Linalool inhibits 22Rv1 prostate cancer cell proliferation and induces apoptosis

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Abstract. Linalool is an unsaturated terpene that can be found in several plants and exhibits various biological activities. The aim of the present study was to investigate the anticancer activity of linalool using the human prostate cancer 22Rv1 cell line. Flow cytometry was employed to study the effects of linalool on the induction of apoptosis, cell cycle progression, loss of mitochondrial membrane potential and cytochrome *c* release, whereas the effects of linalool on apoptosis-associated proteins were investigated by western blot analysis. An efficacy study was conducted using 22Rv1 tumor-bearing mice. The expression of the cell proliferation markers Ki-67 and proliferating cell nuclear antigen (PCNA) in xenograft tumors was evaluated by immunohistochemistry. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was used to study the induction of apoptosis in an *in vivo* model. Linalool exerted an inhibitory effect on 22Rv1 cell proliferation and induced apoptosis in both *in vitro* and *in vivo* models. Western blot analysis indicated that both the mitochondria-mediated intrinsic and death-receptor-mediated extrinsic pathways were involved in the induction of apoptosis. Furthermore, linalool significantly reduced the expression of Ki-67 and PCNA in the 22Rv1 xenograft model. The findings of the present study provide evidence supporting the anti-proliferative effects of linalool on 22Rv1 human prostate cancer cells, and suggest that linalool may be an effective agent for prostate cancer treatment.

Introduction

Prostate cancer is the most common cancer in men and the second leading cause of cancer-associated mortality (1).

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Almost all cases prostate cancer are adenocarcinomas and are characterized by slow growth, displaying no signs or symptoms during the early stages. The 5-year survival rate of localized prostate cancer is ~100%; however, the survival rate decreases to 30% for metastatic prostate cancer (2). The major treatment modalities for prostate cancer include prostatectomy, radiotherapy, hormonotherapy and chemotherapy (3). The treatment options depend on cancer stage, patient's age and potential benefits (for example overall survival). The side effects of the treatment and the development of drug resistance are the major obstacles in prostate cancer management (4). Therefore, seeking novel therapeutic approaches, such as medications derived from natural products, has become a topic of great interest (5).

Herbs and plant extracts are considered as potential candidates for drug development and serve as alternative therapies for cancer treatment with minimal side effects (6). In recent years, plant-based drugs have attracted great interest due to their anticancer properties, and have gradually become a research focus (7). Previous studies indicate that the majority of medicinal and aromatic herbs contain valuable compounds with unique properties (8-10). Several popular drugs and compounds have been isolated from medicinal plants and used to treat various diseases; examples include artemisinin, schisandrin C, paclitaxel, vincristine and vinblastine (11).

Linalool is a natural terpenoid alcohol substance that may be found in several herbs, spices and fruits (12). Linalool has been reported to possess anti-microbial, anti-inflammatory and antioxidant properties (13). Moreover, linalool exhibits anticancer potential against prostate cancer, colon cancer, leukemia and cervical cancer (14,15). The anticancer activity of linalool may be due to its apoptotic effect, oxidative stress induction, cell cycle arrest and immunomodulatory properties (14,15). In DU145 and PC-3 prostate cancer cells, linalool was able to induce cell cycle arrest and the extrinsic death receptor-dependent apoptosis pathway (16). Linalool was found to protect HDFa cells from oxidative stress by inhibiting the phosphorylation of the ERK1, JNK and p38 proteins of the mitogen-activated protein kinase family and the activation of nuclear factor-κB/p65 (17). Linalool also induced Th1 cellular immune response in T-47D cells by stimulating interferon-γ, interleukin (IL)-13, IL-2, IL-21, IL-21R, IL-4,

IL-6sR and tumor necrosis factor (TNF)-α secretion (18). p53 and cyclin-dependent kinase inhibitors were found to be upregulated in linalool-treated leukemia cells (19). In addition, caspase-3 and caspase-9 were activated in linalool-induced glioma cell apoptotic death (20).

In the present study, the anti-proliferative effect and mechanism of action of linalool in prostate cancer 22Rv1 cells were investigated. The efficacy of the compound was evaluated and compared in both an *in vitro* cell line-based model and an *in vivo* xenograft tumor model.

Materials and methods

Materials. The 22Rv1 human prostate cancer cell line was obtained from the Chinese Academy of Sciences Cell Bank (Shanghai, China). Linalool (97% purity), Cell Counting Kit-8 (CCK-8) and the Annexin V-FITC apoptosis detection kit were purchased from Sigma-Aldrich; (cat. no. APOAF); Merck KGaA. The DNA content quantification assay kit and the rhodamine 123 kit were purchased from Nanjing KeyGen Biotech Co., Ltd. The FlowCellect Cytochrome *c* kit (cat. no. FCCH100110) was purchased from Luminex Corporation.

Cytotoxicity assay. Human prostate cancer 22Rv1 cells were maintained in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) medium supplemented with 1 mM sodium pyruvate and 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.). Cells were seeded in clear flat-bottomed 96-well plates at a density of $5x10^3$ cells/well in 90 μl RPMI-1640 growth medium. After the cells adhered to the bottom, linalool in PBS with 1% dimethyl sulfoxide (50, 25, 10, 5, 1 and 0.5 mM) were added and incubated at 37°C with 5% CO_2. After 48 h of treatment, 10 μl CCK-8 were added to each well. Following incubation at 37°C for 4 h, the plates were read at 450 and 630 nm on a plate reader.

Apoptosis. An Annexin V-FITC Apoptosis Detection kit was used to study the apoptosis induction of linalool. Samples were prepared according to the manufacturer's instruction. Briefly, 22Rv1 cells were seeded in 6-well plates at a density of $5x10^5$ cells/well in 1 ml RPMI-1640 growth medium. After the cells adhered to the surface (12 h), they were treated with linalool (2.5 mM) for 24 h. The cells were collected, washed twice with PBS, resuspended in 1X binding buffer (cat. no. B9796, Sigma-Aldrich; Merck KGaA), and stained with Annexin V-FITC conjugate (cat. no. A9210, Sigma-Aldrich, ~50 μg/ml in 50 mM Tris-HCl, pH 7.5, containing 100 mM NaCl) and propidium iodine (PI; cat. no. P2667; Sigma-Aldrich; Merck KGaA; 100 μg/ml in 10 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl) for 10 min at room temperature in the dark. Subsequently, the samples were quantified by a flow cytometer (CyFlow Space; Sysmex Partec GmbH) and analyzed by FloMax cell cycle analysis software, version 2.82 (Quantum Analysis GmbH).

Cell cycle analysis. Cell cycle analysis was performed using a DNA content quantitation assay kit (Nanjing KeyGen Biotech Co., Ltd.). The cells were seeded in a 6-well plate at a density of $5x10^5$ cells/well in 1 ml RPMI-1640

growth medium and treated with linalool (2.5 mM) for 24 h at 37°C. After the treatment, the cells were collected and fixed with 70% ethanol at 4°C overnight. The cells were washed 3 times with PBS and stained with PI in the presence of 1% DNase-free RNase A at 37°C for 30 min prior to flow cytometry analysis. The distribution of cells among the different cell cycle phases was determined using FloMax cell cycle analysis software, version 2.82 (Quantum Analysis GmbH).

Mitochondrial transmembrane potential. The rhodamine 123 kit was used to monitor mitochondrial function in the cells. After linalool (2.5 mM) treatment for 24 h at 37°C, the cells were collected and washed. Subsequently, the cells were resuspended in 1 ml RPMI-1640 medium with 10 μg/ml rhodamine 123 and incubated at 37°C for 10 min. The cells were washed with PBS once and analyzed with a flow cytometer (FloMax 2.82, CyFlow Space; Sysmex Partec GmbH).

Cytochrome c release. The FlowCellect Cytochrome c kit was used to investigate the effect of linalool on cytochrome *c* release. The assay was done according to the manufacturer's instructions. Briefly, following treatment with linalool (2.5 mM) for 24 h at 37°C, the cells were collected and washed. Subsequently, the cells were incubated with 100 μl permeabilization working solution on ice for 10 min, followed by 100 μl fixation working solution at room temperature for 20 min. Thereafter, the cells were washed with 1X blocking buffer once. After centrifugation at 13,000 x g (4°C) for 10 min, the cells were incubated with 100 μl 1X blocking buffer for 30 min at room temperature, followed by addition of 10 μl anti-cytochrome c-FITC and incubation at room temperature for 30 min. After another centrifugation step, blocking buffer (from the kit) was added for flow cytometry analysis (FloMax 2.82, CyFlow Space; Sysmex Partec GmbH).

Western blot analysis. The 22Rv1 cells ($1x10^6$) were seeded in a 25-cm^2 flask and treated with linalool (2.5 mM) for 24 h at 37°C. After the treatment, the cells were collected and lysed with RIPA buffer on ice for 30 min. The protein concentration was determined by a BCA kit (Beyotime Institute of Biotechnology). The proteins (30 μg per lane) were separated on a 10% SDS-PAGE gel and electrotransferred onto a PVDF membrane. The membrane was blocked with 5% bovine serum albumin at room temperature for 1 h, and then immunoblotted with antibodies against caspase-3, cleaved caspase-3 (cat. nos. 9665 and 9664, respectively; both 1:1,000; Cell Signaling Technology, Inc.), caspase-8 (cat. no. SC56070; 1:500 dilution; Santa Cruz Biotechnology, Inc.), cleaved caspase-8 (cat. no. 9748; 1:1,000; Cell Signaling Technology, Inc.), caspase-9, cleaved caspase-9, DR4, DR5 (cat. nos. ab32539, ab2324, ab8414 and ab8416, respectively; all 1:1,000; Abcam), p53 (cat. no. AF0879; 1:1,000; Affinity Biosciences, Inc.), Bcl-2 (cat. no. ab59348; 1:1,000; Abcam), Bax, and β-actin (cat. nos. GB11007 and GB12001, respectively; 1:300 and 1:3,000, respectively; Wuhan Servicebio Technology, Co., Ltd.). The BeyoECL Plus kit (Beyotime Institute of Biotechnology) was used for visualization. The

bands were analyzed by AlphaEaseFC 4.0 software (Genetic Technologies, Inc.).

Efficacy study in nude mice. Male BALB/c nude mice (specific pathogen-free; 6 weeks old, 18-22 g) were purchased from HFK Bioscience Co, Ltd. The animals were fed a standard commercial diet purchased from Beijing Keao Xieli Feed Co., Ltd. The nude mice were housed in the SPF Animal Experiment Center (no. of permit: SYXK K2015-0002) that is specific and pathogen-free with a 12 h light-dark cycle. The room was maintained at a temperature of 18 to 22°C, relative humidity of 40 to 60%. Food and water are accessible at all times.

A 22Rv1 cell suspension ($1x10^7$ cells in 0.1 ml PBS) was inoculated subcutaneously into the rear flanks of the mice. Once xenograft tumors were palpable (~50 mm³), the animals were randomly divided into two groups (five mice/group) and treated with either a solvent control (15% polyethylene glycol 400) or linalool (100 mg/kg body weight) twice a week for 4 weeks via subcutaneous injection (12). Tumor growth was measured twice a week with calipers, and the tumor volume was calculated by the following formula: Length x width x height x 0.5236. Animals were euthanized by cervical dislocation once the xenograft tumor in control groups reached the limit of tumor size (1,600 mm³).

All animal experimental procedures were in compliance with the Guide for the Care and Use of Laboratory Animals at the Kunming Medical University and were approved by the Kunming Medical University Experimental Animal Ethics Committee (approval no. KMMU 2015008).

Immunohistochemistry. All mice were euthanized after the last treatment on the 28th day. Tumor tissues were collected, washed with PBS, fixed in 10% neutral formalin at room temperature overnight, and embedded in paraffin. Paraffin blocks were cut into 5-μm sections. Sections were mounted onto slides and air dried for 30 min. Then the slides were baked at 45°C in an oven overnight. After antigen retrieval at 100°C with 0.01 M pH 6.0 citric acid buffer for 10 min, the slides 'Then the slides were incubated with primary antibodies, Ki-67 (1:250; clone H-300; cat. no. sc-15402; Santa Cruz Biotechnology, Inc.) and proliferating cell nuclear antigen (PCNA; 1:250; clone PC10; cat. no. sc-56; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Slides were washed and incubated with a secondary antibody (1:50 dilution; m-IgGκ BP-HRP; cat. no. sc-516102; Santa Cruz Biotechnology, Inc.) for 30 min at room temperature. Then, the sections were visualized under a light microscope (400x magnification) using a DAKO LSAB detection system (K0679; Dako; Agilent Technologies) and immunosignal quantification was performed with the following formula: (Positive cell number/total cell number) x100%.

Apoptosis in xenograft tumors. To detect apoptotic cell death in xenograft tumors, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed using the *in situ* TUNEL cell apoptosis detection kit (KGA702, KeyGEN BioTECH). Slides were prepared according to the manufacturer's instructions. Three fields of each section were visualized (400x magnification). The immunosignal indices
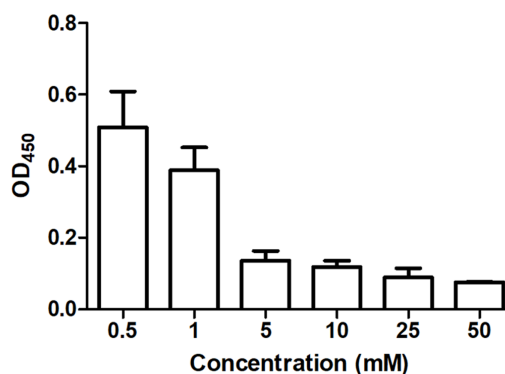

Figure 1. Linalool inhibits 22Rv1 cell proliferation in a dose-dependent manner. OD, optical density.

were calculated as follows: (Apoptotic cell number/total cell number) x100.

Statistical analysis. GraphPad Prism 5 software (GraphPad Software, Inc.) was used for statistical analysis. t-test was used for comparisons between two means. P≤0.05 was considered to indicate a statistically significant difference.

Results

In vitro cytotoxicity. The inhibitory effect of linalool on human prostate cancer cells was evaluated using a cytotoxicity assay. Cell viability was inhibited after 48 h of treatment in a dose-dependent manner (Fig. 1). The half maximal inhibitory concentration in 22Rv1 cells was 3.384±0.118 mM.

Apoptosis. Annexin V/PI staining was used to study the induction of apoptosis by linalool in 22Rv1 cells. The proportion of total apoptotic cells (late and early apoptotic cells, Q2 + Q4, respectively) was significantly higher in the 2.5-mM treatment group compared with that in the untreated cells (P<0.001; Fig. 2A).

Cell cycle arrest. The effect of linalool on cell cycle regulation was investigated by flow cytometry after PI staining. PI binds to the major groove of double-stranded DNA producing a fluorescent signal (ex/em, 488/600 nm). Cells in the S phase have more DNA compared with cells in the G_1 phase. Therefore, the cells take up proportionally more dye and fluoresce more brightly. G_2 cells are approximately twice as bright as G_1 cells. The distribution of 22Rv1 cells after a 24-h treatment is shown in Fig. 2C. It was observed that, after the treatment, the percentage of the cells in the G_0/G_1 phase was increased, whereas the number of cells in the S and G_2/M phases was reduced.

Mitochondrial transmembrane potential. The effect of linalool on the mitochondrial membrane potential in 22Rv1 cells was analyzed using rhodamine 123. Depolarized mitochondria display decreased membrane potential, which is an early characteristic of apoptosis, attributed to the loss of the electrochemical gradient across the mitochondrial membrane. Mitochondrial energization induces rhodamine 123 fluorescence quenching. The rate of fluorescence decay is
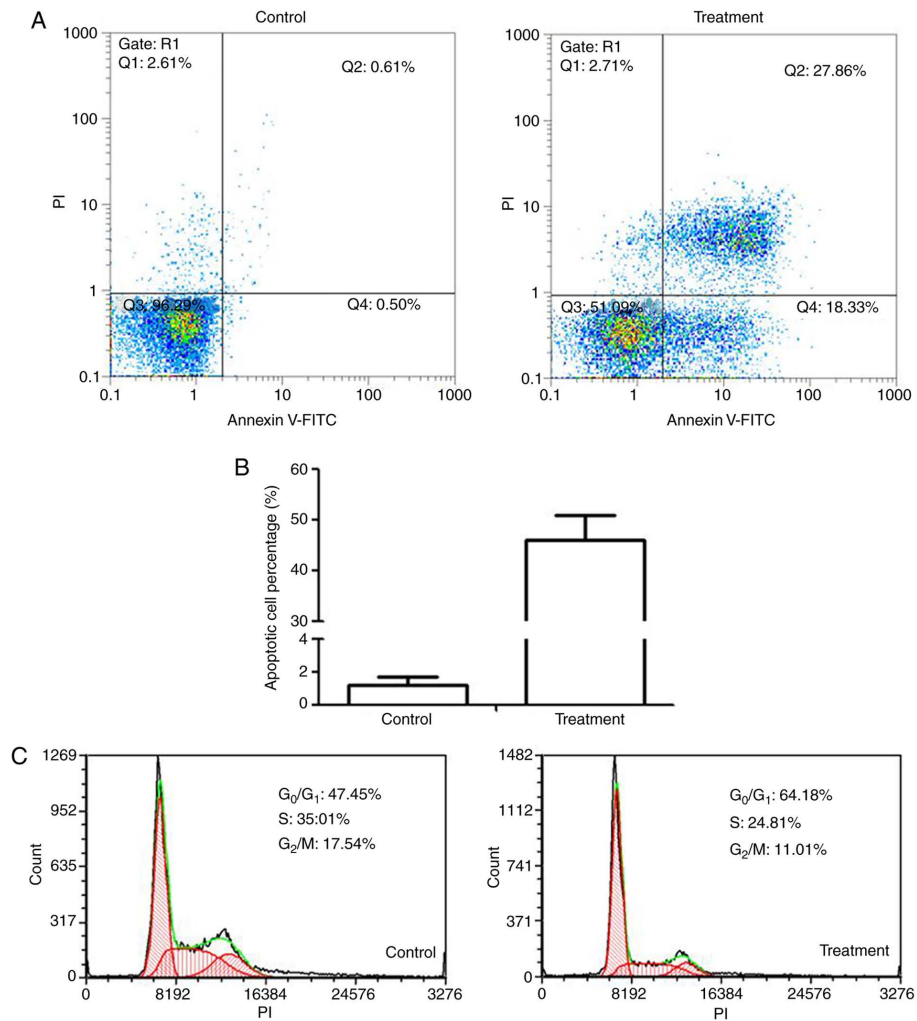
Figure 2. Representative results of apoptosis and cell cycle analysis of linalool (2.5 mM) treatment in 22Rv1 prostate cancer cells. (A) Linalool treatment induced 22Rv1 cell apoptosis. (B) Statistical analysis of apoptosis by flow cytometry. (C) Cell cycle was arrested in G_0/G_1 phase following linalool treatment. PI, propidium iodine.

proportional to the decrease in the mitochondrial membrane potential (21). In the present study, the control group retained 93.9% fluorescence. After treatment with 2.5 mM linalool for 24 h, the fluorescence declined to 80.6% (Fig. 3A). This result indicated that linalool induces apoptosis through the disruption of mitochondria membrane potential.

Cytochrome c release. The release of cytochrome *c* from the mitochondria to the cytosol has been considered as a critical early event leading to caspase-induced apoptosis (22). Further involvement of the intrinsic pathway of apoptosis was investigated. Live cells demonstrate higher levels of cytochrome *c* staining, whereas apoptotic cells with cytochrome *c* release to the cytoplasm demonstrate reduced staining intensity. In the present study, flow cytometry results revealed that the control group retained 96.09% cytochrome *c* in the mitochondria. Linalool-treated 22Rv1 cells had 78.71% cytochrome *c* in the mitochondria, whereas 21.29% was released to the cytoplasm (Fig. 3B). This result indicates that apoptosis induction occurred by means of the intrinsic pathway.

Western blot analysis for apoptosis-related proteins. The mechanism underlying apoptosis induction by linalool was demonstrated by western blot analysis. The expression of Bax, Bcl-2, p53, DR4, DR5, cleaved caspase-3, cleaved caspase-8, caspase-8, cleaved caspase-9 and caspase-9, but not caspase-3, were significantly activated by treatment with 2.5 mM linalool for 24 h (Fig. 4). These results indicated that both the intrinsic mitochondria-dependent and extrinsic death receptor-dependent pathways are involved in apoptosis induction by linalool.

Animal efficacy study. The antitumor effects of linalool were examined in a mouse xenograft model. The maximum xenograft tumor diameter was 17.83 mm in the control group and 13.48 mm in the treatment group. As shown in Fig. 5, xenograft tumor growth was significantly suppressed at the end of treatment in the linalool group compared with the control group (P<0.001). Tumor volumes in the control group were 1208.97, 1309.25, 1394.95, 1477.08 and 1562.78 mm³. Tumor volumes in the treatment group were 431.94, 536.02, 653.48, 696.19 and 749.28 mm³.

Immunohistochemistry. Tumor cell proliferation indices were assessed by detecting PCNA and Ki-67 expression in xenograft tumors. As shown in Fig. 6A, the expression of
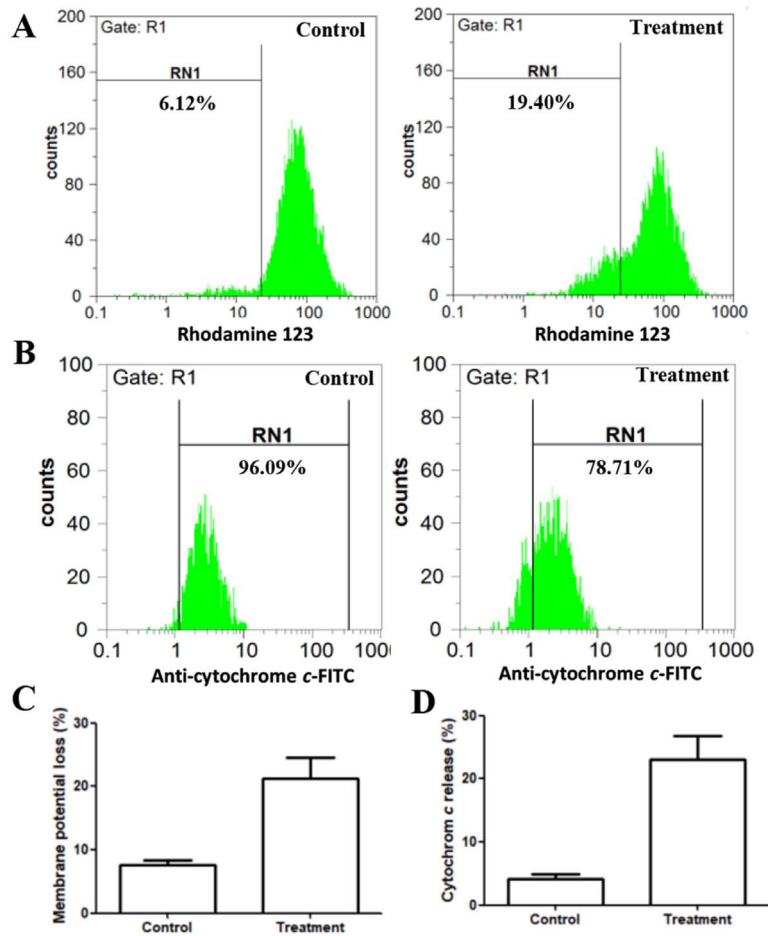
Figure 3. Mitochondrial membrane potential and cytochrome *c* release assay. (A) Flow cytometry analysis of linalool treatment induced mitochondrial membrane potential loss in 22Rv1 cells. (B) Flow cytometry analysis of linalool treatment induced cytochrome *c* release from 22Rv1 cells. (C) Statistical analysis of mitochondrial membrane potential loss. (D) Statistical analysis of cytochrome *c* release.
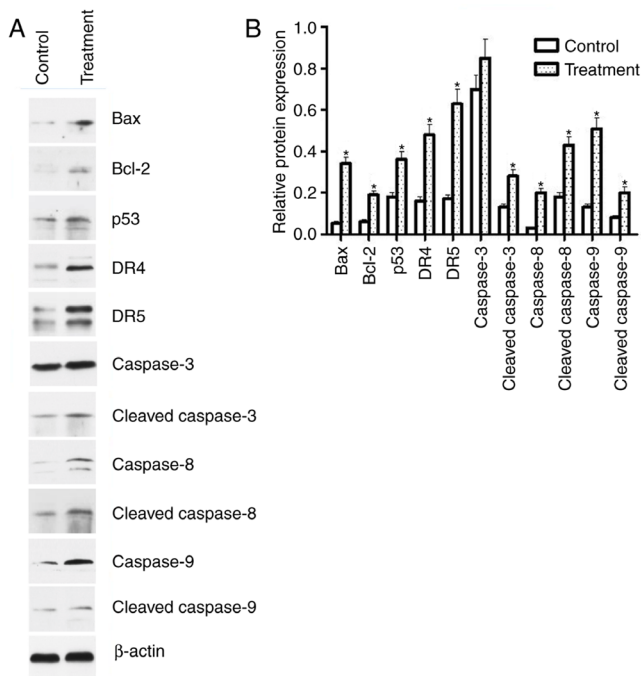

Figure 4. Western blot analysis of apoptosis-related proteins. (A) Protein expression levels in 22Rv1 cells treated with 2.5 mM linalool. (B) Relative protein expression levels in 22Rv1 cells following linalool treatment. Values represent means ± SD (n=3). *P<0.05.
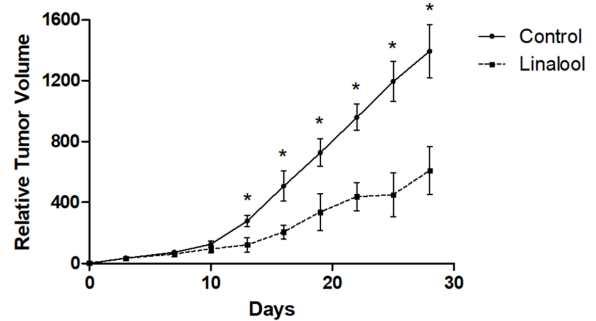

Figure 5. Relative tumor volume growth of 22Rv1-bearing mice treated with linalool (100 mg/kg). Linalool significantly suppressed tumor growth at the end of the treatment (*P<0.001). Values represent means ± SD (n=5).

PCNA and Ki-67 was markedly lower in the treatment group compared with that in the control group. These results are consistent with the tumor growth curves as measured by tumor size, indicating that linalool suppresses tumor growth *in vivo* by inhibiting tumor cell proliferation.

TUNEL assay. A TUNEL assay was performed to determine whether linalool induces apoptotic cell death in xenograft tumors. Positive immunosignals were observed in xenograft tumor sections from mice treated with linalool. Quantitative
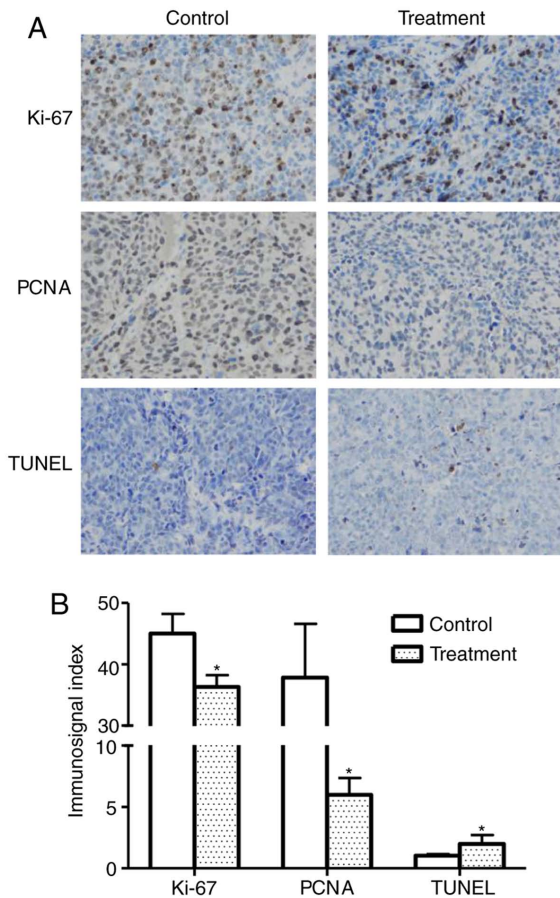
Figure 6. Linalool suppresses cancer cell proliferation and induces apoptosis. (A) Immunohistochemical examination (Ki-67 and PCNA) and TUNEL assay of xenograft tumors (400x magnification). (B) Quantitative analysis of the images. Values represent means ± SD (n=5). *P<0.05. PCNA, proliferating cell nuclear antigen.

analysis of the images (Fig. 6B) indicated that the rate of apoptosis in the linalool treatment group was significantly higher compared with that in the control group.

Discussion

Approximately 80% of men who reach the age of 80 years are diagnosed with prostate cancer. To reduce this health burden, it is important to discover medicinal herb- and phytocompound-based therapies for prostate cancer treatment. Therefore, complementary and alternative therapies are increasingly being used in prostate cancer patients, particularly those that may not be associated with the same side effects as conventional therapy (23). However, several of those therapies have not been thoroughly studied.

Linalool exerts its cytotoxic effects by apoptosis induction and cell cycle arrest. The present study demonstrated that linalool was able to induce cell death via the apoptotic pathway and, thus, may hold promise as a chemotherapeutic agent. Furthermore, linalool was reported to induce G_1 phase arrest in several types of cancer, such as oral cancer (24), leukemia (15) and hepatocellular carcinoma (25). These findings are consistent with results of the present study, indicating that linalool blocks the transition through the G_1 checkpoint to inhibit cancer growth.

p53 is a tumor suppressor protein that is involved in growth arrest, DNA repair and apoptosis induction. p53 is mutated in cell lines from various cancer types (26). In the present study, it was demonstrated that the expression of the p53 protein in the linalool treatment group was higher compared with that in the control group, indicating that linalool may induce apoptosis by regulating p53 activity. Since there is a balance between p53 and androgen receptor expression in prostate cancer progression (27), linalool may also be able to block androgen signaling and play an important role in androgen-resistant prostate cancer treatment.

There are two major apoptotic pathways, namely the mitochondria-mediated intrinsic pathway and the death receptor-mediated extrinsic pathway. Mitochondria play a pivotal role in energy generation and cascade events associated with cell death. The release of cytochrome *c*, a key mitochondrial protein, is a hallmark of apoptosis. Mitochondrial membrane perturbation is a consequence of intrinsic pro-apoptotic signaling. Upon apoptosis induction and accompanying events, such as mitochondrial depolarization, cytochrome *c* along with pro-apoptotic proteins are released into the cytosol, activating the intrinsic pathway. The quantification of cytochrome *c* release may be used to characterize the mitochondrial-dependent pathway. The present study, using rhodamine 123 as a fluorescent probe, demonstrated a slight mitochondrial membrane depolarization in the linalool treatment group. In addition, following linalool treatment, 21.29% of cytochrome *c* was released from the mitochondria into cytosol, whereas only 3.91% cytochrome *c* was released in the control group. Moreover, the intrinsic pathway is partly regulated by Bcl family members, including the negative apoptosis regulatory protein Bcl-2, as well as the pro-apoptotic regulatory protein Bax (28). It was previously reported that prostate epithelial cells with an increased Bax/Bcl-2 ratio are more sensitive to apoptotic stimuli (29). In the present study, the Bax/Bcl-2 ratio was ~2-fold higher in the linalool treatment group compared with that in the control group. These results suggest that the cellular apoptosis induced by linalool treatment is dependent on alterations in the expression of the Bcl-2 family of proteins and is associated with the mitochondrial pathway. Furthermore, stress signals cause the binding of cytoplasmic proteins, such as Bax and Bid, to the outer membrane of the mitochondria to trigger the release of the internal content (30). The mitochondrial protein Bak interacts with Bax and Bid to promote cytochrome *c* release to the cytosol. Cytochrome *c* forms a complex with Apaf-1, which triggers the activation of caspase-9, thereby initiating caspase-3 activation, ultimately leading to apoptosis (31). It was also demonstrated that the expression of cleaved caspase-3 and cleaved caspase-9 are higher in the linalool treatment group compared with the control group. These results are consistent with previous experiments, indicating that linalool may induce 22Rv1 cell apoptosis via the intrinsic pathway.

The extrinsic apoptosis pathway is mediated by death receptors, including Fas, TNF, and TNF-related apoptosis-inducing ligand (TRAIL) receptors (32). The binding of a ligand to its receptors on the target cell triggers multiple receptors to aggregate on its surface. This aggregation recruits the adaptor proteins on the cytoplasmic side of the receptors to form the death-inducing signaling complex (DISC). DISC induces

the activation of caspase-8 and initiates caspase-3 activation to initiate the degradation of the cell (33). Active caspase-8 also mediates the cleavage of the Bid protein, which links the intrinsic and extrinsic apoptotic pathways (34). In addition, there is accumulating evidence that the upregulation of DR4 (TRAIL receptors 1) and DR5 (TRAIL receptors-2) are associated with TRAIL-induced apoptosis (35). The present study demonstrated that DR4, DR5 and cleaved caspase-8 were upregulated in the linalool treatment group. These results indicate that linalool may augment TRAIL-induced apoptosis by increasing the expression of DR4 and DR5 in 22Rv1 cells.

The antiproliferative effect of linalool was studied using a xenograft model. It was demonstrated that linalool significantly suppressed tumor growth in 22Rv1-bearing mice. Furthermore, Ki-67 and PCNA expression in prostatic carcinoma was found to be correlated with Gleason score (36). The overexpression of these markers was also associated with increased serum levels of prostate-specific antigen, lymph node metastases, capsular penetration, seminal vesicle invasion and surgical resection margin positivity (37). Ki-67 and PCNA may be used in early diagnosis of prostate carcinoma and as prognostic markers of prostate cancer recurrence (38). In the present study, both Ki-67 and PCNA were significantly lower in the treatment group compared with the control group, indicating that linalool suppressed tumor cell proliferation and may improve prostate cancer prognosis. In addition, the TUNEL assay detected more apoptotic cells in the linalool treatment group compared with the control group. These data are consistent with the *in vitro* Annexin V-FITC and western blotting experiments of the present study, suggesting that linalool inhibited 22Rv1 cell growth by inducing apoptosis in both *in vitro* and *in vivo* models. Although further studies are required to elucidate the detailed anticancer mechanisms underlying the effects of linalool, these data provide preliminary evidence supporting the use of linalool in the treatment of human prostate cancer and other similar conditions.

In conclusion, the present study demonstrated that linalool can inhibit prostate cancer 22Rv1 cell growth and induce apoptosis both *in vitro* and *in vivo*, and the effects of linalool are mediated by both the intrinsic and extrinsic apoptotic pathways. Therefore, linalool may hold promise as an alternative therapeutic approach to prostate cancer.

Acknowledgements

Funding

Availability of data and materials

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YZ and CQ designed the experiments and interpreted the data. XC, GW and YL performed the experiments and analyzed the results. All authors drafted, reviewed, edited and approved the final manuscript, and agree to be accountable for all aspects of the study.

Ethics approval and consent to participate

All animal experimental procedures were in compliance with the Guide for the Care and Use of Laboratory Animals of the Kunming Medical University (Kunming, China) and were approved by the Kunming Medical University Experimental Animal Ethics Committee (approval no. KMMU 2015008).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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