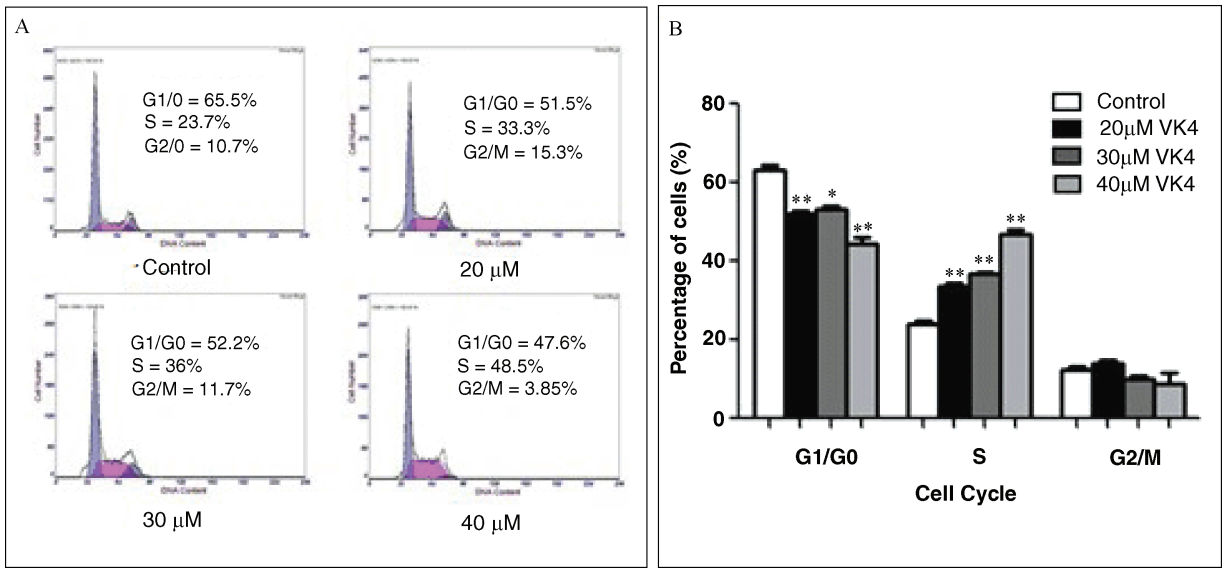

Fig. 2: Effect of VK4 on PC-3 cell cycle distribution. (A) Flow cytometry results of cell cycle phase distribution analysis. PC-3 cells treated with 0, 20, 30, and 40 μM VK4 for 24 h, and were stained with PI for flow cytometric analysis. (B). Data are expressed as Mean ± SE of three independent experiments. X-axis and y-axis represent DNA content and number of cells, respectively. Data are expressed as Mean ± SE of three independent experiments with the similar results. $p < 0.05$, $^{**}p < 0.01$ compared with the control

respectively, with a corresponding decrease in G0/G1 phase cells from 65.6% ± 0.0151 to 51.5% ± 0.0092, 52.2% ± 0.0081, and 47.6% ± 0.0198, respectively (Fig. 2).

2.3. VK4 Induces apoptotic cell death in human prostate carcinoma PC-3 cells

Apoptosis is also an effective mechanism involved in the induction of cell death. Nuclear condensation is one of the most typical characteristics of apoptosis. To investigate the effect of VK4 on induction of apoptosis in PC-3 cells, PC-3 cells were incubated with different concentrations (20, 30, or 40 μM) of VK4 for 24 h. Cells were collected and stained with Hoechst 33258, and then analyzed under a fluorescence microscope. As shown in Fig. 3A , VK4 caused significant nuclear condensation in PC-3 cells, which correlate well with apoptotic features. To further confirm the apoptosis induction effect of VK4, the above PC-3 cells were double stained with Annexin V-FITC and PI to detect apoptosis and necrosis, as the flow cytometry results showed in Fig. 3B. The rate of apoptosis was increased to 1.64 ± 0.0010%, 17.56 ± 0.0121%, and 45.07 ± 0.0202% in the cells treated with 20, 30, or 40 μM VK4 (vs 0.48% in control group), as shown in Fig. 3C.

2.4. VK4 reduced mitochondrial membrane potentials in human prostate carcinoma PC-3 cells

Mitochondria play key roles in regulation of cell death and proliferation through generating reactive oxygen species (ROS) (Schultz and Harrington 2003; Antico et al. 2012; Gottlieb 2001). The mitochondrial apoptotic pathway is associated with ROS generation and mitochondrial membrane potential (MMP) disruption. Recently, several studies showed that VK induced apoptosis through ROS-mediated mitochondrial signaling pathways (Wu et al. 2011; Li et al. 2010). So, we examined the effects of VK4 on PC-3 ROS. The PC-3 cells were treated with DMSO (negative control), or with 20, 30 and 40 μM VK4 for 24 h, intracellular ROS level was measured by using a DCF-DA detection kit, and the DCF fluorescence intensity was measured by flow cytometry. It found that the level of ROS (indicated as DCF fluorescence) in VK4 treated group remained unchanged (Fig. 4A).

The loss of MMP is a hallmark for apoptosis. MMP changes were detected by using Rho-123 staining method. PC-3 cells were exposed to VK4 for 24 h, and then stained with Rho-123. As shown in Fig. 4B, VK4 dose-dependently increased Rho-123 fluorescence to 63.27%, 76.94%, and 84.35% vs 95.59% in control group.

2.5. Effect of VK4 on major mitochondrial apoptosis regulatory proteins

To better understand VK4-induced apoptosis in PC-3 cells, we detected the expression of some major mitochondrial apoptosis regulatory proteins (Bax, Bcl-2, PARP, Cleaved-caspase-3) and release of Cyt-c using Western blot analysis. As shown in Fig. 5, Treatment with VK4 led to upregulation of Bax, PARP, and cleaved-caspase-3, down-regulation of Bcl-2, and induction of the release of cytochrome c. These findings clearly indicate that VK4 induces caspase-dependent apoptosis in PC-3 cells.

3. Discussion

In the present study, the authors showed for the first time the inhibitory effect of VK4 on human prostate cancer cell line PC-3 cells. VK4 dose-dependently inhibited proliferation of PC-3 cells. The cytotoxicity was induced through nuclear DNA fragmentation suggesting that VK4 causes cell death in PC-3 by apoptosis.

We then investigated the mechanism of VK4-induced apoptosis in PC-3 cells. It has been well established that apoptosis is mainly initiated *via* two distinct but convergent pathways: the death receptors and mitochondrial pathways (Akiyoshi et al. 2009; Kawakita et al. 2009). The mitochondrial pathway is controlled by balance of antiapoptotic (e.g., Bcl-2) and pro-apoptotic proteins (e.g., Bax). Previous reports showed that other VK family members, e.g. VK2 and VK3, induced intrinsic apoptosis in Smmc-7721 and MCF-7 cells through up-regulation of Bax/Bcl-2 ratio (Schultz and Harrington 2003). So, we examined the expression of Bcl-2 and Bax protein in VK4 treated PC-3 cells. The results indicated a significant decrease in Bcl-2 expression and increase in Bax expression in PC-3 cells treated with VK4. These findings suggested that VK4 induced apoptosis in PC-3 cells via mitochondrial intrinsic apoptotic pathway.

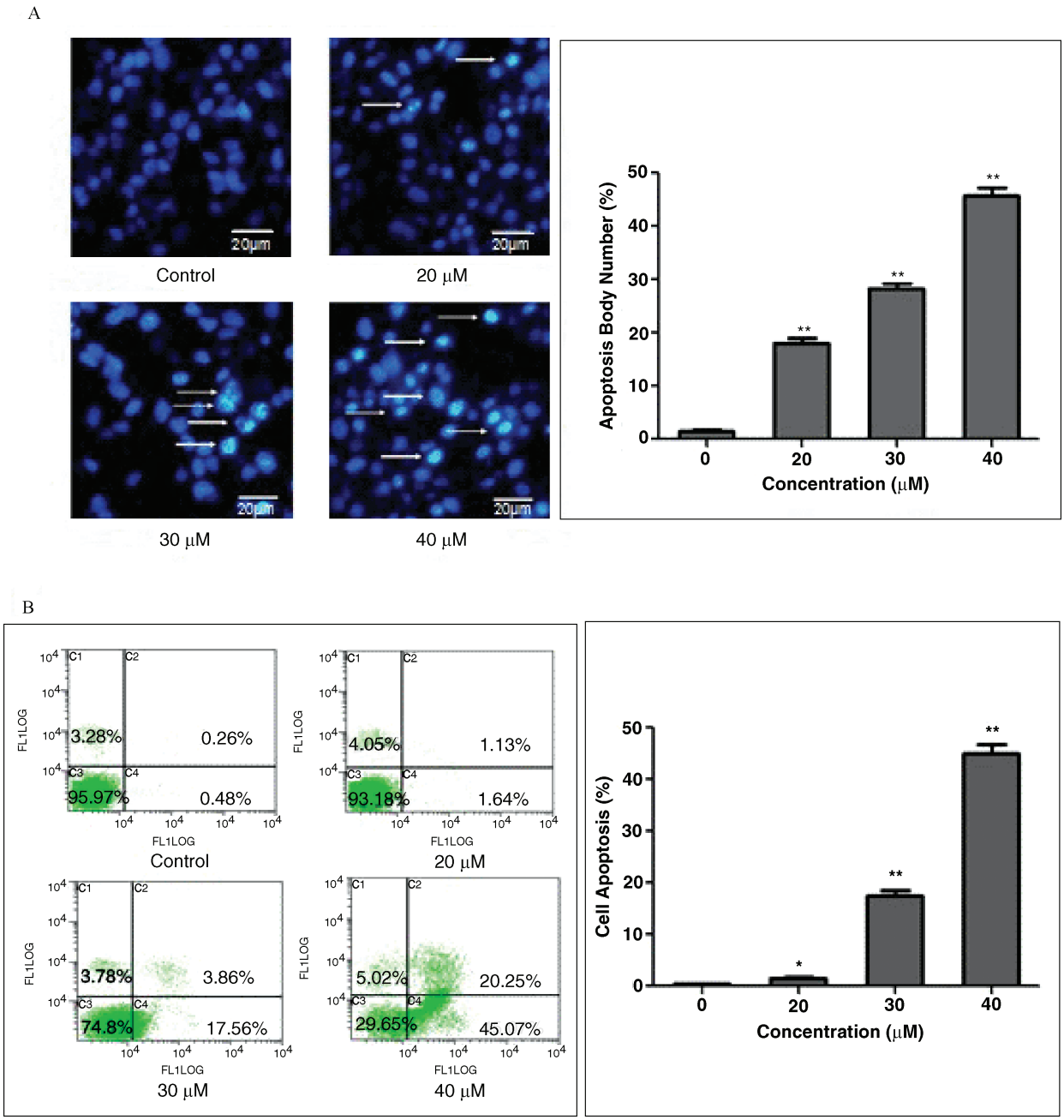

Fig. 3: Induction of apoptosis by VK4 in PC-3cells. (A) Cells were treated with 0, 20, 30, and 40 μM VK4 for 24 h, and stained with Hoechst 33258. The stained cells were observed under a fluorescence microscope. Arrows indicate the condensed and fragmented nuclei. Scale bar = 20 μm. Histograms show percentage of cleaved nuclei counted microscopically from 100 nuclei. Data are expressed as Mean ± SE of three independent experiments with the similar results. $^{**}p < 0.01$ compared with the control. (B) PC-3 cells treated with different concentrations of VK4 for 24 h. The cells were then stained with FITC-conjugated Annexin V and PI for flow cytometric analysis. X-axis and y-axis represent Annexin V-FITC staining and PI, respectively. Cell populations shown in the lower right (Annexin V+/PI-) represents apoptotic cells, upper right (Annexin V+/PI+) represents necrotic cells

Because depolarization in mitochondrial membrane potential is a characteristic feature of apoptosis, we further examined MMP in PC-3 cells. It showed that VK4 significantly reduced MMP that further support mitochondrial apoptotic pathway induced by VK4. A significant reduction in MMP suggests an increased opening state of mitochondrial transmembrane pore (MTP) which can lead the release of cytochrome c. Release of cytochrome c may lead to the activation of caspase-3 (Schultz and Harrington 2003; Gottlieb 2001) which eventually cleave effector proteins including PARP (Soldani and Scovassi 2002). In agreement with above findings, VK4 induced the release of cytochrome c and cleaved caspase-3 and PARP in PC-3 cells. These results further support intrinsic apoptotic pathway. Cytochrome c release from mitochondria is largely mediated by direct or indirect ROS action. Wu and colleagues reported that VK3 stimulated ROS production in human glioma cells (Wu et al. 2011). Contrast to Wu's results, we didn't find significant ROS stimulation in PC-3 cells treated with VK4.

In conclusion, VK4 induced apoptosis in human prostate cancer cell line PC-3 cells *via* the mitochondria-related apoptotic pathway. Our findings suggest new pharmaceutical activity of VK4, and VK4 may become a potential leading drug in the therapy of prostate carcinoma.

4. Experimental

4.1. Chemicals and reagents

VK4 was purchased from the National Institute for Food and Drug Control (Beijing, China). VK4 was dissolved in DMSO to make a mother solution which was stored at −80 °C. Stability of the compound was ver-

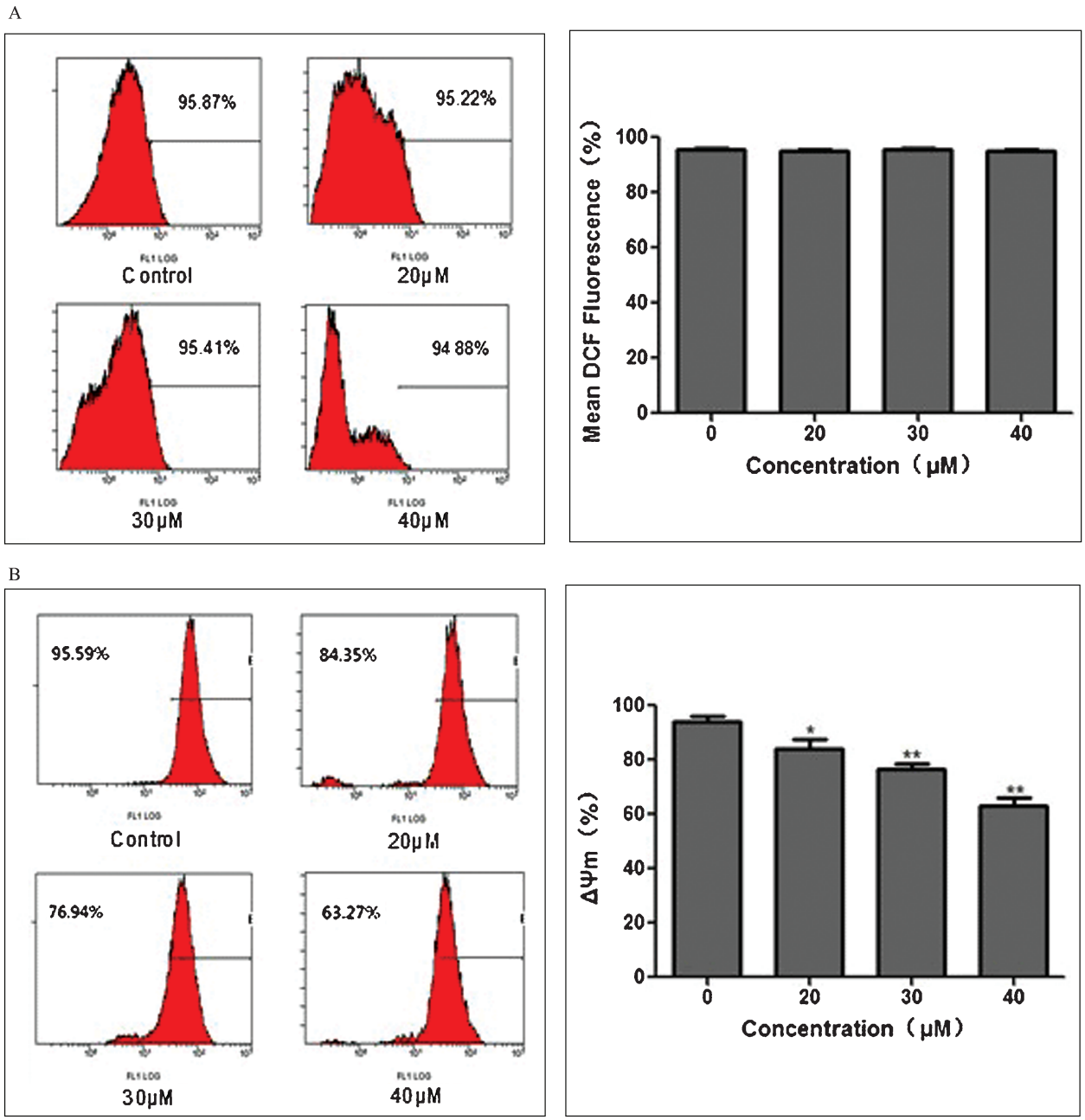

Fig. 4: Flow cytometry analysis of ROS generation (A) and MMP in PC-3 cells (B). PC-3 cells were treated with various concentrations of VK4 for 24 h. Data are expressed as Mean ± SE of three independent experiments with similar results. $*p < 0.05$, $**p < 0.01$

ified by HPLC analysis. Characterized fetal bovine serum was purchased from HyClone Company (USA). MTT [3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], DMEM cell culture medium, trypsin, Rhodamine 123 and Hoechst 33258 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from Sangon Biotech Co., Ltd (Shanghai, China). Annexin V-FITC Apoptosis Detection Kit and Cell Cycle, Apoptosis Analysis Kit, BCA Protein Assay Kit and PVDF membrane were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Rabbit anti-human Bax, Cyt-C, Caspase-3 antibodies, mouse anti-human β-actin, Bcl-2, PARP andtibodies were purchased from Santa Cruz Biotechnology (USA). ECL reagent was purchased from GE (USA).

4.2. Cell culture

Human prostate cancer cell line PC-3 cells were purchased from ATCC (China) and maintained in our lab. The cells were cultured in DMEM supplemented with 10% FBS. Newly thawed cells were passed at least 3 passages before tests.

Mouse splenocytes were freshly isolated from Kunming mice (Central Research Laboratory, Jilin University Bethune Second Hospital, China). The splenocytes were cultured in RPMI 1640 medium supplemented with 10% (v/v) FBS and maintained in a CO_2 incubator (5% CO_2 and 95% air, 95% humidity).

4.3. Cell proliferation assay

MTT method was used to evaluate the effect of VK4 on PC-3 proliferation (Khan et al. 2012). Logarithmic phase PC-3 cells were seeded into 96-well tissue culture plates at a density of 10^5 per well, and incubated in CO_2 incubator (37 °C, 5% CO_2, 95% humidity) for about 6 h. The cells were treated with different concentrations of test compounds for 24 h, and then 20 μl MTT (5 mg/ml, dissolved in PBS) was added into each well and incubated for another 4 h. The medium was then removed and formazan crystals were dissolved by adding 150 μl DMSO into each well. The plate was gently shaken for 15 min and A_{570} data were determined using a microplate reader (Thermo, Vario SKAN Flash). Percentage viability rate (V%) was calculated as follows

$$V\% = A_{570}(\text{treated})/A_{570}(\text{control}) \times 100$$

The IC_{50} values were calculated using Graphpad Prism 5.0.

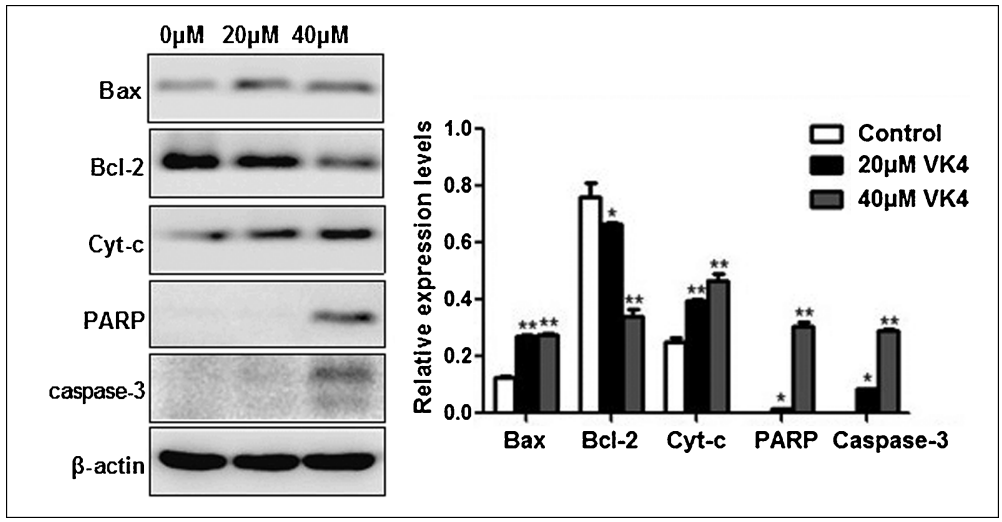

Fig. 5: Effect of VK4 on mitochondrial apoptosis regulators. (A) PC-3 cells were treated with indicated concentrations of VK4 for 24 h. Expression of Bax, Bcl-2, Cyt-c, PARP and cleaved caspase-3 in PC-3 cells were monitored using Western blot assay. β-actin was used as loading control. (B) The data shown are representative of three independent experiments with the similar results. *$p < 0.05$, **$p < 0.01$

4.4. Cell cycle analysis

Cell cycle distribution was evaluated by the flow cytometry method (Rasul et al. 2012). PC-3 cells were treated with test compound or DMSO (as negative control) for 24 h. The cells were trypsinized, rinsed twice with PBS, and then centrifuged (1000 rpm x 5 min, 4 °C). Cell pellets were fixed with 70% ethanol at −20 °C overnight. Then the cells were thoroughly rinsed with PBS and stained with RNase A-containing PI solution (50 μg/ml) for 30 min at 37 °C. Cell suspension was analyzed by flow cytometry (Beckman Coulter, Epics XL-MCL).

4.5. Flow cytometric analysis of apoptosis

PC-3 cells were treated with VK4 or DMSO (as negative control) for 24 h. The cells were collected, washed with PBS, centrifuged (1000 rpm x 5 min) and stained with Annexin V-FITC and PI sequentially as protocols. All experiments were done at room temperature in dark. The Annexin V-FITC/PI double stained cells were analyzed using flow cytometry and data were analyzed using Cell Quest software.

4.6. Measurement of intracellular ROS generation

DCF-DA detection kit was used to determinate ROS generation. Experiments were done according to manufacture's instruction. PC-3 cells were treated with VK4 for specified duration, harvested, suspended in PBS, and then incubated with 10 μM DCF-DA at 37 °C for 30 min. the cells were collected with centrifugation, washed with PBS (3 times), and suspended in 0.5 ml PBS. DCF fluorescence data were measured using flow cytometry.

4.7. Measurement of mitochondrial membrane potential ($\Delta\Psi_m$)

$\Delta\Psi_m$ was determined as reported (Ji et al. 2011). Briefly, after treating with VK4 or DMSO (negative control) for 24 h, PC-3 cells were collected and washed twice with PBS. The cells were incubated with Rhodamine 123 (Rho-123, 10 μg/ml) at 37 °C for 30 min, centrifuged (1000 rpm for 5 min), re-suspended in 200 μl of PBS. Rho-123 fluorescence was determined using flow cytometry with λex and λem wavelengths of 488 and 530 nm, respectively.

4.8. Western blot analysis

PC-3 Cells were exposed to VK4 for 24 h, then collected with centrifugation at 4000 rpm for 5 min, 4 °C. The cell pellets were washed twice with pre-cooled PBS and lyzed with RIPA Lysis Buffer (Beyotime, Shanghai, China). Nuclear and cytosolic and proteins were extracted using cytosolic and nuclear extraction kit (Keygen, China) according to the manufacturer's instructions. The lysate was centrifuged at 12000 rpm for 15 min at 4 °C to collect supernatants. The protein concentrations were determined using Nano Drop 1000 (Thermo Scientific, USA) spectrophotometer. The supernatants (40 μg of proteins) were electrophoresed on 12% SDS-PAGE and Western blotting analyses were done on PVDF membranes. The PVDF membranes were sequentially blocked with 5% (w/v) nonfat milk, washed with tris-buffered saline tween solution (TBST), and then incubated with respective primary antibodies at 4 °C for overnight. After washing three times with TBST, the membranes were incubated with anti-rabbit or anti-mouse horseradish-peroxidase-conjugated secondary antibodies for 1 h. Signals were detected using ECL plus chemiluminescence kit on X-ray film (Millipore Corporation, Billerica, USA).

4.9. Statistical analysis

The data are expressed as Mean ± SEM. Student's *t* test was used to compare test and control values, *P* values of < 0.05 were considered statistically significant.

Acknowledgements: This work was supported by Special Fund for Doctorate Disciplines Construction in Chinese Universities (20112136110002), Fund for key laboratory from Department of Education of Liaoning ProvinceZ (LS2010094) National Natural Science Fund (No.30973577).

References

Akiyoshi T, Matzno S, Sakai M, Okamura N, Matsuyama K (2009) The potential of vitamin K3 as an anticancer agent against breast cancer that acts via the mitochondria-related apoptotic pathway. Cancer Chemother Pharmacol 65: 143–150.

American Cancer Society: (2014) http://www.cancer.org/cancer/prostatecancer/overviewguide/prostate-cancer-overview-key-statistics; http://www.cancer.org/cancer/prostatecancer/overviewguide/index; http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-treating-general-info; http://www.cancer.org/cancer/prostatecancer/overviewguide/prostate-cancer-overview-survival-rates

Antico Arciuch VG, Elguero ME, Poderoso JJ, Carreras MC (2012) Mitochondrial regulation of cell cycle and proliferation. Antioxid Redox Signal 16: 1150–1180.

Baran I, Ganea C, Scordino A, Musumeci F, Barresi V, Tudisco S, Privitera S, Grasso R, Condorelli DF, Ursu I, Baran V, Katona E, Mocanu MM, Gulino M, Ungureanu R, Surcel M, Ursaciuc C (2010) Effects of menadione, hydrogen peroxide, and quercetin on apoptosis and delayed luminescence of human leukemia Jurkat T-cells. Cell Biochem Biophys 58: 169–179.

Bartek J, Lukas J (2007) DNA damage checkpoints: from initiation to recovery or adaptation. Curr Opin Cell Biol 19: 238–245.

Berkner KL (2005) The vitamin K-dependent carboxylase. Annu Rev Nutr 25: 127–149.

Cranenburg C, Schurgers LJ, Vermeer C (2007) Vitamin K: the coagulation vitamin that became omnipotent. Thromb Haemost 98: 120–125.

Gottlieb RA (2011) Mitochondria and apoptosis. Biol Signals Recept 10: 147–161.

Houtgraaf JH, Versmissen J, van der Giessen WJ (2006) A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovasc Revasc Med 7: 165–172.

Ishibashi M, Arai M, Tanaka S, Onda K, Hirano T (2012) Antiproliferative and apoptosis-inducing effects of lipophilic vitamins on human melanoma A375 cells *in vitro*. Biol Pharm Bull 35: 10–17.

Ji YB, Qu ZY, Zou X (2011) Juglone-induced apoptosis in human gastric cancer SGC-7901 cells via the mitochondrial pathway. Experim Toxicol Pathol 63: 69–78.

Kawakita H, Tsuchida A, Miyazawa K, Naito M, Shigoka M, Kyo B, Enomoto M, Wada T, Katsumata K, Ohyashiki K, Itoh M, Tomoda A, Aoki T (2009) Growth inhibitory effects of vitamin K2 on colon cancer cell lines via different types of cell death including autophagy and apoptosis. Int J Mol Med 23: 709–716.

Khan M, Yu B, Rasul A, Al Shawi A, Yi F, Yang H, Ma T (2012) Jaceosidin induces apoptosis in U87 glioblastoma cells through G2/M phase arrest. Evid-Based Complem Altern Med doi: 10.1155/2012/703034.

Kitano T, Yoda H, Tabata K, Miura M, Toriyama M, Motohashi S, Suzuki T (2012) Vitamin K3 analogs induce selective tumor cytotoxicity in neuroblastoma. Biol Pharm Bull 35: 617–623.

Li L, Qi Z, Qian J, Bi F, Lv J, Xu L, Zhang L, Chen H, Jia R (2010) Induction of apoptosis in hepatocellular carcinoma Smmc-7721 cells by vitamin K(2) is associated with p53 and independent of the intrinsic apoptotic pathway. Mol Cell Biochem 342: 125–131.

Mamede AC, Tavares SD, Abrantes AM, Trindade J, Maia JM, Botelho MF (2011) The role of vitamins in cancer: a review. Nutr Cancer 63: 479–494.

Rasul A, Khan M, Yu B, Ma T, Yang H (2011). Xanthoxyletin, a Coumarin Induces S Phase Arrest and Apoptosis in Human Gastric Adenocarcinoma SGC-7901 Cells. Asian Pac J Cancer Prev 12: 1219–23.

Schultz DR, Harrington WJ Jr (2003) Apoptosis: programmed cell death at a molecular level. Semin Arthritis Rheum 32: 345–369.

Shearer MJ. Vitamin K. Lancet 1995; 345: 229–34.

Showalter SL, Wang Z, Costantino CL, Witkiewicz AK, Yeo CJ, Brody JR, Carr BI (2010) Naturally occurring K vitamins inhibit pancreatic cancer cell survival through a caspase-dependent pathway. J Gastroenterol Hepatol 25: 738–744.

Soldani C, Scovassi AI (2002) Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis 7: 321–328.

Vermeer C (2012) Vitamin K: the effect on health beyond coagulation - an overview. Food Nutr Res 56: doi: 10.3402/fnr.v56i0.5329.

Wei G, Wang M, Carr BI (2010) Sorafenib combined vitamin K induces apoptosis in human pancreatic cancer cell lines through RAF/MEK/ERK and c-Jun NH2-terminal kinase pathways. J Cell Physiol 224: 112–119.

Wu J, Chien CC, Yang LY, Huang GC, Cheng MC, Lin CT, Shen SC, Chen YC (2011) Vitamin K3-2,3-epoxide induction of apoptosis with activation of ROS-dependent ERK and JNK protein phosphorylation in human glioma cells. Chem Biol Interact 193: 3–11.