RESEARCH

A YAC-based Contig of 1.5 Mb Spanning the Human Multidrug Resistance Gene Region and Delineating the Amplification Unit in Three Human Multidrug-resistant Cell Lines

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A contig of 21 nonchimeric yeast artificial chromosomes (YACs) has been assembled across 1.5 Mb of the multidrug resistance (MDR) gene region located at 7q21, and formatted with four previously reported probes, six newly isolated probes, and three sequence-tagged sites (STSs) from internal and end fragments of YACs. A physical map of rare cutter restriction enzyme sites across the region was also constructed by pulsed-field gel electrophoretic (PFGE) analysis of four overlapping YAC clones. The amplification unit of this region in different cell lines was then determined by Southern blot analysis on the basis of the physical map and probes. Amplified DNA was located in extrachromosomal elements in human MDR cell lines studied here, and the size of the amplification unit was determined to be discrete in one MDR amplification but variable in others.

The acquisition of multidrug resistance (MDR) *in vitro* is commonly associated with the increased expression of the cellular surface protein, P-glycoprotein, which functions as an energy-dependent drug efflux pump, resulting in a decrease of intracellular drug concentration (Pastan and Gottesman 1987; Bradley et al. 1988; van der Bliek and Borst 1989). Mammalian P-glycoproteins are encoded by families of linked genes, of which there are two known members in humans (*PGY1* and *PGY3* which are also called *MDR1* and *MDR3*, respectively) (Roninson et al. 1984; Ueda et al. 1987; Chin et al. 1989; Bliek and Borst 1989; Lincke et al. 1990), and three members in mice (*mdr1a*, *mdr1b*, and *mdr2*) (Gros et al. 1986a,b; Devault and Gros 1990) and hamster (*pgp1*, *pgp2*, and *pgp3*) (Endicott et al. 1991).

PGY1 and *PGY3* have been mapped to human chromosome 7 (Fojo et al. 1989; Chin et al. 1989), and their three rodent counterparts have been localized on mouse chromosome 5 (Hsu et al. 1989). The entire human MDR locus covers ~230 kb, and the human *PGY1* and *PGY3* genes are closely linked, separated by only 34 kb of intergenic DNA region and transcribed in the same direction (Chin et al. 1989; Lincke et al. 1991; Matsuda et al. 1993). Although no MDR-related genes other than *PGY1* and *PGY3* have been detected by Southern blotting (Riordan et al. 1985; Gros et al. 1986a; Roninson et al. 1986), other genes related to drug resistance might be in the region.

From a mechanistic point of view, two aspects have been shown to be involved in the acquisition of MDR. First, many experimental results indicate that *trans*-activation of the MDR genes is associated with exogenous stimulation, including anticancer agents, carcinogens, and

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retinoic acid (Fairchild et al. 1987; Bates, et al. 1989; Kohno et al. 1989; Mickley et al. 1989). This activation, in turn, appears to be mediated through nuclear *trans*-acting factors (Kohno et al. 1992, 1994; Uchiumi et al. 1993; Asakuno et al. 1994). However, *trans*-activation via chromatin structure rather than *trans*-acting factor seems to play a role in some cases (Kohno et al. 1989). Moreover, our recent study has demonstrated that expression of the *PGY1* promoter-driven chloramphenicol acetyl transferase (CAT) is increased along with expression of the endogenous *PGY1* gene in human cancer cells during the early steps of selection by drugs but is decreased with the onset of *PGY1* gene amplification (Kohno et al. 1994). During the selection of resistant phenotypes by drugs, there appears to be some mechanistic interrelation between transcriptional activation and onset of gene amplification. Thus, it is important to survey for regulatory domains and genome structure across the region contributing to MDR.

A second factor in MDR is the frequent amplification of the *PGY1* gene (Riordan et al. 1985; van der Bliek et al. 1988). However, little is known about the structure of the "amplicon" or the mechanism of gene amplification. In general, amplified DNA is located in either a homogeneous staining region (HSR) or in extrachromosomal elements such as double minute chromosomes (DMs) (Cowell 1982; Stark and Wahl 1984). It has been proposed that some extrachromosomal elements have the potential to integrate into chromosomes, resulting in HSRs (Biedler 1982; Windle et al. 1991). On the other hand, double-strand break-rejoining mechanisms seem to be involved in the generation of HSR (Kuo et al. 1994), and DMs appear to be formed directly from prematurely condensed chromosomes of replicating micronuclei (Sen et al. 1989). Once again, studies of the structure of amplification region may aid in further analysis.

Yeast artificial chromosomes (YACs) (Burke et al. 1987) have proven to be a powerful tool to clone large genomic fragments that cover extensive regions of DNA. As the first step in the isolation of genes, assessment of their correlation with drug resistance, and analysis of the structure of the MDR gene region in MDR cell lines, YAC clones were isolated and assembled to construct a contig spanning 1.5 Mb including the MDR gene region. YACs were obtained from three different libraries (Brownstein et al. 1989; Scherer et al. 1992; Green et al. 1995). Nonchimeric clones

were selected, and the contig was then constructed and mapped further as part of the study; the extent of the amplification unit of the MDR gene region in two independent drug-resistant cell lines was then determined.

RESULTS

Construction of the YAC Contig

Three YAC libraries were used as sources to collect YAC clones carrying the human MDR genes (*PGY1* and *PGY3*). A primer pair was designed from the promoter region of the *PGY1* gene (Table 1) and was used to isolate YAC clones from Washington University total human library and chromosome 7-specific libraries. Additional clones were isolated from the libraries by PCR-based screening (Green and Olson 1990) using STSs produced from the end clones of yWSS167 and yWSS172. Clones were also isolated from another chromosome 7-specific YAC library (Scherer et al. 1992) using a hybridization-based approach (Table 2). The contig was assembled using criteria based on STS content (Fig. 1). Overlaps were detected by dot blot hybridization of probes, including end clones isolated from some YAC clones (Table 1), or by PCR for STSs (Table 1), and were confirmed by Southern blot analysis (data not shown). Clones that had been shown to be chimeric or gave ambiguous results were not analyzed further except for yWSS167 and yWSS2393, because the insert ends of these clones were used to extend the contig. Distances within the contig (Fig. 1) were estimated from the size of the YACs (Table 2), as determined by pulsed-field gel electrophoresis (PFGE).

The order of the DNA markers has been determined unambiguously by contig mapping as D7S2622–D7S574–D7S2625–D7S159–D7S2621–D7S1646–*PGY3*–*PGY1*–D7S2626–D7S2624–D7S2628–D7S1642–D7S2623–D7S2627. The direction of these markers along the chromosome could also be determined by multicolor fluorescence in situ hybridization (FISH) analysis on prophase chromosomes (Fig. 2A). The arrangement of green (HSC7E718, which localizes on 7q21.1, and RFC2, which localizes on 7q11.23) and red (yWSS3740, which also localizes on 7q21.1) signals indicated that the order is cen–HSC7E718–yWSS3740–ter. The order of some of the markers around the contig has been determined as follows: 7cen–D7S165–D7S177–D7S574–7q–ter by somatic cell hybrid analysis

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Table 1. List of previously cloned and newly isolated DNA probes and STSs

DNA probe or STS	D-segment number	Source of DNA segment	Size (kb)	Method of isolation	PCR primer	Source
E515cdA-6	D7S574	HSC7E515	1.0	cDNA, direct selection	—	Scherer et al. (1993)
pA35	D7S159	flow-sorted chromosome 7	4.0	random selection	—	Rommens et al. (1988)
pA85	D7S165	flow-sorted chromosome 7	4.1	random selection	—	Rommens et al. (1988)
pA164	D7S177	flow-sorted chromosome 7	2.0	random selection	—	Rommens et al. (1988)
E66cd2	D7S577	HSC7E66	0.5	cDNA, direct selection	—	Scherer et al. (1993)
E502cd135	D7S569	HSC7E502	0.75	cDNA, direct selection	—	Scherer et al. (1993)
pMDR1-3 ^a	PGY1	pMDR 105	0.8	cDNA	—	Gottesman et al. (1987)
pMDR1-5 ^b	PGY1	pMDR-H3	1.0	Cloning from genomic DNA	—	Kohn et al. (1990)
pE718L	D7S2621	HSC7E718	0.5	bubble PCR	—	this work
pE718R	D7S2622	HSC7E718	0.5	bubble PCR	—	this work
pWSS1336L	D7S2623	yWSS1336	0.5	bubble PCR	—	this work
pWSS1336R	D7S2624	yWSS1336	0.8	bubble PCR	—	this work
pWSS1599L	D7S2625	yWSS1599	0.5	bubble PCR	—	this work
pWCC2393L	D7S2626	yWSS2393	0.9	bubble PCR	—	this work
pWSS3740L	D7S2627	yWSS3740	0.5	bubble PCR	—	this work
pWSS3740R	D7S2628	yWSS3740	0.9	bubble PCR	—	this work
sMDR1	PGY1	—	—	—	5'-AACGGAAGCCAGAACATTCC-3' 5'-AGGCTTCCTGTGGCAAGAG-3' 5'-AGTGGTGTTCAGAAATGGG-3' 5'-CTGTAGCAAAAGTTCATAA-3' 5'-CCGTTTAAATTCAGCTC-3' 5'-ATCTTCTTAATGTGGGTC-3' 5'-GTTTAAAGATGAGTGGTATG-3' 5'-AGATGAGTAGGAAGTGTGC-3'	Kohn et al. (1990)
sMDR3	PGY3	—	—	—	5'-AACGGAAGCCAGAACATTCC-3' 5'-AGGCTTCCTGTGGCAAGAG-3' 5'-AGTGGTGTTCAGAAATGGG-3' 5'-CTGTAGCAAAAGTTCATAA-3' 5'-CCGTTTAAATTCAGCTC-3' 5'-ATCTTCTTAATGTGGGTC-3' 5'-GTTTAAAGATGAGTGGTATG-3' 5'-AGATGAGTAGGAAGTGTGC-3'	Van der Bliek et al. (1988)
sWSS899	D7S1646	yWSS899	—	bubble PCR and sequencing	—	this work
sWSS897	D7S1642	yWSS897	—	bubble PCR and sequencing	—	this work

^aProbe specific for 3' end of PGY1.^bProbe specific for 5' end of PGY1.

Table 2. List of YAC clones used in this study

Total human (Brownstein et al. 1989)			Chromosome 7 (Green et al. 1995)			Chromosome 7 (Scherer et al. 1993)		
YACs	length (kb)	comments ^a	YACs	length (kb)	comments ^a	YACs	length (kb)	comments ^a
yWSS166	770	chimera	yWSS1033	290		HSC7E106	370	
yWSS167	580	chimera	yWSS1336	300		HSC7E307	600	ambiguous
yWSS168	120		yWSS1565	350		HSC7E424	500	ambiguous
yWSS169	220	ambiguous	yWSS1599	280		HSC7E462	450	deletion?
yWSS170	400	chimera	yWSS2136	400, 250	ambiguous	HSC7E481	900	
yWSS171	260	rearrangement	yWSS2393	550	chimera	HSC7E515	540	
yWSS172	240		yWSS3740	590		HSC7E656	850	
yWSS173	310		yWSS3843	200		HSC7E659	220	ambiguous
yWSS174	120	ambiguous	yWSS3908	790		HSC7E661	370	
yWSS175	160		yWSS3931	800		HSC7E708	900	
yWSS176	370, 250	rearrangement	yWSS3953	450		HSC7E718	360	
yWSS177	600, 400	rearrangement				HSC7E734	860	
						HSC7E763	1400	ambiguous
						HSC7E859	500	ambiguous

^aDefinition of comments are as follows: (deletion?) Hybridization signal is not detected by the probes, which is expected to localize inside of the insert, although outside probes hybridize to the clone. Results were confirmed by Southern blotting. Shortening of size has not been determined yet. (Chimera) Determined by hybridization or PCR assay of probes or STSs isolated from end of each clones to hybrid cell panel. (Rearrangement) Unexpected size is detected by Southern blot analysis. (Ambiguous) Hybridization results are not explained simply. They may have some deletion, rearrangement, and/or chimerism.

(Scherer et al. 1994a): 7cen—the region corresponding to the contig constructed here—D7S577–D7S569–7q-ter by YAC contig analysis has been detailed two separate studies (Scherer et al. 1993, 1994b). The complete order of the markers is 7cen–D7S165–D7S177–D7S2622–D7S574–D7S2625–D7S159–D7S2621–D7S1646–PGY3–PGY1–D7S2626–D7S2624–D7S2628–D7S1642–D7S2623–D7S2627–D7S577–D7S569–7q-ter.

Restriction Mapping of the YAC Contig

To confirm overlaps and consistency of the clones and to refine the contig map more accurately, restriction mapping was carried out on four clones, yWSS3740, HSC7E734, HSC7E656, HSC7E515, covering the entire region (Fig. 3), by indirect end labeling methods as described by Burke et al. (1987). Six putative CpG islands (Bird 1986) were observed (Fig. 3). The second one from the left was located very near the 5' end of the *PGY1* gene, and likely is linked to the promoter of this gene (Ueda et al. 1987; Kohno et al.

1990; Uchiumi et al. 1993). Because CpG islands are usually linked to genes, the other five CpG islands may mark new genes that have not been identified yet.

Amplification Unit of MDR Gene Region in MDR Cell Lines

The availability of the YAC map facilitates the study of amplification units of the MDR gene region. Three MDR cell lines were analyzed by Southern blot hybridization using the probes described above (Fig. 4) and the amplification unit in each cell lines were estimated by the relative amplification ratio of the drug resistant cell lines to the parental cell lines according to the calculation described in Methods. The KB-C1 cell line was originally isolated as a colchicine-resistant cell, and the MDR1 region was amplified in an extrachromosomal DNA state (Akiyama et al. 1985; Fojo et al. 1985; Schoenlein et al. 1992). Kst-V100 cells were isolated by stepwise selection with increasing doses of vincristine (up to 100 ng/ml),

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Figure 1 Contig map of YAC clones from the MDR region. Probes and STs are shown at the top (see Table 1). (○) The presence of markers or STs on the corresponding clones. Ambiguous clones, including chimeric, deletion, and rearrangement shown in the Table 2, were not included here.

and Kst-V500 cells were isolated further with higher doses of vincristine (up to 500 ng/ml), respectively (Kohno et al. 1994). Metaphase spreads showed that both Kst-V100 and Kst-V500 had typical aneuploidic chromosomes (Fig. 2B,C). FISH analysis demonstrated that the MDR1 region was amplified in DMs in both Kst-V100 and Kst-V500 cells. Amplification of the *PGY1* gene at its original loci was also observed in Kst-V100 cells.

Figure 5 summarizes the estimation of the amplification unit. The amplification unit of the MDR gene region in the KB-C1 cell was determined to be delimited in a 700- to 1300-kb range, consistent with Southern blot results on PFGE analyses of cellular DNA digested with *NotI* (890 kb; data not shown), as well as with results reported by Schoenlein et al. (1992). The amplification ratio of KB-C1 to parental KB is uniform (16.4 times) across the amplified region.

In contrast with KB-C1 cells, the amplification unit in Kst-V100 and Kst-V500 cells is

longer, >1.5 Mb, with a gradient in the degree of amplification. The amplification ratio of Kst-V100 to parental Kst-6 is 1.5, 2.4, 2.8, 2.7, 2.3, and 2.0 times, and the ratio of Kst-V500 is 1.6, 3.8, 10.0, 7.4, 4.3, and 3.3 times at loci pE718R, pA35, PGY1, pWSS1336R, pWSS1336L, and pWSS3740L, respectively. Although amplification was detected in Kst-V100 at E66cd2 and pA164 loci, which are the outer-most markers used in this study, it was not detected in Kst-V500. These results suggest that the genomic region around the MDR genes is amplified as a huge zone at early stages (Kst-V100) of drug selection, and the unit becomes shorter and shorter during acquisition of higher resistance (Kst-V500). As a result, the ratio of amplification is highest at the middle of the amplification unit and lower at the ends.

DISCUSSION

The structure of human MDR genes *PGY1* and *PGY3* has been examined (Chin et al. 1989; Chen

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Figure 2 (A) Multicolor FISH on prophase chromosomes with pairs of adjacent YACs from the MDR region. FITC-labeled HSC7E718 and rhodamine-labeled yWSS3740 visualized on the same prophase chromosome 7. The arrangement of green and red signals indicates that the order is cen–HSC7E718–yWSS3740–ter. The marker clone (RFC2) was also detected by FITC and indicated by the arrowhead. (B) Detection of the amplified region in drug-resistant cell line, Kst-V100 by in situ hybridization. The photomicrograph shows a representative metaphase spread from Kst-V100 cells hybridized to biotinylated total YAC DNA. The hybridized probe was detected with fluorescence-conjugated avidin and by fluorescence microscopy. The arrows indicate three original copies of the *PGY1* gene, which is also observed in parental KB (Kst-6) cells (data not shown). The signal was found to be much stronger in the region indicated by arrows in Kst-V100 cells than in the parental cells. The arrowheads indicate DMs. Typical images of FISH analysis are presented here, and similar results were observed in >10 fields. (C) Detection of the amplified region in Kst-V500 by in situ hybridization. The arrow indicates three original copies of the *PGY1* gene as described above. Many DMs are observed.

et al. 1990; Lincke et al. 1991) and a long-range physical map of this region is now available. In this study a contig and restriction map spanning the region are based on YACs. Moreover, ambiguous clones, including chimeras, were eliminated from the starting materials to build the contig, and the YAC clones and the map presented here are likely to be reliable sources for further detailed analysis of the region.

Because a physical map spanning 400 kb

around *PGY1* and *PGY3* has been reported previously (Lincke et al. 1991), we have compared our map with it to assess the integrity and consistency of the YAC system. We detected all four *SfiI* clusters (five sites) and three *BssHII* clusters (four sites) reported by Lincke et al. (1991), as well as an additional *BssHII* site in the middle of the *MDR1* gene. This new site probably is not a result of the experimental artifact because we detected this site in three independent YAC clones (Fig. 3). There may be some polymorphism among HepG 2 cell lines used earlier and the somatic cell hybrid cell line 4AF1/106/KO15 used for the YAC library constructed by Scherer et al. (1993). Another explanation for the difference in the restriction maps is that the new *BssHII* site may have been methylated in the study of Lincke et al. (1991) (genomic DNA was mapped). However, yeast DNA (which has no known methylation system) was used in our experiments, and the site is now susceptible to restriction enzyme digestion by *BssHII*.

At least six putative CpG islands were identified from the restriction map. Three MDR-related genes are located in tandem in the mouse and hamster genome (Gros et al. 1986a; Devault and Gros 1990; Endicott et al. 1991). The *PGY1* gene encoding membrane P-glycoprotein is often

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Figure 3 Restriction map of the contig. Restriction sites of three rare-cutter enzymes are shown (*middle*) with the relative positions of *PGY1* and *PGY3* (top) and the four YAC clones used in this analysis (*bottom*). Six putative CpG islands, which are defined as the cluster of two or more CpG-containing rare-cutter restriction sites are indicated by open boxes.

correlated with the acquisition of MDR phenotype in cancer cells, possibly because of the enhanced efflux pump of anticancer agents (Riordan et al. 1985; van der Bliek et al. 1988). P-glycoprotein also has chloride channel activity like the cystic fibrosis transmembrane conductance regulator (CFTR) (Gill et al. 1992; Higgins 1992). Isolation of genes linked to CpG islands thus may be useful in detecting any new MDR-related or ion channel gene.

We present here a molecular basis for two different mechanisms generating DMs. The amplification unit in the KB-C1 cell line was determined as a discrete 890-kb region encompassing the *PGY1* and *PGY3* genes. This is consistent with the results reported by Schoenlein et al. (1992). The amplification unit in the Kst-V500 cell line spans >1.5 Mb, and the middle of the unit, near the *PGY1* gene, was amplified more than the end of the unit. DMs are often observed in KB-C4 cells, which were isolated by stepwise selection to further increasing doses of colchicine from KB-C1 cells (Fojo et al. 1985; Schoenlein et al. 1992). Although DMs have not been observed in the KB-C1 cells, the data reported by Schoenlein et al. (1992) and extended in this study, which show a

Figure 4 Southern blot analysis of the DNA isolated from drug-resistant cell lines. Genomic DNAs were isolated from KB-C1 and Kst-V500 and their parental cell lines, KB and Kst-6, respectively, and subjected to Southern blot analysis as described in Methods. Probes used are indicated by arrows.

Figure 5 Schematic representation of the amplified region around the MDR gene region. Relative copy numbers of each site in the drug-resistant cell line to parental cell lines have been estimated from the results shown in Fig. 4 and are represented by the thickness of the bars shown. Thin bars represent single copy. Markers of each site are shown at the top. The positions of the *PGY1* and *PGY3* genes are also indicated ($\square$). The distances between pA164 and pE718R, and pWSS3740L and E66cd2, have not been determined.

discrete amplification unit of 890 kb, support a model of gene amplification in which small circular or submicroscopic extrachromosomal DNA precedes or multimerizes to form cytologically detectable DMs. Recently, a complete physical map of an amplicon was determined in two cases, the adenosine deaminase gene region (Nonet et al. 1993) and the *N-myc* gene region (Schneider et al. 1992). Both results support the notion that the amplicon in DM-type cells is 500 kb and 1.2 Mb, respectively. A discrete circular configuration would be consistent with our results for KB-C1 cells.

Sen et al. (1989) proposed that DMs are generated from prematurely condensed replicating micronuclei in drug-treated CHO cells. This intriguing proposal would require that micronuclei are generated specifically from the MDR gene region (or perhaps from an adjacent region). The results presented here provide one molecular basis for the model proposed by Sen et al. (1989). An amplification unit can span a large zone of >1.5 Mb at the early step (Kst-V100) during stepwise selection of drug-resistant cell lines, but it is possible that the unit becomes shorter and the amplification ratio increases at later steps (Kst-

V500). This pattern of amplification fits the model proposed by Sen et al. (1989) and is typically observed in HSR-type cells (Stark et al. 1989).

The YAC-based contig should help to analyze additional drug-resistant cell lines. These cell lines could further the analysis of the basis of amplification and could aid in the determination of a possible minimum sequence extent for amplicon.

METHODS

YACs Containing Chromosome 7

YAC clones were isolated from three different libraries; a total human library constructed at the Washington University (Brownstein et al. 1989), a chromosome 7-specific library containing human DNA derived from GM10791 somatic cell hybrid (Green et al. 1995), and a chromosome 7-specific library containing human DNA derived from the somatic cell hybrid cell line 4AF1/106/KO15 (Scherer et al. 1992).

DNA Probes

Detailed descriptions of four previously obtained probes are given in Table 1. The seven new probes were isolated by the bubble-PCR method (Riley et al. 1990).

Synthetic Oligonucleotides

Several primer pairs were prepared for PCR. YAC end clones were isolated by the bubble-PCR method, and the DNA sequence was determined to develop STSs (Olson et al. 1989). The DNA sequences of the oligonucleotides are described in Table 1.

Cell Lines and Cell Cultures

The parent cell line KB-3-1 was subcloned from a human KB epidermoid carcinoma cell line. The colchicine-resistant sublines KB-C1 and KB-C4 were kindly provided by S.I. Akiyama (Akiyama et al. 1985). Kst-6 was isolated from human KB cell lines after stable transfection of pMDR1. The vincristine-resistant sublines, Kst-V100 and Kst-V500, were isolated by increasing the concentration of vincristine in the selection media to 100 or 500 ng/ml (Nacalai Tesque, Inc., Kyoto, Japan), respectively (Kohno et al. 1994). Cells were grown as described previously (Kohno et al. 1988, 1994).

PCR Analysis

Genomic DNA (100 ng) was mixed with 1 μ M primer pairs and 1 unit of *Taq* DNA polymerase (Amersham Co., Buck-

inghamshire, UK). PCR was carried out in 5- μ l aliquots in a DNA Thermal Cycler (Astec Co., Fukuoka, Japan). An initial 10-min denaturation at 92°C was followed by 35 cycles of PCR. Each cycle included 1 min of denaturation at 92°C, followed by 2 min of primer annealing at 55°C, and 2 min of polymerization at 72°C. The last cycle of PCR included an additional 10-min extension at 72°C. Each reaction was analyzed by electrophoresis on a 10% polyacrylamide gel, and PCR products were detected after gel electrophoresis by ethidium bromide staining.

Southern Blot Analysis and Estimation of the Amplification Ratio

Genomic DNA digested with *Hind*III was separated on an 0.8% agarose gel and transferred to a nylon filter. DNA was cross-linked to the filter by UV irradiation. A ³²P-labeled DNA probe was prepared by the random priming method (Feinberg and Vogelstein 1983). Gene amplification was determined by a bioimaging analyzer (BAS 2000, Fuji Film Co., Tokyo, Japan). Radioactivity of the bands hybridized by each probe was measured directly by the BAS 2000 and was normalized by radioactivity of the band hybridized by β -actin probe on the same filter to correct for differences between lanes, which were caused by loading. The amplification ratio was then calculated from the normalized radioactivity of the drug-resistant cell lines divided by that of the parental cell lines.

FISH Analysis

Probe labeling and in situ hybridization conditions were completed as described elsewhere (Lichter et al. 1988). About 200 ng of labeled probes, 2 μ g of total yeast DNA without YAC, 2 μ g of human COT-1 DNA (GIBCO BRL, Gaithersburg, MD), and 4 μ g of salmon sperm DNA were ethanol-precipitated together and redissolved in 10 μ l of hybridization mixture [50% (vol/vol) formamide, 2 $\times$ SSC, 10% dextran sulfate]. After denaturation at 75°C for 5 min, the probes were preannealed at 37°C for 20 min to block signals derived from repetitive sequences and hybridized onto metaphase chromosomes. Following incubation overnight and subsequent posthybridization washes and blocking with Block Ace (Dainippon Pharmaceutical Co., Ltd.) in 4 $\times$ SSC, probes were detected by means of fluorescence isothiocyanate (FITC)-conjugated avidin (Boehringer Mannheim). Chromosomes were counterstained with 0.2 mg/ml of 4',6-diamidino-2-phenylindole (DAPI, Sigma). Prophase chromosomes for the YAC orientation were prepared by the method of Inazawa et al. (1994) using ICRF193 to stop cell cycle to prophase. To determine the orientation of the MDR gene region, two YAC clones, HSCE718 and yWSS3740, were labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively. The digoxigenin-labeled probe was detected with rhodamine-antidigoxigenin (Boehringer Mannheim). The Biotin-labeled *RFC2* gene (a subunit gene of human replication factor C) was used as a marker for its location on 7q11.23 (Okumura et al. 1995). Fluorescence signals were imaged using a Zeiss Axioskop epifluorescence microscope equipped with a cooled charge coupled device (CCD) camera (Photometrics, PXL 1400). Image acquisition was per-

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formed on a Macintosh Quadra 840 AV computer with the software program IPLab (Signal Analytics Co.). The images were then pseudocolored and merged using Adobe Photoshop 2.5J (Adobe Systems, Inc.). DAPI, rhodamine, and FITC images were shown in blue, red, and green, respectively. The merged images of FITC and DAPI were printed directly using a Fuji Pictography 3000 from a Macintosh computer.

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