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Sea cucumber extract TBL-12 inhibits the proliferation, migration, and invasion of human prostate cancer cells through the p38 mitogen-activated protein kinase and intrinsic caspase apoptosis pathway

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Abstract

Background: Sea cucumber is a kind of nutritious echinoderm that has multiple biological activities, including antioxidant, antibacterial, and antitumor activities. However, there is no extensive study on the antitumor effect of sea cucumber extract on prostate cancer (PCa). TBL-12 is a new sea cucumber extract. In this study, we investigated the in vivo anti-PCa effect of TBL-12 and its in vitro effects on the proliferation, apoptosis, migration, and invasion of the human PCa cell lines LNCaP, 22RV1, PC-3, and DU145, and evaluated its possible mechanisms.

Methods: Cell proliferation was analyzed by cell counting kit-8 and colony formation assays. Scratch migration assay and transwell invasiveness assay were used to observe TBL-12 effect on the migration and invasion of PCa cells. Matrix metalloproteinase 2 (MMP-2) and MMP-9 expression and enzymatic activity was determined by Western blot analysis, quantitative reverse-transcription polymerase chain reaction, and gelatin zymography. Apoptosis level was detected by flow cytometry analysis. Western blot analysis was used to analyze p38 mitogen-activated protein kinase (MAPK) and apoptosis pathways. Angiogenic array analysis was used to explore autocrine and paracrine growth factors in PCa cell lines. Xenograft tumor model was built to observe the in vivo anticancer effect.

Results: TBL-12 could significantly inhibit tumor growth in xenograft PCa mice in vivo, and dramatically inhibit the proliferation, colony formation, migration, and invasiveness of PCa cells in vitro ($P < 0.05$ and $P < 0.001$). The expression and enzyme activity of MMP-2 and MMP-9 were significantly suppressed by TBL-12 ($P < 0.01$), and decreased phosphorylation level of p38 in PCa cells was detected ($P < 0.001$). Furthermore, TBL-12 could reinforce the MMP-2/MMP-9 inhibitory effect of SB203580, a specific inhibitor of the p38 MAPK pathway ($P < 0.05$). Besides, TBL-12 could induce the apoptosis of PCa cells by activating caspase-9, caspase-7, and poly(ADP-ribose) polymerase and suppressing survivin, and inhibit

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the secretion of angiogenin, angiopoietin-2, and vascular endothelial growth factor in PCa cells.

Conclusions: Sea cucumber extract TBL-12 could suppress the proliferation and metastasis of human PCa cells by inhibiting MMP-2 and MMP-9 via blocking the p38 MAPK pathway, inducing apoptosis through intrinsic caspase apoptosis pathway and inhibiting the secretion of angiogenic factors. Our findings may be of importance and significance for the research and clinical applications of sea cucumber extract in PCa treatment.

KEYWORDS

apoptosis, matrix metalloproteinases, prostate cancer, sea cucumber extract, tumor metastasis

1 | INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed cancer, second only to lung cancer, and the fifth leading cancer mortality in males worldwide, and its incidence is notably higher in developed countries.[1] At present, treatments for PCa mainly include surgery, radiation, and androgen deprivation therapy. However, eventually the tumors usually become hormone-refractory after long-term treatment, and at this stage, efficient therapies are not available.[2,3] The prognosis of PCa is mainly affected by cancer metastasis.[4] Hence, finding new therapeutic approaches to inhibiting tumor cell metastasis is urgently required.

Sea cucumber is a kind of nutritious echinoderm. It has been proven to have antioxidant, antibacterial, and antitumor effects.[5–10] It contains a variety of active molecules, including holothurin A (HA), philinopside A, philinopside E, stichoposide C, stichoposide D, frondoside A, ds-echinoside A, 24-dehydroechinoside A (DHEA), cucumarioside A2–2, and so forth.[5] Studies have shown that these molecules can inhibit tumor progression through many different mechanisms. For example, frondoside A, isolated from *Cucumaria frondosa*, can inhibit the proliferation of pancreatic cancer, lung cancer, and breast cancer cell lines and increase the expression of the p53 and p21 genes and promote apoptosis via the cas3/7 pathway.[5–10] It also inhibits cell migration and invasion of breast cancer by inhibiting the prostaglandin receptor EP2/EP4 and blocking extracellular signal-regulated kinase 1/2 (ERK1/2) activation.[6,8] Philinopside A and philinopside E, isolated from *Pentacta quadrangularis*, can inhibit receptor tyrosine kinases, such as fibroblast growth factor receptor-1 (FGFR1) and platelet-derived growth factor receptor β (PDGF-β).[11,12] Philinopside E can also inhibit the binding of kinase insert domain-containing receptor and integrin αvβ3 in human microvascular endothelial cells, thus inhibiting the adhesion and migration of tumor cells.[13] Stichoposide C and stichoposide D promote ceramide production and increase the apoptosis of leukemia and colorectal cancer cells by activating the Fas and caspase-8 pathways.[14–16] The antitumor effect of cucumarioside A2-2 lies in the inhibition of the S phase of the cell cycle as well as cell division and proliferation,[17] whereas Ds-echinoside A induces liver cancer Hep-G2 cells to remain in the G0/G1 phase.[18] Ds-echinoside A, HA, and DHEA can also promote TIMP-1 (tissue inhibitor of metalloproteinase-1) expression, inhibit MMP-9 and vascular endothelial growth factor (VEGF) expression, and thus reduce cancer cell angiogenesis and invasion.[19,20] However, most of these studies focused on only a few types of cancers, such as breast cancer, pancreatic cancer, and leukemia. To date, few studies describing the effect of sea cucumber or its components on PCa have been reported,[21–23] and its mechanisms of in vivo or in vitro anticancer/antitumor activities are not fully understood.

TBL-12 is a new sea cucumber extract, a product of Unicorn Pacific Ltd, New Zealand (http://www.tbl12.com/). As the first natural product to be granted orphan drug indication for the treatment of multiple myeloma (MM) by the Food and Drug Administration of the United States, TBL-12 is currently undergoing clinical II trials at the York University Hospital and Mount Sinai Hospital for the treatment of MM. [24] Preliminary studies have found that TBL-12 can inhibit the proliferation of MM1, U266, KMS1, and APP1 cells.[24] However, there are no other reports on the antitumor effect of this natural bioactive compound on other tumors. Since no extensive study on the antitumor effect of sea cucumber on PCa is available, the effect of TBL-12 on the biological activities of PCa cells (eg, proliferation and metastasis) is not fully known, and its possible mechanism is not clear.

Therefore, in this study, we explored the biological effect of TBL-12 on the human PCa cell lines LNCaP, 22RV1, PC-3, and DU145 and the possible molecular mechanisms that may be involved. The in vivo anti-PCa effect of TBL-12 was also observed in a xenograft mouse model. Our results showed that the sea cucumber extract TBL-12 could suppress the proliferation and metastasis of human PCa cells by reducing the enzyme activities of MMP-2/MMP-9 by blocking the p38 mitogen-activated protein kinase (MAPK) pathway and inducing PCa cell apoptosis through the intrinsic caspase apoptosis pathway, and inhibiting secretion of agiogenic factors, which showed that TBL-12 could be a potential promising therapeutic agent for PCa in the future.

2 | MATERIALS AND METHODS

2.1 | Cell lines and culture

The normal prostate epithelial cell line RWPE-1, the normal prostate myofibroblast stromal cell line WPMY-1 as well as the human PCa cell lines LNCaP, 22RV1, PC-3, and DU145 were obtained from the American Type Culture Collection (ATCC). RWPE-1 cells were cultured in

keratinocyte serum-free medium (K-SFM). LNCaP, 22RV1, and PC-3 cells were cultured in RPIM-1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO). WPMY-1 and DU145 were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and 1% penicillin/streptomycin in a 5% CO_2 atmosphere at 37°C. K-SFM, RPIM-1640, DMEM, and FBS were all products of (Gibco, Thermo Fisher Scientific Inc, Waltham, MA).

2.2 | Cell proliferation analysis

Cell proliferation was measured using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies Inc, Kumamoto, Japan). Cells were seeded into a 96-well plate (Corning Incorporated Costar, Corning, NY) with an initial density of 2000 cells/well. After 18 hours of culture at 37°C in a 5% CO_2 atmosphere, cells were treated with TBL-12 (Unicorn Pacific Co, Port Vila, Vanuatu) at concentrations of 30, 60, and 90 μg/mL, based on the outcomes of preliminary experiments. Cells treated with phosphate-buffered saline (PBS) served as a control. In each experiment for each group, three duplicate wells were analyzed. Then, 10 μL CCK-8 solution was added to each well and incubated for another 1 hour before detection. Absorbance at 450 nm was measured every 24 hours for 5 consecutive days by a Tecan Sunrise microplate reader (Tecan, Männedof, Switzerland), and the growth curves were drawn.

2.3 | Colony formation assay

Cells (500 cells/well) were seeded into six-well plates (Corning Incorporated Costar, Corning, NY), cultured overnight and subsequently treated with TBL-12 (30, 60, and 90 μg/mL) for 24 hours, and then the supernatant was changed to the fresh complete culture medium. Cells treated with PBS served as negative control. Cells were cultured for 15 days, and colonies were observed every day. After 15 days of culture, the medium was removed and washed with PBS three times. Cells were fixed with 100% ice-cold methanol for 20 minutes and stained with 0.1% crystal violet for 10 minutes and then washed twice with PBS. Cell colony images were photographed by an inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan). The numbers of colonies with more than 50 cells were counted.

2.4 | Scratch migration assay

Cells were seeded at a density of 3.0×10^5 cells in six-well plates and allowed to reach approximately 90% confluence. After the culture medium was removed, the cell monolayer was carefully scraped with a 10 μL plastic pipet tip to create a scratch, and cell debris was removed by washing twice with PBS. Then, the cells were treated with TBL-12 (concentration 30, 60, and 90 μg/mL) for 24 hours. Cells treated with PBS served as the negative control. The scratch area was photographed at 0 and 24 hours using an inverted fluorescence microscope. The scratch area was gauged by the ImageJ software (National Institutes of Health, Bethesda, MD). The migration rate was calculated with the following formula:

Migration rate = (wound area [0 hours]−wound area [24 hours])/wound area [0 hours]) × 100%.

Data are presented as the mean ± standard deviation (SD) of three independent experiments.

2.5 | Transwell invasiveness assay

An invasiveness test was performed using Transwell chambers (Corning Incorporated Costar, Corning, NY). Matrigel (BD Biosciences, San Jose, CA) was diluted to 1:8 with serum-free RPIM-1640 and DMEM medium, respectively, and 100 μL diluted Matrigel was added to the upper chamber. The chamber was incubated for 3 hours at 37°C to solidify. After incubation, the remaining liquid was removed, and cells were seeded on the upper chamber at a density of 5×10^4 cells/well in 100 μL medium containing TBL-12 (at concentrations of 30, 60, and 90 μg/mL). Cells treated with PBS served as a negative control. Then, 600 μL medium containing 10% FBS was added to the lower chamber. After 24 hours of incubation at 37°C in 5% CO_2, the culture medium was discarded, and the cells on the upper surface that did not cross the membrane were wiped with a cotton swab. The cells that invaded the lower surface of the membrane were fixed with 4% paraformaldehyde for 20 minutes and stained with 0.1% crystal violet for 10 minutes. The numbers of cells were counted with the ImageJ software in six random fields (×100) per membrane and were shown as the mean number of cells per field ± SD of three representative chambers.

2.6 | Flow cytometric analysis of apoptosis

Cell apoptosis was analyzed using the Annexin V Alexa Fluor 488 and Propidium Iodide (PI) Dead Cell Apoptosis kit (Invitrogen, Thermo Fisher Scientific Inc, Waltham, MA). Cells were seeded at a density of 3.0×10^5 cells in six-well plates, treated with TBL-12 (concentration 30, 60, and 90 μg/mL) and cultured until approximately 90% confluence. Cells treated with PBS served as a negative control. After 24 hours of TBL-12 treatment, cells were digested with 0.125% trypsin-ethylenediaminetetraacetic acid (Gibco, Thermo Fisher Scientific Inc) and centrifuged at 1000*g* for 5 minutes at 4°C. Then, the cells were washed twice with cold PBS and resuspended in 100 μL 1× binding buffer with 5 μL annexin V-fluorescein isothiocyanate and 1 μL PI. Cells were incubated in the dark at 20°C for 15 minutes and then analyzed by a Becton Dickinson FACS Calibur instrument (BD Biosciences, San Jose, CA) within 1 hour. The results were analyzed with Kaluza Flow cytometry analysis software (Beckman Coulter Inc, Kraemer Boulevard Brea, CA).

2.7 | Gelatin zymography

The enzyme activities of MMP-2 and MMP-9 were assayed by gelatin zymography. After treatment with TBL-12 (concentrations of 30, 60, and 90 μg/mL) for 24 hours, the culture supernatant was collected. Protein concentration was assayed using bicinchoninic acid (BCA) assay reagent (Beyotime Institute of Biotechnology, Guangzhou, China). The samples were mixed with the loading buffer and applied to a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel containing 0.1% gelatin as a substrate.

After electrophoresis at 100 V for 1.5 hours at 4°C, the gels were washed twice in eluent buffer (2.5% Triton X-100, 50 mmol/L Tris-HCl, 5 mmol/L $CaCl_2$, pH 7. 6) on a shaker for 40 minutes at 4°C and rinsed twice with rinse buffer (50 mmol/L Tris-HCl and 5 mmol/L $CaCl_2$) for 20 minutes. Then, the gels were incubated overnight at 37°C in the incubation buffer (50 mmol/L Tris-HCl, 5 mmol/ $CaCl_2$, 0. 02% Brij-35, pH 7.6). After incubation, the gels were stained with a staining solution (0.05% Coomassie Brilliant Blue, 30% methanol, and 10% acetic acid) for 3 hours and then decolorized for 0.5, 1, and 2 hours with decolorizing solutions A, B, and C (methanol concentrations of 30%, 20%, and 10%, and acetic acid concentrations of 10%, 10%, 5%, respectively). MMP-2 (72 KD) and MMP-9 (92 KD) were visualized as translucent bands.

2.8 | RNA extraction and qRT-PCR

Cells (1×10^6 cells/well) were cultured in six-well plates for 48 hours and treated with TBL-12 (concentration 30, 60, and 90 μg/mL) for 24 hours. Cells treated with PBS served as a negative control. Total RNA was extracted using EZNA HP Total RNA Kit (Omega, Norcross, GA). A total of 1 μg RNA was reverse-transcribed using a Thermo Fisher Scientific Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific), with the following procedure: 25°C for 5 minutes; 42°C for 60 minutes; followed by 70°C for 5 minutes. Quantitative polymerase chain reaction (qPCR) was performed using TB Green Premix Ex Taq II (Takara, Kusatsu, Japan) under the following conditions: 95°C for 30 seconds; 40 cycles of 95°C for 5 seconds and 60°C for 34 seconds; one cycle of 95°C for 15 seconds, 60°C for 1 minute, ending as 95°C for 15 seconds; and standing at 4°C. The primers used for the *MMP-2* and *MMP-9* qPCR analysis were as follows: *MMP-2* forward CTGGCTGTGCAATACCTGAA, reverse CAGGGTCCATAGCTCATCGT; *MMP-9* forward AAGGCCATG CGAACCCC ACG, reverse TGGAACCACGACGCCCT TGC; and *GAPDH* forward AGGCCGGTG CTGAGTATGTC, reverse TGCCTGCT TCACCA CCTTCT. The relative RNA levels were determined with the $2^{-\Delta\Delta Ct}$ method.

2.9 | Western blot analysis

Cells (1×10^6 cells/well) were seeded in six-well plates and treated with TBL-12 (at concentrations of 30, 60, and 90 μg/mL) for 24 hours. Cells were then washed twice with ice-cold PBS. Total proteins were prepared using a total protein extract kit (Keygen, Nanjing, China) according to the manufacturer's instructions. Briefly, the protein concentration was determined with a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). A total of 30 μg protein was loaded on a 10% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride membrane (Pierce Biotechnology Inc, Rockford, IL). The membranes were blocked with 5% (w/v) nonfat dry milk for more than 2 hours, washed with Tris-buffered saline-Tween (TBST) for 10 minutes three times and incubated with the indicated specific antibodies overnight. The primary antibody dilutions used were as follows: MMP-2 (1:200), MMP-9 (1:1000), β-tubulin (1:1000), phospho-Erk1/2 (p-Erk1/2,1:1000), survivin (1:1000), phospho-p38 (1:1000), phospho-stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK; 1:1000), caspase-7 (1:1000), caspase-9 (1:1000), poly(ADP-ribose) polymerase (PARP, 1:1000), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1000). The anti-MMP-2 antibody was a product of Santa Cruz Co (Dallas, TX), and the anti-GAPDH antibody was from Cwbiotech Co (Kangwei, Jiangsu province, China). Other antibodies were all products of Cell Signaling Technology (Boston, MA). After incubation, the membranes were washed with TBST for 10 minutes three times and then incubated with the secondary antibody for 1 hour. After washing with TBST three times, the membrane was visualized with an enhanced chemiluminescence kit (Applygen Technologies, Beijing, China). The density of each band was analyzed with Image PRO Plus software (Image-Pro Plus 4.5, MediaCybernetics, Rockville, MD), and GAPDH served as an internal control.

2.10 | Angiogenic array analysis

After treatment with TBL-12 (concentrations of 30, 60, and 90 μg/mL) for 24 hours, the cell culture supernatant was collected. The quantification of 10 key angiogenic factors was performed using the Quantibody Human Angiogenesis Array (RayBiotech, Inc, Norcross, GA) according to the manufacturer's instructions, including angiogenin, angiopoietin-2 (ANG-2), VEGF, basic FGF (bFGF), heparin-binding epidermal growth factor (HB-EGF), hepatocyte growth factor (HGF), leptin, PDGF-BB, placental growth factor (PIGF), and EGF. Briefly, 100 μL sample diluent was added to each well and incubated at room temperature for 30 minutes to block. After decanting the blocking buffer, 100 μL standard cytokines or samples were added into each well and incubated with the arrays at room temperature for 1 hour. After washing three times, 80 μL Cy3 equivalent dye-conjugated streptavidin was added into each well and incubated for 1 hour in the dark at room temperature. Fluorescent signals were visualized with a laser scanner (Axon GenePix; Molecular Devices, Sunnyvale, CA). Data were analyzed using the microarray analysis software (GenePix Pro 3.0; Axon Instruments Inc, Union City, CA).

2.11 | In vivo tumor xenograft assay

Human PCa cells PC-3 (5×10^6 cells/mouse) were subcutaneously transplanted into immune-deficient nude mice to establish a xenograft tumor model. Mice were orally gavaged with vehicle (water) or TBL-12 (dose 2.5, 5.0, 7.5, 10 mg/kg) on consecutive days for 4 weeks. Tumor growth was monitored and recorded every 3 days, and the tumor volume was calculated using the following formula: volume = 1/2 (length × width^2). Relative tumor volume (*T*/*C*) was used to evaluate the antineoplastic activity of TBL-12, and was calculated using the following formula: $T/C = 1-(V_t-V_o)_{\text{treatment group}}/(V_t-V_o)_{\text{control group}} \times 100\%$, where "$V_t$" and "$V_o$" represent tumor volume at measurement and before TBL-12 administration, respectively. The observation period was 30 days. When the observation ended, all the nude mice were killed, and PCa tumor xenograft samples were resected and fixed in 10% buffered formalin for further analysis. This animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of Zhongshan School of Medicine, Sun Yat-Sen University (Guangzhou, China).

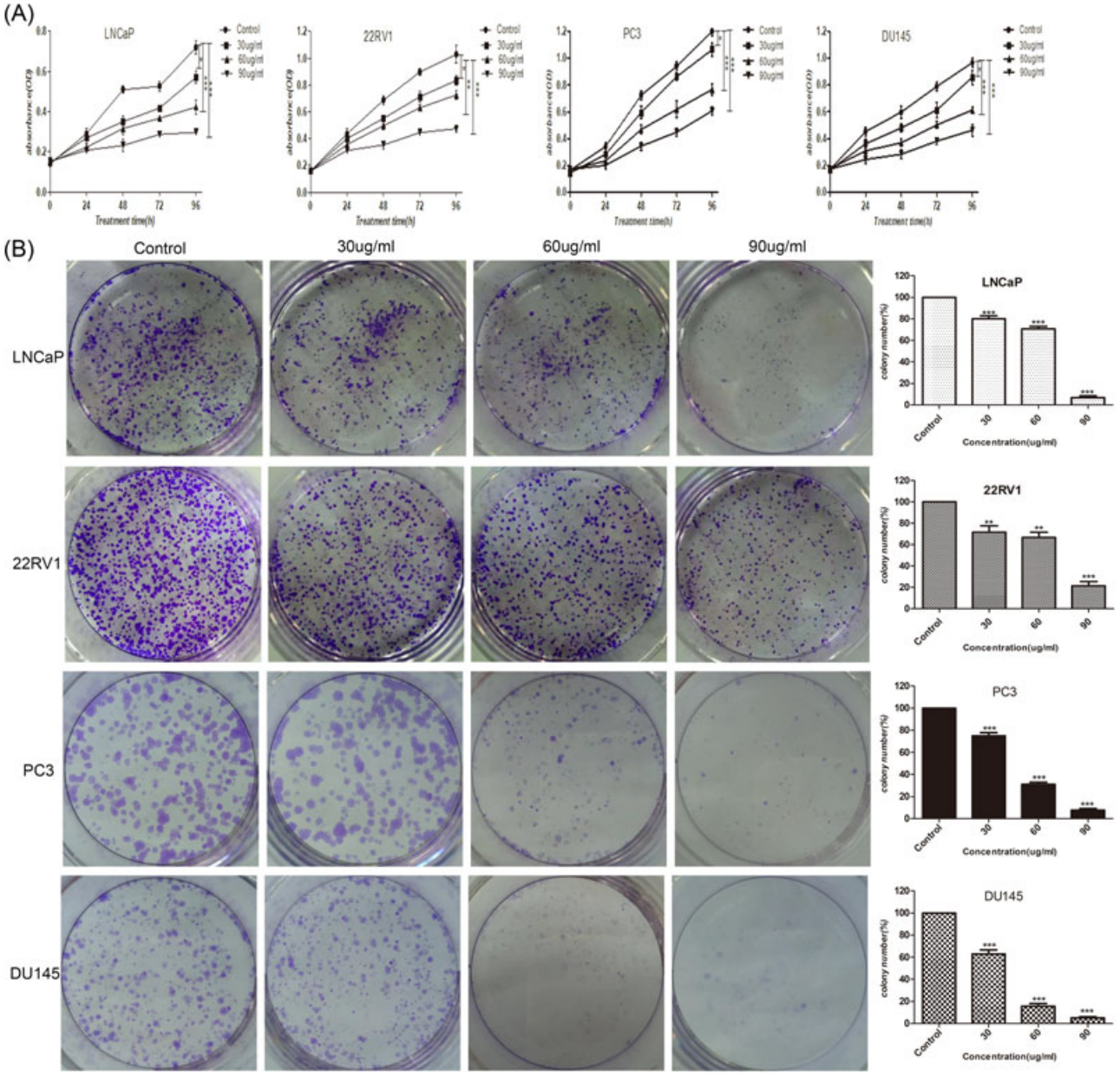

FIGURE 1 Sea cucumber extract TBL-12 suppressed the proliferation and colony formation of prostate cancer cells. A, CCK-8 assay showed that TBL-12 significantly inhibited the growth of prostate cancer cell lines, including LNCap, 22RV1, PC-3, and DU145 cells, in a dose-dependent manner. Treatment with TBL-12 for 48 hours significantly inhibited the proliferation of prostate cancer cells. $^{*}P < 0.05$ compared with the control. B, The colony formation assay showed that TBL-12 markedly reduced the colony formation ability of prostate cancer cells. Representative micrographs (upper) and quantifications (bottom) of the crystal violet-stained cell colonies formed by the indicated PCa cells are shown 15 days after cell inoculation. Colonies containing >50 cells were counted. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control. CCK-8, Cell Counting Kit-8; PCa, prostate cancer. [Color figure can be viewed at wileyonlinelibrary.com]

2.12 | Statistical analysis

Statistical data were evaluated using SPSS24.0 (SPSS Inc, Chicago, IL) and GraphPad Prism5 (GraphPad Software Inc, La Jolla, CA). Measurement data are shown as the mean ± SD of at least three independent experiments. One-way analysis of variance and the Student *t* test were used for statistical analysis. A value of $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | TBL-12 suppressed the proliferation and colony formation ability of PCa cells in vitro

The effect of TBL-12 (concentrations of 30, 60, and 90 μg/mL) on PCa cell proliferation and normal prostate cells was analyzed. The results showed that after TBL-12 treatment, the proliferation of

PCa cells was significantly suppressed ($P < 0.05$; Figure 1A), revealing that TBL-12 could inhibit PCa cell proliferation in a dose-dependent manner. Further colony formation assays confirmed that TBL-12 could significantly inhibit the colony formation ability of PCa cells (Figure 1B). All colony numbers were dramatically lower than the control group when the cells were treated with ≥30 μg/mL TBL-12 ($P < 0.01$) for 24 hours. When treated with 30 and 60 μg/mL TBL-12, the colony formation rates were 80% ± 5% and 71% ± 4% per field in LNCaP cells, 72% ± 10% and 67% ± 9% in 22RV1 cells, 75% ± 5% and 31% ± 4% in PC-3 cells, and 63% ± 6% and 16% ± 4% in DU145 cells, respectively ($P < 0.05$). When treated with 90 μg/mL TBL-12, the colony formation rates dramatically dropped to 7% ± 3% per field in LNCaP cells, 21% ± 7% in 22RV1 cells, 8% ± 3% in PC-3 cells and 5% ± 2% in DU145 cells ($P < 0.001$). In addition, to verify the effect of TBL on normal cells, we observed the toxicity of TBL on the normal prostate epithelial cell line RWPE-1 and the normal prostate myofibroblast stromal cell line WPMY-1 with CCK-8 and found that there was no significant toxic effect of TBL-12 on normal prostate cells (Figure S1).

3.2 | TBL-12 inhibited the migration and invasiveness of PCa cells in vitro

To understand the effects of TBL-12 on the migration ability of PCa cells, the cells were treated with TBL-12 (concentrations 30, 60, and 90 μg/mL) for 24 hours, and the inhibitory effect on migration was analyzed by a scratch migration assay. The results showed that TBL-12 significantly suppressed the migration of PCa cells ($P < 0.05$; Figure 2A). The migration rates were 23% ± 2% to 8% ± 3% of LNCaP cells, 19% ± 3% to 2% ± 3% of 22RV1 cells, 53% ± 10.82% to 12.33% ± 4.04% of PC-3 cells, and 54% ± 6.25% to 9% ± 1% of DU145 cells (Figure 2A). The effect of TBL-12 on the invasiveness of PCa cells was further analyzed using Transwell chambers, and the cells that invaded through the polycarbonate membrane of the Boyden chamber were counted. The results showed that TBL-12 also significantly suppressed the invasiveness of PCa cells ($P < 0.05$; Figure 2B). After treatment with TBL-12 for 24 hours, the numbers of invasive cells were significantly decreased from 152 ± 8 cells to 9 ± 7 cells per field in the LNCaP cell line, from 512 ± 33 to 22 ± 13 per field in the 22RV1 cell line, 1085 ± 101 cells to 29 ± 18 cells per field in the PC-3 cell line and from 516 ± 38 to 10 ± 9 per field in the DU145 cell line (Figure 2B). These results showed that TBL-12 could dramatically inhibit the migration and invasiveness of PCa cells in vitro.

3.3 | TBL-12 restrained the growth of PC-3 xenograft tumors in vivo

To further confirm the antitumor activity of TBL-12 in vivo, a PC-3 xenograft tumor model was established in nude mice. After TBL-12 treatment of only 6 days, we observed that TBL-12 significantly reduced tumor volume at the dose of 5.0 mg/kg ($P < 0.05$ for tumor volume, Figure 3A and $P < 0.001$ for tumor weight at Day 28; Figure 3C). The mice did not show significant loss of body weight except for the 10.0 mg/kg group. Mice body weight was attenuated only at a dose of 10.0 mg/kg and after administration for 12 days (Figure 3B; $P < 0.05$). At the end of the observation (Day 28), the mice were killed, and the tumors were weighed and photographed. As shown in Figure 3C, when the mice were administered a dose of 10.0 mg/kg for 4 weeks, tumor growth was significantly inhibited by approximately 79.8% compared with the vehicle treatment group ($P < 0.001$). A dose higher than 5 mg/kg could generate significant suppression of tumor growth, and these results confirmed the in vivo antitumor effect of TBL-12 in PCa xenograft mice.

3.4 | TBL-12 decreased the expression and activity of MMP-2 and MMP-9 in PCa cells

MMPs are crucial for extracellular matrix (ECM) degradation, and ECM degradation is vital for cell migration and invasiveness. Therefore, to further illustrate the mechanism of the TBL-12 effect on PCa cell migration and invasiveness, we further investigated the effects of TBL-12 on MMP expression and function. As shown in Figure 4A and 4B, Western blot analysis and quantitative reverse-transcription PCR (RT-qPCR) analysis revealed that TBL-12 significantly inhibited the expression of MMP-2 and MMP-9 ($P < 0.05$). After treatment with TBL-12 (30, 60, and 90 μg/mL) for 24 hours, MMP-2 and MMP-9 activity, messenger RNA, and protein expression levels in PCa cells were notably suppressed ($P < 0.05$; Figure 4). In LNCaP and 22RV1 cells, MMP-2 and MMP-9 expression was more markedly inhibited by TBL-12 than in PC-3 and DU145 cells. In PC-3 cells, the expression of MMP-2 was more markedly inhibited than that of MMP-9, whereas in DU145 cells, the inhibition effect of MMP-9 was larger than that of MMP-2 (Figure 4A and 4B). Moreover, the enzyme activities of MMP-9 and MMP-2 were also remarkably suppressed by TBL-12, as shown by gelatin zymography analysis ($P < 0.05$; Figure 4C). Taken together, these results suggested that TBL-12 could inhibit PCa cell metastasis via the downregulation of MMP-2 and MMP-9.

3.5 | TBL-12 inhibited MMP-2/MMP-9 via the p38 MAPK signaling pathway

The p38 MAPK pathway is main pathway that regulates MMP-2 and MMP-9 levels, so we explored the effect of TBL-12 treatment on the phosphorylation of p38, JNK1/2, and ERK1/2 in PCa cells. Although the levels of p38, JNK, and ERK were not significantly changed, their phosphorylation was dramatically altered. After treatment with TBL-12 (concentrations of 30, 60, and 90 μg/mL) for 24 hours, the phosphorylation of p38 MAPK was significantly inhibited in all PCa cell lines, as shown in Figure 5A ($P < 0.001$). Additionally, the p-JNK1/2 level was dramatically enhanced in LNCaP, PC-3 and DU145 cells but was not changed in 22RV1 cells (Figure 5A). The level of p-ERK1/2 was enhanced in PC-3 and DU145 cells but not in LNCap and 22RV1 cells (Figure 5A). Western blot analysis of AKT and p-AKT, the key factor of the phosphoinositide-3-kinase/protein kinase B (PI3K/Akt) pathway, showed negative results (Figure S2),

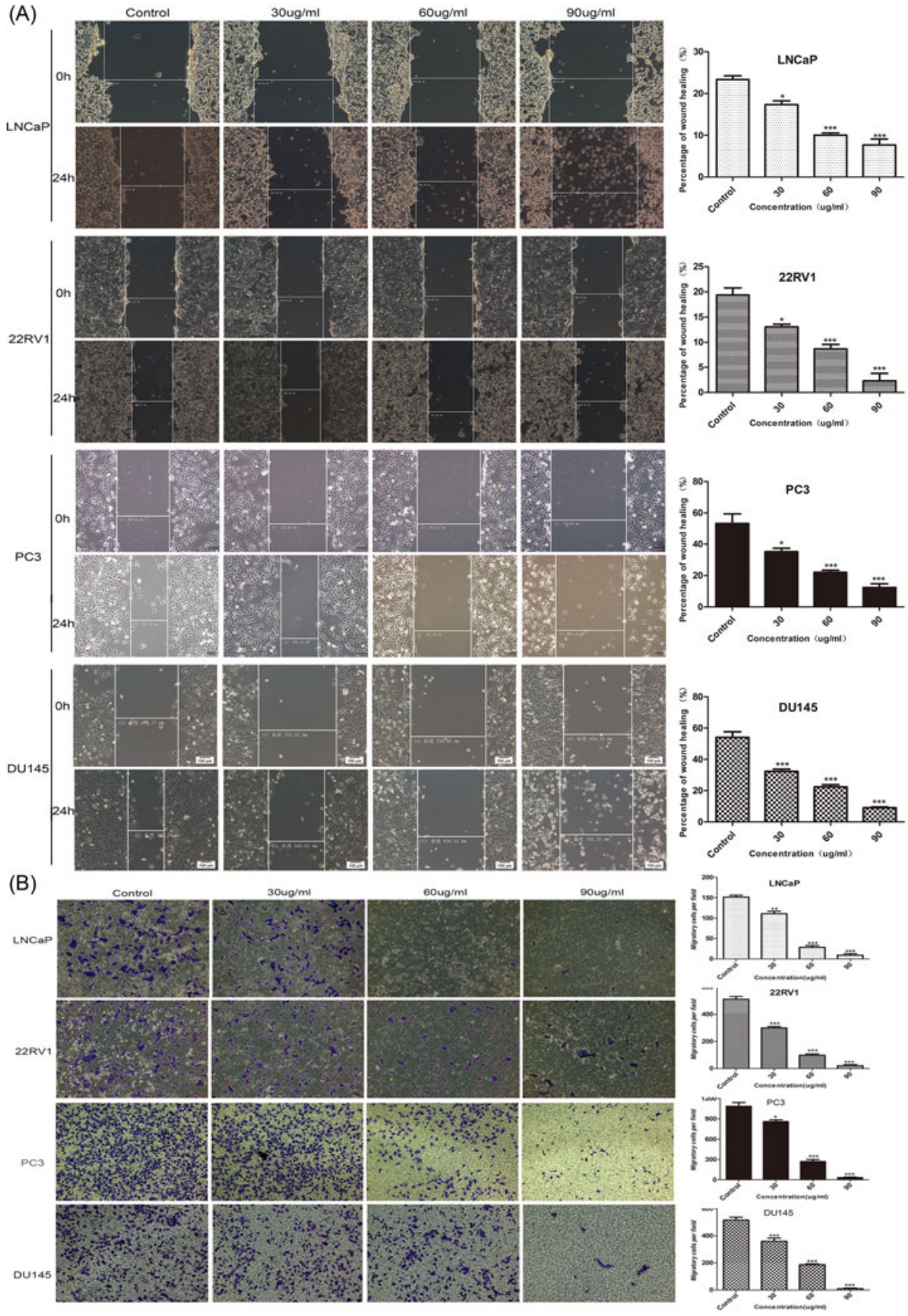

FIGURE 2 TBL-12 suppressed the migration and invasiveness of prostate cancer (PCa) cells. A, Scratch migration assay showed that TBL-12 significantly suppressed the cell migration ability of prostate cancer cells. Cell mobility was measured at 0 and 24 hours (phase contrast ×100). B, Transwell invasiveness assays showed that TBL-12 could dramatically inhibit the invasiveness of PCa cells. Cells that invaded the lower surface of the membrane were stained with 0.1% crystal violet and counted (×100). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control. [Color figure can be viewed at wileyonlinelibrary.com]

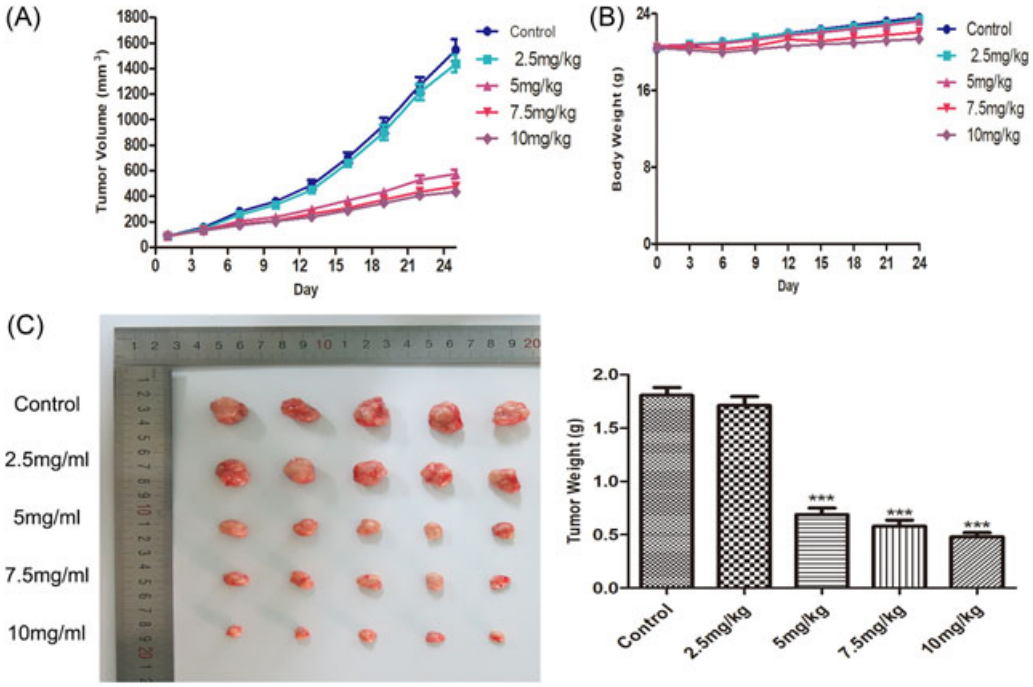

FIGURE 3 TBL-12 suppressed prostate cancer growth in vivo. Prostate cancer cell PC-3 cells (5 million per mouse) were subcutaneously transplanted into immune-deficient nude mice to establish a xenograft tumor model. Mice were orally gavaged with water or TBL-12 for 4 weeks. A, Tumor volumes were measured every 3 days to assess the effect of TBL-12 on tumor growth. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control group. B, The body weight of the mice was monitored every 3 days to observe TBL-12 toxicity. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control group. C, Xenografts were resected, and the weights of the xenograft tumors were measured at the end of the observation period. $^{***}P < 0.001$ compared with the control group. [Color figure can be viewed at wileyonlinelibrary.com]

indicating that PI3K/Akt was not a major regulatory pathway of MMP-2 and MMP-9 in PCa cells treated with TBL-12.

To confirm the regulatory effect of TBL-12 on the p38 MAPK signaling pathway, PCa cells were further treated with or without a p38 inhibitor (50 μM SB203580) for 24 hours, and MMP-2/MMP-9 activity was analyzed by gelatin zymography assay (Figure 5B). Gelatin zymography analysis confirmed that MMP-2 and MMP-9 activity could be significantly suppressed by TBL-12, whereas SB203580 could reinforce this effect in all PCa cell lines ($P < 0.001$; Figure 5B). MMP-2 and MMP-9 enzyme activity was significantly suppressed by SB203580 together with 30 μg/mL of TBL-12 in PCa cells, and showing a synergistic effect with TBL-12 ($P < 0.05$; Figure 5B). Therefore, TBL-12 may inhibit the migration and invasiveness of human PCa cells by the downregulation of MMP-2/MMP-9 via the suppression of the p38 MAPK signaling pathway.

3.6 | TBL-12 promotes cell apoptosis through the caspase-9/caspase-7/PARP pathway

Proliferation analysis revealed that TBL-12 suppressed the proliferation and colony formation ability of PCa cells. To further elucidate the mechanism, apoptosis levels were determined by flow cytometry. We found that TBL-12 could enhance PCa cell apoptosis (Figure 6A). The proportion of apoptotic cells increased significantly from 1.5% ± 1.7% to 21.8% ± 1.5% of LNCaP cells, from 2.8 ± 2.5% to 71.1 ± 6.5% of 22RV1 cells, from 6.7% ± 0.8% to 15.4% ± 0.4% of PC-3 cells, and from 4.0 ± 2.6% to 70.7 ± 2.1% of DU145 cells ($P < 0.001$; Figure 6A) after treatment with TBL-12 for 24 hours. Further analysis of the apoptosis signaling pathways confirmed that caspase family factors were activated by TBL-12 treatment, while the apoptosis inhibition gene survivin was downregulated, as shown by Western blot analysis (Figure 6B). Western blot analysis showed that the expression of the precursors including pro-caspase-9, pro-caspase-7, PARP, and survivin were downregulated, whereas simultaneously, the expression of cleaved caspase-9, caspase-7, and PARP were upregulated (Figure 6B). Because caspase-7 is cleaved by the apoptotic initiators caspase-9 and is responsible for cleaving downstream substrates such as PARP,[25] and survivin is one of the antiapoptotic proteins that could bind active caspase-7 and prevent cascade from being cleaved,[26] these results confirmed that TBL-12 could inhibit PCa cell proliferation and promote cell apoptosis through the activation of the intrinsic caspase-9/caspase-7/PARP apoptosis pathway.

3.7 | TBL-12 downregulated angiogenic growth factors in PCa cells

To understand the role of TBL-12 on the regulation of angiogenic growth factors, 10 human angiogenic growth factors, including angiogenin, ANG-2, EGF, bFGF, HB-EGF, HGF, leptin, PDGF-BB, PIGF, and VEGF, in the TBL-12-treated PCa cell culture supernatant were measured by angiogenic array analysis. The results showed that among these

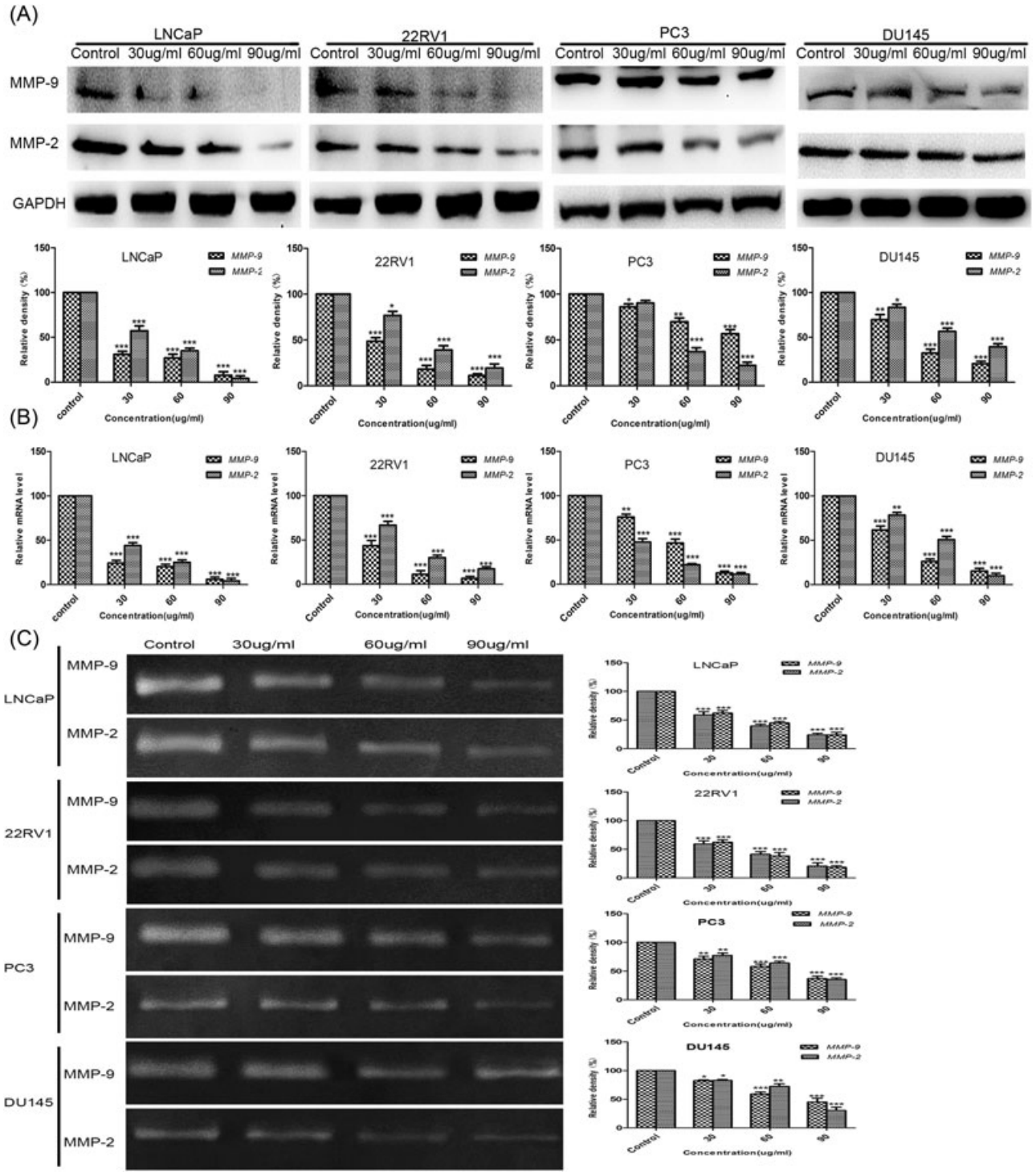

FIGURE 4 TBL-12 suppressed MMP-2 and MMP-9 expression and activity in prostate cancer cells in vitro. Western blot analysis and RT-qPCR assays were used to detect the protein and mRNA expression of MMP-2 and MMP-9 in prostate cancer cells. Expression levels were normalized to GAPDH expression. The error bars represent standard deviation (SD) values calculated from three parallel experiments. A, Western blot analysis showed that MMP-2 and MMP-9 protein expression was significantly suppressed after 24 hours of TBL-12 treatment. B, RT-qPCR analysis showed that MMP-2 and MMP-9 expression was accordingly suppressed at the mRNA level after 24 hours of TBL-12 treatment. C, Gelatin zymography analysis of MMP-2 and MMP-9 activity showed that TBL-12 could remarkably inhibit the enzyme activities of MMP-9 and MMP-2. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MMP, matrix metalloproteinase; mRNA, messenger RNA; RT-qPCR, quantitative reverse-transcription polymerase chain reaction

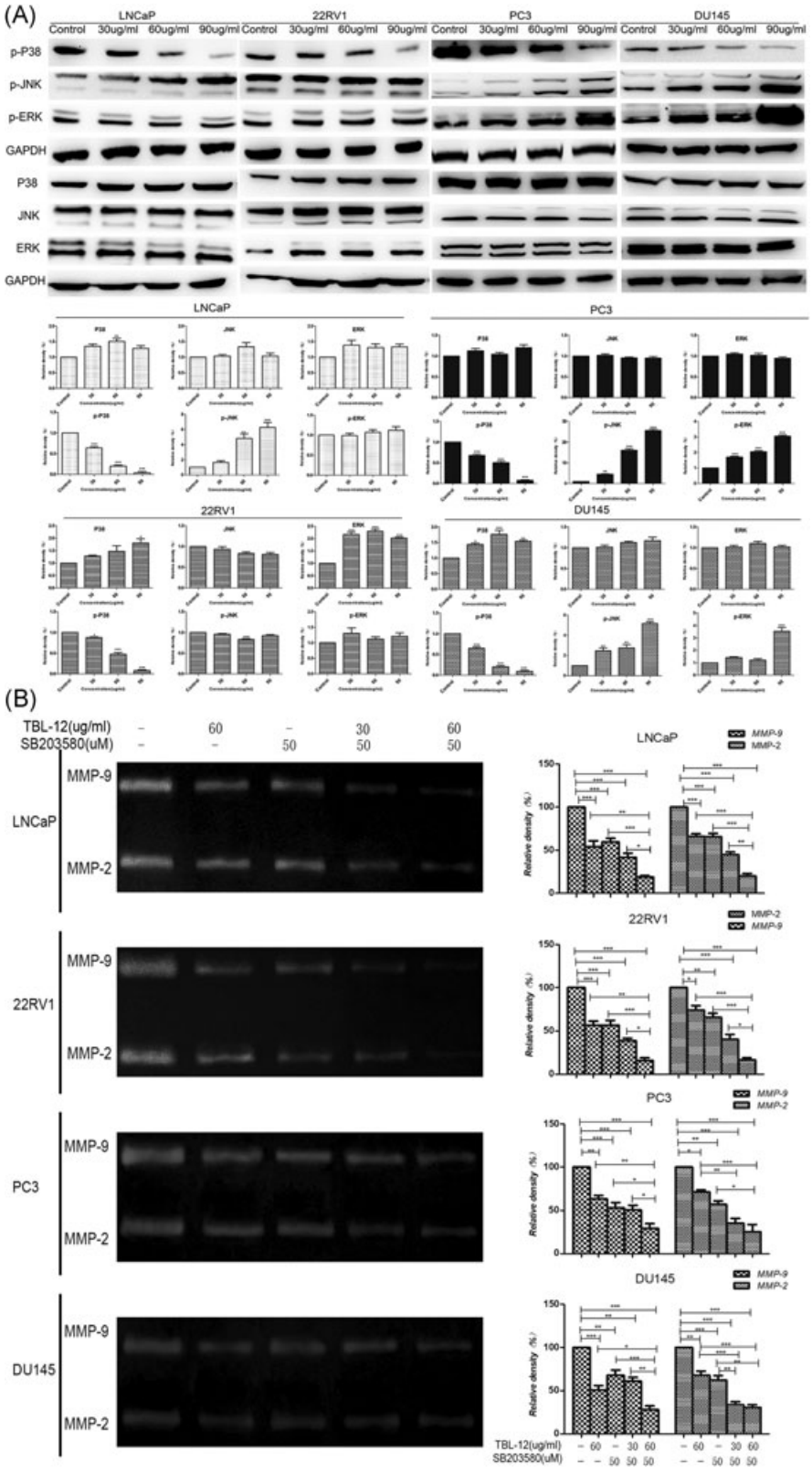

FIGURE 5 TBL-12 suppressed MMP-2 and MMP-9 activity via the p38 MAPK signaling pathway in prostate cancer cells. A, Western blot analysis of the phosphorylation levels of p38, JNK1/2, and ERK1/2 in prostate cancer cells showed that TBL-12 could significantly downregulate p-p38 levels in all four PCa cell lines, whereas p-JNK1/2 and p-ERK1/2 levels varied in different PCa cells. B, Gelatin zymography analysis confirmed that the p38 inhibitor SB203580 could synergistically enhance the suppression effect of MMP-2 and MMP-9 activity with TBL-12 in PCa cells. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control. ERK, extracellular signal-regulated kinase; JNK, c-Jun NH2-terminal kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase

angiogenic factors, EGF, PIGF, HB-EGF, leptin, bFGF, HGF, and PDGF-BB levels were extremely low (<5 pg/mL) in all four PCa cell lines after 24 hours of culture, and there was no significant difference between the PBS-treated control group and the TBL-12-treated groups ($P > 0.05$). However, angiogenin levels were decreased in LNCaP, PC-3, and DU145 cells ($P < 0.001$, Figure 7), but not in 22RV1 cells. The ANG-2 level was decreased in 22RV1 and DU145 cells; 60 μg/mL TBL-12 significantly inhibited ANG-2 levels in 22RV1 cells, and 90 μg/mL TBL-12 inhibited ANG-2 in DU145 cells ($P < 0.05$; Figure 7). VEGF was decreased in 22RV1 and DU145 cells but not in LNCap and PC-3 cells (Figure 7). In

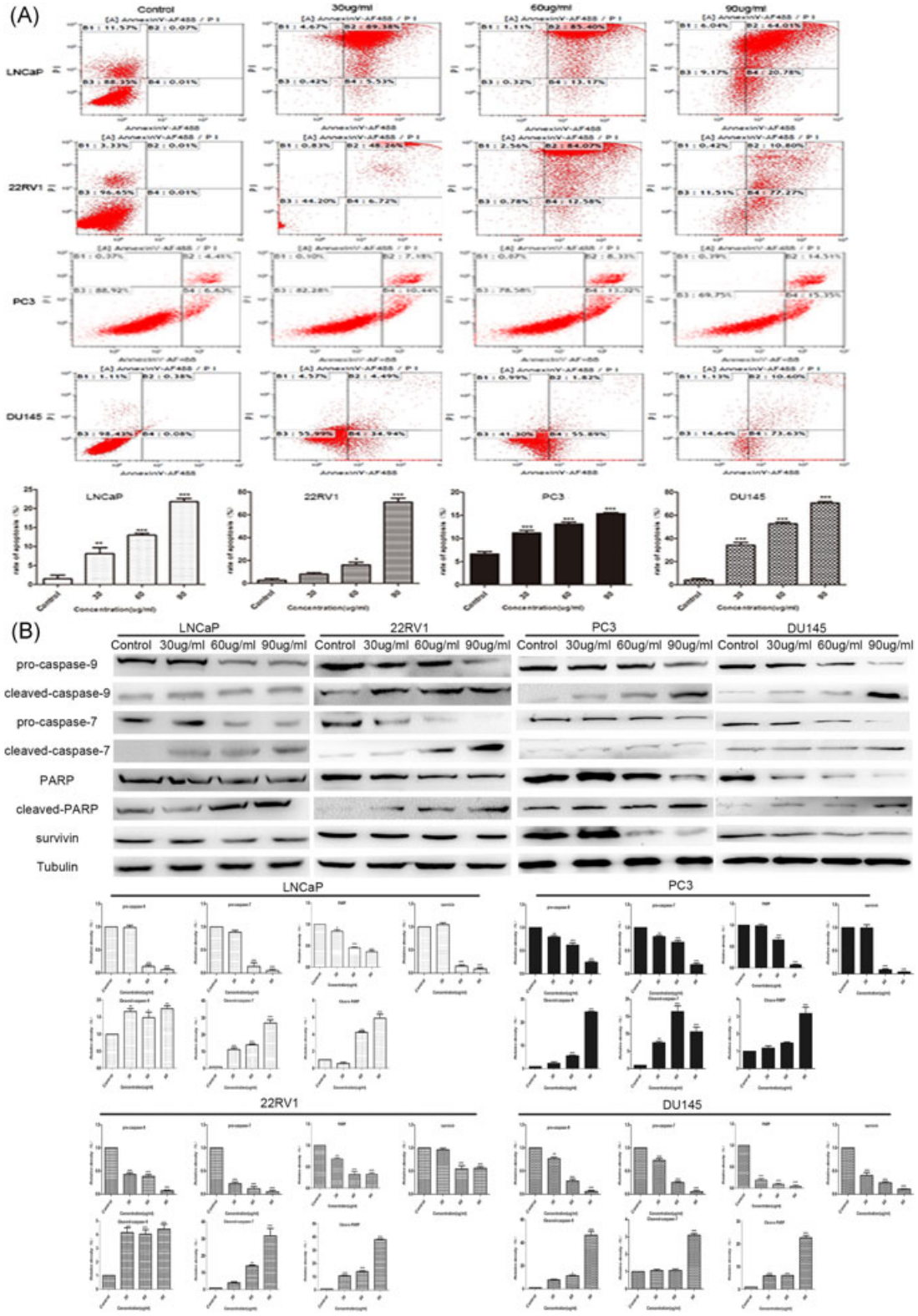

FIGURE 6 TBL-12 promoted the apoptosis of prostate cancer cells via the caspase-9/caspase-7/PARP pathway. A, Flow cytometry was used to analyze the apoptosis of TBL-12-treated prostate cancer cells. An Annexin V Alexa Fluor 488 and Propidium Iodide (PI) Dead Cell Apoptosis kit was used to detect apoptosis after 24 hours of TBL-12 treatment. The early apoptotic cells were stained with annexin $V^{+}PI^{-}$. TBL-12 treatment significantly enhanced the proportion of apoptotic cells. Cells treated with PBS served as the negative control. ***$P < 0.001$. B, Key factors of the caspase family were analyzed by Western blot analysis. Tubulin was used as an internal control. TBL-12 decreased the levels of pro-caspase-9, pro-caspase-7, precursor PARP, and survivin and upregulated cleaved caspase-9, caspase-7, and cleaved PARP levels in prostate cancer cells. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the control. PARP, poly (ADP-ribose) polymerase. [Color figure can be viewed at wileyonlinelibrary.com]

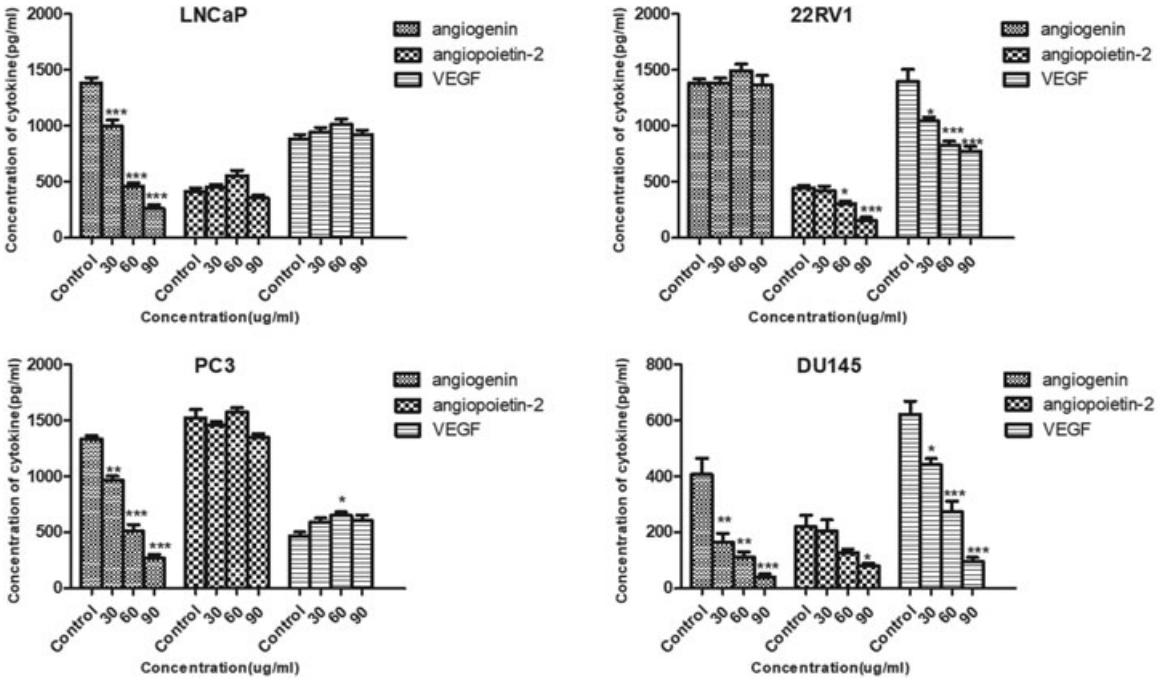

FIGURE 7 TBL-12 downregulated the level of angiogenin, ANG-2, and VEGF in prostate cancer cells. Quantification of 10 key angiogenic factors (angiogenin, ANG-2, EGF, bFGF, HB-EGF, HGF, leptin, PDGF-BB, PIGF, and VEGF) in the cell culture supernatant was performed by angiogenic array analysis. Angiogenin, ANG-2 and VEGF levels were significantly suppressed by 24 hours of TBL-12 treatment. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control. ANG-2, angiopoietin-2; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; HB-EGF, heparin-binding epidermal growth factor; HGF, hepatocyte growth factor; PDGF-BB, platelet-derived growth factor BB; PIGF, placental growth factor; VEGF, vascular endothelial growth factor

PC-3 cells, VEGF was slightly increased when treated with 60 μg/mL TBL-12 ($P < 0.05$; Figure 7). The results showed that TBL-12 could influence some angiogenic growth factors, suppressing tumor angiogenesis by inhibiting the secretion of angiogenin, ANG-2, and VEGF from PCa cells.

4 | DISCUSSION

Metastasis and tumor relapse remain a serious problem for the long-term survival of PCa patients, and a lack of efficient therapy is another major problem for advanced PCa treatment.[1,2] Recently, natural marine drugs have shown the potential to be curative for several cancers.[5–10] In recent years, at least 14 000 natural compounds with pharmacological activities have been isolated from marine animals and plants.[27] There are many bioactive molecules in the body wall of sea cucumber, such as sea cucumber saponin, 18 kinds of taurine, chondroitin sulfate, acid mucopolysaccharide, and sphingolipid. Those components have antioxidant, antibacterial, and antitumor biological activities.[10,28] In this study, the effects of a new sea cucumber extract, TBL-12, were explored on PCa cells.

First, we observed the effect of TBL-12 on the growth and metastasis of PCa cells. Four representative PCa cell lines, including the androgen receptor-negative cell lines PC-3 and DU145 and the androgen receptor-positive cell lines LNCaP and 22RV1, were selected to investigate the effect of TBL-12 on PCa. Two normal prostate cell lines, the normal prostate epithelial cell line RWPE-1 and the normal prostate myofibroblast stromal cell line WPMY-1, were selected to observe the toxic effect of TBL-12 treatment. We found that TBL-12 could significantly inhibit the proliferation and colony formation ability of PCa cells in vitro ($P < 0.05$; Figure 1) and, at the same time, had no obvious cytotoxic effect on the normal prostate cell lines WPMY and RWPE-1 (Figure S1). The scratch migration assay and Transwell invasiveness assay results revealed that TBL-12 could also suppress the migration and invasiveness of PCa cells in vitro ($P < 0.05$; Figure 2). When treated with a dose of 30 μg/mL for 24 hours, TBL-12 significantly suppressed the migration of all four PCa cell lines ($P < 0.05$; Figure 2), whereas at 24 hours, 30 μg/mL TBL-12 did not inhibit the proliferation of PCa cells ($P > 0.05$; Figure 1), indicating that the migration and invasiveness suppression effect was not the result of proliferation inhibition. To further confirm the in vivo antitumor effect of TBL-12, we built a xenograft PCa model in nude mice using PC-3 cells. We observed that a dose higher than 5 mg/kg per mouse could significantly suppress tumor growth, reducing tumor volume and tumor weight ($P < 0.05$; Figure 3). Only the dose of 10.0 mg/kg administered for 12 days attenuated mouse body weight (Figure 3B, $P < 0.05$), indicating that TBL-12 had very low toxicity. Therefore, these results confirmed that the sea cucumber extract TBL-12 could inhibit PCa growth and metastasis in vitro and in vivo and has the potential to be a promising natural drug for PCa treatment.

We further explored the potential mechanism of TBL-12 bioactivity. Since the degradation of the ECM by tumor cells is a committed

step in tumor cell metastasis and MMPs are the most important proteolytic enzymes that degrade ECM,[29] we deduce that TBL-12 could affect MMP expression and thus suppress the migration and invasiveness of PCa cells. Therefore, MMP-2 and MMP-9 expression levels were detected by RT-qPCR, Western blot analysis and gelatin zymography analysis. Consistent with the report in human hepatocellular liver carcinoma cells (HepG2),[30] our results verified that sea cucumber extract TBL-12 could inhibit the expression and activity of MMP-2 and MMP-9 in PCa cells ($P < 0.05$; Figure 4), suggesting that MMP-2/MMP-9 is an important target of TBL-12.

Previous studies proved that MMP-2 and MMP-9 are mainly regulated by the MAPK, PI3K-Akt, and nuclear factor-κB signaling pathways in PCa.[31–34] Therefore, in this study, we analyzed the key factors of the MAPK pathway and found that p38/MAPK may be the major pathway of TBL-12 action. The PI3K/Akt pathway was also evaluated, but the results were negative (Figure S2). Because p38/MAPK are key members of the MAPK family and play crucial roles in cell differentiation, cell growth, and apoptosis,[35] we deduce that p38 may be one of the main targets of TBL-12, so we further detected the level of p38 phosphorylation using Western blot analysis. The results confirmed that p38 phosphorylation was dramatically downregulated after TBL-12 treatment (Figure 5A). Interestingly, the phosphorylation levels of the other two factors, JNK1/2 and ERK1/2, were upregulated in some of the PCa cell lines (Figure 5A). The p-JNK1/2 level was dramatically enhanced in LNCaP, PC-3, and DU145 cells but was not changed in 22RV1 cells (Figure 5A). The level of p-ERK1/2 was enhanced in PC-3 and DU145 cells but not enhanced in LNCap and 22RV1 cells (Figure 5A). Because ERK and JNK are also involved in many physiological processes, such as cell proliferation and apoptosis, and the cross-talk between signaling pathways is widespread in cancer cells,[36,37] we presume that the phenomena of the upregulation of p-JNK1/2 and p-ERK1/2 may be related to the activation of other signaling pathways.

To further prove the role of p38 in TBL-12-mediated MMP-2/MMP-9 inhibition, PCa cells were treated with the p38 inhibitor SB203580, and the results showed that MMP-2 and MMP-9 enzyme activity was significantly suppressed together with TBL-12 in PCa cells, showing a synergistic effect ($P < 0.05$; Figure 5B). Therefore, TBL-12 inhibits PCa cell migration and invasiveness by suppressing the expression of MMP-2 and MMP-9 through the p38/MAPK pathway.

To understand the proliferation inhibition mechanism of TBL-12, we further analyzed the apoptosis level of TBL-12-treated PCa cells by flow cytometry. The results confirmed that TBL-12 could increase the apoptosis level of all four PCa cell lines ($P < 0.05$; Figure 6A). Western blot analysis of key apoptosis factors showed that TBL-12 enhanced the expression of cleaved caspase-9, cleaved caspase-7, and cleaved PARP, whereas it also decreased the expression of precursor PARP and the inhibitor of apoptosis protein survivin (Figure 6B). Therefore, TBL-12 could inhibit PCa cell proliferation and promote apoptosis through the intrinsic caspase-9/caspase-7/PARP apoptosis pathway.

We also analyzed the effect of TBL-12 on angiogenic growth factor levels in PCa cells to understand the role of TBL-12 in angiogenic regulation. Because EGF, PIGF, HB-EGF, leptin, bFGF, HGF, and PDGF-BB levels were extremely low in the culture supernatant of PCa cells, there was no obvious effect of TBL-12. We found that angiogenin levels were decreased in LNCaP, PC-3, and DU145 cells (Figure 7), but no marked downregulation was observed in 22RV1 cells. ANG-2 levels were decreased in 22RV1 and DU145 cells ($P < 0.05$; Figure 7) but not in LNCap and PC-3 cells. VEGF was decreased in 22RV1 and DU145 cells but not in LNCap and PC-3 cells (Figure 7). These results indicated that the antitumor activity of TBL-12 also lies in the inhibition of growth factor levels and suppression of tumor angiogenesis by inhibiting the secretion of angiogenin, ANG-2, and VEGF from PCa cells, and the antiangiogenic effect of TBL-12 on different PCa cell lines varied. Since TBL-12 is sea cucumber extract which act as a compound preparation, further consideration will be taken to the purification and identification of the active components in TBL-12 in our future study.

5 | CONCLUSIONS

In summary, this study proved that the new sea cucumber extract TBL-12 dramatically inhibited the proliferation of PCa cells, suppressed their migration and invasiveness, induced cell apoptosis, and inhibited the secretion of angiogenic growth factors such as angiogenin, ANG-2, and VEGF. These findings show that TBL-12 could be a promising therapeutic candidate for PCa treatment. It was also found that the antitumor mechanism of TBL-12 includes the suppression of PCa cell proliferation via the induction of the endogenous apoptosis pathway and the inhibition of PCa cell migration and invasiveness by repressing MMP-2/MMP-9 expression via the downregulation of the p38 MAPK signaling pathway. Our study may be of importance and significance to research and clinical applications of sea cucumber extract in PCa treatment, especially advanced and refractory PCa treatment.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394-424. https://doi.org/10.3322/caac.21492

2. Gomella LG, Petrylak DP, Shayegan B. Current management of advanced and castration resistant prostate cancer. *Can J Urol*. 2014;21(supp 1):1-6.
3. Chang AJ, Autio KA, Roach M, III, Scher HI. High-risk prostate cancer-classification and therapy. *Nat Rev Clin Oncol*. 2014;11(6):308-323.
4. Choi SY, Ryu J, You D, Hong JH, Ahn H, Kim CS. Simple risk assessment in prostate cancer patients treated with primary androgen deprivation therapy: the Korean cancer study of the prostate risk classification. *Int J Urol*. 2019;26(1):62-68.
5. Kelly MS. Echinoderms: their culture and bioactive compounds. *Prog Mol Subcell Biol*. 2005;39:139-165.
6. Ma X, Kundu N, Collin PD, Goloubeva O, Fulton AM. Frondoside A inhibits breast cancer metastasis and antagonizes prostaglandin E receptors EP4 and EP2. *Breast Cancer Res Treat*. 2012;132(3):1001-1008.
7. Al Shemaili J, Mensah-Brown E, Parekh K, et al. Frondoside A enhances the antiproliferative effects of gemcitabine in pancreatic cancer. *Eur J Cancer*. 2014;50(7):1391-1398.
8. Al Marzouqi N, Iratni R, Nemmar A, et al. Frondoside A inhibits human breast cancer cell survival, migration, invasion and the growth of breast tumor xenografts. *Eur J Pharmacol*. 2011;668(1-2):25-34.
9. Roginsky AB, Ding XZ, Woodward C, et al. Antipancreatic cancer effects of a polar extract from the edible sea cucumber, *Cucumaria frondosa*. *Pancreas*. 2010;39(5):646-652.
10. Janakiram N, Mohammed A, Rao C. Sea cucumbers metabolites as potent anti-cancer agents. *Mar Drugs*. 2015;13(5):2909-2923.
11. Tong Y, Zhang X, Tian F, et al. Philinopside A, a novel marine-derived compound possessing dual anti-angiogenic and anti-tumor effects. *Int J Cancer*. 2005;114(6):843-853.
12. Tian F, Zhang X, Tong Y, et al. PE, a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities in vitro and in vivo. *Cancer Biol Ther*. 2005;4(8):874-882.
13. Tian F, Zhu C, Zhang X, et al. Philinopside E, a new sulfated saponin from sea cucumber, blocks the interaction between kinase insert domain-containing receptor (KDR) and αvβ3 integrin via binding to the extracellular domain of KDR. *Mol Pharmacol*. 2007;72(3):545-552.
14. Yun SH, Park ES, Shin SW, et al. Stichoposide C induces apoptosis through the generation of ceramide in leukemia and colorectal cancer cells and shows in vivo antitumor activity. *Clin Cancer Res*. 2012;18:5934-5948.
15. Yun SH, Park ES, Shin SW, et al. By activating Fas/ceramide synthase 6/p38 kinase in lipid rafts, stichoposide D inhibits growth of leukemia xenografts. *Oncotarget*. 2015;6(29):27596-27612.
16. Stoica BA, Movsesyan VA, Knoblach SM, Faden AI. Ceramide induces neuronal apoptosis through mitogen-activated protein kinases and causes release of multiple mitochondrial proteins. *Mol Cell Neurosci*. 2005;29:355-371.
17. Menchinskaya ES, Pislyagin EA, Kovalchyk SN, et al. Antitumor activity of cucumarioside A2-2. *Chemotherapy*. 2013;59(3):181-191.
18. Zhao Q, Xue Y, Wang J, et al. In vitro and in vivo anti-tumour activities of echinoside A and ds-echinoside A from *Pearsonothuria graeffei*. *J Sci Food Agric*. 2012;92(4):965-974.
19. Zhao Q, Liu Z, Xue Y, et al. Ds-echinoside A, a new triterpene glycoside derived from sea cucumber, exhibits antimetastatic activity via the inhibition of NF-kappaB-dependent MMP-9 and VEGF expressions. *J Zhejiang Univ Sci B*. 2011;12(7):534-544.
20. Alaniz L, García M, Cabrera P, et al. Modulation of matrix metalloproteinase-9 activity by hyaluronan is dependent on NF-kappaB activity in lymphoma cell lines with dissimilar invasive behavior. *Biochem Biophys Res Commun*. 2004;324(2):736-743.
21. Correia-da-Silva M, Sousa E, Pinto MMM, Kijjoa A. Anticancer and cancer preventive compounds from edible marine organisms. *Semin Cancer Biol*. 2017;46:55-64.
22. Cuong NX, Vien LT, Hoang L, et al. Cytotoxic triterpene diglycosides from the sea cucumber *Stichopus horrens*. *Bioorg Med Chem Lett*. 2017;27(13):2939-2942.
23. Cuong NX, Vien LT, Hanh TTH, et al. Cytotoxic triterpene saponins from *Cercodemas anceps*. *Bioorg Med Chem Lett*. 2015;25(16):3151-3156.
24. Chari A, Mazumder A, Lau K, Catamero D, Galitzeck Z, Jagannath S. A phase II trial of TBL-12 sea cucumber extract in patients with untreated asymptomatic myeloma. *Br J Haematol*. 2018;180(2):296-298.
25. Fernandes-Alnemri T, Takahashi A, Armstrong R, et al. Mch3, a novel human apoptotic cysteine protease highly related to CPP32. *Cancer Res*. 1995;55(24):6045-6052.
26. Suzuki Y, Nakabayashi Y, Nakata K, Reed JC, Takahashi R. X-linked inhibitor of apoptosis protein (XIAP) inhibits caspase-3 and -7 in distinct modes. *J Biol Chem*. 2001;276(29):27058-27063.
27. Adrian T. Novel marine-derived anti-cancer agents. *Curr Pharm Des*. 2007;13(33):3417-3426.
28. Wargasetia TL, Widodo. Mechanisms of cancer cell killing by sea cucumber-derived compounds. *Invest New Drugs*. 2017;35(6):820-826.
29. Simpson-Haidaris PJ, Rybarczyk B. Tumors and fibrinogen. The role of fibrinogen as an extracellular matrix protein. *Ann N Y Acad Sci*. 2001;936(1):406-425.
30. Zhao Q, Xue Y, Liu Z, et al. Differential effects of sulfated triterpene glycosides, holothurin A1, and 24-dehydroechinoside A, on anti-metastasic activity via regulation of the MMP-9 signal pathway. *J Food Sci*. 2010;75(9):H280-H288.
31. Huang H, Du T, Xu G, et al. Matrine suppresses invasion of castration-resistant prostate cancer cells by downregulating MMP-2/9 via NF-κB signaling pathway. *Int J Oncol*. 2017;50(2):640-648.
32. Shen KH, Hung SH, Yin LT, et al. Acacetin, a flavonoid, inhibits the invasion and migration of human prostate cancer DU145 cells via inactivation of the p38 MAPK signaling pathway. *Mol Cell Biochem*. 2010;333(1-2):279-291.
33. Tian Y, Guan Y, Jia Y, Meng Q, Yang J. Chloride intracellular channel 1 regulates prostate cancer cell proliferation and migration through the MAPK/ERK pathway. *Cancer Biother Radiopharm*. 2014;29(8):339-344.
34. Xu S, Zhou W, Ge J, Zhang Z. Prostaglandin E2 receptor EP4 is involved in the cell growth and invasion of prostate cancer via the cAMP-PKA/PI3K-Akt signaling pathway. *Mol Med Rep*. 2018;17(3):4702-4712.
35. Ono K, Han J. The p38 signal transduction pathway activation and function. *Cell Signal*. 2000;12(1):1-13.
36. Wang L, Xiao Y, Ping Y, et al. Integrating multi-omics for uncovering the architecture of cross-talking pathways in breast cancer. *PLOS One*. 2014;9(8):e104282.
37. Perner S, Cronauer MV, Schrader AJ, Klocker H, Culig Z, Baniahmad A. Adaptive responses of androgen receptor signaling in castration-resistant prostate cancer. *Oncotarget*. 2015;6(34):35542-35555.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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