Triptolide inhibits benign prostatic epithelium viability and migration and induces apoptosis via upregulation of microRNA-218

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Abstract
Benign prostatic hypertrophy (BPH) has become a troublesome disease for elder men. Triptolide (TPL) has been reported to be a potential anticancer agent. However, the potential effects of TPL on BPH have not been shown out. BPH-1 cells were treated with different concentrations of TPL and/or transfected with microRNA-218 (miR-218) inhibitor, pc-survivin, sh-survivin, or their corresponding controls (NC). Thereafter, cell viability was determined by CCK-8 assay. Cell migration was accessed by modified two-chamber migration assay. Cell apoptosis was checked by propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining. In addition, messenger RNA (mRNA) and protein levels were detected using quantitative real-time polymerase chain reaction (qRT-PCR) and western blot analysis, respectively. BPH-1 cell viability and migration were significantly decreased, while cell apoptosis and expression of miR-218 were statistically enhanced by TPL (P < 0.05 or P < 0.01). However, downregulation of miR-218 increased cell viability and migration, while decreased cell apoptosis compared with the negative control group (P < 0.05 or P < 0.01). Furthermore, the expression of cell cycle–related proteins and cell apoptosis–related proteins were also led to the opposite results with NC. In addition, we found that miR-218 negatively regulated the expression of survivin (P < 0.01) and suppression of survivin significantly enhanced cell apoptosis (P < 0.01). Moreover, the results demonstrated that TPL could inactivate mammalian target of rapamycin (mTOR) pathway, while inhibition of miR-218 alleviated the effects. TPL inhibits viability and migration of BPH-1 cells and induces cell apoptosis and also inactivates mTOR signal pathway via upregulation of miR-218. This study provides evidence for the further studies representing triptolide as a potential agent in the treatment of human BPH.

Keywords
apoptosis, BPH-TPL, migration, miR-218, survivin, viability

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Introduction
Benign prostatic hyperplasia (BPH), also known as prostatic hypertrophy (prostate enlargement), is a common benign disease among middle-aged and elderly men and can also influence quality of daily life and sleeping patterns.¹ ² There are various kinds of treatment strategies for the patients with BPH, such as surgical, prostatectomy, and laser.³ ⁴ Except these, nowadays some medicines exert...
notable effects on BPH, such as α-adrenergic receptor antagonists, inhibitors of the 5α-reductase enzyme, and various phytotherapies. Even though most of these treatments are available and effective, many patients are tormented by these side effects or the surgery complications. Therefore, new medicine or therapy is urgently needed for the treatment of BPH.

Triptolide (TPL), a diterpenoid triepoxide extracted from the traditional Chinese herb *Tripterygium wilfordii*, has been widely used in inflammatory and autoimmune diseases, such as myeloid leukemia, colon carcinoma, pancreatic cancer, nephritis, and rheumatoid arthritis. Due to the effects of TPL on multiple biological and pharmacological activities, such as antioxidant, anti-tumor, anti-proliferative, immunosuppressive, and anti-inflammatory properties, TPL is currently under clinical trials. Previous studies demonstrated that TPL effectively inhibited the development of BPH induced by testosterone in a rat model. However, the effect of TPL on the treatment of BPH is still unclear.

Considerable research works have been devoted to determine the effect of TPL on diseases through regulation of microRNAs (miRNAs). MiRNAs which refer to a class of small (~22 nucleotides) non-coding RNAs can regulate gene expression by directing their target mRNAs and exert various effects. Previous studies have demonstrated that deregulation of miRNAs affected various activities, such as cell viability, migration, and apoptosis in many cancers. Another interesting finding is that microRNA-218 (miR-218) downregulated expression in human malignancies and it has been treated as a suppressor of tumor metastasis and is correlated with clinical stage. Therefore, we hypothesized that miR-218 might affect the response of BPH-1 cells to TPL.

In our study, we aimed to determine the effects of TPL on the prostatic epithelial BPH-1 cells through regulation of miR-218. The treatment with TPL resulted in regulation of cell growth in the human benign prostatic epithelium cell line tested, representing the first use of this approach on prostate cancer cell lines in vitro. This study provides support for the further studies representing triptolide as a potential therapeutic pharmaceutical agent in the treatment of human BPH.

**Materials and methods**

**Cell culture and treatment**

Cells of a BPH epithelial cell line (BPH-1) were provided by the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in BPH-1 culture medium consisting of RPMI 1640 medium supplemented with testosterone 20 ng/mL, transferrin 5 µg/mL, sodium selenite 5 ng/mL, insulin 5 µg/mL, 1% penicillin/streptomycin, and 20% fetal bovine serum (FBS; Life Science, Logan, UT, USA) and maintained at 37°C with air of 5% CO₂. The above chemicals and TPL used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA). TPL was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and then was diluted in phosphate-buffered saline (PBS) solution into different concentrations in cell culture medium.

**CCK-8 assay**

Cell viability was measured by a Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Gaithersburg, MD, USA). In brief, BPH-1 cells were seeded in 96-well plates with 5000 cells/well. After stimulation, 10-µL CCK-8 solution was added to each culture medium with different concentrations of TPL (0, 50, 100, 150, and 200 nM), and then results were, respectively, determined at different time points after treatment (24 and 48 h) at 37°C in humidified 95% air and 5% CO₂. The absorbance was detected by Microplate Reader (Bio-Rad; Hercules, CA, USA) under the optical density (OD) at 450 nm.

**Apoptosis assay**

Propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining purchased from Sigma-Aldrich were used for cell apoptosis analysis. BPH-1 cells were washed in PBS and then fixed in 70% ethanol (Sigma-Aldrich). After that, fixed cells were washed twice in PBS and stained in PI/FITC-Annexin V in the presence of 50 µg/mL RNase A (Sigma-Aldrich). After incubation for 1 h at room temperature in the dark, FACS (Beckman Coulter, Fullerton, CA, USA) was used for flow cytometry analysis according to the manufacturer’s instructions. The data were analyzed using FlowJo software.
**Migration assay**

Cell migration was determined using a modified two-chamber migration assay with a pore size of 8 μm (Bedford, MA, USA). Cells suspended in 200 μL of serum-free medium were seeded on the upper compartment of 24-well Transwell culture chamber, and 600 μL of complete medium was added to the lower compartment. After incubation at 37°C, cells were fixed with methanol. Non-traversed cells were carefully removed from the upper surface of the filter with a cotton swab. Traversed cells on the lower side of the filter were stained with crystal violet and counted.

**MiRNA transfection**

MiR-218 inhibitor and the negative control (NC) were synthesized by GenePharma Co. (Shanghai, China). Cell transfections were conducted using Lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer’s protocol. The primer sequence of miR-218 inhibitor is as follows: 5′-ACAUGG UUAGAUCAAGCACAA-3′. The sequence of the NC is 5′-CAGUACUUUUGUGUAGUACAA-3′.

**Transfection and generation of stably transfected cell lines**

Short-hairpin RNA-directed against human survivin and the full length of survivin were ligated into the pcDNA3.1 plasmid (GenePharma Co.) and were referred as sh-survivin and pc-survivin, respectively. The Lipofectamine 3000 reagent (Life Technologies Corporation) was used for the cell transfection according to the manufacturer’s instructions. The plasmid carrying a non-targeting sequence was used as NC of sh-survivin and pc-survivin. The Lipofectamine 3000 reagent (Life Technologies Corporation) was used for the cell transfection according to the manufacturer’s instructions. The plasmid carrying a non-targeting sequence was used as NC of sh-survivin and pc-survivin. The stably transfected cells were selected by the culture medium containing 0.5 mg/mL G418 (Sigma-Aldrich). After approximately 4 weeks, G418-resistant cell clones were established.

**qRT-PCR**

Total RNA was extracted from cells using Trizol reagent (Life Technologies Corporation) according to the manufacturer’s instructions. The TaqMan MicroRNA Reverse Transcription Kit and TaqMan Universal Master Mix II with the TaqMan MicroRNA Assay were used for measuring expression of miR-218, and U6 (Applied Biosystems, Foster City, CA, USA) was used as internal control.

**Western blot**

The protein used for western blot was extracted using radioimmunoprecipitation assay lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Guangzhou, China). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer’s instructions. Primary antibodies included anti-p16 antibody (ab51243), anti-p21 antibody (ab109520), anti-Cyclin D1 antibody (ab134175), anti-Bcl-2 antibody (ab32124), anti-pro-caspase-3 antibody (ab32150), anti-cleaved-caspase-3 antibody (ab32042), anti-Bax antibody (ab182733), anti-survivin antibody (ab192675), anti-β-actin antibody (ab115777) from Abcam (Cambridge, UK); anti-totally 70kDa ribosomal protein S6 kinase (t-p70S6K) antibody (2708), anti-phosphorylation of 70kDa ribosomal protein S6 kinase (p-p70S6K) antibody (2893), anti-totally mammalian target of rapamycin (t-mTOR) antibody (2976) from Cell Signaling Technology (Beverly, MA, USA). All these primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1000. Primary antibodies were incubated with the membrane at 4°C overnight, followed by wash and incubation with secondary antibody (goat anti-rabbit, IgG ab6721; Abcam) marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the polyvinylidene difluoride (PVDF) membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μL Immobilon Western Chemiluminescent HRP Substrate (Millipore, Danvers, MA, USA) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad).

**Statistical analysis**

All data are shown as means ± standard deviation (SD). Statistical differences among at least three experiment groups were performed using Graphpad 6.0 statistical software (GraphPad Prism, San
The $P$ values were calculated using a one-way analysis of variance (ANOVA). If $P < 0.05$, it means statistically significant.

**Results**

**TPL inhibits cell viability and induces cell apoptosis**

In the first part, we tested the effects of TPL on the viability and apoptosis of BPH-1 cells. As shown in Figure 1(a), the results showed that, for time internal 24h, cell viability was significantly decreased when TPL concentration was or above 100 nM, while for time internal 48h, the changing concentration point was 50 nM. The protein expression of p21 and p16 was significantly upregulated and Cyclin D1 was statistically increased after treatment with TPL compared with the control group (Figure 1(b); $P < 0.05$). Similar results were obtained using western blot (Figure 1(c)). In addition, the results in Figure 1(d) show that TPL significantly enhanced the apoptosis of BPH-1 cells compared with the control group ($P < 0.01$). The expression of cleaved-caspase-3 and Bax were markedly overexpressed, while Bcl-2 showed downregulated expression after treatment with TPL compared with the control (Figure 1(e)). The results indicated that TPL significantly inhibited cell viability in a dose- and time-dependent manner and enhanced cell apoptosis of BPH-1 cells.

**TPL inhibits migration of BPH-1**

Ma et al.\(^9\) found that TPL can suppress the migration of human pancreatic cancer cells. To test whether TPL can also affect the migration of BPH-1 cells, we performed the effects of TPL on BPH-1 cell migration. As shown in Figure 2, the results demonstrated that the migration of BPH-1 cells was significantly decreased after treatment with TPL compared with the control group ($P < 0.05$). These results indicated that TPL inhibited cell migration in BPH-1 cells.

**TPL upregulates the expression of miR-218**

Previous studies showed that TPL had effects on the lymphocytic leukemia cell lines through the regulation of miRNAs.\(^{22}\) Among these identified miRNAs, miR-218 has been previously implicated as a tumor suppressor.\(^{23}\) In order to investigate whether effects of TPL on BPH-1 cells were through the regulation of miR-218, we measured the expression of miR-218 in BPH-1 cells under different concentrations of TPL. The results in Figure 3 demonstrated that compared with control group, treatment with TPL 100 and 200 nM statistically increased the expression of miR-218 (both $P < 0.05$). However, no significant difference was found at the lower concentration (50nM) between the TPL treatment group and the control group. It means that TPL upregulated the expression of miR-218 in a dose-dependent manner.

**TPL inhibits the growth of BPH-1 and induces cell apoptosis via upregulation of miR-218**

Transfecting miR-218 inhibitor was used to test whether the effects of TPL on BPH-1 cells was through regulation of miR-218. The results in Figure 4(a) show that miR-218 inhibitor significantly downregulated the expression of miR-218 in BPH-1 cells compared with the NC group ($P < 0.01$), implying high transfection efficiency. The cell viability was significantly increased by treatment with TPL plus miR-218 inhibitor compared with treatment with TPL plus NC (Figure 4(b); $P < 0.05$). In addition, the protein expression of p16 and p21 was significantly decreased, while expression of Cyclin D1 was statistically increased after treatment with TPL plus miR-218 inhibitor compared with treatment with TPL plus NC (Figure 4(c) and (d); $P < 0.05$ or $P < 0.01$). BPH-1 cell apoptosis was significantly decreased after treatment with TPL plus miR-218 inhibitor compared with treatment with TPL plus NC (Figure 4(e); $P < 0.01$). The related protein cleaved-caspase-3 and Bax were obviously downregulated, while the protein Bcl-2 was markedly upregulated in the treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC (Figure 4(f)). BPH-1 cells migration after treatment with TPL plus miR-218 inhibitor was significantly increased compared with the group treatment with TPL plus NC (Figure 4(g); $P < 0.05$). Moreover, TPL significantly decreased the expression of survivin compared with control ($P < 0.01$), while miR-218 downregulation enhanced the expression of survivin compared with NC ($P < 0.05$; Figure 4(h)). These results revealed that TPL could inhibit cell viability and migration and induce cell apoptosis through upregulation of miR-218 in BPH-1 cells.
MiR-218 negatively regulates expression of survivin and suppression of survivin enhances cell apoptosis

Several studies have identified that survivin is one of the most important miR-218 key targets and it was downregulated by miR-218. This information hints us that miR-218 might play important roles in expression of survivin. The results in Figure 5(a) and (b) show that downregulation of miR-218 could promote expression of survivin compared to the NC.
Furthermore, we overexpressed or downregulated the expression of survivin. As expected, the expression of survivin was significantly increased by transfection with pc-survivin and was significantly decreased by transfection with sh-survivin (Figure 5(c) and (d); \( P < 0.01 \)). The further study in Figure 5(e) demonstrated that sh-survivin significantly induced cell apoptosis and the similar results from western blot demonstrated that cleaved-caspase-3 and Bax were remarkably overexpressed, while Bcl-2 showed downregulated expression by downregulation of survivin (Figure 5(f)). It means that miR-218 negatively regulates expression of survivin and suppression of survivin enhanced cell apoptosis.

**TPL inactivates mTOR signal pathway through upregulation of miR-218**

The results in Figure 6(a) demonstrated that TPL significantly decreased the phosphorylation of mTOR and p70S6K compared with the control group (both \( P < 0.05 \)), while treatment with TPL plus miR-218 inhibitor increased the expression of phosphorylation of mTOR and p70S6K compared with the group treatment with TPL plus NC (both \( P < 0.01 \)). In addition, downregulation of miR-218 activated mTOR signal pathway. The western blot results also presented that the expression of p-mTOR and p-p70S6K was markedly downregulated in the treatment with TPL compared with the control group, while p-mTOR and p-p70S6K both obviously showed upregulated expression by treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC (Figure 6(b)).

**Discussion**

In this study, we investigated whether TPL has potential effects on the treatment of BPH, as well as the underlying mechanisms. We found that TPL decreased cell viability and migration and increased cell apoptosis through upregulation of miR-218. These effects might be by inactivation of mTOR signal pathway.

BPH has become a common disease for the elder men. However, the pathology of BPH is not clearly elucidated and the associated clinical symptoms are much complicated.\(^4\) BPH surgery can cause other side effects, such as bleeding.\(^3,26\) Therefore, more effective medicine and therapies are needed for the treatment of BPH. In recent years, traditional medicine has become popular for treatment of diseases. TPL, which is purified from a Chinese herb *Tripterygium wilfordii* was reported to display antitumor effects.\(^13\) An increasing number of evidence demonstrated that TPL could inhibit cancer cell migration, invasion, and metastasis, such as in leukemia,\(^27\) in oral cancer,\(^28\) and in colon cancer.\(^29\) Similarly, our results also demonstrated that TPL can inhibit cell viability and migration in BPH-1 cells and induce cell apoptosis compared with control (Figures 1(a) and (d) and 2). In addition, cell
Figure 4. Effects of TPL and miR-218 on cell viability, migration, and apoptosis in BPH-1 cells. (a) The expression of miR-218 was significantly decreased under transfection of miR-218 inhibitor compared with transfection of the negative control (NC) group. (b) Cell viability was increased by treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL and NC by CCK-8 assay. (c, d) Western blot was used to determine the expression level of p16, p21, and Cyclin D1. The expression of p21 and p16 were upregulated, while the expression of Cyclin D1 was downregulated under the treatment with TPL plus miR-218 inhibitor compared with the treatment with TPL plus NC. (e) Cell apoptosis was detected by flow cytometry analysis. Cell apoptosis was significantly decreased under the treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC. (f) Western blot was used for exploring apoptosis-associated proteins in BPH-1 cells. Western blot of pro-caspase-3, cleaved-caspase-3, Bcl-2, and Bax were tested to β-actin, the loading control. TPL and miR-218 inhibitor inhibited the expression of caspase-3 and Bax, while induced the expression of Bcl-2 compared with the group treatment with TPL plus NC. (g) Cell migration was accessed by modified two-chamber migration assay. Cell migration was significantly increased under the treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC. Each point represented the mean ± SD of triplicates. (h) Survivin expression was significantly decreased by TPL, while enhanced by the group with TPL and transfection with miR-218 inhibitor. Each experiment was performed three times. *P < 0.05; **P < 0.01.
viability was significantly decreased with increasing concentrations of TPL (Figure 1(a)). p21 and p16 and Cyclin D1 are important cell cycle regulators. p16 and p21 act as cell inhibitors and Cyclin D1 plays as a positive regulator and leads to cell cycle progression. Western blot results from Figure 1(b) and (c) demonstrated that the expression of p16 and p21 increased, while expression of Cyclin D1 decreased by treatment with TPL. Increasing expression of p16 and p21 and reducing expression of Cyclin D1 indicated that TPL inhibited the BPH-1 cell viability.

Based on what we have found in the experiment, we further explored the potential possible mechanisms. Previous studies revealed TPL affected on cell growth through regulation of miRNAs expression, such as miR-142-5p and miR-181a, miR-21, and miR-30. Among all these identified
miRNAs, miR-218 has been found to play an important role in the cell growth and can be treated as a novel potential biomarker for gastric cancer detection. Moreover, miR-218 inhibited invasion and metastasis of gastric cancer and inhibition of miR-218 can increase cell viability. Therefore, we hypothesized that TPL might affect BPH-1 cell growth through regulation of miR-218. Further experiments were performed to verify this hypothesis. Results in our studies demonstrated that the expression of miR-218 was upregulated in treatment with TPL (Figure 3). In addition, after transfection with miR-218 inhibitor, we found that the cell viability and the migration were increased, while the cell apoptosis was decreased compared with the group of TPL plus NC (Figure 4(b) and (g)). Our results were also supported by the western blot that the expression of p16 and p21 were reduced, while the expression of Cyclin D1 was increased after treated with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC (Figure 4(c) and (d)). Similar results were also found by the results from Xia et al. who revealed that glioma cell viability was increased after transfection with miR-218 inhibitor. Moreover, BPH-1 cell apoptosis was significantly decreased after treatment with TPL plus miR-218 inhibitor (Figure 4(e)). This was consistent with the previous studies that miR-218 overexpression was observed to suppress glioma cell apoptosis. Caspase-3 and Bax execute the program of cell apoptosis through several signal pathways. In our study, the expression of cleaved-caspase-3 and Bax were observed downregulation, while expression of Bcl-2 was increased by treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC (Figure 4(f)). Therefore, we found that downregulation of miR-218 increased viability and migration and inhibited cell apoptosis.

Survivin, which belongs to the inhibitor of apoptosis protein family, is observed in most of the human tumors but is rarely found in terminally differentiated normal cells. Survivin was found to be regulated by the expression of miR-218 in tumor cell lines. Therefore, we detected the expression of survivin in BPH-1 cells according to its important role in apoptosis and its close factor related to miR-218. Consistent with previous reports, we found that in our study that expression of survivin was upregulated by miR-218 inhibitor (Figure 5(a) and (b)). Then, we investigated the roles of survivin in BPH-1 cells. After overexpression or suppression of survivin in BPH-1 cells, cell apoptosis was analyzed. Apoptosis of BPH-1 cells (Figure 5(e)) and also the related protein cleaved-caspase-3 and Bax was significantly overexpressed, while the Bcl-2 was downregulated by downregulation of survivin (Figure 5(f)). The results demonstrated that suppressing the effects of miR-218 on BPH-1 cell...
apoptosis might be through regulation of survivin. Therefore, TPL can upregulate the expression of miR-218 and decrease the expression of survivin and induce apoptosis in BPH-1 cells.

mTOR signal pathway is often activated in cancer due to genetic alterations of the genes implicated in this pathway and had also shown to cooperate in prostate cancer progression. p70S6K is a serine/threonine kinase regulated by mTOR pathway, which plays an important role in controlling of cell cycle, growth, and survival. Results of western blot revealed that phosphorylation of mTOR and p70S6K was inhibited by TPL, while phosphorylation of mTOR and p70S6K were enhanced by the treatment with TPL plus miR-218 inhibitor compared with the group treatment with TPL plus NC. It demonstrated that TPL inhibited phosphorylation of mTOR and p70S6K and then through this to inactivate the signal pathway of mTOR. The result provided a possible explanation about how TPL can regulate BPH-1 cell growth through upregulation of the expression of miR-218.

In conclusion, TPL could inhibit the BPH-1 cells viability and migration and induce apoptosis through upregulation of the expression of miR-218 and inactivate mTOR signal pathway. Our data provided new evidence for the mechanism of the effects of TPL on the treatment of BPH. However, further research should be performed to examine the safety and side effects of TPL for the treatment of BPH to support the therapeutic choice and the clinical judgment.

Declaration of conflicting interests

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References

1. Acquaye J and Borofsky MS (2018) Investigational procedures in benign prostatic hypertrophy. Current Opinion in Urology 28(3): 315–321.
2. AHRQ Technology Assessments (2004) Treatments for Benign Prostatic Hyperplasia. Rockville (MD): Agency for Healthcare Research and Quality.
3. Nair SM, Pimentel MA and Gilling PJ (2016) A review of laser treatment for symptomatic BPH (benign prostatic hyperplasia). Current Urology Reports 17(6): 45.
4. Sun F, Crisostomo V, Baez-Diaz C, et al. (2016) Prostatic artery embolization (PAE) for symptomatic benign prostatic hyperplasia (BPH): Part 1, pathological background and clinical implications. Cardiovascular and Interventional Radiology 39(1): 1–7.
5. Kaplan SA (2015) Alpha-blocker therapy: Current update. Reviews in Urology 7(Suppl. 8): S34–S42.
6. Bullock TL and Andriole GL Jr (2006) Emerging drug therapies for benign prostatic hyperplasia. Expert Opinion on Emerging Drugs 11(1): 111–123.
7. Chen F, Liu Y, Wang S, et al. (2013) Triptolide, a Chinese herbal extract, enhances drug sensitivity of resistant myeloid leukemia cell lines through down-regulation of HIF-1α and Nrf2. Pharmacogenomics 14: 1305–1317.
8. Liu Y, Xiao E, Yuan L, et al. (2014) Triptolide synergistically enhances antitumor activity of oxaliplatin in colon carcinoma in vitro and in vivo. DNA and Cell Biology 33(7): 418–425.
9. Ma JX, Sun YL, Wang YQ, et al. (2013) Triptolide induces apoptosis and inhibits the growth and angiogenesis of human pancreatic cancer cells by downregulating COX-2 and VEGF. Oncology Research 20(8): 359–368.
10. Chen BJ (2001) Triptolide, a novel immunosuppressive and anti-inflammatory agent purified from a Chinese herb Tripterygium wilfordii Hook F. Leukemia & Lymphoma 42(3): 253–265.
11. Hamid KS, Reza KA, Ranjbar SH, et al. (2013) A systematic review of the antioxidant, anti-diabetic, and anti-obesity effects and safety of triphala herbal formulation. Journal of Medicinal Plants Research 7: 831–844.
12. Guo Q, Nan XX, Yang JR, et al. (2013) Triptolide inhibits the multidrug resistance in prostate cancer cells via the downregulation of MDR1 expression. Neoplasma 60(6): 598–604.
13. Johnson SM, Wang X and Evers BM. (2011) Triptolide inhibits proliferation and migration of colon cancer cells by inhibition of cell cycle regulators and cytokine receptors. Journal of Surgical Research 168(2): 197–205.
14. Ziaei S and Halaby R (2016) Immunosuppressive, anti-inflammatory and anti-cancer properties of triptolide: A mini review. Avicenna Journal of Phytomedicine 6: 149–164.
15. Meng C, Zhu H, Song H, et al. (2014) Targets and molecular mechanisms of triptolide in cancer therapy. Chinese Journal of Cancer Research = Chung-kuo Chen Chiu 26(5): 622–666.
16. Kiviharju TM, Lecane PS, Sellers RG, et al. (2012) Antiproliferative and proapoptotic activities of triptolide (PG490), a natural product entering clinical trials, on primary cultures of human prostatic epithelial cells. Clinical Cancer Research 8: 2666–2674.
17. Wang YR, Xu Y, Jiang ZZ, et al. (2017) Triptolide reduces prostate size and androgen level on testosterone-induced benign prostatic hyperplasia in Sprague Dawley rats. Chinese Journal of Natural Medicines 15(5): 341–346.
18. Huang X, Yang M and Jin J. (2012) Triptolide enhances the sensitivity of multiple myeloma cells to dexamethasone via microRNAs. Leukemia & Lymphoma 53(6): 1188–1195.
19. MacKenzie TN, Mujumdar N, Banerjee S, et al. (2013) Triptolide induces the expression of miR-142-3p: A negative regulator of heat shock protein 70 and pancreatic cancer cell proliferation. Molecular Cancer Therapeutics 12(7): 1266–1275.
20. Bartel DP (2004) MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116(2): 281–297.
21. Luo JW, Wang X, Yang Y, et al. (2015) Role of micro-RNA (miRNA) in pathogenesis of glioblastoma. European Review for Medical and Pharmacological Sciences 19(9): 1630–1639.
22. Meng HT, Zhu L, Ni WM, et al. (2011) Triptolide inhibits the proliferation of cells from lymphocytic leukemia cell lines in association with down-regulation of NF-kB activity and miR-16-1*. Acta Pharmacologica Sinica 32(4): 503–511.
23. Zhou W, Wang J, Man WY, et al. (2015) siRNA silencing EZH2 reverses cisplatin-resistance of human non-small cell lung and gastric cancer cells. Asian Pacific Journal of Cancer Prevention 16(6): 2425–2430.
24. Kogo R, How C, Chaudary N, et al. (2015) The micro-RNA-218–Survivin axis regulates migration, invasion, and lymph node metastasis in cervical cancer. Oncotarget 6(2): 1090–1100.
25. Alajez NM, Lenarduzzi M, Ito E, et al. (2011) miR-218 suppresses nasopharyngeal cancer progression through downregulation of survivin and the SLIT2-ROBO1 pathway. Cancer Research 71(6): 2381–2391.
26. Kahokehr A and Gilling PJ (2014) Enucleation techniques for benign prostate obstruction: Which one and why. Current Opinion in Urology 24(1): 49–55.
27. Reno TA, Kim JY and Raz DJ (2015) Triptolide inhibits lung cancer cell migration, invasion, and metastasis. The Annals of Thoracic Surgery 100(5): 1817–1824; discussion 1824–1825.
28. Yang CY, Lin CK, Lin GJ, et al. (2016) Triptolide represses oral cancer cell proliferation, invasion, migration, and angiogenesis in co-inoculation with U937 cells. Clinical Oral Investigations 21: 419–427.
29. Oliveira A, Beyer G, Chugh R, et al. (2015) Triptolide abrogates growth of colon cancer and induces cell cycle arrest by inhibiting transcriptional activation of E2F. Laboratory Investigation: A Journal of Technical Methods and Pathology 95(6): 648–659.
30. de Andrade BA, Leon JE, Carlos R, et al. (2012) Immunohistochemical expression of p16, p21, p27 and cyclin D1 in oral nevi and melanoma. Head and Neck Pathology 6(3): 297–304.
31. Tanaka N, Odaiguma T, Mimura M, et al. (2001) Expression of Rb, pRb2/p130, p53, and p16 proteins in malignant melanoma of oral mucosa. Oral Oncology 37(3): 308–314.
32. Bachmann IM, Straume O and Akslen LA. (2004) Altered expression of cell cycle regulators Cyclin D1, p14, p16, CDK4 and Rb in nodular melanomas. International Journal of Oncology 25(6): 1559–1565.
33. Li H, Hui L, Xu W, et al. (2012) Triptolide modulates the sensitivity of K562/A02 cells to adriamycin by regulating miR-21 expression. Pharmaceutical Biology 50(10): 1233–1240.
34. Yang Q, Sun M, Chen Y, et al. (2017) Triptolide protects podocytes from TGF-β-induced injury by preventing miR-30 downregulation. American Journal of Translational Research 9: 5150–5159.
35. Li BS, Zhao YL, Guo G, et al. (2012) Plasma microRNAs, miR-223, miR-21 and miR-218, as novel potential biomarkers for gastric cancer detection. PLoS ONE 7(7): e41629.
36. Tie J, Pan Y, Zhao L, et al. (2010) MiR-218 inhibits invasion and metastasis of gastric cancer by targeting the Robo1 receptor. PLoS Genetics 6(3): e1000879.
37. Xia H, Yan Y, Hu M, et al. (2013) MiR-218 sensitizes glioma cells to apoptosis and inhibits tumorigenicity by regulating ECOP-mediated suppression of NF-κB activity. Neuro-Oncology 15: 413–422.
38. Lin Y, Peng N, Li J, et al. (2013) Herbal compound triptolide synergistically enhanced antitumor activity of amino-terminal fragment of urokinase. Molecular Cancer 12: 54.
39. Liu X, Gao R, Dong Y, et al. (2010) Survivin gene silencing sensitizes prostate cancer cells to selenium growth inhibition. BMC Cancer 10: 418.
40. Shariat SF, Lotan Y, Saboorian H, et al. (2004) Expression of survivin gene is associated with features of hormone-refractory prostate carcinoma. Cancer Therapeutics 70 and pancreatic cancer cell proliferation. Molecular Cancer Research 12: 54.
41. Heinonen H, Nieminen A, Saarela M, et al. (2008) Deciphering downstream gene targets of PI3K/mTOR/p70S6K pathway in breast cancer. BMC Genomics 9: 348.
42. Kinkade CW, Castillo-Martin M, Puzio-Kuter A, et al. (2008) Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a preclinical mouse model. Journal of Clinical Investigation 118(9): 3051–3064.