Chin J Integr Med 2019 Aug;25(8):598-603

Chinese Journal of Integrative Medicine

Available online at link.springer.com/journal/11655
Journal homepage: www.cjim.cn/zxyjhen/zxyjhen/ch/index.aspx
E-mail: cjim_en@cjim.cn

Original Article

Pien Tze Huang (片仔癀) Overcomes Doxorubicin Resistance and Inhibits Epithelial-Mesenchymal Transition in MCF-7/ADR Cells*

CHEN Xi[1,2], QI Fei[1,3], SHEN A-ling[1,3], CHU Jian-feng[1,3], Thomas Joseph Sferra[4], CHEN You-qin[1,4], and PENG Jun[1,3]

ABSTRACT **Objective**: To evaluate the effect of Pien Tze Huang (片仔癀, PZH) on breast cancer chemoresistance and related epithelial-mesenchymal transition (EMT) and investigate the underlying mechanisms. **Methods**: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was used to determine the cell viability. Adriamycin (ADR) staining observed by fluorescence microscope was performed to detect the accumulation of ADR. Transwell assay was used to analyze the cell migration and invasion. Western-blot was performed to detect the protein expression of related genes. **Results**: MCF-7/ADR cells were resistant to ADR treatment, and PZH treatment inhibited the viability of MCF-7/ADR cells in a dose-dependent manner. PZH treatment also increased the intercellular accumulation of ADR and down-regulated the expression of ABCG2 and ABCB1 in MCF-7/ADR cells (P<0.05). In addition, PZH treatment inhibited EMT, migration and invasion of MCF-7/ADR cells (P<0.05). Moreover, PZH suppressed activation of transforming growth factor β1 (TGF-β) signaling in MCF-7/ADR cells (P<0.05). **Conclusion**: PZH treatment can effectively overcome chemoresistance via down-regulating ABCG2, ABCB1 and inhibit EMT in ADR resistant human breast cancer cells via suppression of the TGF-β1 pathway.

KEYWORDS Pien Tze Huang, Chinese medicine, breast cancer, chemoresistance, epithelial-mesenchymal transition

Breast cancer is one of the most commonly diagnosed cancers worldwide and the leading cause of cancer-related deaths among women, with an estimated 1.7 million new diagnoses and 0.5 million deaths each year.[1] Current treatments for breast cancer often involve surgery in combination with chemotherapeutic drugs such as adriamycin (ADR, also known as doxorubicin), 5-fluorouracil (5-FU), cyclophosphamide, paclitaxel and docetaxel.[2,3] However, prolonged use of these chemotherapeutic drugs often generate multi-drug resistance in various cancers, in addition to a high level of toxicity against normal cells. Therefore, it is essential to develop novel chemotherapeutics.

A common mechanism of multi-drug resistance is the overexpression of adenosine triphosphate-binding cassette (ABC) family of transporter proteins, such as breast cancer resistance protein (BCRP/ABCG2) and ABCB1 associated protein, which protects cells from chemotherapeutics-induced damage by increasing the efflux of these compounds.[4-6] Drug resistance is also characterized by the acquisition of epithelial-mesenchymal transition (EMT), a process where epithelial cells lose polarity and cell adhesion, and then acquire the characteristic properties of mesenchymal cells.[7,8] It has shown that EMT is strongly correlated with drug resistance in various kinds of human cancer.[9-11] Chemoresistance-induced EMT confers cancer cells the properties of migration and invasion, leading to cancer progression and metastasis.[12] Drug resistance and EMT could be regulated by various cytokines and growth factors, such as transforming

*Supported by the National Natural Science Foundation of China (No. 81673721), Natural Science Foundation of Fujian Province (No. 2018j01352), and the Developmental Fund of Chen Keji Integrative Medicine (No. CKJ2014004)

1. Academy of Integrative Medicine, Fujian University of Traditional Chinese Medicine, Fuzhou (350122), China; 2. Department of Oncology, Fuzhou General Hospital of Nanjing Military Command, Fuzhou (350003), China; 3. Fujian Key Laboratory of Integrative Medicine on Geriatric, Fujian University of Traditional Chinese Medicine, Fuzhou (350122), China; 4. School of Medicine, Case Western Reserve University, Cleveland, OH (44106), USA
Correspondence to: Dr. PENG Jun, Tel: 86-591-22861303, E-mail: pjunlab@hotmail.com

DOI: https://doi.org/10.1007/s11655-018-2992-4

growth factor β1 (TGF-β1).[13,14] Thus, inhibiting chemoresistance and related EMT via suppression of TGF-β signaling might be a potential novel therapeutic strategy against cancers.

Chinese medicine (CM) has recently received significant attention in the field of cancer therapy, due to the benefits of decreased chemoresistance and fewer side-effects.[15] As a well known CM formula that was first prescribed more than 450 years ago in the Ming Dynasty, Pien Tze Huang (片仔癀, PZH) exhibited significantly properties of heat clearing, detoxification, dissipation of hard mass, detumescence and analgesia,[16] resulting from its main ingredients including *Moschus*, *Calculus Bovis*, *Snake Gall* and *Radix Notoginseng*. Based on CM theory, PZH has been widely used to clinically treat traumatic injuries and a variety of inflammatory diseases, particularly hepatitis.[16-18] More importantly, in China and Southeast Asia, PZH has also been used as a folk remedy to treat patients with various types of human cancer, including colorectal cancer (CRC). Our previous studies indicated that PZH treatment significantly suppressed tumor growth via the promotion of cancer cell apoptosis,[19,20] the inhibition of cell proliferation,[20-23] tumor angiogenesis[21,23] and lymphangiogenesis,[24] which is probably mediated by modulation of multiple signaling pathways. The suppression of PZH on tumor growth in CRC was further confirmed in both osteosarcoma cancer and ovarian cancer from other group's studies.[25,26] Recent studies also indicated that PZH treatment significantly inhibited the growth of colorectal cancer stem cells via suppression Notch signaling pathway.[27,28] More importantly, PZH treatment significantly suppressed the metastasis of colorectal cancer cells via suppression TGF-β signaling pathway,[29,30] as well as overcome drug resistance in colorectal cancer and osteosarcoma cancer.[31,32] To further elucidate the molecular mechanisms of its tumoricidal activities, in the present study we used a ADR resistant human breast cancer cell line (MCF-7/ADR) to evaluate the effect of PZH on breast cancer chemoresistance and related EMT, as well as on expression of ABCG2, ABCB1 and the activation of TGF-β1 pathway.

METHODS

Materials and Reagents

Roswell Park Memorial Institute (RPMI)-1640 medium, fetal bovine serum (FBS) and penicillin-streptomycin were obtained from Thermo Fisher Scientific (USA). N-cadherin and ABCB1 antibodies were purchased from Abcam (China). ABCG2 antibody was purchased from Sangon Biotech (China). TGF-β and β-actin antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies were provided by Cell Signaling Technology (USA). Transwell chambers were obtained from Corning Life Sciences (USA). BD BioCoat Matrigel Invasion Chamber was purchased from BD Bioscience (USA). All the other chemicals, unless otherwise stated, were obtained from Sigma Chemicals (USA).

Preparations of PZH

PZH was obtained from and authenticated by Zhangzhou Pien Tze Huang Pharmaceutical Co. Ltd., China (lot No. 201602001). Stock solutions of PZH were prepared by dissolving the PZH powder in phosphate-buffered saline (PBS) at a concentration of 20 mg/mL. The working concentrations of PZH were made by diluting the stock solution in the cell culture medium.

Cell Culture

Human breast cancer MCF-7 cells and MCF-7/ADR cells were obtained from Nanjing KeyGen Biotech. Co. Ltd. (China). MCF-7 cells were cultured in RPMI-1640 complete medium containing 10% (v/v) FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in a 37 ℃ humidified incubator with 5% CO_2. MCF-7/ADR cells were cultured in RPMI-1640 complete medium supplemented with 100 μg/mL ADR.

Evaluation of Cell Viability

Cell viability of MCF-7 and MCF-7/ADR cells was assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. Cells were seeded in 96-well plates at a density of 3×10^3 cells/well in 100 μL medium and treated with various concentrations of ADR (0.5–8 μmol/L) for 48 h or PZH (0.25–0.75 mg/mL) for 24 h. MTT assay was subsequently performed by addition of 100 μL MTT (0.5 mg/mL in PBS) to each well, and then incubated for 4 h at 37 ℃. The resulting purple-blue MTT formazan precipitate was dissolved in 100 μL dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using an enzyme linked immunosorbent assay (ELISA) reader (BioTek, Model ELX800, USA).

Measurement of ADR Accumulation

MCF-7 and MCF-7/ADR cells were seeded in 12-well plates at a density of 1×10^5 cells/mL, and

Chin J Integr Med 2019 Aug;25(8):598-603

MCF-7/ADR cells were treated with various concentrations of PZH (0.25–0.75 mg/mL) for 24 h. Both MCF-7 and MCF-7/ADR cells were then treated with 5 μmol/L ADR and incubated for an additional 1 h at 37 ℃. ADR accumulation was halted by cooling on ice, and cells were washed with ice-cold PBS prior to observation under a fluorescence microscope (200×).

Measurement of Cell Migration and Invasion

Cell migration assay was performed using transwell cell culture chambers with 8 μm pore filters (Corning Life Sciences, USA). Cells were treated with various concentrations of PZH for 24 h, then resuspended and seeded into the inserts at a density of 5×10^4 cells/well containing 200 μL serum-free RPMI-1640. The inserts were placed within a 24-well chamber with RPMI-1640 media containing 10% (v/v) FBS. Cells were cultured for 12 h at 37 ℃, and the top surface of the transwell membrane was scraped to remove non-migratory cells. Migrated cells were fixed and stained with crystal violet. The mean number of migrated cells per field was assessed by counting 5 random fields under a phase-contrast microscope (Leica, Germany) at a magnification of 200×. Cell invasion assays were similarly performed, with the exception that the upper chambers were coated with Matrigel Matrix (BD Biosciences, USA).

Western Blot Analysis

MCF-7 and MCF-7/ADR cells were seeded into 25 cm^2 culture flasks at a density of 1.5×10^5 cells/mL and treated with various concentrations of PZH for 24 h. Cells were lysed with cell lysis buffer containing protease and phosphatase inhibitor cocktails, and the resulting total protein concentrations were determined by BCA assay. Equal amounts of proteins were resolved on 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and electroblotted. The PVDF membranes were blocked with blocking buffer and probed with primary antibodies against N-cadherin, TGF-β, ABCB1, ABCG2 or β-actin (1:1000 dilution) overnight at 4 ℃, then incubated with the appropriate HRP-conjugated secondary antibody (1:5000). Protein bands were subsequently detected using enhanced chemiluminescence.

Statistical Analysis

Statistical analysis of the data was performed with independent Student-*t* test between two groups or one-way ANOVA among 3 and more groups using SPSS 18.0 software. Data were presented as mean ± strandard deviation ($\bar{x}\pm s$) of 3 individual experiments. Differences with $P<0.05$ were considered statistically significant.

RESULTS

PZH Overcomes ADR Resistance in Human Breast Cancer Cells

ADR treatment had no significant inhibitory effect on MCF-7/ADR cell viability, while the viability of parental MCF-7 cells was remarkably reduced by ADR treatment ($P<0.05$, Figure 1A). However, PZH (0.25–0.75 mg/mL) treatment significantly decreased MCF-7/ADR cell viability with a dose-dependent manner (Figure 1B).

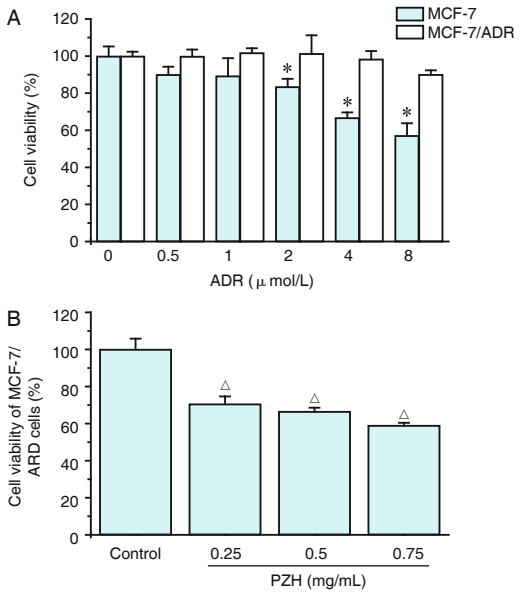

Figure 1. Effect of PZH on Viability of ADR Resistant Human Breast Cancer Cells by MTT Assay (n=3, $\bar{x}\pm s$)

Notes: *$P<0.05$ *vs.* MCF-7/ADR cells; △$P<0.05$ *vs.* control group

PZH Increases Intracellular Drug Accumulation via Reducing Expression of ABCB1 and ABCG2 in MCF-7/ADR Cells

As shown in Figure 2, the extent of intracellular ADR was significantly increased after PZH treatment. As shown in Figure 3, drug resistance induced the elevated expression of ABCB1 and ABCG2 in MCF-7/ADR cells ($P<0.05$), which however was significantly inhibited by PZH (0.5 and/or 0.75 mg/mL) treatment ($P<0.05$).

PZH Inhibits EMT and Metastatic Abilities in MCF-7/ADR Cells

As shown in Figure 3, PZH (0.75 mg/mL) treatment profoundly inhibited the drug resistance-induced elevation in N-cadherin expression ($P<0.05$). MCF-7/ADR cells displayed a significant

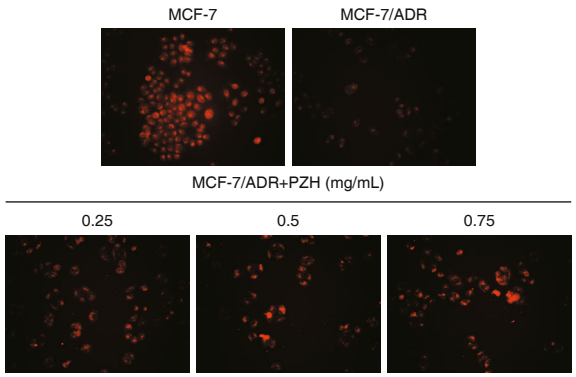

Figure 2. Effect of PZH on Accumulation of ADR in MCF-7/ADR Cells

Notes: Intracellular accumulation of ADR was determined by fluorescence microscope and photographed at magnification of ×200 after treatment with the indicated concentration of PZH for 24 h. Red fluorescence represents ADR. Images were representatives of 3 independent experiments.

increase in both migration and invasion, compared to parental MCF-7 cells (P<0.05). However, PZH (0.25–0.75 mg/mL) treatment significantly reduced metastatic capacities of MCF-7/ADR cells (Figure 4).

PZH Suppresses Activation of TGF-β1 Pathway in MCF-7/ADR Cells

As shown in Figure 3, ADR resistance significantly enhanced TGF-β1 expression (P<0.05).

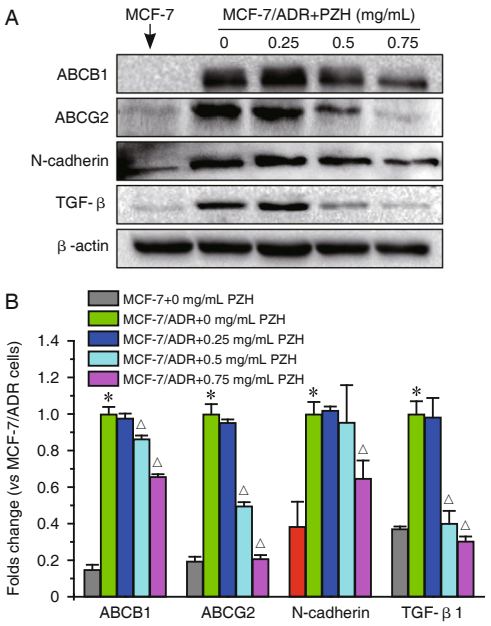

Figure 3. Effect of PZH on Expressions of ABCB1, ABCG2, N-cadherin and TFG-β1 in MCF-7/ADR Cells by Western Blot Analysis

Notes: MCF-7/ADR cells were treated with indicated concentration of PZH for 24 h. (A) Images were representatives of three independent experiments. (B) The integrated density of bands were determined by ImageLab Software. *P<0.05 *vs*. untreated MCF-7 cells; △P<0.05 *vs*. untreated MCF-7/ADR cells.

However, PZH (0.5 and 0.75 mg/mL) significantly suppressed drug resistance-induced activation of

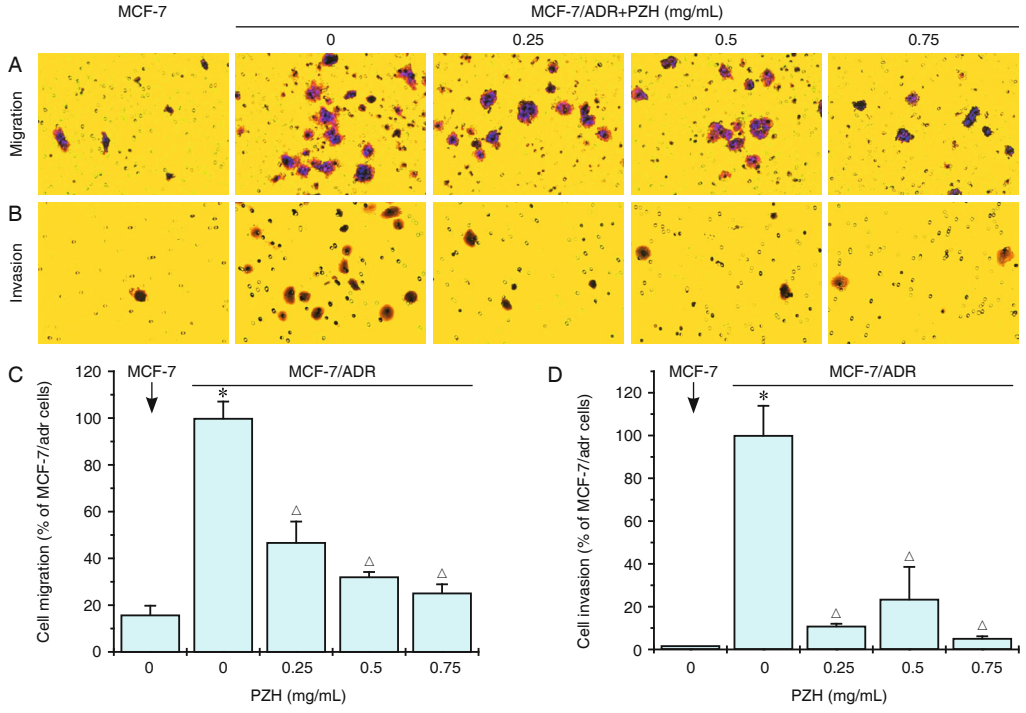

Figure 4. Effect of PZH on Migration and Invasion of MCF-7/ADR Cells

Notes: MCF-7/ADR cells were treated with indicated concentrations of PZH for 24 h. The migration (A and C) and invasion (B and D) of MCF-7/ADR or parental MCF-7 cells was determined using transwell cell culture chambers. Cells were stained with crystal violet and the photographs were taken at a magnification of ×200. The data were normalized to the migration or invasion of MCF-7/ADR cells ($\bar{x} \pm s$, n=3). *P<0.05 *vs*. parental MCF-7 cells; △P<0.05 *vs*. MCF-7/ADR cells without PZH treatment.

TGF-β1 pathway (P<0.05).

DISCUSSION

Chemotherapy remained to be one of the most major treatments for various malignancies, including breast cancer. However, the development of novel and effective therapeutic strategies which can overcome multi-drug resistance still remains a significant challenge. Recently, it is indicated that the treatment of CM significantly decreased cancer drug resistance caused by current used chemotherapy drugs in various cancers.[33-35] PZH is a well-known CM formula which has long been used as a remedy for various cancers in China and Southeast Asia. We recently demonstrated that PZH can inhibit the growth and metastasis of 5-FU resistant colorectal cancer HCT-8/5-FU cells via modulation of TGF-β signaling pathway,[31] suggesting that PZH can overcome drug resistance in human CRC. In the present study, we used MCF-7/ADR cell line to further elucidate the effects of PZH on ADR resistance in breast cancer. We found that PZH significantly inhibited the viability of ADR resistant MCF-7/ADR cells in a dose-dependent manner.

Since overexpression of ABC family of transporter proteins, such as breast cancer resistance protein (BCRP/ABCG2) and ABCB1 associated protein is one of the most common mechanisms of multi-drug resistance by protecting cells from chemotherapeutics-induced damage via increasing the efflux of these compounds.[4-6] Therefore, we evaluated the effect of PZH treatment on accumulation of ADR and expression of ABCG2 and ABCB1. We found that PZH increased the accumulation of ADR and down-regulated the expressions of cancer drug resistance proteins ABCG2 and ABCB1, thereby demonstrating the ability of PZH in inhibiting chemoresistance in human breast cancer cells.

EMT is a key process involved in cancer drug resistance.[9-11] In addition, it has been demonstrated that ADR-resistant MCF-7 cells exhibit typical phenotypes of EMT, as well as the increased expression of mesenchymal cell markers and decreased formation of desmosomes and tight junctions.[11] Data in the present study showed that PZH treatment significantly inhibited chemoresistance-induced characteristics of EMT in MCF-7/ADR cells, as evidenced by the decreased expression of mesenchymal cell marker N-cadherin. Moreover, PZH significantly inhibited chemoresistance-enhanced migrative and invasive capacities of breast cancer cells. The involvement of EMT in cancer is mediated by multiple intracellular pathways, including TGF-β1 signaling pathway. Cancer drug resistance significantly increased the expression levels of TGF-β1 in breast cancer MCF-7/ADR cells, which was also significantly suppressed by PZH treatment.

In conclusion, we demonstrated that PZH can effectively overcome ADR resistance via down-regulating ABCG2, ABCB1 and inhibit EMT in breast cancer MCF-7/ADR cells via suppression of the TGF-β1 pathway.

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Author Contributions

Peng J designed research and revised paper; Chen X, Shen AL, Qi F and Chen YQ performed research; Chu JF analyzed data; Chen X and Shen AL wrote the paper; Sferra TJ reivised paper.

REFERENCE

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
2. Del Mastro L, De Placido S, Bruzzi P, De Laurentiis M, Boni C, Cavazzini G, et al. Fluorouracil and dose-dense chemotherapy in adjuvant treatment of patients with early-stage breast cancer: an open-label, 2×2 factorial, randomised phase 3 trial. Lancet 2015;385:1863-1872.
3. Martín M, Ruiz A, Ruiz Borrego M, Barnadas A, González S, Calvo L, et al. Fluorouracil, doxorubicin, and cyclophosphamide (FAC) versus FAC followed by weekly paclitaxel as adjuvant therapy for high-risk, node-negative breast cancer: results from the GEICAM/2003-02 study. J Clin Oncol 2013;31:2593-2599.
4. Higgins CF. Multiple molecular mechanisms for multidrug resistance transporters. Nature 2007;446:749-757.
5. Zhu MM, Tong JL, Xu Q, Nie F, Xu XT, Xiao SD, et al. Increased JNK1 signaling pathway is responsible for ABCG2-mediated multidrug resistance in human colon cancer. PLoS One 2012;7:e41763.
6. Szakács G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006;5:219-234.
7. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871-890.
8. Kalluri R, Weinberg RA. The basics of epithelial-

mesenchymal transition. J Clin Invest 2009;119:1420-1428.
9. Rosanò L, Cianfrocca R, Spinella F, Di Castro V, Nicotra MR, Lucidi A, et al. Acquisition of chemoresistance and EMT phenotype is linked with activation of the endothelin A receptor pathway in ovarian carcinoma cell. Clin Cancer Res 2011;17:2350-2360.
10. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010;29:4741-4751.
11. Işeri OD, Kars MD, Arpaci F, Atalay C, Pak I, Gündüz U. Drug resistant MCF-7 cells exhibit epithelial-mesenchymal transition gene expression pattern. Biomed Pharmacother 2011;65:40-45.
12. Turley EA, Veiseh M, Radisky DC, Bissell MJ. Mechanisms of disease: epithelial-mesenchymal transition-dose cellular plasticity fuel neoplastic progression? Nat Clin Pract Oncol 2008;5:280-290.
13. Xu J, Lamouille S, Derynck R. TGF-β-induced epithelial to mesenchymal transition. Cell Res 2009;19:156-172.
14. Allendorph GP, Read JD, Kawakami Y, Kelber JA, Isaacs MJ, Choe S. Designer TGF-β superfamily ligands with diversified functionality. PLoS One 2011;6:e26402.
15. Qi F, Zhao L, Zhou A, Zhang B, Li A, Wang Z, et al. The advantages of using traditional Chinese medicine as an adjunctive therapy in the whole course of cancer treatment instead of only terminal stage of cancer. BioSci Trends 2015;9:16-34.
16. Chinese Pharmacopoeia Commission. Pharmacopoeia of the Peoples Republic of China. Vol. 1. Beijing: Chinese Medical Science and Technology Press; 2010:573-575.
17. Lee KK, Kwong WH, Chau FT, Yew DT, Chan WY. Pien Tze Huang protects the liver against carbon tetrachlorideinduced damage. Pharmacol Toxicol 2002;91:185-192.
18. Chan WY, Chau FT, Lee KK, Kwong WH, Yew DT. Substitution for natural musk in Pien Tze Huang does not affect its hepatoprotective activities. Hum Exp Toxicol 2004;23:35-47.
19. Lin JM, Wei LH, Chen YQ, Liu XX, Hong ZF, Sferra TJ, et al. Pien Tze Huang induced apoptosis in human colon cancer HT-29 cells is associated with regulation of the Bcl-2 family and activation of caspase 3. Chin J Integr Med 2011;17:685-690.
20. Zhuang Q, Hong F, Shen A, Zheng L, Zeng J, Lin W, et al. Pien Tze Huang inhibits tumor cell proliferation and promotes apoptosis via suppressing the STAT3 pathway in colorectal cancer mouse. Int J Oncol 2012;40:1569-1574.
21. Shen AL, Hong F, Liu LY, Lin JM, Zhuang QC, Hong ZF, et al. Effects of Pien Tze Huang on angiogenesis *in vivo* and *in vitro*. Chin J Integr Med 2012;18:431-436.
22. Shen A, Chen Y, Hong F, Lin J, Wei L, Hong Z, et al. Pien Tze Huang suppresses IL-6-inducible STAT3 activation in human colon carcinoma cells through induction of SOCS3. Oncol Rep 2012;28:2125-2130.
23. Shen A, Lin J, Chen Y, Lin W, Liu L, Hong Z, et al. Pien Tze Huang inhibits tumor angiogenesis in a mouse model of colorectal cancer via suppression of multiple cellular pathways. Oncol Rep 2013;30:1701-1706.
24. Lin JM, Feng JY, Jin YY, Yan ZK, Lai ZJ, Peng J. Pien Tze Huang suppresses VEGF-C-mediated lymphangiogenesis in colorectal cancer. Oncol Rep 2016;36:3568-3576.
25. Fu Y, Zhang L, Hong Z, Zheng H, Li N, Gao H, et al. Methanolic extract of Pien Tze Huang hnduces apoptosis signaling in human osteosarcoma MG63 cells via multiple pathways. Molecules 2016;21:283
26. He F, Wu HN, Cai MY, Li CP, Zhang X, Wan Q, et al. Inhibition of ovarian cancer cell proliferation by Pien Tze Huang via the AKT-mTOR pathway. Oncol Lett 2014;7:2047-2052.
27. Wei L, Chen P, Chen Y, Shen A, Chen H, Lin W, et al. Pien Tze Huang suppresses the stem-like side population in colorectal cancer cells. Mol Med Rep 2014;9:261-266.
28. Qi F, Wei L, Shen A, Chen Y, Lin J, Chu J, et al. Pien Tze Huang inhibits the proliferation, and induces the apoptosis and differentiation of colorectal cancer stem cells via suppression of the Notch1 pathway. Oncol Rep 2016;35:511-517.
29. Lin W, Zhuang Q, Zheng L, Cao Z, Shen A, Li Q, et al. Pien Tze Huang inhibits liver metastasis by targeting TGF-β signaling in an orthotopic model of colorectal cancer. Mol Rep 2015;33:1922-1928.
30. Shen A, Lin W, Chen Y, Liu L, Chen H, Zhuang Q, et al. Pien Tze Huang inhibits metastasis of human colorectal carcinoma cells via modulation of TGF-β/ZEB/miR-200 signaling network. Int J Oncol 2015;46:685-690.
31. Shen A, Chen H, Chen Y, Lin J, Lin W, Liu L, et al. Pien Tze Huang overcomes multidrug resistance and epithelial-mesenchymal transition in human colorectal carcinoma cells via suppression of TGF-β pathway. Evid Based Complement Alternat Med 2014;2014:679436.
32. Zhang Y, Wang Q, Niu S, Liu J, Zhang L. Pien Tze Huang induces apoptosis in multidrug-resistant U2OS/ADM cells via downregulation of Bcl-2, survivin and P-gp and upregulation of Bax. Oncol Rep 2014;31:763-770.
33. Ji XM, Wu ZC, Liu GW, Yu HY, Liu H, Wang ZT, et al. Wenxia Changfu Formula induces apoptosis of lung adenocarcinoma in a transplanted tumor model of drug-resistance nude mice. Chin J Integr Med 2016;22:752-758.
34. Sui H, Pan SF, Feng Y, Jin BH, Liu X, Zhou LH, et al. Zuo Jin Wan reverses P-gp-mediated drug-resistance by inhibiting activation of the PI3K/Akt/NF-κB pathway. BMC Complement Altern Med 2014;14:279.
35. Li WB, Li Y, Yu C, He YM. Reversal of multidrug resistance by the Chinese medicine Yiqi Jianpi Huaji Decoction and the mechanism of action in human gastric cancer SGC7901/VCR cells. Evid Based Complement Alternat Med 2015;2015:390812.

(Accepted July 14, 2017; First Online August 22, 2018)
Edited by YUAN Lin