Lobaplatin Inhibits Prostate Cancer Proliferation and Migration Through Regulation of BCL2 and BAX

Hongwen Cao¹, Yigeng Feng¹, Lei Chen¹, and Chao Yu¹

Abstract
Lobaplatin is a diastereometric mixture of platinum (II) complexes, which contain a 1,2-bis (aminomethyl) cyclobutane stable ligand and lactic acid. Previous studies have showed that lobaplatin plays inhibiting roles in various types of tumors. However, the role of lobaplatin in prostate cancer remains unknown. Cell viability was detected by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide assay. Cell proliferation was detected by cell colony formation assay. Cell migration and invasion were determined by transwell migration and invasion assay. Cell apoptosis was detected by flow cytometry. The messenger RNA and protein expression levels were detected by quantitative real-time polymerase chain reaction and Western blot. Lobaplatin treatment inhibits cell viability, cell proliferation, cell migration, and invasion, while promotes cell apoptosis of prostate cancer cell lines DU145 and PC3. Meanwhile, lobaplatin treatment regulates apoptosis by downregulation of BCL2 expression and upregulation of BAX expression levels. Our study suggests lobaplatin inhibits prostate cancer proliferation and migration through regulation of BCL2 and BAX expression.

Keywords
lobaplatin, prostate cancer, proliferation and migration, BCL2, BAX

Introduction
Prostate cancer is one of the most common cancers in the world and leads to the second most cancer-related death for US men.¹ There are more than 3.3 million patients with prostate cancer in the United States,² and diagnosis of prostate cancer in United States was estimated 220 000 yearly.³ More importantly, the increasing occurrence in developing countries including China has been noted in recent years.⁴ Patients with prostate cancer are usually diagnosed by prostate-specific antigen (PSA) testing, although the efficacy of PSA screening is under debates.⁵ Treatment for prostate cancer include surgery, radiation therapy, cryotherapy (cryosurgery), hormone therapy, chemotherapy, and vaccine treatment,⁶ while the opinions always vary depending on disease extent and patient characteristics, such as age, comorbidity, and personal preferences.⁷ Advances in understanding the epidemiology and pathogenesis of prostate cancer have been made to develop potential novel treatments. However, in clinical management, patients are likely to relapse after primary therapy, which is the most important problem.⁷ Therefore, more efforts are needed to explore the molecular mechanism, which regulates prostate cancer progress for more effective therapies and improved clinical outcomes.

Lobaplatin is the third-generation platinum complex originally developed by the German firm ASTA Medica (Degussa).⁸ With the DNA-alkylating activity, lobaplatin induces the DNA–drug adducts (interstrand Pt-GG and Pt-AG cross-links) formation, blocks DNA replication and transcription, and further inhibits gene expression in tumor cells, which holds the similar mechanism of action to other platinum drugs.⁹ Lobaplatin has numerous advantages for clinical development, such as high antitumor activity, favorable toxicity profile, good solubility, and stability in water.¹⁰ Previous studies demonstrated that lobaplatin plays antitumor activation in various kinds

¹ Surgical Department I (Urology Department), LONGHUA Hospital Shanghai University of Traditional Chinese Medicine, Shanghai, China

Received 16 March 2019; received revised 10 April 2019; accepted 23 April 2019

Corresponding Authors:
Lei Chen and Chao Yu, Surgical Department I (Urology Department), LONGHUA Hospital Shanghai University of Traditional Chinese Medicine, No. 725 Wanping Road South, Xuhui District, Shanghai City 200032, China. Email: chenleijoe@126.com; yuchao97@sina.com
of tumors, including breast cancer, esophageal squamous cell carcinoma, gastric carcinoma, lung cancer, melanoma, and ovarian cancer. However, until now, no evidence has showed the role of lobaplatin in prostate cancer.

In the present study, we explored the role of lobaplatin in prostate cancer. We found that after lobaplatin treatment, the cell viability, cell proliferation, cell migration, and invasion of prostate cancer cell lines DU145 and PC3 were significantly inhibited, while cell apoptosis was significantly promoted. Mechanically, lobaplatin treatment inhibited BCL2 expression levels and increased BAX expression level. These data indicate the potential role of lobaplatin for clinical treatment of patients with prostate cancer.

Methods and Materials

Cell Lines

The human prostate cancer cell lines, DU145 and PC3, were purchased by Cell Bank of Shanghai (China) and cultured in Roswell Park Memorial Institute 1640 (Invitrogen, Carlsbad, California) in the presence of 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 g/mL streptomycin in a humidified 5% (vol/vol) atmosphere of CO2 at 37°C incubator. The cells were verified by Short Tandem Repeat method.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

According to the manufacturer’s instructions, total RNA was isolated from DU145 and PC3 cell lines using TRizol reagent (Invitrogen). Complementary DNA was synthesized using the PrimeScript real-time reagent kit (TaKaRa, Dalian, China). The primers used for detecting the messenger RNA (mRNA) expression levels of BCL2 and BAX are as follows: BCL2: forward 5'-GCC CTG TGG ATG ACT GAG TA-3', reverse 5'-TTC AGA GAC AGC CAG GAG AAA-3'; BAX: forward 5'-GCT GGA CAT TGG ACT TCC TC-3', reverse 5'-GGC GTC CCA AAG TAG GAG AG-3'; VIM: forward 5'-GAG CTA CGT GAC TAC GTC CAC C-3', reverse 5'-GTT CTT GAA CTC GGT GTT GAT G-3'. The mRNA expression level of GAPDH was used for normalization, and the relative

Figure 1. Lobaplatin inhibits prostate cancer cell proliferation. (A) DU145 and (B) PC3 cells were treated with lobaplatin and subjected to MTT assay as indicated. Data are mean (SD) of 3 independent experiments and each measurement in triplicate (*P < .05, **P < .01). MTT indicates 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-h-tetrazolium bromide; SD, standard deviation.
expression levels of BCL2, BAX, and VIM were measured by 2−ΔΔCT method.

3-(4,5-Dimethyl-2-thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide Assay and Colony Formation Assay

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-h-tetrazolium bromide (MTT) assay was performed according to the manufacturer’s protocol. Briefly, 3–5 × 10^5 cells/well were cultured in 96-well plates for 24, 48, or 72 hours. At the end of culture, 50 μL of the MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours, followed by adding 200 μL of dimethyl sulfoxide to each well and incubating for 15 minutes. The absorbance was read using a microplate reader (SpectraMAX Plus, Molecular Devices, Sunnyvale, California) at a wavelength of 570 nm.

For colony formation assays, 1000 cells were seeded into 6-well plates to culture for 14 days. Then, the cells were fixed using 70% ethanol and stained with 10% Giemsa (Sigma-Aldrich, St. Louis, Missouri). Only colonies containing >50 cells were counted. All experiments were carried out in triplicate.

Cell Proliferation Assay and Apoptosis Analysis

DU145 and PC3 cells were seeded at the 1 × 10^5/well into 6-well plates. The total cell number of each well was counted 3 times on days 1, 2, 3, 4, and 5 days using the Z1 particle counter (Beckman Coulter, Inc, Brea, California).

To perform apoptosis assay, cells were plated at a density of 2 × 10^6 cells per 6 cm dish and treated with 15 μM lobaplatin or the solvent control. 12 or 24 hours later, the cells were pelleted by centrifugation at 1500 rpm for 5 minutes and washed twice with phosphate buffer saline. Then, cells were stained according to the protocol of the Annexin V/PI kit, followed by flow cytometry evaluation.

Migration and Invasion Assays

The transwell system (24 wells, 8 mm pore size with polycarbonate membrane) and Matrigel were used according to the
manufacturer’s protocols for invasion assays. Total of $1 \times 10^5$/well cells were seeded into the upper chamber with serum-free opti-MEM medium. In the lower compartment, opti-MEM containing 10% FBS was used as a chemoattractant. 48 hours later, cells remained in the upper chamber were scrapped out. Matrigel membranes were fixed with ice-cold methanol and stained with 0.1% crystal violet solution. Five randomly fields were selected under a light microscope to count the number of cells migrated to the lower side.

For the migration assay, cells were seeded in the upper chambers without Matrigel. The rest of assay was performed as the invasion assay.

**Western Blot**

Cells were lysed in radioimmunoprecipitation assay buffer (Sigma) and electrophoresed in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. The proteins were transferred to polyvinylidene fluoride membranes (Millipore, Billerica, MA), followed by blocking with 5% milk at room temperature. Then, the membrane was incubated with anti-BCL2 or BAX primary antibody at 4°C overnight, followed by incubation with horseradish peroxidase-conjugated secondary antibody at room temperature for 1 hour. Finally, signals were visualized by enhanced chemiluminescence (Pierce, Wisconsin). β-Actin (CST) was used as control.

**Statistical Analysis**

All data analyzed as the mean (standard deviation SD) are from at least 3 separate experiments. The statistical significance was determined using Student $t$ test, 1- or 2-way analysis of variance analysis followed by a Bonferroni post hoc test; $P < .05$ was considered significant.

**Results**

**Lobaplatin Inhibits Cell Viability of Prostate Cancer Cells**

To explore the role of lobaplatin in prostate cancer cell proliferation, we treated DU145 and PC3 cells with different doses of lobaplatin and detected cell viability by MTT assay at different time points (12, 24, and 48 hours). As shown in Figure. 1A and B, the cell viability of DU145 or PC3 significantly decreased long with the increased concentrations of lobaplatin, indicating that lobaplatin could inhibit cell viability of DU145 or PC3 in a dose-dependent manner. In addition, cell viability of the 2 prostate cancer cell lines significantly decreased at 48 hours compared with that at 12 hours at the low dose of lobaplatin (5 μM). These data demonstrate that lobaplatin inhibits cell viability of prostate cancer cells, which is as similar as the effect of cisplatin and oxaliplatin (Figure 2A and B).

**Lobaplatin Inhibits Proliferation of Prostate Cancer Cells**

We next detected the influence of lobaplatin on prostate cancer cell proliferation. The cell numbers of DU145 or PC3 were

**Lobaplatin Inhibits Cell Proliferation of Prostate Cancer Cells**

We next detected the influence of lobaplatin on prostate cancer cell proliferation. The cell numbers of DU145 or PC3 were
much lower after lobaplatin treatment (15 μM; Figure 3A and B). The cell colony formation ability of DU145 or PC3 was also significantly inhibited by lobaplatin treatment (Figure 3C and D). These data indicate that lobaplatin inhibits proliferation of prostate cancer cells.

**Lobaplatin Induces Cell Apoptosis of Prostate Cancer Cells**

We next determined cell apoptosis after lobaplatin treatment. DU145 and PC3 prostate cancer cell lines were treated with 15 μM lobaplatin for 12 or 24 hours. The percentage of DU145 and PC3 cell apoptosis significantly increased (from approximately 4% to 7%) after lobaplatin treatment (Figure 4A and B) and 24 hours (from approximately 5% to 18%; Figure 5A and B). These data indicate that lobaplatin has the ability to induce apoptosis in prostate cancer cell lines.

**Lobaplatin Suppresses Cell Migration and Invasion of Prostate Cancer Cells**

We next detected the influence of lobaplatin on prostate cancer cell migration and invasion, which are important for cancer progress. DU145 and PC3 cells were treated with 15 μM lobaplatin, followed by cell migration and invasion assay. As shown in Figure 6A and B, lobaplatin treatment significantly inhibited cell migration and invasion of the 2 cell lines, demonstrating that lobaplatin inhibits prostate cancer cell migration and invasion.

**Lobaplatin Regulates BCL2, BAX, and VIM Expression Levels**

Given that lobaplatin promotes prostate cancer cell apoptosis and suppresses migration, we next detected whether lobaplatin has the ability to regulate apoptosis and epithelial–mesenchymal transition (EMT)-related gene expression. DU145 and PC3 cell lines were treated with 15 μM lobaplatin for 48 hours. The mRNA level of BCL2 and VIM significantly decreased after lobaplatin treatment, while BAX mRNA level increased in both of the prostate cancer cell lines (Figure 7A and B). Western blot analysis showed similar decreased BCL2 and VIM protein levels and increased BAX protein levels after lobaplatin treatment (Figure 7C). These data demonstrate that lobaplatin promotes prostate cancer cell apoptosis and suppresses migration through regulating BCL2, BAX, and VIM expression levels.
Lobaplatin is the third-generation water-soluble platinum compounds, appears to be more stable, less toxic, with a better therapeutic index, and may overcome tumor resistance as compared with the first and second generation of platinum compounds.17,18 Because of superior pharmacokinetic parameters in Chinese populations versus Western populations,10,19 lobaplatin has been approved in China for the treatment of chronic myelogenous leukemia, metastatic breast, and small cell lung cancer.20,21 However, there is still no report about the application of lobaplatin in prostate cancer treatment. In the present study, we explored the role of lobaplatin in prostate cancer. We found that lobaplatin treatment inhibits cell viability, cell proliferation, cell migration, and invasion of prostate cancer cell lines DU145 and PC3, while promoted cell apoptosis. Lobaplatin treatment regulates the expression levels of apoptosis and EMT-related genes, BCL2, BAX, and VIM. To the best of our knowledge, this is the first study to explore the role of lobaplatin in prostate cancer.

Cell migration and invasion into surrounding tissue and vasculature is a crucial step for tumor development22,23, so a pressing goal in tumor biology is how to inhibit the activity of migration and invasion of tumor cells. In this study, we provided evidence that lobaplatin treatment inhibits cell migration and invasion of DU145 and PC3 cell lines, indicating that lobaplatin may inhibit prostate cancer via inhibiting cell migration and invasion.

Apoptosis is an ordered and orchestrated programmed cell death that occurs in physiological and pathological conditions, which plays crucial roles in maintaining survival/death balance in cells.24 During the process of antitumor activity, there always be apoptosis of the tumor cells.25 Defect in apoptosis can promoted cancer development, while enhanced apoptosis may inhibit cancer progress.26 We found that lobaplatin treatment significantly increased the percentage of apoptosis in both DU145 and PC3 cell lines, indicating that lobaplatin plays important roles in promoting cell apoptosis.

BCL-2 (B-cell lymphoma 2) is one of BCL-2 family members that regulates cell death by either inducing or inhibiting apoptosis.27,28 Bcl-2 has clinical significance in lymphoma, specifically be considered to inhibit apoptosis during tumor development.29 In human colon cancer cell line HCA-7, PGE2

**Discussion**

Lobaplatin is the third-generation water-soluble platinum compounds, appears to be more stable, less toxic, with a better therapeutic index, and may overcome tumor resistance as compared with the first and second generation of platinum compounds. Because of superior pharmacokinetic parameters in Chinese populations versus Western populations, lobaplatin has been approved in China for the treatment of chronic myelogenous leukemia, metastatic breast, and small cell lung cancer. However, there is still no report about the application of lobaplatin in prostate cancer treatment. In the present study, we explored the role of lobaplatin in prostate cancer. We found that lobaplatin treatment inhibits cell viability, cell proliferation, cell migration, and invasion of prostate cancer cell lines DU145 and PC3, while promoted cell apoptosis. Lobaplatin treatment regulates the expression levels of apoptosis and EMT-related genes, BCL2, BAX, and VIM. To the best of our knowledge, this is the first study to explore the role of lobaplatin in prostate cancer.
treatment leads to increased clonogenicity, inhibits programmed cell death via inducing BCL-2 expression levels. ABT-199, a potent and selective BCL-2 inhibitor, inhibits BCL-2 dependent tumor growth in vivo, and leads to tumor lysis within 24 hours in 3 patients with refractory chronic lymphocytic leukemia, indicating the promise of BCL-2 inhibition in the treatment of BCL-2–dependent hematomal cancers. The expression levels of BCL-2 were increased in miR-143-downregulated cervical tissues as compared with normal cervical tissues. Overexpression of miR-143 in HeLa cells resulted in downregulated Bcl-2 expression, while miR-143 knockdown upregulated Bcl-2 expression, indicating that miR-143 plays crucial roles in the pathogenesis of cervical cancer through regulating BCL-2 expression. BAX (Bcl-2–associated X protein) functions as an apoptotic activator, showed lower expression in breast cancer cells. The interaction of BAX with p53 and caspase-3 is associated with active apoptosis in breast cancer cells. The dissociation of BAX from Bcl-xL and activation of caspase families were essential for quercetin caused apoptotic processes in human prostate cell line (LNCaP). In our study, the most important question is the mechanism of how lobaplatin promotes apoptosis. We detected the mRNA and protein levels of apoptosis-related genes, BCL2 and BAX. BCL2 expression levels decreased, while BAX expression levels increased after lobaplatin treatment, indicating that lobaplatin promotes apoptosis via regulating apoptosis-related genes expression.

It is important to note that the effects of lobaplatin in prostate cancer were performed in 2 androgen receptor negative prostate cancer cell lines (DU145 and PC3), while androgen receptor positive prostate cancer cell lines, such as 22Rv1, should be used for further detection. More importantly, the data are from prostate cancer cell lines in vitro, no in vivo data. The prostate cancer mouse model and the clinical samples should be used to verify the effects of lobaplatin in prostate cancer, which will provide theoretical basis for the potential use of lobaplatin for the treatment of patient with prostate cancer.

Taken together, we found that lobaplatin treatment significantly inhibits the cell viability, cell proliferation, cell migration, and cell invasion of prostate cancer cell lines DU145 and PC3 and promotes cell apoptosis of the 2 cell lines. Mechanically, lobaplatin treatment inhibited BCL2 and VIM expression levels and increased BAX expression level. These data indicate the potential role of lobaplatin for clinical treatment of patients with prostate cancer.

Conclusion

Lobaplatin inhibits prostate cancer proliferation and migration, promotes cell apoptosis through regulation of BCL2 and BAX. These data indicate the potential role of lobaplatin for clinical treatment of patients with prostate cancer.

Authors’ Note

Hongwen Cao and Yigeng Feng contributed equally to this study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by The Third Batch of Young Chinese Name Training Program of LongHua Hospital Shanghai University of Traditional Chinese Medicine (Chen Lei; subject number: RC-2017-01-14); Shanghai Municipal Health and Family Planning Commission Special Subject of Chinese Medicine Research (subject number: 2016JP014); and National TCM Clinical Research Base Dragon Medicine Scholars (nursery plan) of LONGHUA Hospital Shanghai University of Traditional Chinese Medicine (subject number: LYTD-56).

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9-29.
2. Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66(4):271-289.
3. Bresalier RS, Kopetz S, Brenner DE. Blood-based tests for colorectal cancer screening: do they threaten the survival of the FIT test? Dig Dis Sci. 2015;60(3):664-671.
4. Chen W, Zheng R, Zeng H, Zhang S, He J. Annual report on status of cancer in China, 2011. Chin J Cancer Res. 2015;27(1):2-12.
5. Smith RA, Andrews K, Brooks D, et al. Cancer screening in the United States, 2016: a review of current American Cancer Society guidelines and current issues in cancer screening. CA Cancer J Clin. 2016;66(2):96-114.
6. Heidenreich A, Bastian PI, Bellmunt J, et al. EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol. 2014;65(1):124-137.
7. Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD, Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. JAMA. 1999;281(17):1591-1597.
8. Perabo FG, Muller SC. New agents for treatment of advanced transitional cell carcinoma. Ann Oncol. 2007;18(5):835-843.
9. McKeage MJ. Lobaplatin: a new antitumour platinum drug. Expert Opin Investig Drugs. 2001;10(1):119-128.
10. Peng Y, Liu YE, Ren XC, et al. A phase I clinical trial of dose escalation of lobaplatin in combination with fixed-dose docetaxel for the treatment of human solid tumours that had progressed following chemotherapy. Oncol Lett. 2015;9(1):67-74.
11. Wu X, Tang P, Li S, et al. A randomized and open-label phase II trial reports the efficacy of neoadjuvant lobaplatin in breast cancer. Nat Commun. 2018;9(1):832.
12. Du L, Fei Z, Song S, Wei N. Antitumor activity of Lobaplatin against esophageal squamous cell carcinoma through caspase-dependent apoptosis and increasing the Bax/Bcl-2 ratio. Biomed Pharmacother. 2017;95:447-452.
13. Li Y, Liu B, Yang F, et al. Lobaplatin induces BGC-823 human gastric carcinoma cell apoptosis via ROS-mitochondrial apoptotic pathway and impairs cell migration and invasion. Biomed Pharmacother. 2016;83:1239-1246.

14. Li JR, Sun Y, Liu L. Radioactive seed implantation and lobaplatin chemotherapy are safe and effective in treating patients with advanced lung cancer. Asian Pac J Cancer Prev. 2015;16(9):4003-4006.

15. Yang F, Yu Y, Lei Q, et al. Lobaplatin arrests cell cycle progression, induces apoptosis and impairs migration and invasion in B16-F10 melanoma cell line in vitro. Biomed Pharmacother. 2015;69:402-408.

16. Sun X, Lou LG, Sui DH, Wu XH. Preclinical activity of lobaplatin as a single agent and in combination with taxanes for ovarian carcinoma cells. Asian Pac J Cancer Prev. 2014;15(22):9939-9943.

17. Dilruba S, Kalayda GV. Platinum-based drugs: past, present and future. Cancer Chemother Pharmacol. 2016;77(6):1103-1124.

18. Monneret C. Platinum anticancer drugs. From serendipity to rational design. Ann Pharm Fr. 2011;69(6):286-295.

19. Welink J, Pechstein B, van der Vijgh WJ. Determination of the two diastereoisomers of lobaplatin (D-19466) in plasma ultrafiltrate of cancer patients with a normal or an impaired kidney or liver function by high-performance liquid chromatography with ultraviolet detection. J Chromatogr B Biomed Appl. 1996;675(1):107-111.

20. Lobaplatin. D 19466. Drugs R&D. 2003;4(6):369-372.

21. Ali I, Wani WA, Saleem K, Haque A. Platinum compounds: a hope for future cancer chemotherapy. Anticancer Agents Med Chem. 2013;13(2):296-306.

22. Entschladen F, Drell TL, Lang K, Joseph J, Zaenker KS. Tumour-cell migration, invasion, and metastasis: navigation by neurotransmitters. Lancet Oncol. 2004;5(4):254-258.

23. Bozzuto G, Ruggieri P, Molinari A. Molecular aspects of tumor cell migration and invasion. Ann Ist Super Sanita. 2010;46(1):66-80.

24. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer. 2005;5(3):231-237.

25. Hassan M, Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N. Apoptosis and molecular targeting therapy in cancer. Biomed Res Int. 2014;2014:10845.

26. Wong RS. Apoptosis in cancer: from pathogenesis to treatment. J Exp Clin Cancer Res. 2011;30:87.

27. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science. 1984;226(4678):1097-1099.

28. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14;18) translocation. Cell. 1986;47(1):19-28.

29. Yang J, Liu X, Bhalla K, et al. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science. 1997;275(5303):1129-1132.

30. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN. Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res. 1998;58(2):362-366.

31. Souers AJ, Levendor JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202-208.

32. Liu L, Yu X, Guo X, et al. miR-143 is downregulated in cervical cancer and promotes apoptosis and inhibits tumor formation by targeting Bcl-2. Mol Med Rep. 2012;5(3):753-760.

33. Pluta P, Smolewski P, Pluta A, et al. Significance of Bax expression in breast cancer patients. Pol Przegl Chir. 2011;83(10):549-553.

34. Lee DH, Szczepanski M, Lee YJ. Role of Bax in quercetin-induced apoptosis in human prostate cancer cells. Biochem Pharmacol. 2008;75(12):2345-2355.