

• BASIC RESEARCH •

## P-glycoprotein is not involved in pathway of anti-Fas/Fas-induced apoptosis in KBv200 cells

Qiu-Liang Wu, Xing-Ping Wu, Yong-Ju Liang, Li-Ming Chen, Yan Ding, Li-Wu Fu

Qiu-Liang Wu, Department of Pathology, Sun Yat-Sen University Cancer Center, Guangzhou 510060, Guangdong Province, China  
Xing-Ping Wu, Clinical Laboratory, Sun Yat-Sen University Cancer Center, Guangzhou 510060, Guangdong Province, China  
Yong-Ju Liang, Li-Ming Chen, Yan Ding, Li-Wu Fu, Research Department, Sun Yat-Sen University Cancer Center, Guangzhou 510060, Guangdong Province, China

Supported by the Natural Science Foundation of Guangdong Province, No. 021813 and National Natural Science Foundation of China, No. 30371659

Correspondence to: Professor Li-Wu Fu, Cancer Center, Sun Yat-Sen University, Guangzhou 510060, Guangdong Province, China. fulw@gzsums.edu.cn

Telephone: +86-20-873-43163 Fax: +86-20-873-43392

Received: 2004-06-15 Accepted: 2004-07-15

### Abstract

**AIM:** To verify whether P-glycoprotein (P-gp) could induce cell resistance to apoptosis by inhibiting caspase-8 and caspase-3.

**METHODS:** Human KB cells, either drug-sensitive or with multidrug resistance (MDR) phenotype caused by overexpression of P-gp (KBv200 cells), were treated with anti-Fas (CH-11 monoclonal antibody) to induce apoptosis. Cytotoxicity was detected by MTT assay. Symptoms of cell death were assessed by morphological observation after Hoechst33258 staining, activation of caspase-8 and caspase-3 was measured by Western blotting.

**RESULTS:** Compared with KB cells, the resistance of KBv200 cells to VCR (vincristine) was about 51-fold higher. Anti-Fas (CH-11) induced cytotoxicity and apoptosis in both sensitive KB cells and MDR phenotype KBv200 cells. The IC<sub>50</sub> of CH-11 in KB cells was similar to that in KBv200 cells. CH-11 induced similar apoptotic rates in both KB cells and KBv200 cells, which could be classified as caspase-dependent apoptotic pathway. Verapamil (VRP) did not affect CH-11-mediated apoptosis in KBv200 cells.

**CONCLUSION:** Expression of P-glycoprotein does not induce resistance to caspase-8 and -3 activation or anti-Fas-induced cell apoptosis.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

**Key words:** P-glycoprotein; Apoptosis; CH-11; Fas; Verapamil

Wu QL, Wu XP, Liang YJ, Chen LM, Ding Y, Fu LW. P-glycoprotein is not involved in pathway of anti-Fas/Fas-

induced apoptosis in KBv200 cells. *World J Gastroenterol* 2005; 11(23): 3544-3548

<http://www.wjgnet.com/1007-9327/11/3544.asp>

### INTRODUCTION

Multidrug resistance (MDR) is a kind of resistance of cancer cells to multiple classes of chemotherapeutic drugs. MDR is a major obstacle to treating patients with cancer and is often the result of overexpression of a 170-kDa plasma membrane glycoprotein known as P-glycoprotein (P-gp)<sup>[1]</sup>. Tumor cells are susceptible to apoptosis in response to drugs, the susceptibility of cells to undergo apoptosis may ultimately determine its drug sensitivity. In other words, resistance to apoptosis is an important factor in drug resistance, ultimately determining the cellular response to drug treatment<sup>[2,3]</sup>.

Many chemotherapeutic drugs function in a caspase-dependent manner, by activating the main effector caspase-3. Caspase-3 can be activated via two main apoptotic pathways described sometimes as receptor-dependent and mitochondrial-dependent pathways. Mitochondrial-dependent pathway, which is mainly triggered by stress and cytotoxic drugs, involves the release of cytochrome *c* from mitochondria. Stimulation of the mitochondrial membrane in this process is not yet fully understood. Cytochrome *c* in synergy with ATP allows a conformational change of Apaf-1 to occur. Apaf-1 binds to caspase-9, which in turn activates caspase-3. The receptor-dependent pathway is triggered by ligation of death receptors that are members of the tumor necrosis factor (TNF) superfamily (i.e., Fas receptor) and characterized by an intracellular death domain. The death domain attracts the intracellular adaptor protein FADD that in turn recruits procaspase-8 to the death-inducing signaling complex (DISC). At the DISC, procaspase-8 is cleaved and yields active caspase-8, activating in turn caspase-3<sup>[4-7]</sup>. Cytotoxic drugs have been shown to induce receptor, which then mediates cell death through the activation of receptor-dependent pathways such as Fas/Fas-Ligand<sup>[8]</sup>.

Recently, it has been postulated that P-gp, apart from actively effluxing drugs from cells, may protect them against apoptosis by inhibiting caspase-8 and caspase-3, as P-gp-positive cells have been found to resist cell death induced by UV irradiation, and ligation of cell surface death receptors Fas and TNF<sup>[9-11]</sup>. Smyth *et al*<sup>[12]</sup>, and Cuello *et al*<sup>[13]</sup>, postulated that successful treatment of P-gp<sup>+</sup> MDR tumors can be achieved using chemotherapeutic agents that can function in the absence of caspase-3 activation. However, the molecular mechanisms underlying this novel function

of P-gp remain unknown.

Apoptosis by Fas/Fas L (anti-Fas) is triggered via activating caspase-8 and in turn activating caspase-3<sup>[8,14]</sup>.

The present study was to verify the hypothesis that there is a correlation between P-gp overexpression and caspase-8 and caspase-3 inhibition.

## MATERIALS AND METHODS

### Materials

Verapamil (VRP), vincristine (VCR), Hoechst 33258 and 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Company. RPMI 1640 was purchased from Gibco BRL. Mouse monoclonal antibodies (MAbs) against caspase-8 (Ab-3) and caspase-3 (E-8) and rabbit polyclonal antibody against P-gp (mdr1) were purchased from Santa-Cruz Biotechnology (Santa Cruz, CA). PARP (c-20) was purchased from Pharmingen (San Diego, CA). Peroxidase-conjugated anti-mouse and anti-rabbit IgG were purchased from Calbiochem (La Jolla, CA). Anti-Fas monoclonal antibody (CH-11) was obtained from Immunotech Co.

### Cell lines and cell culture

KBv200 cells, a classic multidrug resistant human epidermoid carcinoma cell line expressing high levels of P-gp, were cloned from parental drug-sensitive KB cells by stepwise exposure to increasing doses of vincristine and ethylmethane sulfonate (EMS) mutagenesis. KBv200 cells and parental sensitive KB cells were obtained from Chinese Academy of Medical Sciences, Beijing. KBv200 cells and KB cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, benzylpenicillin (50 kU/L), and streptomycin (50 mg/L) at 37 °C in a humidified atmosphere of 50 mL/L CO<sub>2</sub>+95% air<sup>[15-17]</sup>.

### Cytotoxicity assay

Sensitivity to anticancer agents such as VCR was determined in triplicate using MTT cell viability assay as previously described<sup>[18]</sup>. The 50% inhibitory concentration (IC<sub>50</sub>) was determined as the drug concentration causing 50% reduction in cell viability. The degree of resistance was calculated by dividing the IC<sub>50</sub> for MDR cells by that for parental sensitive cells. The fold-reversal of MDR was calculated by dividing the IC<sub>50</sub> for cells to anticancer drug in the absence of modulator (VRP) by that in the presence of modulator (VRP).

### Western blot analysis

The cells were treated with desired Fas antibody in the presence or absence of VRP (10 µmol/L) for 24 h. After treatment, whole-cell lysates were extracted with lysis buffer containing 1% Triton-100, 50 mmol/L sodium chloride, 50 mmol/L sodium fluoride, 20 mmol/L Tris (pH 7.4), 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L sodium vanadate, 0.2 mmol/L phenylmethylsulfonyl fluoride and 0.5% Nonidet P-40. Western blotting was carried out as described previously<sup>[19]</sup>. In brief, equal amounts of cell lysate (25 µg) were solubilized in 6-12% SDS-PAGE and transferred onto nitrocellulose membranes. After being

blocked with non-fat milk, membranes were incubated with the appropriately diluted primary antibody. Then, membranes were incubated with a horseradish peroxidase-conjugated secondary antibody. Proteins were detected by the enhanced chemiluminescence detection system (Amersham, Aylesbury, UK).

### Apoptosis measurement

Cells were treated with anti-Fas (CH-11) in the presence or absence of VRP (10 µmol/L) for 48 h, both floating and trypsinized adherent cells were collected, washed with phosphate-buffered saline, fixed with 10% paraformaldehyde for 30 min, and incubated in Hoechst 33258 (Sigma) at room temperature for 30 min (final concentration, 30 µg/mL). Nuclear morphology was examined using fluorescence microscopy with standard excitation filters. Apoptotic cells stained brightly and displayed condensed and fragmented nuclei, normal cells showed an even distribution of the stain throughout the nucleus with flocculated chromatin. The percentage of apoptotic cells was calculated, all cells from four random microscopic fields at 400× magnification were counted<sup>[20]</sup>.

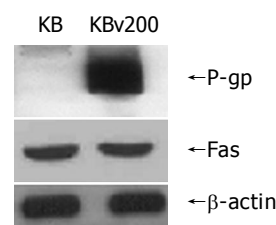
### Statistical analysis

All experiments were repeated at least twice and differences were determined using Student's *t*-test. *P*<0.05 was considered statistically significant.

## RESULTS

### Characterization of Fas and P-glycoprotein-expressing in KBv200 and KB cells

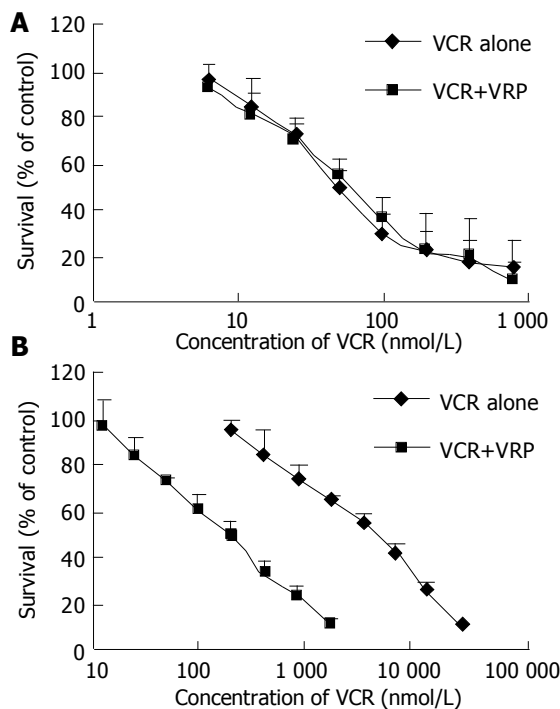
As drug resistance is correlated with decreased Fas receptor expression in hematopoietic MDR cell lines<sup>[21,22]</sup>, Fas expression was examined in these cells. Drug sensitive KB cells and resistant KBv200 cells showed similar levels of Fas expression (Figure 1). A very strong band of P-gp expression was observed in MDR KBv200 cells, while there was no P-gp expression in their parental drug-sensitive KB cells.



**Figure 1** Western blot analysis of P-gp and Fas expression in KBv200 cells and KB cells.

### VRP effectively reversed resistance of KBv200 cells to VCR

To determine the resistance of KBv200 cells to VCR, we compared the cytotoxicity of VCR between KBv200 and their parental sensitive KB cells. IC<sub>50</sub> of VCR to KB and KBv200 cells was 69.8 nmol/L and 3 577.2 nmol/L, respectively (Figures 2A and B). The resistance of KBv200



**Figure 2** VRP enhanced cytotoxicity of VCR to KB cells (A) and KBv200 cells (B).

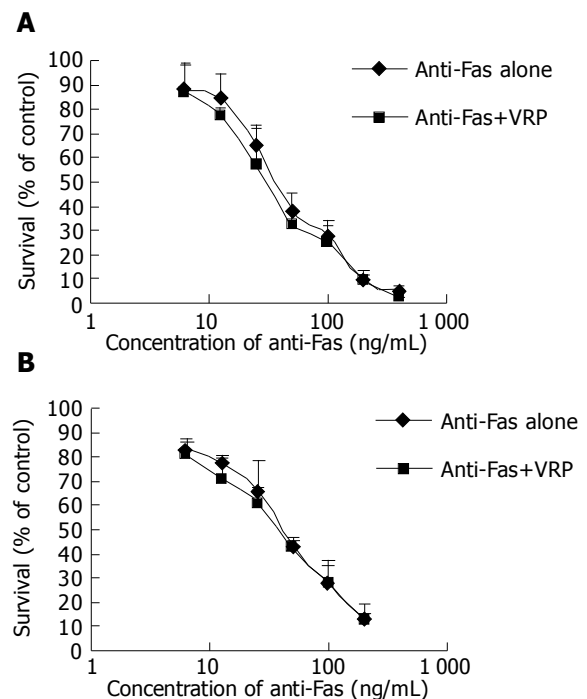
cells to VCR was approximately 51.2-fold higher than that of the parental sensitive KB cells in our experimental system. The cells were incubated with 10  $\mu\text{mol/L}$  of VRP and a full range of concentrations of VCR. At the concentration of 10  $\mu\text{mol/L}$ , VRP lowered the  $\text{IC}_{50}$  of VCR to 189.6 nmol/L in KBv200 cells (Figure 2B). This gave 18.9-fold reversal of MDR. In the sensitive KB cells, however, the  $\text{IC}_{50}$  of VCR was 65.4 nmol/L in the presence of VRP at the concentration of 10  $\mu\text{mol/L}$  (Figure 2A). These results suggested VRP was very effective on reversing MDR *in vitro*.

#### **VRP did not enhance the sensitivity of anti-Fas (CH-11) to P-gp positive KBv200 cells**

CH-11 induced a similar cytotoxicity to both P-gp positive KBv200 cells and their parental P-gp negative KB cells. The  $\text{IC}_{50}$  was  $39.3 \pm 3.6$  (ng/mL) and  $38.5 \pm 4.2$  (ng/mL) for KBv200 and KB cells, respectively ( $P > 0.05$ ). Furthermore, 10  $\mu\text{mol/L}$  of VRP did not significantly enhance the sensitivity of KBv200 cells to anti-Fas (CH-11, Figure 3).

#### **KBv200 cells did not show resistance to anti-Fas (CH-11)-mediated apoptosis and VRP did not sensitize KBv200 cells to anti-Fas (CH-11)-mediated apoptosis**

To examine whether P-gp positive cells failed to undergo apoptosis induced by anti-Fas/Fas, apoptosis was assessed by Hoechst 33258 staining. Treatment with 20 ng/mL anti-Fas (CH-11) resulted in significant apoptosis in parental KB cells, as well as in drug-resistant KBv200 cells. Condensed and fragmented nuclei were found, and normal cells showed an even distribution of the stain throughout the nucleus with flocculated chromatin. The apoptotic rate was 39.0% and 40.5% in KB and KBv200 cells respectively after treatment with 20 ng/mL CH-11 for 24 h. Anti-Fas (CH-11)/Fas induced similar apoptosis in P-gp positive KBv200



**Figure 3** VRP did not enhance cytotoxicity of anti-Fas to KB cells (A) and KBv200 cells (B).

cells and parental sensitive KB cells. Moreover, treatment with CH-11 (20 ng/mL) resulted in similar cell apoptosis with or without VRP (10  $\mu\text{mol/L}$ ). The results are shown in Figure 4 and Table 1.

**Table 1** Effect of VRP on the apoptotic rate in cells treated with CH-11 (mean $\pm$ SD)

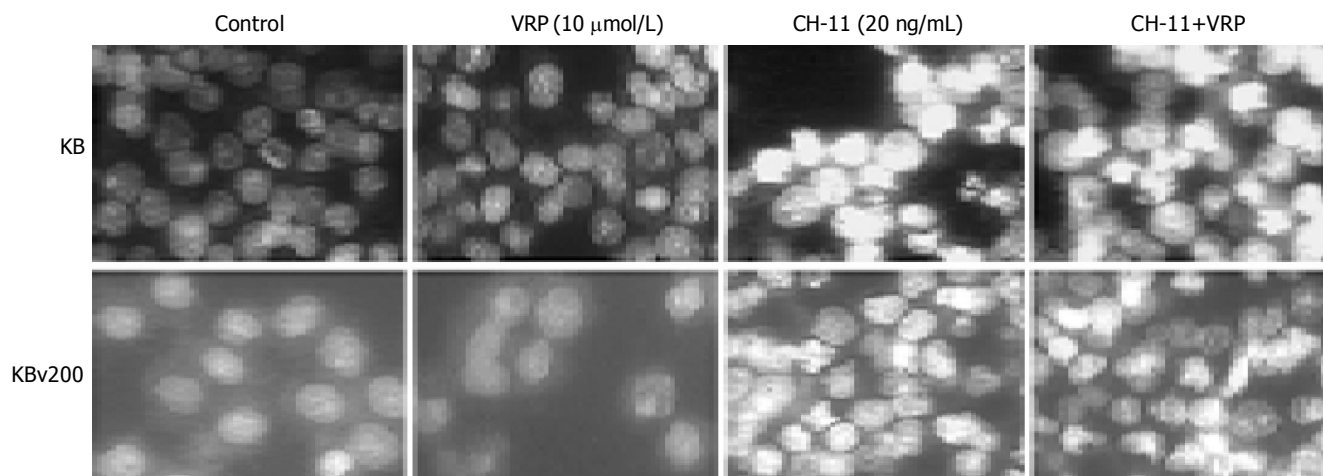
	Control	VRP (10 $\mu\text{mol/L}$ )	CH-11 (20 ng/mL)	VRP+CH-11
KB	$3.5 \pm 1.3$	$3.0 \pm 0.8$	$39.0 \pm 6.1$	$40.3 \pm 6.8$
KBv200	$2.8 \pm 1.0$	$3.5 \pm 1.3$	$40.5 \pm 3.4$	$41.3 \pm 3.4$

#### **VRP did not sensitize activation of caspase-8 and -3 in KBv200 cells**

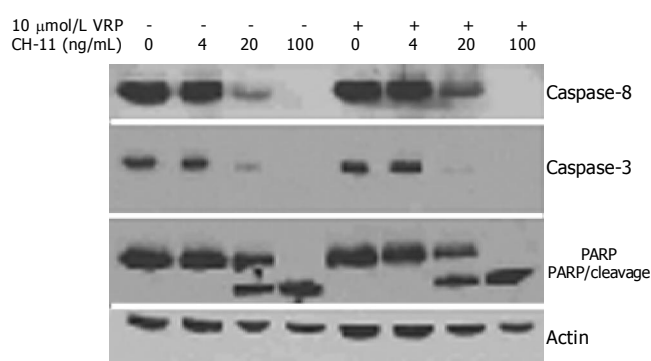
The induction of apoptosis by anti-Fas was assessed by Western blot. VRP did not induce apoptosis alone. Treatment with 20 ng/mL anti-Fas (CH-11) resulted in significantly reduced levels of caspase-8 and -3 in KBv200 cells. KBv200 cells did not demonstrate greater CH-11 mediated-caspase-8 and -3 activation in the presence of 10  $\mu\text{mol/L}$  VRP (Figure 5). These results were in agreement with the apoptosis assessed by Hoechst 33258 staining.

## **DISCUSSION**

Following the introduction of more effective drugs and the development of better-designed chemotherapy strategies, the treatment of cancer has improved. However, the development of multidrug resistance (MDR) is still a problem and remains an obstacle to successful chemotherapy. Many cellular changes have been associated with the development of MDR, including expression of ATP-binding cassette



**Figure 4** Cell morphology and DNA fragmentation.



**Figure 5** Western blot analysis of caspase-8 and -3 in KBv200 cells after treatment with anti-Fas antibody (CH-11) in the presence (+) or absence (-) of VRP for 24 h at 37 °C.

(ABC) transport proteins, multidrug resistance-associated protein (MRP1), and P-glycoprotein. Resistance mediated by these proteins is associated with altered drug transport, resulting in decreased intracellular drug accumulation<sup>[21-25]</sup>.

P-gp has been demonstrated to efflux a wide range of structurally and functionally diverse compounds<sup>[22,24]</sup>. Recently it has been proposed that in addition to mediating drug resistance through drug transport, P-glycoprotein may also play a role in resistance to apoptosis<sup>[26]</sup>. Resistance to apoptosis is an important factor in drug resistance, ultimately determining the cellular response to drug treatment. This supports the suggestion that P-glycoprotein can protect cells against apoptotic stimuli that function in a caspase-dependent manner by transporting a key caspase or adaptor molecules out of cells or by inhibiting caspase activation through decreasing intracellular ATP or altering intracellular pH<sup>[27,28]</sup>. On the other hand, P-gp-expressing cells are not resistant to caspase-independent cell death mediated by pore-forming proteins and granzyme B as well as by other factors<sup>[10,29,30]</sup>.

However, P-gp does not interfere with the formation of DISC since equivalent amounts of pro-caspase-8 are associated with the Fas-receptor<sup>[31]</sup>. Cullen *et al.*<sup>[19,27]</sup>, showed that MDR mediated by MRP and P-gp does not correlate

with resistance to Fas-mediated cell death or to caspase-3 activation. Sikora *et al.*<sup>[32]</sup>, also found that there is no correlation between P-gp expression and resistance to caspase-3-dependent apoptosis induced by curcumin and UVC, at least in HL-60 cells.

Our results tended to exclude that the multidrug transporter P-gp played an important role in the inhibition of apoptosis by inhibiting activation of caspase-8 and -3. In fact, anti-Fas/Fas induces similar cytotoxicity and apoptotic rate in P-gp overexpressing KBv200 cells as in their parental sensitive KB cells. In addition, the P-gp inhibitor VRP strongly reverses the resistance to VCR and VCR-induced apoptosis in KBv200 cells. But verapamil does not increase the cytotoxicity and the rate of apoptosis induced by anti-Fas antibody in the same cells. KBv200 cells do not exhibit greater caspase-8 and -3 degradation in the presence of 10 μmol/L VRP.

Notarbartolo *et al.*<sup>[33]</sup>, and Campone *et al.*<sup>[34]</sup>, have shown that P-gp-expressing HL-60 cells are resistant to caspase-3-dependent apoptosis due to the presence of XIAP in these cells. This suggests that the novel family of IAPs may have a responsibility in such a process.

In conclusion, overexpression of P-gp in KBv200 cells does not influence the apoptotic pathway leading to activation of caspase-8 which in turn activates caspase-3.

## ACKNOWLEDGMENTS

We thank Professor Liu YS (Chinese Academy of Medical Sciences, Beijing, China) for providing KB and KBv200 cell lines.

## REFERENCES

- 1 Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 1989; **58**: 137-171
- 2 Inoue S, Salah-Eldin AE, Omoteyama K. Apoptosis and anticancer drug resistance. *Hum Cell* 2001; **14**: 211-221
- 3 Tolomeo M, Simoni D. Drug resistance and apoptosis in cancer treatment: development of new apoptosis-inducing agents active in drug resistant malignancies. *Curr Med Chem*

- Anticancer Agents* 2002; **2**: 387-401
- 4 **Adams J**. The proteasome: structure, function, and role in the cell. *Cancer Treat Rev* 2003; **29** Suppl 1: 3-9
  - 5 **Brown JM**, Wilson G. Apoptosis genes and resistance to cancer therapy: what does the experimental and clinical data tell us? *Cancer Biol Ther* 2003; **2**: 477-490
  - 6 **Hu W**, Kavanagh JJ. Anticancer therapy targeting the apoptotic pathway. *Lancet Oncol* 2003; **4**: 721-729
  - 7 **Kim R**, Tanabe K, Uchida Y, Emi M, Inoue H, Toge T. Current status of the molecular mechanisms of anticancer drug-induced apoptosis. The contribution of molecular-level analysis to cancer chemotherapy. *Cancer Chemother Pharmacol* 2002; **50**: 343-352
  - 8 **Poulaki V**, Mitsiades CS, Mitsiades N. The role of Fas and FasL as mediators of anticancer chemotherapy. *Drug Resist Updat* 2001; **4**: 233-242
  - 9 **Ding Z**, Yang X, Pater A, Tang SC. Resistance to apoptosis is correlated with the reduced caspase-3 activation and enhanced expression of antiapoptotic proteins in human cervical multidrug-resistant cells. *Biochem Biophys Res Commun* 2000; **270**: 415-420
  - 10 **Johnstone RW**, Cretney E, Smyth MJ. P-glycoprotein protects leukemia cells against caspase-dependent, but not caspase-independent, cell death. *Blood* 1999; **93**: 1075-1085
  - 11 **Saini KS**, Walker NI. Biochemical and molecular mechanisms regulating apoptosis. *Mol Cell Biochem* 1998; **178**: 9-25
  - 12 **Smyth MJ**, Krasovskis E, Sutton VR, Johnstone RW. The drug efflux protein, P-glycoprotein, additionally protects drug-resistant tumor cells from multiple forms of caspase-dependent apoptosis. *Proc Natl Acad Sci USA* 1998; **95**: 7024-7029
  - 13 **Cuello M**, Ettenberg SA, Nau MM, Lipkowitz S. Synergistic induction of apoptosis by the combination of trail and chemotherapy in chemoresistant ovarian cancer cells. *Gynecol Oncol* 2001; **81**: 380-390
  - 14 **Petak I**, Houghton JA. Shared pathways: death receptors and cytotoxic drugs in cancer therapy. *Pathol Oncol Res* 2001; **7**: 95-106
  - 15 **Liang YJ**, Fu LW, Feng HL, He LY, Feng GK, Yang XP, Pan QC. Establishment of the model of KBv200 nude mice xenograft and the studies on its characterization of multidrug resistance. *Zhongguo Yaolixue Tongbao* 2000; **16**: 705-707
  - 16 **Fu L**, Liang Y, Deng L, Ding Y, Chen L, Ye Y, Yang X, Pan Q. Characterization of tetrandrine, a potent inhibitor of P-glycoprotein-mediated multidrug resistance. *Cancer Chemother Pharmacol* 2004; **53**: 349-356
  - 17 **Jiang XH**, Wong BC, Yuen ST, Jiang SH, Cho CH, Lai KC, Lin MC, Kung HF, Lam SK. Arsenic trioxide induces apoptosis in human gastric cancer cells through up-regulation of p53 and activation of caspase-3. *Int J Cancer* 2001; **91**: 173-179
  - 18 **Hishikawa K**, Oemar BS, Tanner FC, Nakaki T, Luscher TF, Fujii T. Connective tissue growth factor induces apoptosis in human breast cancer cell line MCF-7. *J Biol Chem* 1999; **274**: 37461-37466
  - 19 **Cullen KV**, Davey RA, Davey MW. Drug resistance does not correlate with resistance to Fas-mediated apoptosis. *Leuk Res* 2001; **25**: 69-75
  - 20 **Friesen C**, Fulda S, Debatin KM. Deficient activation of the CD95 (APO-1/Fas) system in drug-resistant cells. *Leukemia* 1997; **11**: 1833-1841
  - 21 **Fu LW**, Zhang YM, Liang YJ, Yang XP, Pan QC. The multidrug resistance of tumour cells was reversed by tetrandrine *in vitro* and in xenografts derived from human breast adenocarcinoma MCF-7/adr cells. *Eur J Cancer* 2002; **38**: 418-426
  - 22 **Yang HH**, Ma MH, Vescio RA, Berenson JR. Overcoming drug resistance in multiple myeloma: the emergence of therapeutic approaches to induce apoptosis. *J Clin Oncol* 2003; **21**: 4239-4247
  - 23 **Fojo T**, Bates S. Strategies for reversing drug resistance. *Oncogene* 2003; **22**: 7512-7523
  - 24 **Lee CH**. Reversing agents for ATP-binding cassette (ABC) transporters: application in modulating multidrug resistance (MDR). *Curr Med Chem Anticancer Agents* 2004; **4**: 43-52
  - 25 **Sparreboom A**, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. *Drug Resist Updat* 2003; **6**: 71-84
  - 26 **Miao ZH**, Ding J. Research advances on circumventing tumor multidrug resistance. *Aizheng* 2003; **22**: 886-892
  - 27 **Cullen K**, Davey R, Davey M. The drug resistance proteins, multidrug resistance-associated protein and P-glycoprotein, do not confer resistance to Fas-induced cell death. *Cytometry* 2001; **43**: 189-194
  - 28 **Johnstone RW**, Ruefli AA, Smyth MJ. P-glycoprotein: more than a drug pump? *Today Life Sci* 1999; **11**: 66-72
  - 29 **Ruefli AA**, Smyth MJ, Johnstone RW. HMBA induces activation of a caspase-independent cell death pathway to overcome P-glycoprotein-mediated multidrug resistance. *Blood* 2000; **95**: 2378-2385
  - 30 **Ruefli AA**, Bernhard D, Tainton KM, Kofler R, Smyth MJ, Johnstone RW. Suberoylanilide hydroxamic acid (SAHA) overcomes multidrug resistance and induces cell death in P-glycoprotein-expressing cells. *Int J Cancer* 2002; **99**: 292-298
  - 31 **Ruefli AA**, Tainton KM, Darcy PK, Smyth MJ, Johnstone RW. P-glycoprotein inhibits caspase-8 activation but not formation of the death inducing signal complex (disc) following Fas ligation. *Cell Death Differ* 2002; **9**: 1266-1272
  - 32 **Piwocka K**, Bielak-Mijewska A, Sikora E. Curcumin induces caspase-3-independent apoptosis in human multidrug-resistant cells. *Ann N Y Acad Sci* 2002; **973**: 250-254
  - 33 **Notarbartolo M**, Cervello M, Dusanochet L, Cusimano A, D'Alessandro N. Resistance to diverse apoptotic triggers in multidrug resistant HL60 cells and its possible relationship to the expression of P-glycoprotein, Fas and of the novel anti-apoptosis factors IAP (inhibitory of apoptosis proteins). *Cancer Lett* 2002; **180**: 91-101
  - 34 **Campone M**, Vavasseur F, Le Cabellec MT, Meflah K, Vallette FM, Oliver L. Induction of chemoresistance in HL-60 cells concomitantly causes a resistance to apoptosis and the synthesis of P-glycoprotein. *Leukemia* 2001; **15**: 1377-1387