The reversal of antineoplastic drug resistance in cancer cells by β-elemene

Guan-Nan Zhang1, Charles R. Ashby Jr.1,†, Yun-Kai Zhang1, Zhe-Sheng Chen1* and Huiqin Guo2*

Abstract
Multidrug resistance (MDR), defined as the resistance of cancer cells to compounds with diverse structures and mechanisms of actions, significantly limits the efficacy of antitumor drugs. A major mechanism that mediates MDR in cancer is the overexpression of adenosine triphosphate (ATP)-binding cassette transporters. These transporters bind to their respective substrates and catalyze their efflux from cancer cells, thereby lowering the intracellular concentrations of the substrates and thus attenuating or even abolishing their efficacy. In addition, cancer cells can become resistant to drugs via mechanisms that attenuate apoptosis and cell cycle arrest such as alterations in the p53, check point kinase, nuclear factor kappa B, and the p38 mitogen-activated protein kinase pathway. In this review, we discuss the mechanisms by which β-elemene, a compound extracted from Rhizoma zedoariae that has clinical antitumor efficacy, overcomes drug resistance in cancer.

Keywords: Rhizoma zedoariae, β-Elemene, Multidrug resistance

Background
Curcuma belongs to the Zingiberaceae family and is a medicinal plant that distributes worldwide. The plants of this genus are mainly grown in southeastern Asia, Brazil, and Australia [1]. Recently, approximately 20 Curcuma species have been discovered in China [2]. The Chinese Pharmacopoeia indicates that Rhizoma zedoariae is the dry rhizome derived from Curcuma wenyujin [1], Curcuma phaeocaulis [2], and Curcuma kwangsiensis [3]. Rhizoma zedoariae has been used as an anti-microbial, anti-inflammatory, anti-proliferative, and antitumor drug [3–7]. β-elemene [(1S,2S,4R)-2,4-diisopropenyl-1-methyl-1-vinylcyclohexane], a naturally occurring compound isolated from Rhizoma zedoariae, is approved for use in Chinese medicine to treat a variety of cancers, including leukemia and brain, breast, prostate, ovarian, cervical, and lung cancers [8–14]. The structure of β-elemene is shown in Fig. 1. β-elemene does not produce significant or problematic toxicity and is well tolerated by patients [12]. It has been postulated that the anticancer effect of β-elemene is due to the induction of apoptosis and cell cycle arrest [8, 14].

Currently used antitumor drugs can produce their therapeutic action via a number of distinct mechanisms. For example, antitumor drugs can (1) damage DNA or inhibit DNA replication, (2) inhibit DNA and RNA synthesis, (3) interfere with RNA transcription, (4) inhibit protein synthesis, (5) interfere with hormone homeostasis, and (6) disrupt cellular microtubules via stabilization or destabilization [15]. However, it is well established that cancer cells can become resistant to many antitumor drugs; this phenomenon is known as multidrug resistance (MDR) [16]. MDR occurs when cancer cells become resistant to a variety of drugs that have distinct structures and mechanisms of action [17]. A number of mechanisms have been reported to produce MDR, including altered activity of specific enzyme systems such as glutathione S-transferase (GST) and topoisomerase, which can attenuate the efficacy of anticancer drugs [18, 19]. In addition, alterations in the levels of proteins that control apoptosis can occur; such alterations decrease the efficacy of anticancer drugs by inducing apoptosis [20]. A well-documented mechanism that produces or elicits MDR is the
overexpression of adenosine triphosphate (ATP)-binding cassette (ABC) transporters, such as the ABCB1 (P-glycoprotein, P-gp/MDR1), ABCCs [multidrug resistance-associated proteins (MRPs)], and ABCG2 transporters (BCRP/MXR/ABCP) [21]. These transporters use energy obtained from the hydrolysis of ATP to remove or efflux compounds from cancer cells, thereby significantly lowering their intracellular concentrations and attenuating their efficacy [22]. The ABC transporter P-gp is expressed by cancer cells derived from epithelial cells of the colon, liver, adrenal gland, and pancreas and has been reported to produce resistance to a broad spectrum of anticancer drugs, including anthracyclines, vinca alkaloids, etoposide, and taxanes [23–26]. In addition, the overexpression of other ABC transporters, such as ABCCs and ABCG2, can also produce MDR in cancer cells [21, 27–29]. Numerous studies indicate that the blockade of the efflux function of specific ABC transporters by various compounds can resensitize resistant cancer cells to specific antitumor drugs by increasing the intracellular concentration of these transporters. However, this approach has been hampered because inhibitors of the efflux activity of ABC transporters have produced severe adverse effects and toxic drug–drug interactions [30].

One approach that has been used to overcome MDR in cancer cells is to find or synthesize compounds that can block the efflux action of the ABC transporters without producing significant toxic effects [31]. Over the last four decades, three generations of ABC transporter inhibitors have been developed in an attempt to overcome MDR in cancer cells. One of the first-generation drugs, verapamil, a calcium channel blocker, was the first drug shown to inhibit the efflux function of P-gp [32]. However, clinical data indicated that verapamil produced significant toxic effects at concentrations required to overcome P-gp-mediated resistance [33]. In addition, verapamil and other first-generation compounds were shown to inhibit the activities of various cytochrome P450 (CYP450) enzymes, thereby increasing the likelihood of adverse and toxic drug–drug interactions [34–36]. To overcome the aforementioned limitations of first-generation compounds, second-generation compounds were developed [37, 38]. Second-generation P-gp modulators included PSC833 and biricodar (VX-710). Compared with first-generation compounds, these P-gp inhibitors were reported to be more potent in reversing MDR and less toxic [37, 39]. Among second-generation P-gp modulators, PSC833 was the most well characterized, and it was used in clinical trials in combination with doxorubicin [DOX, also known as adriamycin (ADM)], vincristine, vinblastine, paclitaxel (TAX), or mitoxantrone to treat MDR cancer [37, 39]. PSC833 alone did not produce significant adverse effects, but it increased the likelihood of toxic effects when used with antitumor drugs. For example, a significant pharmacokinetic interaction was observed when PSC833 was administered in combination with DOX and TAX [40, 41], thus requiring a reduction in the doses of the anticancer drugs. It was subsequently shown that second-generation P-gp inhibitors significantly decreased the excretion of various drugs, thereby increasing the incidence of significant adverse effects [41]. In addition, to overcome the problematic drug–drug interactions, the doses of these drugs had to be decreased, which attenuated their efficacy.

Third-generation P-gp inhibitors such as tariquidar (XR9576), laniquidar (R101933), and LY335979, were developed to overcome the limitations associated with second-generation inhibitors [42–45]. Although third-generation P-gp inhibitors were significantly efficacious in vitro, these compounds are not used clinically because in vivo and preclinical studies indicated a lack of efficacy and/or significant adverse effects [46].

It has been well-established that certain compounds extracted from various natural sources have antitumor efficacy [47, 48]. However, relatively few studies have reported the effects of naturally derived compounds on the activities and functions of ABC transporters. Given the important role of ABC transporters in mediating MDR, we recently reported the in vitro effect of the compound β-elemene, extracted from the plant Rhizoma zedoariae, on MDR in cancer cells [49]. In addition, this compound may overcome MDR via other mechanisms [50]. Therefore, in this review, we discuss the mechanisms by which β-elemene surmounts MDR. Figure 2 illustrates the mechanisms by which β-elemene mediates the reversal of MDR in cancer cells.

Antitumor efficacy of β-elemene

β-Elemene has been purported to inhibit the proliferation of cancer cells by inducing apoptotic cell death and cell cycle arrest [8, 51, 52]. The dysfunction or blockade of apoptosis has been proposed to play a role in abnormal cell proliferation, thus initiating the carcinogenic

![Fig. 1 Structure of β-elemene](image-url)
processes involved in tumor proliferation, angiogenesis, and metastasis [53]. Apoptosis can be initiated by the activation of the intrinsic and extrinsic pathways. The activation of the intrinsic pathway disrupts the balance between pro-survival proteins (e.g., Bcl-2 and Bcl-xL) and pro-apoptotic proteins of the Bcl-2 family (e.g., Bax and Bak), which triggers the release of cytochrome c from the mitochondrial outer membrane [54]. In contrast, the extrinsic pathway is activated by the binding of specific molecules to death receptors such as FAS receptor (FasR), tumor necrosis factor receptor 1 (TNFR1), death receptor 3 (DR3), and death receptor 4/death receptor 5 (DR4/DR5) [55]. Numerous studies indicate that apoptosis is an important therapeutic target for cancer treatment [56]. There are studies indicating that β-elemene affects the apoptotic process in cancer cells. For example, β-elemene significantly inhibits the growth and proliferation of various types of T24 bladder cancer cell lines by decreasing the expression of the anti-apoptotic proteins Mta-1, Survivin, and Bcl-xL [57]. In addition, β-elemene significantly inhibits the proliferation of lung and prostate cancer cells by increasing the release of cytochrome c and the activation of caspases-3, -7, and -9 and of poly(ADP-ribose) polymerase (PARP) and by decreasing the expression of Bcl-2 [58].

There are data suggesting that β-elemene triggers cell cycle arrest by activating the p38 mitogen-activated protein kinase (MAPK) pathway. For example, in C6 glioblastoma cells, β-elemene significantly increases the fraction of C6 cells at the G0/G1 phase [48]. The cell cycle-arresting action of β-elemene was associated with an increase in the phosphorylation of p38 MAPK, whereas this effect was reversed by the inhibition of p38 MAPK [52]. Furthermore, in non-small cell lung cancer (NSCLC) and epithelial cell lines, β-elemene significantly arrested the cell cycle at the G2-M phase by decreasing the expression of Cyclin B1 and phospho-Cdc2 (Thr-161) and by increasing the expression of P27 (kip) and phospho-Cdc (Tyr-15) [11]. Recently, Zhao et al. [59] reported that β-elemene can significantly inhibit the proliferation of NSCLC cells by inhibiting extracellular signal-regulated kinases (ERK1/2) and the adenosine monophosphate-activated protein kinase α (AMPKα)-mediated transcription factor Sp1 and then by decreasing the protein expression of DNA (cytosine-5)-methyltransferase 1 (DNMT1). In addition, the action of β-elemene on the
proliferation of NSCLC cells was reversed by the overexpression of DNMT1, and the inhibition of Akt signaling and DNMT1 expression by metformin can potentiate the effects of β-elemene [59]. In A2780/CP ovarian carcinoma cells (which are resistant to cisplatin), β-elemene induces cell cycle arrest at the G2/M phase by decreasing the expression of B1 and Cdc2 and by increasing the expression of p53, p27, and growth arrest and DNA-damage-inducible protein (GADD45) [8].

It is well documented that radiation therapy plays an important role in the treatment of radiation-sensitive tumors [60]. The overexpression of peroxiredoxin 1 (Prx-1), a critical regulator of redox in cancer cells, has been reported to abrogate the response of cancer cells to radiation therapy [60]. Thus, reducing the expression of Prx-1 is a promising way to sensitize tumor cells to radiation therapy. In this study, Li et al. [60] demonstrated that 45 mg/kg β-elemene restores the sensitivity of A549 tumor cells to radiation. Reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting assays indicated that the potentiation of radiation therapy by β-elemene was mediated by the down-regulation of Prx-1.

**Reversal of MDR by β-elemene**

**Reversal of ABC transporter-mediated MDR by β-elemene**

As mentioned above, one of the well-documented mechanisms responsible for MDR is the overexpression of P-gp. Therefore, our laboratory conducted a study to examine the in vitro effect of β-elemene on P-gp-induced MDR in drug-resistant and non-drug-resistant parental cell lines [49]. We determined the effect of β-elemene on the cytotoxic effects of the antitumor drugs colchicine, vinblastine, and TAX (P-gp substrates) in parenteral KB-3-1 cells and in KB-C2 cells overexpressing P-gp. β-elemene (100 μmol/L) significantly increased the efficacy (i.e., the cytotoxicity) of P-gp substrate drugs in KB-C2 cells but not in parenteral KB-3-1 cells. For example, the responses to TAX, vinblastine, and colchicine in KB-C2 cells were 648-, 11.7-, and 720-fold lower, respectively, than those in parental KB-3-1 cells. β-elemene (100 μmol/L) significantly lowered the magnitude of resistance to TAX, vinblastine, and colchicine (by 4.6-, 5.4-, and 1.1-fold, respectively) in KB-C2 cells. The results of accumulation and efflux assays indicated that β-elemene potentiated the cytotoxic action of the antitumor drugs that were P-gp substrates by blocking their efflux via P-gp, thereby increasing their intracellular concentrations (by 3.6-fold) in KB-C2 cells overexpressing P-gp. It is possible that β-elemene could potentiate the actions of antitumor drugs by decreasing the expression of P-gp protein. However, the incubation of KB-C2 with β-elemene for 24, 48, or 72 h did not significantly alter the expression of P-gp. Overall, our results suggested that β-elemene potentiated the actions of P-gp substrate drugs by inhibiting the efflux function of P-gp. Our study did not rule out the possibility that β-elemene could produce its potentiating action by decreasing the insertion of the P-gp transporter into the cell membrane.

Similar to our findings, Xu et al. [61] reported that β-elemene (30 μmol/L) significantly potentiated (6.38-fold) the action of DOX in DOX-resistant MCF-7 cells. Furthermore, it was shown that β-elemene (30 μmol/L) significantly increased the intracellular accumulation of DOX and the compound Rh123, which is a substrate of P-gp. Interestingly, Xu et al. [61] showed that β-elemene (30 μmol/L) significantly decreased P-gp protein expression, suggesting that the action of β-elemene is due in part to this mechanism. This finding was in contrast to our results indicating that β-elemene did not significantly alter the expression of P-gp. The exact explanation for this discrepancy is unknown, but it may be due to differences in the cell lines used (MCF-7/DOX versus KB-C2 and HEK293/ABCB1) [61].

β-Elemene significantly increases the suppressive effect of DOX and docetaxel (Doc) on the growth and proliferation of the resistant MCF-7/Adr and MCF-7/Doc breast cancer cell lines [7]. β-elemene may attenuate MDR by influencing MDR-related microRNA expression and subsequently regulating the target genes phosphatase and tensin homolog (PTEN) and P-gp, which are responsible for the proliferation of resistant breast cancer cells [7].

**β-Elemene reverses MDR by promoting apoptosis of resistant cancer cells**

It is known that mechanisms that inhibit or attenuate the induction of cancer cell apoptosis produce resistance to certain antitumor drugs [20]. The induction of apoptosis by antitumor drugs has been shown to trigger a significant generation of reactive oxygen species (ROS) and disruption of mitochondrial membrane potential [62]. Numerous studies suggest that apoptosis involves the complex interaction and regulation of many genes and proteins [56]. The suppressor gene p53 is a key mediator of apoptosis-induced cellular transformation [63, 64]. Following DNA damage by certain antitumor drugs, p53 is activated, resulting in G1 phase arrest and, subsequently, apoptosis [56, 65]. Thus, the loss of p53 or inactivation of the p53 pathway could contribute to drug resistance [63, 66]. In addition, proteins whose expression is regulated by p53, such as B cell lymphoma-2 (Bcl-2) and Bax, are also involved in mediating resistance to antitumor drugs [67]. Indeed, proteins categorized as anti-apoptotic Bcl-2 members are transcriptionally up-regulated in cancer cells and are associated with resistance to the antitumor drugs DOX, TAX, cisplatin, mitoxantrone, and
etoposide. Thus, the down-regulation (as well as inactivation) of anti-apoptotic Bcl-2 proteins can augment apoptosis, thereby increasing the efficacy of antitumor drugs in resistant cancer cells. For example, it has been postulated that the reversal mechanism of β-elemene (6 μg/mL) in the ADM-resistant human breast cancer cell line MCF-7/ADM may result from a decrease in the expression of Bcl-2 [68]. In the parental ovarian cancer cell line A2780 and the cisplatin-resistant cell line A2780/CP70, β-elemene (0.6 μmol/L) significantly enhanced cisplatin cytotoxicity in the drug-resistant cell lines (by 60-fold) [69]. These results suggested that β-elemene acts by abrogating the expression of the excision pathway that repairs protein cross-complementation group 1 (ERCC1) protein. In addition, the level of the X-linked inhibitor of apoptosis protein (XIAP) is significantly decreased by β-elemene (0.6 μmol/L) [69, 70]. In ovarian carcinoma cells resistant to cisplatin, the combination of β-elemene and cisplatin can stimulate the activity of caspases-3, -8, and -9 as well as the cleavage of caspase-9, whereas the expression of Bcl-2 and Bcl-xl was decreased [70]. Thus, based on the aforementioned results, β-elemene restores the sensitivity of resistant ovarian cancer cells to cisplatin by attenuating DNA repair activity and promoting apoptosis. Furthermore, β-elemene significantly augments the antisurvival activity of cisplatin in human bladder cancer cells by inducing cell apoptosis via a caspase-dependent mechanism [69, 70]. Wang et al. [11] reported that β-elemene significantly augments cisplatin-induced inhibition of the growth of a NSCLC cell line by inducing cell cycle arrest. Similarly, β-elemene significantly increased the suppressive effect of cisplatin on the growth and proliferation of NSCLC H460 and A549 cell lines [14]. It was postulated that the potentiation of the efficacy of cisplatin by β-elemene was due to inducing cell cycle arrest in NSCLC cells at the G2/M phase by increasing checkpoint kinase 2 (CHK2) expression and reducing Cdc2 activity [71]. In addition, the combination of β-elemene and cisplatin significantly decreased the protein levels of Cyclin B1 and Cdc25C and increased the levels of P21, P27, and GADD45 in cancer cells [71]. Furthermore, a meta-analysis of clinical data suggested that the combination of β-elemene and cisplatin was more efficacious than cisplatin alone in treating NSCLC and significantly improved the quality of life of the patients [22].

Endocrine therapy plays a critical role in the treatment of estrogen receptor (ER)-positive breast cancer, and the lack of ER expression is associated with a decrease in the efficacy of endocrine-based therapy [72]. In MCF7/TAX cell lines, which do not express ER-α, 10 μg/mL of β-elemene restored the sensitivity of MCF7/TAM cell lines to TAX. RT-PCR and Western blotting assays indicated that the reversal of TAX resistance by β-elemene was mediated by up-regulating the expression of ER-α mRNA via the MAPK pathway [73].

The protein nuclear factor-κB (NF-κB) is associated with the development of chemo-resistance in various cancer cells by inducing the overexpression of anti-apoptotic proteins [74]. Thus, inhibition of NF-κB-mediated responses may be a promising strategy for potentiating the response to antitumor drugs. The incubation of ADM-resistant cells (SGC7901/ADM gastric cancer cells) with β-elemene significantly decreased the expression of NF-κB [75]. In addition, β-elemene significantly increased the apoptotic rate of SGC7901/ADM cells by inhibiting or attenuating NF-κB activity [75].

β-Elemene reverses MDR by reducing the stemness of cancer cells

Glioblastoma stem-like cells (GSCs) play an important role in tumor development, recurrence, and chemo-resistance [76]. It has been postulated that impairing stemness and enhancing differentiation could decrease GSC-associated chemo-resistance [76]. Fu et al. [75] reported that β-elemene significantly impaired the stemness of GSC spheres, dispersed GSC spheres, and reduced the proliferation of GSCs in vitro and in vivo. In addition, they also found that β-elemene in GSC spheres and xenografts can significantly decrease the expression of CD133 and ABCG2 and increase the expression of glial fibrillary acidic protein (GFAP). Furthermore, β-elemene can also restore the sensitivity of GSCs to temozolomide [76]. The β-elemene-mediated potentiation of the efficacy of temozolomide on glioblastoma cells results from activation of the glia maturation factor β (GMFβ)/MAPK 3/6/p38 pathway [77]. In addition, it has been reported that the antitumor effect of β-elemene was also mediated by GMFβ-dependent inactivation of the ERK1/2-Bcl-2/survivin pathway, which suggests that β-elemene is a promising chemosensitizer for temozolomide against glioblastoma tumors [77].

In addition, the combination of β-elemene and TAX, compared with TAX alone, significantly inhibited the proliferation of MB-468 cells by decreasing the expression of Cyclin B1 and increasing the expression of P27 (kip1) [78]. Furthermore, the combination of β-elemene (20 or 50 μg/mL) and etoposide phosphate (VP-16, 15 μg/mL) increased the likelihood of apoptosis compared with VP-16 alone (16.57 or 21.98 vs. 6.25%) [79]. The potentiation of the efficacy of VP-16 by β-elemene may be mediated by the increase of Bax, p53, and p21 and the suppression of Cyclin D1 [79].

Conclusions

β-Elemene plays an important role in overcoming MDR via multiple mechanisms. It is possible that the reversal
action of β-elemene could result from (1) a blockade of the efflux function of P-gp, (2) a decrease in the protein levels of P-gp, (3) an induction of apoptosis or cell cycle arrest, or (4) a decrease in NF-κB signaling activity. Finally, it may be of interest to determine if β-elemene alters the expression and/or efflux activity of other ABC transporters. Currently, the in vitro and in vivo data obtained for β-elemene suggest that it may be useful in treating certain MDR cancers. However, controlled clinical trials will be required to determine if β-elemene will significantly increase the efficacy of various chemotherapeutic drugs in reversing MDR in cancer patients.

**Authors’ contributions**

GNZ, RAC, and ZSC conceived the concept and contributed to writing the manuscript. YKZ, RAC, and HG contributed to revising the manuscript. All authors read and approved the final manuscript.

**Author details**

1. Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John’s University, Queens, NY 11439, USA.
2. Department of Thoracic Surgery, Peking Union Medical College Hospital, Beijing 100730, P. R. China.

**Acknowledgements**

This work was supported by St. John’s University Research Seed Grant (No. 579-1110-7002) to Zhe-Sheng Chen.

**Compliance with ethical guidelines**

The authors declare that they have no competing interests.

**Received:** 11 June 2015 **Accepted:** 26 July 2015

**Published online:** 14 September 2015

**References**

1. Lim CB, Ky N, Ng HM, Hamza MS, Zhao Y. *Curcuma wenyujin* extract induces apoptosis and inhibits proliferation of human breast cancer cells in vitro and in vivo. *Integr Cancer Ther. 2010;9(1):36–49.*

doi:10.1177/1534735409359773.

2. Lai EY, Chyau CC, Mau JL, Chen CC, Lai YJ, Shih CF, et al. Antimicrobial activity and cytotoxicity of the essential oil of *Curcuma zedoaria*. *Am J Chin Med. 2004;32(2):281–90.*

doi:10.1142/S0192415X0400193X.

3. Tohda C, Nakayama N, Hatanaka F, Komatsu K. Comparison of anti-inflammatory effects of *β-elemene* and *curcumin* rhizoma in mice. *Nat Prod Res. 2006;20(7):680–5.*

doi:10.1080/14786410500462900.

4. Park SD, Jung JH, Lee HW, Kwon YM, Chung KH, Kim MG, et al. *Zedoaria rhiza* and *curcumin* inhibits platelet-derived growth factor-induced proliferation of human hepatic myofibroblasts. *Int Immunopharmacol. 2005;5(3):555–69.*

doi:10.1016/j.intimp.2004.11.003.

5. Zhang J, Zhang H, Chen L, da Sun W, Mao C, Chen W, et al. β-Elemene reverses chemoresistance of breast cancer via regulating MDR-related miRNA expression. *Cell Physiol Biochem. 2014;34(6):2027–37.*

doi:10.1159/000366398.

6. Lee RX, Li QQ, Reed E. β-Elemene effectively suppresses the growth and survival of both platinum-sensitive and -resistant ovarian tumor cells. *Anticancer Res. 2012;32(8):3103–13.*

7. Zheng S, Yang H, Zhang S, Wang X, Yu L, Lu J, et al. Initial study on naturally occurring products from traditional Chinese herbs and vegetables for chemoprevention. *J Cell Biochem Suppl. 1997;27:106–12.*

8. Zhou HY, Shen JK, Hsu JS, Qu YM, Luo QZ. Experimental study on apoptosis induced by elemene in glioma cells. *Ai Zheng. 2003;22(9):959–63* (in Chinese).

9. Wang G, Li X, Huang F, Zhao J, Ding H, Cunningham C, et al. Antitumor effect of beta-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death. *Cell Mol Life Sci. 2005;62(7–8):881–93.*

doi:10.1007/s00018-005-0017-3.

10. Li X, Wang G, Zhao J, Ding H, Cunningham C, Chen F, et al. Antiproliferative effect of beta-elemene in chemoresistant ovarian carcinoma cells is mediated through arrest of the cell cycle at the G2-M phase. *Cell Mol Life Sci. 2005;62(7–8):894–904.*

doi:10.1007/s00018-005-0027-1.

11. Zhao J, Li QQ, Zou B, Wang G, Li X, Kim JE, et al. In vitro combination characterization of the new anticancer plant drug beta-elemene with taxanes against human lung carcinoma. *Int J Oncol. 2007;31(2):241–52.*

12. Li QQ, Wang G, Zhang M, Cuff CF, Huang L, Reed E. beta-Elemene, a novel plant-derived antineoplastic agent, increases cisplatin chemosensitivity of lung tumor cells by triggering apoptosis. *Oncol Rep. 2009;22(1):161–70.*

13. Kishi S, Ueda T. Chemotherapy. *Nihon Rinsho.* 2014;72(3):364–7 (in Japanese).

14. Gottesman MM. How cancer cells evade chemotherapy: sixteenth Richard and Hindi Rosenthal Foundation Award Lecture. *Cancer Res. 1993;53(4):747–54.*

15. Gillet JP, Gottesman MM. Mechanisms of multidrug resistance in cancer. *Methods Mol Biol. 2010;596:47–76.*

doi:10.1007/978-1-60761-416-6_4.

16. Seitz G, Bonin M, Fuchs J, Poths S, Ruck P, Warnmann SW, et al. Inhibition of glutathione-S-transferase as a treatment strategy for multidrug resistance in childhood HbDombeyosarcoma. *Int J Oncol. 2010;36(2):491–500.

17. Lewis AD, Forrester LM, Hayes JD, Wareing CJ, Carmichael J, Harris AL, et al. Glutathione S-transferase isoenzymes in human tumours and tumour derived cell lines. *Br J Cancer. 1989;60(3):327–31.*

18. Reed JC. Regulation of apoptosis by bcl-2 family proteins and its role in cancer and chemoresistance. *Curr Opin Oncol. 1995;7(6):541–4.*

19. van Veen HW, Konings WN. The ABC family of multidrug transporters in microorganisms. *Biochim Biophys Acta. 1998;1365(1–2):231–6.*

20. Wang B, Peng XX, Sun R, Li J, Zhan XR, Wu L, et al. Systematic review of beta-elemene injection as adjunctive treatment for lung cancer. *Chin J Integr Med. 2012;18(11):813–23.*

doi:10.1007/s11655-012-1271-9.

21. Roninson IB, Chin JE, Choi KG, Gros P, Housman DE, Fojo A, et al. Overexpression of a transporter gene in a multidrug-resistant human KB carcinoma cells. *Proc Natl Acad Sci USA. 1986;83(12):4538–42.*

22. van der Velden AM, Baas F, Ten Houte de Lange T, Kooiman PM, Van der Velde-Koerts T, Borst P. The human mdr3 gene encodes a novel P-glycoprotein homologue and gives rise to alternatively spliced mRNAs in liver. *EMBO J. 1987;6(11):3325–31.*

23. de Bruin MH, Van der Bliek AM, Bedrij JL, Borst P. Differential amplification and disproportionate expression of five genes in three multidrug-resistant Chinese hamster lung cell lines. *Mol Cell Biol. 1986;6(12):4717–22.*

24. Gros P, Raymond M, Bell J, Housman D. Cloning and characterization of a second member of the mouse mdr gene family. *Mol Cell Biol. 1988;8(7):2770–8.*

25. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science. 1992;258(5088):1650–4.*

26. Guo Y, Kotova E, Chen ZS, Lee K, Hopper-Borge E, Belinsky MG, et al. MRPP8, ATP-binding cassette C11 (ABCC11) is a cyclic nucleotide efflux pump and a resistance factor for fluoropyrimidines 2′,3′-dideoxy‑cytidine and 9′-(2′-deoxy‑phosphoryl‑methoxyethyl)‑adenine. *J Biol Chem. 2003;278(32):29059–14.*

doi:10.1074/jbc.M305409200.

27. Robert J, Jarry C. Multidrug resistance reversal agents. *J Med Chem. 2006;49(23):4805–17.*

doi:10.1021/jm060318a.

28. Krishna R, Mayer LD. Liposomal doxorubicin circumspects PSC 833-free drug interactions, resulting in effective therapy of multidrug-resistant solid tumors. *Cancer Res. 1997;57(23):5246–53.*

29. Wu CP, Calogaco AM, Ambudkar SV. Reversal of ABC drug transporter-mediated multidrug resistance in cancer cells: evaluation of current strategies. *Curr Mol Pharmacol. 2008;1(2):93–105.*
32. Tsurow T, Iida H, Tsukagoshi S, Sakurai Y. Overcoming of vincristine resistance in P388 leukemia in vivo and in vitro through enhanced cytotoxicity of vincristine and vinblastine by verapamil. Cancer Res. 1981;41(5):1967–72.

33. Ozols RF, Cunnion RE, Klecker RW Jr, Hamilton TC, Ostchega Y, Parrillo JE, et al. Verapamil and adriamycin in the treatment of drug-resistant ovarian cancer patients. J Clin Oncol. 1987;5(4):641–7.

34. Dantzig AH, de Alwis DP, Burgess M. Considerations in the design and development of transport inhibitors as adjuncts to drug therapy. Adv Drug Deliv Rev. 2003;55(1):133–50.

35. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y. Potentiation of vincristine and vinblastine by verapamil. Cancer Res. 1983;43(3):2267–72.

36. Shukla S, Chen ZS, Ambukar SV. Tyrosine kinase inhibitors as modulators of ABC transporter-mediated drug resistance. Drug Resist Update. 2012;15(1–2):70–80. doi:10.1016/j.drup.2012.01.005.

37. Boesch D, Muller K, Fourtier-Marzanedo A, Loor F. Restoration of daunorubicin retention in multidrug-resistant P388 cells by submicromolar concentrations of SDZ PSC 833, a nonimmunosuppressive cyclosporin derivative. Exp Cell Res. 1991;196(1):26–32.

38. Germann UA, Shlyakhter D, Mason VS, Martin TM, Ostchega Y, Parrillo JE, et al. Verapamil plus adriamycin in the treatment of drug-resistant ovarian cancer patients. J Clin Oncol. 1987;5(4):641–7.

39. Zhang JN, Zhai D, Chen L, Zhuang Y, Wang RQ. Humanin peptide suppresses apoptosis by interfering with Bax activation. Nature. 2003;423(6938):456–61. doi:10.1038/nature01627.

40. Aouali N, El Btaouri H, Dumontet C, Eddabra L, Malagarie-Cazenave S, Madoulet C, et al. Accumulation of lactosylceramide and overexpression of ABCG2 in human colon cancer cells. J Cell Mol Med. 2010;14(5):1031–40. doi:10.1111/j.1749-6632.2009.09309.x.

41. Lhomme C, Joly F, Walker JL, Lissoni AA, Nicoletto MO, Manikhas GM, et al. Humanin and its synthetic analogs. Med Oncol. 2013;30(1):488. doi:10.1007/s11010-012-1350-7.

42. Yao YQ, Xu YH, Lu J, Zhou HY, Wang YZ. Effect of p38 MAPK on elemene-induced cell cycle arrest in C6 glioblastoma cells. Zhonghua Yi Xue Za Zhi. 2008;88(1):56–61 (in Chinese).

43. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer. 2005;5(3):231–7. doi:10.1038/nrc1560.

44. Guo B, Zhai D, Cabezas E, Welsh K, Nouraini S, Sattar Watt A, et al. Humanin peptide suppresses apoptosis by interfering with Bax activation. Nature. 2003;423(6938):456–61. doi:10.1038/nature01627.

45. Guns ES, Bullock PL, Reimer ML, Dixon R, Bally M, Mayer LD. Assessment of anti-neoplastic effect of elemene on prostate cancer cells and other types of solid tumour cells. J Pharm Pharmacol. 2010;62(8):1018–27. doi:10.1200/JCO.2010.11.135x.

46. Kim TE, Lee H, Lim KS, Lee S, Yoon SH, Park KM, et al. Effects of HM30181, a P-glycoprotein inhibitor, on human sarcoma cells. Oncol Rep. 2011;25(4):1161–7. doi:10.3892/or.2011.1188.

47. Aouali N, El Btaouri H, Dumontet C, Eddabra L, Malagarie-Cazenave S, Madoulet C, et al. Accumulation of lactosylceramide and overexpression of ABCG2 in human colon cancer cells. J Cell Mol Med. 2010;14(5):1031–40. doi:10.1111/j.1749-6632.2009.09309.x.

48. Germann UA, Shlyakhter D, Mason VS, Martin TM, Ostchega Y, Parrillo JE, et al. Verapamil plus adriamycin in the treatment of drug-resistant ovarian cancer patients. J Clin Oncol. 1987;5(4):641–7.

49. Guo HQ, Zhang GN, Wang YJ, Zhang YK, Sodani K, Talele TT, et al. Role of p53 and Bcl-2 in the sensitivity of cell line A549 to chemotherapeutic drugs. Zhonghua Jie He He Xi Hu Xi Za Zhi. 2008;33(3):191–7 (in Chinese).

50. Lu JJ, Dang YY, Huang M, Xu WS, Chen XP, Wang YT. Anticancer properties of terpenoids isolated from Zhonghuaxia Cucumaria—a review. J Ethnopharmacol. 2012;132(2):406–11. doi:10.1016/j.jep.2012.07.009.
72. Ward A, Shukla K, Balwierz A, Soons Z, Konig R, Sahin O, et al. microRNA-519a is a novel oncomir conferring tamoxifen resistance by targeting a network of tumor-suppressor genes in ER+ breast cancer. J Pathol. 2014. doi:10.1002/path.4363.

73. Zhang B, Zhang X, Tang B, Zheng P, Zhang Y. Investigation of elemene-induced reversal of tamoxifen resistance in MCF-7 cells through oestrogen receptor alpha (ERalpha) re-expression. Breast Cancer Res Treat. 2012;136(2):399–406. doi:10.1007/s10549-012-2263-6.

74. Wang Z, Xia Q, Cui J, Diao Y, Li J. Reversion of P-glycoprotein-mediated multidrug resistance by diallyl trisulfide in a human osteosarcoma cell line. Oncol Rep. 2014;31(6):2720–6. doi:10.3892/or.2014.3154.

75. Fu TH, Li JY, Jing YY, Sun PJ, Bai X. Effect of elemene on reversing chemoresistance to adriamycin in human stomach cancer cell line. Zhong Yao Cai. 2013;36(4):601–3 (in Chinese).

76. Zhu TZ, Li XM, Luo LH, Song ZQ, Gao X, Li ZQ, et al. beta-Elemene inhibits stemness, promotes differentiation and impairs chemoresistance to temozolomide in glioblastoma stem-like cells. Int J Oncol. 2014;45(2):699–709. doi:10.3892/ijo.2014.2448.

77. Zhu TZ, Li XM, Luo LH, Xu YH, Cao P, Liu Y, et al. beta-Elemene inhibits proliferation through crosstalk between glia maturation factor beta and extracellular signalregulated kinase 1/2 and impairs drug resistance to temozolomide in glioblastoma cells. Mol Med Rep. 2014;10(2):1122–8. doi:10.3892/mmr.2014.2273.

78. Gai DY, Gao X, Wu XH, Hong TT. Synergistic effect of beta-elemene injection combined paclitaxel injection on human breast cancer MB-468 cells: an in vitro study. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2013;33(7):978–82 (in Chinese).

79. Zhang F, Xu L, Qu X, Zhao M, Jin B, Kang J, et al. Synergistic antitumor effect of beta-elemene and etoposide is mediated via induction of cell apoptosis and cell cycle arrest in non-small cell lung carcinoma cells. Mol Med Rep. 2011;4(6):1189–93. doi:10.3892/mmr.2011.537.