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Primer-dependent Amplification of *mdr*1 mRNA by Polymerase Chain Reaction

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We have developed a sensitive reverse transcription (RT)-polymerase chain reaction (PCR) to evaluate multidrug resistance in various tissues, using seven *mdr*-specific primer sets. *mdr*1 mRNA expression was noted in 16/197 samples. However, the apparent degree of *mdr*1 mRNA expression depended on the sequence of the primers employed. These findings suggest that more than two sets of primers should be used for effective RT-PCR.

Key words: *mdr*1 mRNA — Polymerase chain reaction — Cancer

P-Glycoprotein (P-GP) is known to be present in the membranes of cancer cells resistant to adriamycin, Vinca alkaloids, colchicine and other agents.[1-3] The prognosis of cancer patients showing a large quantity of P-GP in the tumor cell membranes is poor.[4-9] Thus, it is important to evaluate the degree of multi-drug resistance (MDR) in cancer cells prior to treatment with anticancer drugs. MDR can be evaluated at either the protein level or gene level. Although immunohistochemistry is useful for detection of P-GP-bearing cancer cells, the methodology does not give any useful information about MDR if expression of P-GP is below the detectable level. On the other hand, it is possible to detect the gene encoding P-GP (*mdr*1 mRNA) if reverse transcription (RT) and polymerase chain reaction (PCR) are utilized. To determine which pair of primers is most sensitive for evaluation of MDR, we first synthesized six pairs of *mdr*1-specific primers and one pair of *mdr*3-specific primers using a DNA synthesizer (Cyclone, USA) (Fig. 1).[10,11] Then, total RNA was isolated from KB-8-5, a KB variant, established in the laboratory of Dr. I. Pastan according to the manual by Maniatis *et al.*[1,12] RT was carried out as follows. After the total RNA had been heated at 95°C for 10 min, a mixture containing 2 μg of total RNA, 10 μl of 5× buffer (BRL, USA), 10 μl of dNTP (2.5 m*M* each), 5 μl of random primer (40 ng/μl), 200 U/μl reverse transcriptase and 22 μl of double-distilled water (DDW) was incubated at 37°C for 60 min. After termination of the reaction in iced water, 10 μl of the mixture was used for subsequent PCR. cDNA aliquots equivalent to 0.2 μg of total RNA were used for enzymatic amplification by PCR using 0.6 unit of Taq polymerase (Perkin Elmer Cetus) in the presence of 1 μM various *mdr*-specific primers (Fig. 1) and β_2-microglobulin-specific primers (1556–1575 and 3558–3677 in the genomic sequence) (the size of the amplified band: 261 bp).[13]

Thirty cycles of PCR were carried out in a 100 μl volume using a thermal cycler. Each cycle included 1 min of denaturation at 94°C, followed by 5 min of primer annealing and extension at 55°C, 60°C or 65°C. As shown in Fig. 2, detection of *mdr*-specific amplified cDNA bands was dependent upon the primer sequence. The most sensitive set of primers was set F, followed by set E. Weaker bands were recognized for primer set B and G. No amplified cDNA bands were recognized for primer sets C and D in agarose gel. Though we chose 55°C, 60°C or 65°C for annealing and 65°C for extension, multiple repeated experiments produced similar results. The reason for the dependence of effective amplification on a particular sequence of primers remains to be clarified. At the same time, we performed Southern blot hybridization of *mdr* cDNA, and the results parallelled the degree of amplification of various sets of primers shown by PCR (data not shown). We used ethidium bromide for visualization of the amplified DNA bands. Thus, this RT-PCR was as sensitive as Northern hybridization. However, PCR was much more sensitive than slot blot hybridization or Northern hybridization, when PCR was carried out in the presence of [α-^{32}P]dCTP.[14]

We then carried out RT-PCR of various fresh tissues using primers A, E and F. When the three *mdr*1-specific amplified DNA bands were visualized by ethidium bromide staining, *mdr*1 mRNA was judged to be present. As shown in Table I, we recognized amplified *mdr*1-specific bands in agarose gel for placenta (5/5), adrenal (1/1), adenoma of the adrenal (1/1), carcinoma of the thyroid (2/44), malignant lymphoma (2/21), colonic cancer

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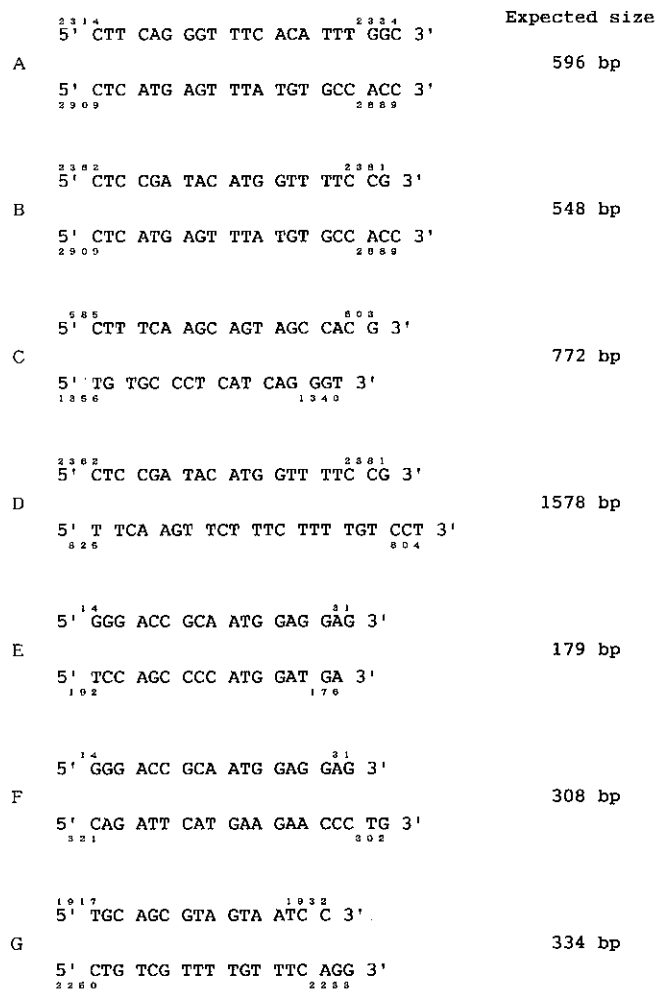

Fig. 1. *mdr*1 and *mdr*3-specific primers used in this study. The positions were counted from the start site of *mdr*1 or *mdr*3 cDNA. The primer sets A, B, D, E, F and G are *mdr*1-specific, and primer set C is *mdr*3-specific.

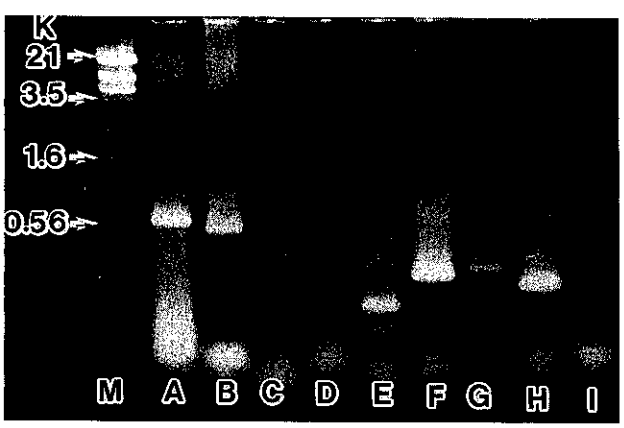

Fig. 2. PCR products separated in 2% agarose gel. Total RNA was converted to cDNA by reverse transcriptase and the cDNA was subjected to PCR at 94°C for denaturation, at 65°C for annealing and at 65°C for primer extension. M, DNA size marker; A, primer set A; B, primer set B; C, primer set C; D, primer set D; E, primer set E; F, primer set F; G, primer set G; H, β_2-microglobulin-specific primer set; I, no primer.

(3/6), carcinoma of the uterus (1/2), and hepatoma (1/2). We could not find *mdr*1 mRNA expression in peripheral mononuclear cells from healthy volunteers. These data correlated well with the level of P-GP expression assessed by immunohistochemistry.[15] None of the positive cases had been treated with anti-cancer drugs.

Table I. Detection of *mdr*1 mRNA by RT-PCR

Tissue	Detection frequency
Thyroid	0/8
Placenta	5/5
Liver	0/2
Tonsil	0/2
Brain	0/2
Heart	0/1
Pancreas	0/1
Adrenal	1/1
Peripheral mononuclear cells	0/2
Adenoma of the thyroid	0/25
Meningioma	0/1
Hyperthyroidism	0/7
Pleomorphic adenoma	0/1
Adenoma of the adrenal	1/1
Pheochromocytoma	0/1
Thymoma	0/1
Adenoma of the cecum	0/1
Hemangioma of the brain	0/1
Carcinoma of the thyroid	2/44
Malignant lymphoma	2/21
Mammary ca.	0/41
Leukemia	0/3
Lung ca.	0/4
Ovarian ca.	0/10
Colonic ca.	3/6
Pancreas ca.	0/1
Ca. of the uterus	1/2
Hepatoma	1/2
Total	16/197

Expression of *mdr*1 mRNA was examined by RT-PCR using primer sets A, E and F. When the three *mdr*1-specific amplified bands were visualized by ethidium bromide staining, *mdr*1 mRNA was assessed to be present.

We have thus demonstrated mdr1-specific amplified bands by RT-PCR, and conclude that the detection depends on the sequence of the primers employed. Our finding suggest that more than two sets of primers should be used for effective RT-PCR.[16]

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