

# Detection of multidrug resistant gene 1 in pancreatic cancer

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**BACKGROUND:** The results are conflicting in detecting P-glycoprotein (P-gp) in pancreatic cancer. The aim of this study was to detect the expression of multidrug resistant gene 1 (MDR1) in pancreatic cancer cell lines.

**METHODS:** MDR1 mRNA and P-gp were detected by reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical assay (IHC) in three pancreatic cancer cell lines SW1990, CAPAN-1 and P3. P-gp functions were evaluated through the rhodamine extrusion test.

**RESULTS:** Two of the three cell lines expressed MDR1 positively at different levels. The rhodamine extrusion test showed that the percentage of positive cells in MDR (+) cells was significantly lower than that in MDR1 (-) cells. The results of IHC, RT-PCR and the rhodamine extrusion test were consistent with each other.

**CONCLUSION:** All of these methods are reliable in the detection of MDR1 in pancreatic cancer tissue, thus providing a guide for clinical chemotherapy of pancreatic cancer.

(*Hepatobiliary Pancreat Dis Int* 2004; 3: 307-310)

**KEY WORDS:** pancreatic cancer; drug-resistance; immunohistochemical assay

## Introduction

The expression of multidrug resistant gene 1 (MDR1) is a major factor for failure of chemotherapy in cancer patients.<sup>[1]</sup> The expression of P-gp has been found in neoplastic and normal tissues such as the adrenals,<sup>[2]</sup> kidney,<sup>[3]</sup> liver,<sup>[4]</sup> pancreas,<sup>[5]</sup> large intestine,<sup>[6]</sup> and jejunum<sup>[7]</sup> as well as in several cancer cell lines,<sup>[8,9]</sup> before or after induction by cyto-

toxic agents. Conflicting results have been reported in studies regarding P-gp in pancreatic cancer. One study reported the absence of P-gp in pancreatic cancer,<sup>[10]</sup> whereas another study reported its expression in the majority of pancreatic cancer samples.<sup>[11]</sup> We detected the expression of MDR1 in three pancreatic cancer cell lines through RT-PCR and IHC as well as the functional assessment of P-gp by the rhodamine extrusion test, thus providing an experimental basis for clinical detection of MDR1 in pancreatic cancers.

## Methods

### Materials

Human pancreatic cancer cell lines SW1990 and CAPAN-1 were provided by the PLA General Hospital, Beijing, China, and the Department of Pathology of Peking Union Medical College Hospital, Beijing provided P3. These cell lines were cultured in PRMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin solution. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### Detection of MDR1 mRNA

Total RNA was isolated in reference to the TRIzol reagent description. After the cell lines grew to 90% confluence, total RNA was extracted from the cell lines by using TRIzol reagent (Life Technologies, GIBCO BRL, Rockville, USA). The total RNA was quantitated by measuring its absorbance at 260 nm. Equal amounts of RNA were reverse transcribed through RT-PCR kit (DingGuo Co., China). Each tube contained a total volume of 50 µl of PCR reaction mixture, including 8 µl of cDNA, 5 µl of MDR1 primer pairs, 1 µl of β-actin primer pairs, 4.5 µl of buffer, 1 µl of randomized primer pairs, 1 µl of Taq enzyme, 1 µl of dNTP, and 29.5 µl of diethyl pyrocarbonate (DEPC)-treated distilled water. After an initial denaturation in a programmed thermocycler at 94 °C for 5 minutes, PCR was carried out for 30 cycles following the thermal profile: denaturing at 94 °C for 45 seconds, annealing at 65 °C for 45 seconds, and extension at 72 °C for 45 seconds, with an extra 10-minute extension for the last cycle. Af-

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This study was supported by a grant from the Excellent Young Teachers Foundation of the Ministry of Education, China.

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ter completion of the amplification cycles, 5  $\mu$ l of each PCR product was treated through electrophoresis at 100 V for 20 minutes on a 1% agarose gel, together with a 100-bp DNA marker. Both target and control ( $\beta$ -actin) gene sequences were coamplified in the same tube. Gene expression was normalized to  $\beta$ -actin transcript; this was noted for relative expression level (REL); REL = densitometric value of target gene/densitometric value of  $\beta$ -actin. The primers of MDR1 and  $\beta$ -actin were designed as Genbank, MDR1 upper stream 5'-ACTGAGCCTGGAGGGAAGA-3', down stream 5'-CCAC-CAGAGAGCTGAGTTCC-3',  $\beta$ -actin upper stream 5'-AACTGGGACATGGAGAAAATC-3', down stream 5'-AGGAAGGAAGGCTGGAAGAGTGC-3'.

### Detection of P-gp by IHC

Immunohistochemical assay was performed with the strept avidin-biotin complex (SABC) technique. After the cell lines were 90% confluence under a microscope, the slides were washed in a phosphate-buffered saline (PBS) buffer, fixed with acetone, and incubated with 3% H<sub>2</sub>O<sub>2</sub>. The slides were incubated again with normal goat serum at room temperature for 20 minutes to block unspecific binding before adding specific monoclonal P-gp antibodies (overnight, 4 °C). After washing with PBS buffer, biotinylated goat anti-rat immunoglobulin (IgG) secondary antibodies were added at 37 °C for 20 minutes. After washing with PBS buffer and incubation with SABC at 37 °C for 20 minutes, the slides were incubated with dimethylamino-azobenzene butter (DAB) to dye.

### Evaluation of P-gp functions by rhodamine extrusion test<sup>[12]</sup>

The cell lines were harvested in a logarithmic growth phase with 0.25% trypsin and re-suspended in RPMI 1640 medium at  $1 \times 10^6$ /ml with 0.15  $\mu$ g/ml of rhodamin, and was incubated at 37 °C for 30 minutes in the dark. After incubation, the cells were washed twice with and re-suspended in ice-cold rhodamin-free RPMI 1640 medium with 5  $\mu$ mol/L Ver. The accumulation of rhodamine in the cells were analyzed using flow cytometry.

### Statistical analysis

The data were analyzed using SPSS 10.0 software.

## Results

### Expression of MDR1 mRNA

The expression of MDR1 mRNA was termed as the OD ratio of MDR1/ $\beta$ -actin. MDR1/ $\beta$ -actin <0.05 was defined negative (-) and MDR1/ $\beta$ -actin >0.05 was positive (+). The expression of MDR1 mRNA was significantly higher in SW1990 than in CAPAN-1; how-

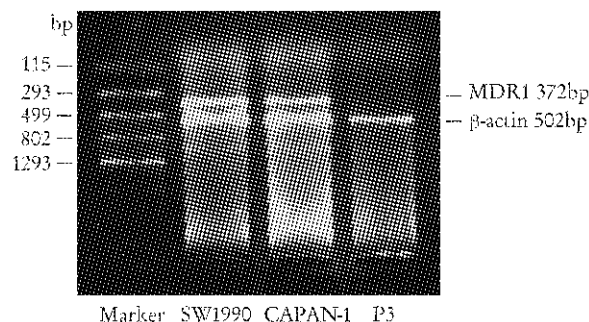


Fig. 1. Expression of MDR1 mRNA in three pancreatic cell lines.

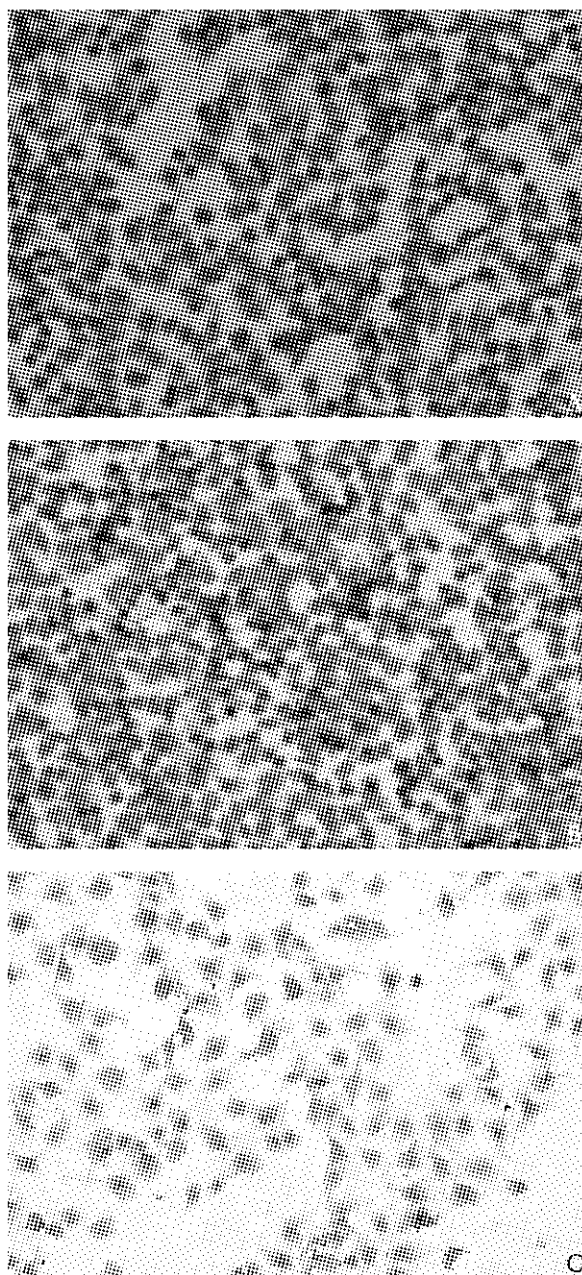


Fig. 2. Expression of P-gp in three cell lines. A; SW1990 (original magnification  $\times 100$ ); B; CAPAN-1 (original magnification  $\times 100$ ); C; P3 (original magnification  $\times 200$ ).

**Table.** Results of rhodamine extrusion test in pancreatic cell lines

Cell lines	Percentage of positive cells (%) *
SW1990	58.3
CAPAN-1	79.0
P3	99.5

\* :  $P < 0.01$ .

ever, P3 expressed no MDR1 (Fig. 1).

### Expression of IHC

The expression of P-gp was termed as - (<5%), + (5%-30%), ++ (30%-75%), +++ (>75%) according to the percentage of positively stained cells. The expression of P-gp in SW1990, CAPAN-1 and P3 was ++, +, -, respectively (Fig. 2).

### Rhodamine extrusion test

Rhodamine, a type of fluorescent dye, entered cells passively, whereas P-gp, a membrane protein with energy dependent drug pump, extruded rhodamine out of the cells. Thus P-gp function was assessed by detecting positive cells percentage via flow cytometry. The results indicated that P-gp function in SW1990 was stronger than that in CAPAN-1; however, P3 did not show P-gp function (Table).

### Discussion

The concept of MDR first proposed by Biedle and Riehm<sup>[13]</sup> in 1970 refers to tumor cells resistance to a variety of drugs with different structures and functions. Recent basic and clinical studies have shown that chemo-resistance of most tumors are related to the expression of the MDR1 gene,<sup>[14,15]</sup> and its protein P-gp, a cell membrane protein with energy dependent drug pump, which can pump a lot of agents out of cells.<sup>[16]</sup> Thus lowering the concentration of drugs in cells can lead to resistance.

It was reported that most malignant tumors expressed MDR1 differently.<sup>[17]</sup> Pancreatic cancer is a common digestive tumors with poor prognosis, also with a characteristic of being insensitive to chemotherapy and radiotherapy.<sup>[18]</sup> The reported effective rate of chemotherapy for patients with no chance of surgery is only 30%.<sup>[19]</sup> Few studies concentrated on the expression of MDR1 in pancreatic cancer.<sup>[11]</sup>

MDR1 detection methods in pancreatic cancer include RT-PCR, IHC, and functional test, which are accurate and significant in clinical practice. In this study, two out of three cell lines expressed MDR1, which was consistent with that reported elsewhere,<sup>[21]</sup> suggesting possible reasons for failure of chemotherapy in patients with pancreatic cancer. Moreover, pancreatic cancer cell lines expressed MDR1 differently, and P-gp function

was positively related to the levels of MDR1 expression. These indicated why chemotherapeutic efficacy is greatly different in different patients with pancreatic cancer. This is why individual use of chemotherapy is necessary clinically.

Many factors influence the results of MDR1 detection, and the methods used in such detection accounts for much. Nowadays, various methods are used in the detection of MDR1, including RT-PCR<sup>[21]</sup> and IHC.<sup>[22]</sup> RT-PCR, quantifying the MDR1 at mRNA level, is a more sensitive but complicated method with increased false positive results. Compared with RT-PCR, IHC is simple but less sensitive. Both methods have limitations, and their combination is crucial for reliable results. In addition, the rhodamine extrusion test is also significant for the assessment of P-gp function.<sup>[23]</sup> In our study, the results of the three methods were consistent with each other. Therefore we presume that all these methods are reliable in the detection of MDR1 in pancreatic cancer tissues. Consequently chemotherapy regimen should be carefully designed according to the expression of MDR1 and MDR-related drugs should be ruled out for patients with positive MDR.

### Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

### References

- 1 Pirker R, Wallner J, Geissler K, Linkesch W, Haas OA, Bettelheim P, et al. MDR1 gene expression and treatment outcome in acute myeloid leukemia. *J Natl Cancer Inst* 1991;83:708-712.
- 2 Scheffer GL, Scheper RJ. Drug resistance molecules: lessons from oncology. *Novartis Found Symp* 2002;243:19-31; discussion 31-37, 180-185.
- 3 Beck WT, Grogan TM, Willman CL, Cordon-Cardo C, Parham DM, Kuttesch JF, et al. Methods to detect P-glycoprotein-associated multidrug resistance in patients' tumors: consensus recommendations. *Cancer Res* 1996;56:3010-3020.
- 4 Grude P, Conti F, Menecier D, Louvel A, Houssin D, Weill B, et al. MDR1 gene expression in hepatocellular carcinoma and the peritumoral liver of patients with and without cirrhosis. *Cancer Lett* 2002;186:107-113.
- 5 Pileri SA, Sabatini E, Falini B, Tazzari PL, Gherinzoni F, Michieli MG, et al. Immunohistochemical detection of the multidrug transport protein P170 in human normal tissues and malignant lymphomas. *Histopathology* 1991;19:131-140.
- 6 Ho GT, Moodie FM, Satsangi J. Multidrug resistance 1 gene (P-glycoprotein 170): an important determinant in gastrointestinal disease? *Gut* 2003;52:759-766.
- 7 Fromm MF. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. *Adv Drug Deliv Rev* 2002;54:1295-1310.
- 8 Zhou XJ, Chen WT, Li Q, He RG. Establishment and bio-

- logical characteristics of cisplatin resistant cell line from human tongue squamous cell carcinoma Tca8113. *Shanghai Kou Qiang Yi Xue* 2001;10:31-34.
- 9 Yu DS, Ma CP, Chang SY. Establishment and characterization of renal cell carcinoma cell lines with multidrug resistance. *Urol Res* 2000;28:86-92.
  - 10 Benard J, Bourhis J, Riou G. Clinical significance of multiple drug resistance in human cancers. *Anticancer Res* 1990;10:1297-1302.
  - 11 Suwa H, Ohshio G, Arai S, Imamura T, Yamaki K, Manabe T, et al. Immunohistochemical localization of P-glycoprotein and expression of the multidrug resistance-1 gene in human pancreatic cancer; relevance to indicator of better prognosis. *Jpn J Cancer Res* 1996;87:641-649.
  - 12 Ludescher C, Thaler J, Drach D, Drach J, Spitaler M, Gatteringer C, et al. Detection of P-glycoprotein in human tumor samples using rhodamine 123. *Br J Haematol* 1992;82:161-168.
  - 13 Biedler JL, Riehm H. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross-resistance, radioautographic, and cytogenetic studies. *Cancer Res* 1970;30:1174-1184.
  - 14 Petracca L, Onori P, Sferra R, Lucchetta MC, Liberati G, Grassi M, et al. MDR (multidrug resistance) in hepatocarcinoma clinical-therapeutic implications. *Clin Ter* 2003;154:325-335.
  - 15 Gariboldi MB, Ravizza R, Riganti L, Meschini S, Calcabrini A, Marra M, et al. Molecular determinants of intrinsic resistance to doxorubicin in human cancer cell lines. *Int J Oncol* 2003;22:1057-1064.
  - 16 Fedoruk MN, Gimenez-Bonafe P, Guns ES, Mayer LD, Nelson CC. P-glycoprotein increases the efflux of the androgen dihydrotestosterone and reduces androgen responsive gene activity in prostate tumor cells. *Prostate* 2004;59:77-90.
  - 17 Fillpits M, Suchomel RW, Dekan G, Stiglbauer W, Haider K, Depisch D, et al. Expression of the multidrug resistance-associated protein (MRP) gene in colorectal carcinomas. *Br J Cancer* 1997;75:208-212.
  - 18 Nazli O, Tansug T, Bozdog AD, Cln N, Kaymak E. Locoregional chemotherapy in pancreatic cancer. *Hepatogastroenterology* 1999;46:479-482.
  - 19 Rauch DP, Maurer CA, Aebi S, Pampallona S, Friess H, Ludwig CU, et al. Activity of gemcitabine and continuous infusion fluorouracil in advanced pancreatic cancer. *Oncology* 2001;60:43-48.
  - 20 Lu Z, Kleeff J, Shrikhande S, Zimmermann T, Korc M, Friess H, et al. Expression of the multidrug-resistance 1 (MDR1) gene and prognosis in human pancreatic cancer. *Pancreas* 2000;21:240-247.
  - 21 Cai X, Wang T, Shi L. Expression and clinical implication of multidrug resistance gene and multidrug resistant-associated protein gene in patients with hypopharyngeal squamous cell carcinoma. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 1999;34:173-176.
  - 22 Raspollini MR, Pinzani P, Pazzagli M, Baroni G, Taddei A, Amunni G, et al. Multidrug resistance in ovarian cancer: comparing an immunocytochemical study and ATP-tumor chemosensitivity assay. *J Chemother* 2002;14:518-525.
  - 23 Wuchter C, Leonid K, Ruppert V, Schrappe M, Buchner T, Schoch C, et al. Clinical significance of P-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. *Haematologica* 2000;85:711-721.

*Received March 3, 2003*

*Accepted after revision February 28, 2004*