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• ORIGINAL RESEARCH •

Taxotere resistance in SUIT Taxotere resistance in pancreatic carcinoma cell line SUIT 2 and its sublines

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Abstract

AIM: **To investigate the specific mechanisms of intrinsic and acquired resistance to taxotere (TXT) in pancreatic adenocarcinoma (PAC).**

METHODS: **MTT assay was used to detect the sensitivity of PAC cell line SUIT-2 and its sublines (S-007, S-013, S-020, S-028 and TXT selected SUIT-2 cell line, S2/TXT) to TXT. Mdr1 (P-gp), multidrug resistance associated protein (MRP), lung resistance protein (LRP) and β-tubulin isotype gene expressions were detected by RT-PCR. The functionality of P-gp and MRP was tested using their specific blocker verapamil (Ver) and indomethacin (IMC), respectively. The transporter activity of P-gp was also confirmed by Rhodamine 123 accumulation assay.**

RESULTS: **S-020 and S2/TXT were found to be significantly resistant to TXT(19 and 9.5-fold to their parental cell line SUIT-2, respectively). RT-PCR demonstrated strong expression of Mdr1 in these two cell lines, but weaker expression or no expression in other cells lines. MRP and LRP expressions were found in most of these cell lines. The TXT-resistance in S2-020 and S2/TXT could be reversed almost completely by Ver, but not by IMC. Flow cytometry showed that Ver increased the a ccumulation of Rhodamine-123 in these two cell lines. Compared with S-020 and SUIT-2, the levels of β-tubulin isotype II, III expressions in S-2/TXT were increased remarkably.**

CONCLUSION: **The both intrinsic and acquired TXT-related drug resistance in these PAC cell lines is mainly mediated by P-gp, but had no relationship to MRP and LRP express ions. The increases of β-tubulin isotype II, III might be collateral changes that occur when the SUIT-2 cells are treated with TXT.**

Subject headings pancreatic neoplasms/pathology; tumor cells, cultured/drug effects; paclitaxel/analogs & derivatives; paclitxael/pharm acology; drug resistance, multiple; drug resistance, neoplasm

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INTRODUCTION

Up to now, pancreatic adenocarcinoma (PAC) is still one of the leading causes of cancer death in the world, although meat progress has been made in the treatment of this disease[1,2]. Most patients with PAC are in its advanced stages and surgically unresectable at the time of diagnosis, and for those who are resected, the risk of recurrence is very high[3,4]. Consequently chemotherapy still is an alternative strategy for patients with non-resectable PAC[5-8]. However, the response to most forms of chemotherapy achieved so far is generally quite limited, and is related in part to the resistance to these chemical agents[1,5,9]. To date, some specific mechanisms of drug resistance have been elucidated, among which the best understood are increased expressions of mdr1-encoded p-glycoprotein (P-gp)[10-14], multidrug resistance protein (MRP)[15-17] and lung resistance protein (LRP)[18-20]. These membrane transporter proteins play important roles in multiple drug resistance (MDR) involving increased drug efflux and intracellular drug entrapment and/or redistribution.

Taxotere (TXT) is a member of the family of taxanes and is more potent than paclitaxel with regard to the promotion of the polymerization of tubulin, inhibition of depolymerization, and it inhibits cell replication and has greater antitumor activity in many *in vitro* and *in vivo* tumor model systems[21-24]. The drug has displayed significant antitumor efficacy against breast, lung and ovarian cancer s in clinical trials[25-28], but some clinical studies do not support the use of Taxane in advanced PAC[29,30]. The studies on mechanisms by which cells acquire resistance to Taxane demonstrated that the drug is a substrate for the P170 multidrug resistance pump that is able to confer resistance to a wide variety of naturally derived hydrophobic substances[16,29,31]. Therefore, the cells selected with Taxane were found to exhibit cross-resistance to a variety of other hydrophobic drugs and have elevated levels of P-gp[32]. Other possible mechanisms of resistance to TXT the include the alteration in microtubulin composition and/or dynamics[33,34] increased protein kinase C-α and -γ expression[35] and overexpression of Bc1-2[36], and SP-gp[37]. But the exact mechanism of TXT- resistance in PAC is still not clear[32,38]. The specific mechanisms in different tumors might be different.

The mechanisms of refractoriness to chemotherapy of PAC are not fully understood. Published reports provide confl icting information regarding the expression of this MDR phonotype in human PAC[16,39]. Whether t he intrinsic and acquired TXT-resistance share the same MDR mechanism is unclear, which might be important for designing a chemotherapeutic regiment and investigating appropriate reversal agents. This study was designed to investigate the mechanisms of intrinsic and acquired resistance to T XT in PAC cell line SUIT-2 and its sublines.

MATERIALS AND METHODS

Chemical reagents

Taxotere (Rh0ne-Poulenc Rorer Pharmaceuticals Inc.) was stored as 10 mmol·L^{-1} stock solution in absolute ethanol at -20℃. These solutions were further diluted in the mediu m used in the cell culture immediately before each experiment. Final dilutions of 0.5-3.5 nmol·L^{-1} TXT were used for the experiments to detect the sensitivity of SUIT-2 and its sublines. Verapamil (Ver) was purchased from

American Reagent Laboratory, Inc. and Trypsin, EDTA was purchased from Gibco. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), rhodamine (Rho) 123 (2-[6-Amino-3-imino-3H-xanthen-9-yl] benzoic acid methyl ester), indomethacin (IMC) (1-[P-chl orobenzoyl]-5-methoxy-2-methylindole-3-acetic acid), McCoy's 5A Medium [modified], DMEM (phenol free) medium, Fetal calf serum (FCS), sodium pyruvate, MEM amino acids (50X), MEM amino acids (100X), MEM Vitamins, L-glutamine (200 mmol·L^{-1}) penicillin-streptomycin, serine, a sparagine sodium bicarbonate were purchased from Sigma.

Cell cultures

Human pancreatic cancer cell line SUIT-2 was established and supplied by Iwamura. Its sublines, including S2-007, S2-013, S2-020, S2-028, were cloned by soft agar culture and shown to have different metastatic potential[40]. These cell lines were cultured and the TXT resistant SUIT-2 cell line (S2/TXT) was develop ed as described previously[41].

MTT colorimetric assay

The MTT colorimetric assay was performed as described previously[41]. Briefly, when the cells of SUIT-2 and its sublines reached 85%-90% confluency, they were detached from the flasks with 0.25% trypsin and resuspended in the culture medium. Cells were grown within 9 6-well microtitre plates (Costar) in 2×10^{10} cells·L^{-1} of culture medium each well and acclimated for 6 h, and 100 μL of various concentrations of drugs diluted in culture medium was added. To study the effect of Ver and IMC on TXT cytotoxicity, 1 μmol·L^{-1} Ver or 10 μmol·L^{-1} IMC was added with this drug. Five duplicate wells were used for each determination. The plates were incubated for 72 h when the control cells reached 90% confluency, and 30 μL of MTT in PBS solution was then added to each well and the plates were incubated for another 4 h. The medium and MTT solution were then aspirated and 150 μL of dimethyl sulfoxide (DM SO) (Sigma) was added. The plates were read on Bio-Tek Microplate reader EL 800 (Bio-Tek Instruments, Inc). Fractioo of cell proliferation was defined as the ratio of optical density volume to that of controls. The IC_{50} was defined as the concentration of the drugs required to reduce the absorbance by 50% in treated cells as compared to that of the controls.

Rho-123 accumulation assay

Rho-123 accumulation was determined by flow cytometry. Briefly, SUI T -2, S-020 and S2/TXT cells in logarithmic growth phase were harvested with trypsin and resuspended in phenol red-free DMEM medium at 1×10^{9} cells·L^{-1}. Aliquots of 1 ml cell suspension were preincubated with or without Verapamil for 45 min and then 200 μg·L^{-1} Rho-123 dissolved in DMEM was added and incubated for 40 min. After incubation, cells were washed and resuspended in ice-cold Rho-free DMEM with 5 μmol·L^{-1} Ver. The accumulation of Rho-123 in cells was analyzed with flow cytometry. Ten thousand cells per sample were analyzed. Cells of these cell lines, which had not been exposed to Rho-123, were used to determine the background of autofluorescence.

RT-PCR

The isolation of total RNA was based on the method of Chomczynski *et al*[40]. Equal amounts of RNA were reversed transcribed using SuperScript™ O ne-step™ RT-PCR System (Life Technologies). Twenty-five μL PCR mixed in each tube containing: 0.5 μL RT/Tag Mix, 3 μL of 5 mmol·L^{-1} $MgSO_4$, 5 μL diethy pyrocarbonate (DEPC, Sigma)-treated distilled water, 3 μL mixed primer pairs, 12.5 μL 2X Reaction Mix and 1 μg Template RNA in DEPC water. After an initial denaturation in a programmable thermocycler at 94℃ for 2 min, PCR was carried out for 30 cycles with the thermal profile: denaturing at 94℃ for 30s, annealing at 55℃ for 30 s and extension at 72℃ for 1 min with an extra-10 min extension for the last cycle. After completion of the amplification cycles, 5 μL of each PCR product was electrophoresed at 60 V for 1.5 hrs on a 1.2% agarose gel (GIBCOBRL) in Trizma base and Glacial acetic acid EDTA buffer. Both target and control (β-actin) gene sequen ces were coamplified in the same tube. Gene expression was normalized to β-actin transcript; this was noted for REL for relative expression levelg(REL=densitometric value of studied gene/densitometric value of â-actin). The specific primers for mdr1, MRP and LRP used in this study were as described as before [41]. The beta-tubulin isotype sequences were designed as follows: The sense primer sequence was 5'-CAA CAG CAC GGC CAT CCA GG-3'. The antisense primer sequences were M40 (Cla ss I): 5'-AAG GGG CAG TTG AGT AAG ACG G-3', β9 (Class II): 5'-GTA GAA AG A CCA TGC TTG GG-3', β4 (Class III): 5'- CTT GGG GCC CTG GGC CTC CGA-3', 5β (C lass I Va): 5'-AAG TAG CCA GAG GTA AAG CGA G-3', β2 (Class IVb): 5'-CTT TCC CCA GTG AC T GAA GG-3'. β-actin was used as control. Its sense primer was: 5'- TGA CG G GGT CAC CCA CAC TGT GCC CAT CTA-3'; and antisense primer was: 5'- CTA GAA GCA T TT GCG GTG GAC GAT GGA GGG-3'.

RESULTS

Sensitivity of SUIT-2 and its sublines to TXT

To identify for intrinsic TXT-resistant PAC cell lines, SUIT-2 and its sublines (S2-007, S2-013, S2-0 20 and S2-028)) were investigated as to their sensitivity to TXT as by MTT assay. The in nate sensitivity of SUIT-2 and its sublines to TXT were found. S2-020 cells we re most resistante to TXT compared with its parental cell line SUIT-2 (IC_{50} 0.85 nmol·L^{-1}), and other cell lines. IC_{50} of S2-020 (16.2 nmol ·L^{-1}) was 19-fold to that of SUIT-2. The second most resistant cell line was found to be S2-007 (IC_{50}: 1.82 nmol·L^{-1}). The other cell lines had no significant resistance to TXT. The IC_{50} of S2-013 and S2-028 were 0.75 and 1.2 nmol·L^{-1}, respectively. IC_{50} of the acquired TXT resistant cell line established from SUIT-2 was 8.1 nmol·L^{-1}, 9.5- fold to its parental cell line SUIT-2 (Figure 1).

Reversal effects of Ver and IMC on the resistance to TXT

In order to elucidate the function of drug transporter pump P-gp and MRP expressed in the cell line S2-020 and S2/TXT, their corresponding blockers Ver and IMC were used respectively. Figure 2A shows that Ver at a concentration of 1ìmol·L^{-1} can almost completely reverse the resistance of S2-020 and S 2/T XT to TXT, but the same concentration of Ver had no sensitizing effect to SUIT- 2, which did not express P-gp. IMC, a specific modulator of MRP, had no reversal effect on the TXT-resistance found in these cell lines, although they all expressed MRP (Figure 2B).

Rho 123 accumulation and efflux

Accumulation and efflux of Rho-123, which is related to the transporter activity of P-gp, from SUIT-2, S2/TXT and S-020 cells as tested by flow cytometry. The accumulation of Rho-123 in SUIT-2 cells is much higher than that of S-020 and S2/TXT cells. The addition of 5 μmol·L^{-1} Ver led to significantly increased intracellular Rho 123 levels in the TXT-resistant S2-020 and S2/TXT cells but not in the TXT-sensitive SUIT-2 cells and its other cell sublines.

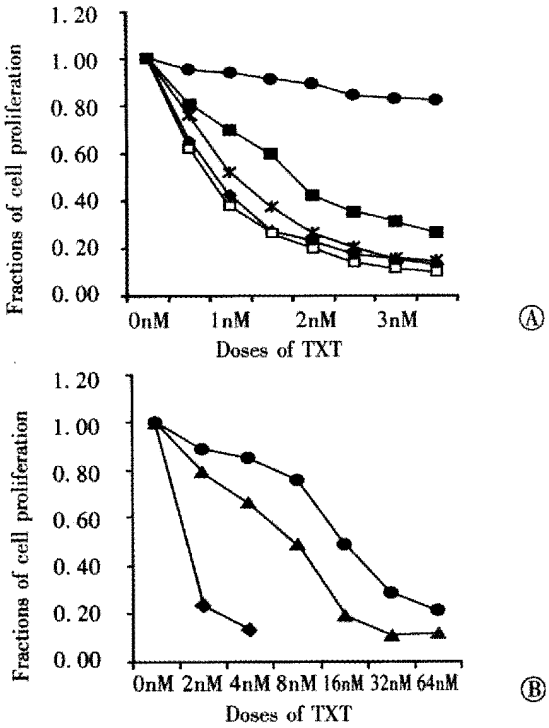

Figure 1 Dose-response curves of SUIT-2 and its subl ines for taxotere. ◆: SUIT-2, ■: S2-007,: S2-013, ●: S2-020, : S2-028, △: S2 /TXT.

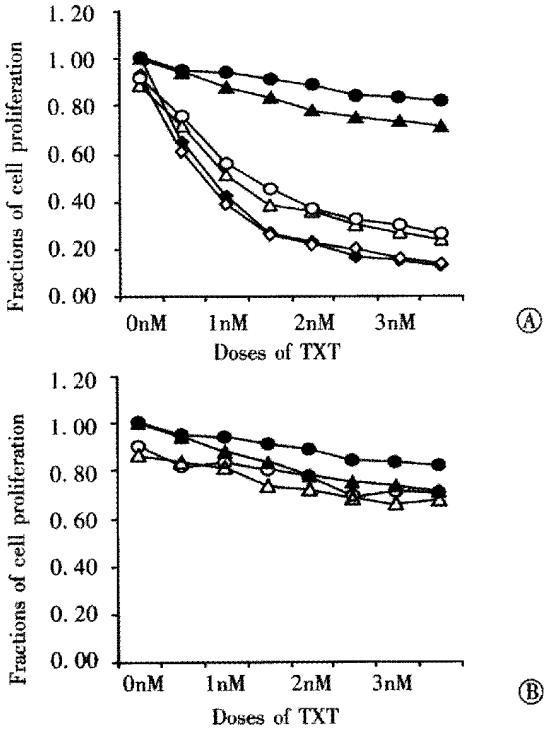

Figure 2 Reversal effects of Verapamil and Indomethacin on TXT -resistance. S2/TXT (▲), and S2-020 cell lines (●) were compared to SUIT- 2 (◆). Ver (Figure 2A) and IMC (Figure 2B) have been shown different sensitizing effects of TXT-resistance in S2/TXT and S2-020 (open symbols).

Expressions of mdr1, MRP, and LRP

The expressions of three major drug transporter pump genes mdr1, MRP, L RP were studied by RT-PCR. Figure 3 shows that there is a strong expression of mdr1 in both TXT-resistant cell line S2-020 and S2/TXT, no expressions in their parental cell line SUIT-2 and other two subline S2-013 and S2-028, which are most sensitive to TXT. MRP and LRP expressed in most of these cell lines, but no relationship was found between their expression and TXT-resistance.

Expressions of β-tubulin isotypes

β-tubulin isotype transcript analysis was performed by using isotype-specific primers and the RNA from SUIT-2 and its two TXT-resist ant cell line S2-020 and S2/TXT. Densitometric analysis of expression levels of each isotype was quantitated relative to the expression of the control gene β actin by calculating the ratio of the target gene to the control gene PCR product. As shown in Figure 4, the βI and β I Vb were the predominant transcript in all the three cell lines. A 2.4-fold increase of β II and a 2.3-fold increase of β III compared to SUIT-2 cell line were seen in S2/TXT cell line.

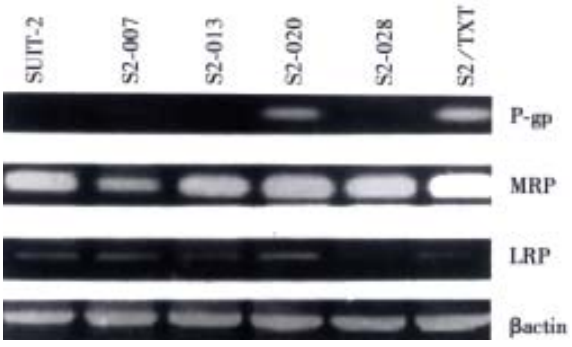

Figure 3 Expressions of mdr1, MRP, LRP in SUIT-2 and its sublines by RT-PCR analysis.

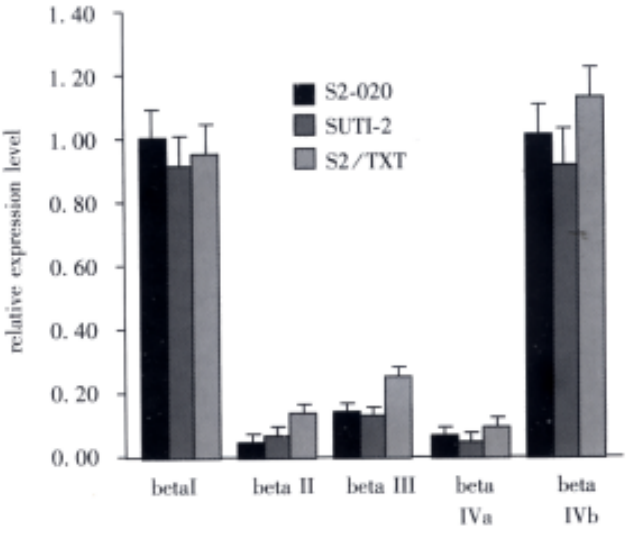

Figure 4 The RT-PCR analysis of the β tubulin isotype transcripts for the SUIT-2 and its two TXT- resistant cell lines. The relative expression levels of transcripts were expressed as a ratio of denitomet ric value of studied gene to that of β actin.

DISCUSSION

An increasing use of Taxanes in the treatment of variety of cancer including advanced PAC, has highlighted the need to elucidate the mechanism responsible for the development of resistance to this kind of drugs. Multidrug resistance may develop through a variety of mechanisms, which may include the alterations of extracellular drug efflux, intracellular extrapment or redistribution, drug detoxification, nuclear target and apoptotic response[42]. With respect to taxane-related resistance, many studies have shown that it is often associated with the expression of mdr1[29,32], but the expression of the mdr1 gene only accounts for part of the resistance mechanisms to taxanes. In

a study by Dumontet *et al*, only 44% of resistant clones were found to express the mdr1 gene, and accumulation studies with labeled PTX did not show altered accumulation in mdr1 negative clones[33]. Such observations indicate that there must be other mechanisms involved in taxanes resistance. Whether or not other drug transporters including MRP and LRP are also involved in this mechanism is not clear. There are reports that cell lines with MRP or LRP phenotype also confer a low level of resistance to taxane[43], though most findings showed that taxane-resistance is independent of MRP expression[44,45].

In this study, both intrinsic and acquired TXT resistant cell lines, which were derived from the same parental cell line, have expression of mdr1 gene, and also these resistances can be reversed almost completely by a P-gp blocker Verapamil. Active drug transporter pump in these TXT-resistant cells was also shown by Rho-123 accumulation assay. Though most of cell lines expressed MRP and LRP as show n by RT-PCR, they had no relationship with the TXT-resistance in these cell lines. To confirm that no MRP mediated TXT resistance exists in these cell lines, we used Indomethacin, a specific inhibitor of MRP[46] and found no sensitizing effect to TXT cytotoxity. LRP positive cell line SUIT-2 had the same sensitivity as LRP negative S2-028 cell lines, showing that there is n o active LRP mediated TXT-resistance. These data indicated that the TXT-related drug resistance in these cell lines is mainly mediated by P-gp. The high incidence of MRP and LRP expressions in these PAC cell lines might be involved in other kinds of intrinsic drugs resistance. Recently, other studies on the mechanisms involve d in taxane-resistance have been focused on the composition and the mutations in â-tubulin isotypes[32,33,47-49]. The possible mechanisms involved in the induction of resistance to taxanes may include altered metabolism and/or subcellular distribution, altered interaction between the tubulin-binding agents and microtubules, and altered response to cell cycle arrest by mitotic blockage.

The taxanes are unique among tubulin-targeted cytotoxins so far as they bind to polymerized tubulin only[24,50]. Direct photoaffinity labeling has demonstrated that taxane binds preferentially to the β subunit of the microtubule[51]. Sequence analysis identified the photoaffinity labeled amino acid residues β270 and β364 as important modulators of paclitaxel's interaction with the tubulin molecule[47]. The direct proofs that altering the isotype composition of a cell affects its sensitivity to any antimitotic drug have been reported, but the results were conflicting[32,33,47,48,52]. Virtually all major β-tubulin isotypes have been reported to be changed in various drug resistant mutants[32-34,4 7-49,52], and this runs counter to the idea that specific isotype of β-tubulin confers altered sensitivity to these drugs. Currently, more direct evidences t hat altered expressions of β-tubulin isotype in taxane-resistance cells contribute to their resistance phenotype have been demonstrated. Blade *et al* [32] by transfecting Chinese hamster ovary cells with cDNA encoding epitopetagged class I,II, and IV b â tubulins found that the production of βI, βII, or βIVb tubulin had no effect on the sensiti vity of the cells to PTX. However, Kavallaris *et al*[53] designed antisense phosphrothioate oligode oxynucleotides targeted against resistant lung cancer cells, and demonstrated that a decrease in class IIIβ tubulin mRNA and protein expression which corresponded to a 39% decrease sensitivity to PTX.

Different mechanism of taxane-related drug resistance may be tumor (or cell line)-dependent or even cell-dependent. Tumor histologic origination and heterogeneity of tumor cells may be responsible for this difference[33]. Since P-gp was often expressed in normal pancreatic tissue[54], P-gp mediated TXT resistance can be found natively in PAC or be readily induced by their subst rates. This study showed the changes of â-tubulin isotype not only in the P-gp-negative taxanes-resistance cells as in other studies[47,48], but also in P-gp-positive cells. However, the S2-020/TXT cells with both P-gp expression and changes of tubulin isotype profile did not show an additive or synergetic effects on TXT-resistance resistant to TXT compared with only P-gp-positive cell line S2-020. On the other hand, this resistance can be fully reversed by P-gp reversal agent Verapamil, suggesting that the change of β-tubulin isotypes might be a collateral change and not necessarily responsible for TXT-resistance in this cell line. Since many critical cellular functions, such as cell movement, mitosis, and maintenance of cell structure are associated with cytoskeletal elements in which the microtubular system plays a major role, it is not clear whether the changes of tubulin in this TXT selected cell line are involved in other biologic behavior of cells, such as cell mobility and inva siveness[55-58], which need to be further investigated.

The different expression of P-gp or tubulin mutation may also be related to the means by which the resistant cells were selected. Multiple step selected cells often present high levels of taxane-resistance mediated by P-gp, while single -step selection yields low level taxane-resistance cells with tubulin mutations[59], since severe tubulin mutations are very likely to affect cell survival and will be lost during the selection. The cells with di fferent mechanisms of taxane-resistance may have different characteristic. TXT-related drug resistan ce mediated by P-gp often presents a cross-resistance to other natural chemical agents, which is different from the taxane-resistance induced by the changes of â-tubulin isotype profile, the latter was often only cross-resistant to other kinds of anti-microtubule agents, such as Vinca alkaloids[33]. However, additive and synergetic effects between Vinorelbine and TXT on human lung cancer[60] seems that their combinations are not contraindicated. Another study has shown that resistance to taxanes can be reduced by increasing the duration of exposure in P-gp expressing cells, but no t in the taxane-resistance cell line which does not express P-gp[61]. Since the resistance was highly drug specific and none of the cell lines was resistant to all drugs, identifying specific mechanism of drug resistance phenotype in certain tumor is helpful in selecting non-cross-resistant regiments and appropriate reversal agents.

REFERENCES

1 Wang XP. Studies on pancreatic diseases in China: current status and prospective. *Shijie Huaren Xiaohua Zazhi*, 2000;8:843-846
2 Zhou ZH,Song MZ.Current therapies of pancreatic cancer.*Shijie Huaren Xiaohua Zazhi*,2000;8:214-215
3 Ridwelski K, Meyer F, Ebert M, Malfertheiner P, Lippert H. Prognostic parameters determining survival in pancreatic carcinoma and, in particular, after palliative treatment. *Dig Dis*, 2001;19:85-92
4 Xiang HS. Surgical treatment of pancreatic carcinoma with portal vein and liver matastasis. *Shijie Huaren Xiaohua Zazhi*, 1998;6(Suppl 7):507-508
5 Permert J, Hafstrom L, Nygren P, Glimelius B; SBU-group. Swedish Council of Technology Assessment in Health Care. A systematic overview of chemotherapy effects in pancreatic cancer. *Acta Oncol*, 2001;40:361-370
6 Wagman R, Grann A.Adjuvant therapy for pancreatic cancer: current treatment approaches and future challenges. *Surg Clin North Am*, 2001;81:667-681
7 Zhou HC, Wang CJ, Tian FZ. Perioperative local chemotherapy in patients with pancreatic carcinoma. *Shijie Huaren Xiaohua Zazhi*, 2000; 8(Suppl 8):61
8 Jia L, Yuan SZ. Progress of treatment of advanced pancreatic carcinoma with gemcitabine. *Shijie Huaren Xiaohua Zazhi*, 1999;7:985-986
9 Tsubouchi H, Takao S, Aikou T.Sensitivity of human pancreatic adenocarcinomatumor lines to chemotherapy, radiotherapy, and hyperthermia. *Hum Cell*, 2000;13:203-212
10 Lu Z, Kleeff J, Shrikhande S, Zimmermann T, Korc M, Friess H, Buchler MW. Expression of the multidrug-resistance 1 (mdr1) gene and prognosis in human pancreatic cancer. *Pancreas*, 2000;21:240-247
11 Liu ZM, Shou NH. Expression significance of mdr1 gene in gastric carcinoma tissue. *Shijie Huaren Xiaohua Zazhi*, 1999;7:145-146
12 Xu BH, Zhang RJ, Lu DD, Chen XD, Wang NJ. Expression of mdrl gene code d Pglycoprotein in hepatocellular carcinoma and its clinical significance. *Huaren Xiaohua Zazhi*, 1998;6:783-785
13 Zhang LJ, Chen KN, Xu GW, Xing HP, Shi XT. Congenital expression of m dr-1 gene in tissues of carcinoma and its relation with pathomorphology and prognosis. *World J Gastroenterol*, 1999;5:53-56
14 Kong XB, Yang ZK, Liang LJ, Huang JF, Lin HL. Overexpression of P-gly coprotein in hepatocellular carcinoma and its clinical implication.

World J Gastroenterol, 2000;6:134-135
15 Kornmann M, Danenberg KD, Arber N, Beger HG, Danenberg PV, Korc M.Inhibition of cyclin D1 expression in human pancreatic cancer cells is associated with increased chemosensitivity and decreased expression of multiple chemoresistance genes. *Cancer Res*, 1999 15; 59:3505-3511
16 Liang Y, Meleady P, Cleary I, McDonnell S, Connolly L, Clynes M. Selection with melphalan or paclitaxel (Taxol) yields variants with different patterns of multidrug resistance, integrin expression and *in vitro* invasiveness. *Eur J Cancer*, 2001;37:1041-1052
17 Yu LF, Wu YL, Zhang YP. Reversal of drug resistance in the vincristin E resistant human gastric cancer cell lines MKN28/VCR by emulsion of seminal oil of Brucea J avanica. *Shijie Huaren Xiaohua Zazhi*, 2001; 9:376-378
18 Fan K, Fan D, Cheng LF, Li C. Expression of multi drug resistance-related markers in gastric cancer. *Anticancer Res*, 2000;20:48 09-4814
19 Liu ZM, Shou NH, Jiang XH. Expression of lung resistance protein in patients with gastric carcinoma and its clinical significance. *World J Gastroenterol*, 2000;6:433-434
20 Aszalos A, Ross DD. Biochemical and clinical aspects of efflux pump related resistance to anti-cancer drugs. *Anticancer Res*, 1998;18:2937-2944
21 Sherman WH, Fine RL. Combination gemcitabine and docetaxel therapy in advanced adenocarcinoma of the pancreas. *Oncology*, 2001; 60:316-321
22 Rougier P, Adenis A, Ducreux M, de Forni M, Bonneterre J, Dembak M, Clouet P, Lebecq A, Bai lle P, Lefresne-Soulas F, Blanc C, Armand JP.A phase II study: docetaxel as first-line chemotherapy for advanced pancreatic adenocarcinoma. *Eur J Cancer*, 2000;36:1016-1025
23 Correia JJ, Lobert S. Physiochemical aspects of tubulin-interacting antimitotic drugs. *Curr Pharm Des*, 2001;7:1213-1228
24 Snyder JP, Nettles JH, Cornett B, Downing KH, Nogales E. The binding conformation of Taxol in -tubulin: A model based on electron crysta llographic density. *Proc Natl Acad Sci USA*, 2001;98:5312-5316
25 Mattson K.Neoadjuvant chemotherapy with docetaxel in non—small cell lung cancer. *Semin Oncol*, 2001;28(Suppl 9):33-36
26 Khayat D, Chollet P, Antoine EC, Monfardini S, Ambrosini G, Benhammouda A, Mazen MF, Sorio R, Borg-Olivier O, Riva A, Ramazeilles C, Azli N. Phase II study of sequential administration of docetaxel followed by doxorubicin and cyclophosphamide as first-line chemotherapy in metastatic breast cancer. *J Clin Oncol*, 2001;19:3367-3375
27 Gandara DR, Lara PN Jr, Goldberg Z, Lau DH. Integration of new chemotherapeutic agents into chemoradiotherapy for stage III non—small cell lung cancer: focus on docetaxel. *Semin Oncol*, 2001;28(Suppl 9):26-32
28 Baselga J, Tabernero JM. Weekly docetaxel in breast cancer: applying clinical data to patient therapy. *Oncologist*, 2001;6 (Suppl 3):26-29
29 Okada S, Sakata Y, Matsuno S, Kurihara M, Sasaki Y, Ohashi Y, Taguchi T. Phase II study of docetaxel in patients with metastatic pancreatic cancer: a Japanese cooperative study. Cooperative Group of Docetaxel for Pancreatic Cancer in Japan. *Br J Cancer*, 1999;80:438-443
30 Graziano F, Cascinu S.Docetaxel chemotherapy for pancreatic cancer: Do results support certainty? Italian Group for the Study of Gastrointestinal Tract Carcinomas. *J Clin Oncol*, 2000;18:445-446
31 Kapoor P, Ghosh A, Madhubala R.Isolation of a taxol-resi stant Leishmania donovani promastigote mutant that exhibits a multidrug-resistant phenotype. *FEMS Micro biol Lett*, 1999;176:437-441
32 Blade K, Menick DR, Cabral F. Overexpression of class I, II or IVb bet a-tubulin isotypes in CHO cells is insufficient to confer resistance to paclitaxel. *J Cell Sci*, 1999; 112:2213- 2220
33 Dumontet C, Duran GE, Steger KA, Beketic-Oreskovic L, Sikic BI. Resistance mechanisms in human sarcom a mutants derived by single-step exposure to paclitaxel (Taxol). *Cancer Res*, 1996; 56:1091-1097
34 Kapoor P, Ghosh A, Madhubala R. Isolation of a taxol-resistant Leishmania donovani promastigote mutant that exhibits a multidrug-resistant phenotype. *FEMS Microbiol Lett*, 1999;176:437-441
35 Chen L, Burger RA, Zaunbrecher GM, Cheng H, Lincoln AJ, Mallarino MC, Monk BJ, Khan SA. Protein kinase C isoform expression and activity alter paclitaxel resistance *in vitro*. *Gynecol Oncol*, 1999;72:171-179
36 Nehme A, Varadarajan P, Sellakumar G, Gerhold M, Niedner H, Zhang Q, Lin X, Christen RD. Modulation of docetaxel-induced apoptosis and cell cycle arrest by all- trans retinoic acid in prostate cancer cells. *Br J Cancer*, 2001;84:1571-1576
37 Childs S, Yeh RL, Hui D, Ling V. Taxol resistance mediated by transfection of the liver-specific sister gene of P-gly coprotein. *Cancer Res*, 1998;58:4160-4167
38 Gan Y, Wientjes MG, Au JL. Relationship between paclitaxel activity and pathobiology of human solid tumors. *Clin Cancer Res*, 1998;4: 2949-2955
39 Miller DW, Fontain M, Kolar C, Lawson T. The expression of multidrug resistance-associated protein (MRP) in pancreatic adeno carcinoma cell lines. *Cancer Lett*, 1996;107:301-306
40 Yang X, Staren ED, Howard JM, Iwamura T, Bartsch JE, Appert HE. Invasiveness and MMP expression in pancreatic carcinoma. *J Surg Res*, 2001;98:33-39
41 Liu B, Staren ED, Iwamara T, Appert HE, Howard JM. Effects of Taxotere on Invasive Potential and Multidrug Re sistance Phenotype in Pancreatic Carcinoma Cell Line SUIT-2. *World J Gastroenterol*, 2001; 7:143-148
42 Stavrovskaya AA. Cellular mechanisms of multidrug resistance of tumor cells. *Biochemistry*, 2000;65:95-106
43 Vanhoefer U, Cao S, Minderman H, Toth K, Scheper RJ, Slovak ML, Rustum YM. PAK-104P, a pyridine analogue, reverses paclitaxel and doxorubicin resistance in cell lines and nude mice bearing xenografts that overexpress the multidrug resistance protein. *Clin Cancer Res*, 1996;2:369-377
44 Dumontet C, Sikic BI. Mechanisms of action of and resistance to antitubulin agents: microtubule dynamics, drug transport, and cell death. *J Clin Oncol*, 1999; 17:1061-1070
45 Mechetner E, Kyshtoobayeva A, Zonis S, Kim H, Stroup R, Garcia R, Park er RJ, Fruehauf JP. Levels of multidrug resistance (mdr1) P-glycoprotein expression by human breast cancer co rrelate with *in vitro* resistance to taxol and doxorubicin. *Clin Cancer Res*, 1998;4:389-398
46 Draper MP, Martell RL, Levy SB.Indomethacin-mediated reversal of multidrug resistance and drug efflux in human and murine cell lines overexpressing MRP, but not P-glycoprotein. *Br J Cancer*, 1997;75: 810-815
47 Giannakakou P, Sackett DL, Kang YK, Zhan Z, Buters JT, Fojo T, Poruchynsky MS. Rao S, Orr GA, Chaudhary AG, Kingston DG, Horwitz SB. Paclitaxel-resistant human ovarian cancer cells have mutant beta-tubulins that exhibit impaired paclitaxel-driven polymerization. *J Biol Chem*, 1997;272:17118-17125
48 Ranganathan S, Benetatos CA, Colarusso PJ, Dexter DW, Hudes GR. Altered beta-tubulin isotype expression in paclitaxel-resistant human prostate carcinoma cells. *Br J Cancer*, 1998;77:562-566
49 Carles G, Braguer D, Dumontet C, Bourgarel V, Goncalves A, Sarrazin M, Rognoni JB, Briand C. Differentiation of human colon cancer cells changes the expression of beta-tubulin isotypes and MAPs. *Br J Cancer*, 1999;80:1162-1168
50 Veitia R, David S, Barbier P, Vantard M, Gounon P, Bissery MC, Fellous A.Proteolysis of microtubule associated protein 2 and sensitivity of pancreatic tumours to docetaxel. *Br J Cancer*, 2000;83:544-549
51 Rao S, Orr GA, Chaudhary AG, Kingston DG, Horwitz SB. Characterization of the taxol binding site on the microtubule. 2-(m-Azidobenzoyl)taxol photolabels a peptide (amino acids 217-231) of beta-tubulin. *J Biol Chem*, 1995;270:20235-20238
52 Burkhart CA, Kavallaris M, Band Horwitz S.The role of beta-tubulin isotypes in resistance to antimitotic drugs. *Biochim Biophys Acta*, 2001; 1471:O1-9
53 Kavallaris M, Burkhart CA, Horwitz SB. Antisense oligonucleotides to class III beta-tubulin sensitize drug-resistant cells to Taxol. *Br J Cancer*, 1999;80:1 020-1025
54 Sugawara I, Akiyama S, Scheper RJ, Itoyama S. Lung resistance protein (LRP) expression in human normal tissues in comparison with that of mdr1 and MRP. *Cancer Lett*, 1997;112:23-31
55 Fowler RE, Fookes RE, Lavin F, Bannister LH, Mitchell GH. Microtubules in Plasmodium falciparum merozoites and their impo rtance for invasion of erythrocytes. *Parasitology*, 1998;117:425-433
56 Ballestrem C, Wehrle-Haller B, Hinz B, Imhof BA.Actin-dependent lamellipodia formation and microtubule-dependent tail retraction control-directed cell migration. *Mol Biol Cell*, 2000;11:2999-3012
57 Otto T, Lummen G, Be A, Rubben H, Raz A.Tumor cell motility. A novel therapeutic target in bladder carcinoma, experimental and clinical results. *Adv Exp Med Biol*, 1999;462:469-476
58 Yvon AM, Wadsworth P, Jordan MA. Taxol suppresses dynamics of individual microtubules in living human tumor cells. *Mol Biol Cell*, 1999;10:947-959
59 Gonzalez-Garay ML, Chang L, Blade K, Menick DR, Cabral F. A beta-tubulin leucine cluster involved in microtubule assembly and paclitaxel resistance. *J Biol Chem*, 1999;274:23875-23882
60 Miller VA.Docetaxel (Taxotere) and vinorelbine in the treatment of adv anced non-small cell lung cancer: preliminary results of a phase I/II trial. *Semin Oncol*, 1997;24:S14-17
61 Zhan Z, Scala S, Monks A, Hose C, Bates S, Fojo T. Resistance to paclitaxel mediated by P-lycoprotein can be modulated by changes in the schedule of administration. *Cancer Chemother Pharmacol*, 1997; 40: 245-250

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