Chemosensitivity Assessed by Collagen Gel Droplet Embedded Culture Drug Sensitivity Test, and MDR1, MRP1, and MRP2 mRNA Expression in Human Colorectal Adenocarcinomas

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Purpose. To evaluate chemosensitivity and its correlation with expression levels of the multidrug resistant transporter (MDR1) and the multidrug resistance-associated proteins 1 and 2 (MRP1, MRP2) mRNA in human colorectal adenocarcinomas.

Methods. Colorectal adenocarcinomas were obtained as surgical samples from 25 patients. The chemosensitivity of 12 anticancer drugs was assessed by the collagen gel droplet embedded culture drug sensitivity test (CD-DST). The expression levels of MDR1, MRP1, and MRP2 mRNA in colorectal adenocarcinomas were also evaluated by real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR).

Results. The chemosensitivity was successfully evaluated for 16 of 25 patients, and the anticancer drugs were effective against the samples showing a relatively high growth rate. Gemcitabine hydrochloride was found to be more promising than those often prescribed for the treatment of colorectal adenocarcinoma. There was no correlation of the mRNA expression levels of MDR1 and MRP1 with the chemosensitivity of any anticancer drugs tested, but mitomycin C was found to be more effective for the colorectal adenocarcinoma with relatively high expression of MRP2 mRNA.

KEY WORDS: CD-DST; chemosensitivity; gemcitabine; human colorectal adenocarcinomas; MDR1; mitomycin C; MRP1; MRP2.

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INTRODUCTION

It has been well accepted that chemosensitivity tests are useful for optimizing the chemotherapeutic treatment of cancer by selecting anticancer drugs and/or therapeutic methods suitable for each patient, and various tests have been developed and studied for the last 20 years. The subrenal capsule (SRC) assay was developed in the early 1980s (1), but has not been popular due to its low efficiency, high cost, and long time required for assessment. Various *in vitro* chemosensitivity tests have been developed including the human tumor clonogenic assay (HTCA) (2–4), thymidine incorporation assay (TIA) (5,6), succinic dehydrogenase inhibition (SDI) assay (7), Methylthiazoletetrazolium (MTT) assay (8,9), and histoculture drug response assay (HDRA) (10–12). However, these assays have not yet been adopted on a widespread clinical basis due to a variety of problems. For example, HTCA requires a relatively large specimen, and the success rate is not high. TIA also needs a large number of cells. The SDI and MTT assays have often failed due to a low success rate of primary culture and contamination of fibroblasts. HDRA sometimes required a higher concentration of anticancer drugs than clinically achievable concentrations, and the mode of action might differ from the clinical situation (10,11). To overcome these problems, the collagen gel droplet embedded culture drug sensitivity test (CD-DST) was developed in 1997 (13,14). By embedding and culturing tumor cells in 30 μl collagen gel droplets, assessment is possible using a relatively small number of cells (3×10^3 cell/30 μl) with a high success rate of *in vivo*-like primary three-dimensional culture. The imaging analysis system enables the selective quantification of tumor colonies without inaccuracy caused by fibroblast contamination. Consequently, an excellent predictability for clinical response and usefulness have been demonstrated using breast cancer (15), lung cancer (16–19), gastric cancer (20), and carcinomas of the pancreas and biliary tract (21).

In this paper, the CD-DST method has been applied for the assessment of the chemosensitivity of human colorectal adenocarcinomas obtained from 25 patients. The chemosensitivity was evaluated against 12 anticancer drugs, including those often prescribed for patients with colorectal adenocarcinomas, that is, 5-fluorouracil (5-FU), 7-ethyl-10-hydroxycamptothecin (SN-38)—which is an active metabolite of irinotecan hydrochloride (CPT-11)—mitomycin C (MMC), and cisplatin (CDDP). Additionally, doxorubicin hydrochloride (DXR), docetaxel (TXT), paclitaxel (TXL), methotrexate (MTX), cyclophosphamide monohydrate (CPA), gemcitabine hydrochloride (GEM), vindesine sulfate (VDS), and etoposide (VP-16) were tested as potential anticancer drugs for colorectal adenocarcinomas. Three members of the ATP-binding cassette (ABC) transporter superfamily, the multidrug resistant transporter MDR1 and the multidrug resistance-associated proteins 1 and 2 (MRP1, MRP2), are expressed in various types of tumor, and it has been recognized that they confer multidrug resistance to tumors through enhanced drug efflux (22–24). Herein, the mRNA expression levels of MDR1, MRP1, and MRP2 in adenocarcinomas were evaluated by real-time quantitative RT-PCR (25,26), and the correlation between expression levels and chemosensitivity was analyzed.

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MATERIALS AND METHODS

Materials

SN-38 was a gift from Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), TXT was from Aventis Pharma Ltd. (Vitry sur Seine, France), GEM was from Eli Lilly & Co. (Indianapolis, IN, USA), and VDS was from Shionogi & Co., Ltd. (Osaka, Japan). 5-FU, MMC, DXR, TXL, CPA, and VP-16 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CDDP and MTX were from Sigma-Aldrich Co. (St. Louis, MO, USA). Other chemicals used were of the highest purity available.

Human Colorectal Adenocarcinomas

Colorectal adenocarcinomas were obtained as surgical samples from 25 patients with primary colorectal adenocarcinoma diagnosed at Kobe University Hospital. They had never undergone the cancer chemotherapy. The samples were obtained immediately after resection, quickly stripped of connective tissue, and stored at 4°C in culture medium for evaluation of the chemosensitivity for 12 anticancer drugs, which was conducted within 24 h. Chemosensitivity was successfully evaluated by the CD-DST method described below for 16 of 25 samples (8 men and 8 women). The age range was 29 to 75 years with an average of 61.5 ± 3.4. Aliquots of the remaining samples were snap-frozen and stored at –80°C for the assay of mRNA levels, of MDR1, MRP1, and MRP2. Informed consent was obtained from all subjects prior to their participation in the study. The protocol was approved by the Institutional Review Board of Kobe University Hospital.

Chemosensitivity of Human Colorectal Adenocarcinomas Assessed by the CD-DST Method

The CD-DST was conducted as described previously (13,14). Briefly, the Type I collagen solution (Cellmatrix type CD, Nitta Gelatin Inc., Osaka, Japan), 10 x Ham's F-12 medium, and a reconstitution buffer (Nitta Gelatin Inc.) were mixed together at a ratio of 8:1:1. The cell suspension prepared was added to the collagen mixture at a final concentration of 1×10^5 cells/ml. This collagen-cell mixture was dropped into 6-well plates (Nalge Nunc International, Rochester, NY, USA) at a volume of 30 μl per collagen droplet and subjected to gelation in a CO_2 incubator at 37°C for 1 h. In each well, 3 ml/well of DF medium [DF(10), Nissui Pharmaceutical Inc., Tokyo, Japan] containing 10% fetal bovine serum (Lot. No. AGM7413, HyClone, Logan, UT, USA) was overlaid on day 0. After overnight incubation (on day 1), the anticancer drug was added, at final concentrations of 1.0 μg/ml (7.69 μM) for 5-FU, 0.03 μg/ml (0.0750 μM) for SN-38, 0.03 μg/ml (0.0897 μM) for MMC, 0.2 μg/ml (0.667 μM) for CDDP, 0.02 μg/ml (0.0345 μM) for DXR, 0.1 μg/ml (0.116 μM) for TXT, 0.1 μg/ml (0.117 μM) for TXL, 0.3 μg/ml (0.660 μM) for MTX, 0.2 μg/ml (0.717 μM) for CPA, 0.4 μg/ml (1.33 μM) for GEM, 0.01 μg/ml (0.0117 μM) for VDS, and 1.0 μg/ml (1.70 μM) for VP-16. The concentrations used for 5-FU, MMC, CDDP, DXR, VDS, and VP-16 were those often used for CD-DST (13–21). The concentrations of SN-38, TXT, TXL, MTX, CPA, and GEM were determined as the quotient of the area under the concentration–time curve by 24 h, the exposure time for anticancer drugs in CD-DST. After 24 h (on day 2), each well was washed twice with 3 ml/well of warmed complete Hank's balanced salt solution (HBSS, Cat. No. CD H-6136, Sigma-Aldrich Co.), and 4 ml/well of PCM-2 medium (Nitta Gelatin Inc.) was overlaid, and the cells were cultured for 7 days. On day 9, 50 μg/ml of neutral red solution was added, and incubation was continued for 2 h. After removal of the solution, cells were fixed with 10% formalin buffered at neutral pH. The plates were immersed in water in a tray for 10 min without agitation, and then air-dried and subjected to evaluation by imaging analysis. The growth rate of the tumor cells was evaluated as the ratio of total volume of tumor colonies after the culture for 7 days to that before the culture in the untreated group, and the data where the growth rate was more than 0.8 were successfully adopted. The chemosensitivity was evaluated based on the ratio of total volume of tumor colonies in the drug-treated group (T) to that in the untreated group (C). One drug untreated group was set per five treated groups. The assay was conducted in triplicate, and the average values of T/C% after the culture for 7 days were adopted as an index of chemosensitivity. The values of CV% were 1.9–31.1%. For the prediction of clinical response, a T/C% less than or equal to 50% was considered effective and that greater than 60% not effective.

MDR1, MRP1, and MRP2 mRNA Levels in Human Colorectal Adenocarcinomas Assessed by Real-time Quantitative RT-PCR

The expression levels of MDR1, MRP1, and MRP2 mRNA were measured for 13 of 16 samples by real-time quantitative RT-PCR analysis (25,26). Total RNA was extracted from the samples using an RNeasy Mini kit (QIAGEN, Hilden, Germany) and an RNase-Free DNase Set (QIAGEN) according to the manufacturers' protocols. In each run of the assay, mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the target protein (i.e., MDR1, MRP1, or MRP2) was analyzed in 5-fold serially diluted samples from authentic colorectal adenocarcinomas cell line, Caco-2, and the mRNA levels of MDR1, MRP1, and MRP2 were expressed relative to the concentration of GAPDH mRNA. GAPDH was selected as an endogenous RNA control to normalize for differences in the amount of total RNA. The primer pairs and TaqMan probes for MDR1, MRP1, and MRP2 mRNA were designed using the Primer Express 1.0 program (PE Applied Biosystems, Foster City, CA, USA) and published elsewhere (25,26). Using a series of MDR1-overexpressing derivative cell lines of HeLa, it had been confirmed that this assay system gave reasonable values of mRNA concentrations corresponding to those of proteins (27). Primers and the TaqMan probe for GAPDH were purchased from Applied Biosystems (TaqMan GAPDH Control Reagent Kit).

Statistical Analysis

Correlations among the T/C% values were tested using Spearman's (rank) correlation test provided that the variances of groups were not similar, and Spearman's correlation coefficients, ρ, and associated probabilities, p, were calculated. Associated probability values of less than 0.05 were considered significant for correlations.

RESULTS

Chemosensitivity of Human Colorectal Adenocarcinomas Assessed by the CD-DST Method

The chemosensitivity was successfully evaluated by the CD-DST method for 16 of 25 patients (64%), but 9 patients gave the value of growth rate less than 0.8. The growth rate varied among the patients from 0.8 (patients no. 7, 22, and 24) to 8.0 (patient no. 3), and the mean value was 2.42 ± 0.47. Table I lists the T/C% values against 12 anticancer drugs for colorectal adenocarcinomas obtained from 16 patients. The average values of T/C% were 68.1%, 67.0%, 75.7%, 90.6%, 81.8%, and 84.9% for 5-FU, SN-38, MMC, CDDP, DXR, and VP-16, respectively. Additionally, the chemosensitivity was assessed herein for TXT, TXL, MTX, CPA, GEM, and VDS, and they were 71.4%, 74.7%, 118.5%, 107.4%, 49.5%, and 77.3%, respectively. MTX and CPA were ineffective whereas GEM was the most effective of the 12 anticancer drugs tested here. Anticancer drugs were effective for patients no. 1, 3, 8, 9, 14, 17, 18, 20, and 21. For patients no. 8 and 21, GEM was effective only. In contrast, more than a 1.5-fold enhancement of tumor growth by anticancer drugs was observed for patients no. 9 and 24. Figure 1 shows the relationship between the growth rates and the T/C% values for seven anticancer drugs; 5-FU, SN-38, MMC, CDDP, DXR, TXT, and TXL. A significant correlation was observed for these anticancer drugs. The correlation was not evaluated for MTX, CPA, GEM, VDS, and VP-16, due to small numbers of samples.

Table II lists Spearman's correlation coefficients and associated probabilities between the T/C values of 5-FU, SN-38, MMC, CDDP, DXR, TXT, and TXL. A significant correlation was observed for 16 of 21 pairs. 5-FU and DXR showed a correlation of T/C% with all other anticancer drugs tested. The correlation was not evaluated for MTX, CPA, GEM, VDS, and VP-16.

MDR1, MRP1, and MRP2 mRNA Levels in Human Colorectal Adenocarcinomas

The expression levels of MDR1, MRP1, and MRP2 mRNA were measured for 13 of 16 samples by real-time quantitative RT-PCR analysis. The average values of relative concentrations of MDR1, MRP1, and MRP2 mRNA (/GAPDH mRNA) were 3.18 ± 1.28, 2.28 ± 0.57, and 0.07 ± 0.02, respectively. A relatively high level of MDR1 mRNA expression was detected in patient no. 8 with 11.62 and patient no. 22 with 14.70. In contrast, patients no. 7, 14, 17, and 24 showed relatively low levels: 0.39, 0.41, 0.53, and 0.59, respectively. However, the T/C% values against DXR, TXT, TXL, and VDS, well-defined substrates of MDR1, were not always higher in the patients with higher levels of MDR1 mRNA. Table III indicates the relationship between the T/C% values for 5-FU, SN-38, MMC, CDDP, DXR, TXT, and TXL and the expression levels of mRNAs for MDR1, MRP1, and MRP2. A significant negative correlation was observed only between the T/C% values for MMC and MRP2 mRNA level ($p = 0.034$).

DISCUSSION

The chemosensitivity test, CD-DST, was developed in 1997 (13,14). Compared with other such tests, this method has the advantage of requiring only a small number of cells, of enabling the selective quantification of tumor colonies without fibroblast contamination, and of using concentrations of anticancer drugs similar to those used clinically, resulting in excellent predictability for clinical response, as has been demonstrated using various human tumors (15–21). With the CD-DST method, the exposure concentration and time of anticancer drugs were determined by comparative evaluation with clinical results (13,14,21). These concentrations are

Table I. The T/C% of 12 Anticancer Drugs Assessed by the CD-DST Method in 16 Patients with Colorectal Adenocarcinomas

Patient No.	T/C%*											
	5-FU	SN-38	MMC	CDDP	DXR	TXT	TXL	MTX	CPA	GEM	VDS	VP-16
1	38.9	64.6	38.5	49.3								
3	22.3	40.1	20.5	59.6	32.2	17.6	15.1	81.9	97.6	9.2	20.9	18.8
4	79.1	74.0	106.0	120.3	100.1	72.7	82.2	91.8	102.2	56.8	79.1	112.4
7	101.3	84.0	111.4	114.0	106.1							
8	92.9	89.1	94.8	99.0	80.5	84.5	72.6	94.4	108.3	32.3		
9	71.3	124.4	134.7	170.2	140.4	60.8	47.5	216.1	139.5	40.7	79.7	101.8
11	67.4	82.8	82.9	81.6	66.3	54.1	54.2					
14	66.0	33.6	85.2	94.9	74.3	33.9	40.2	95.4	92.8	31.7	56.6	62.8
16	80.6	83.0	72.4	84.7	81.2	102.4	123.8					
17	50.4	37.3	36.8	53.2	42.8	50.5	45.8					
18	75.0	46.7	53.5	74.0								
20	60.5	42.5	46.6									
21	73.4	67.1	72.2	73.4	76.4	67.1	66.3	94.1	92.4	47.5	76.7	67.7
22	61.7	61.2	75.4	101.6	84.7	89.5	119.3	92.6	89.5	70.1	126.3	104.0
23	65.9	54.1	63.7	63.3	77.1	63.5						
24	83.3	87.7	116.1	119.8	100.9	160.6	154.6	181.4	137.0	107.5	102.2	126.5
Mean	68.1	67.0	75.7	90.6	81.8	71.4	74.7	118.5	107.4	49.5	77.3	84.9
SE	4.9	6.1	8.0	8.3	7.6	10.5	12.7	17.9	7.1	10.5	12.6	14.1

5-FU; 5-fluorouracil; SN-38, 7-ethyl-10-hydroxycamptothecin; MMC, mitomycin C; CDDP, cisplatin; DXR, doxorubicin HCl; TXT, docetaxel; TXL, paclitaxel; MTX, methotrexate; CPA, cyclophosphemide monohydrate; GEM, gemcitabine HCl; VDS, vindesine sulfate; VP-16, etoposide.

* The value represents the mean of T/C% estimated from three independent collagen gel drops.

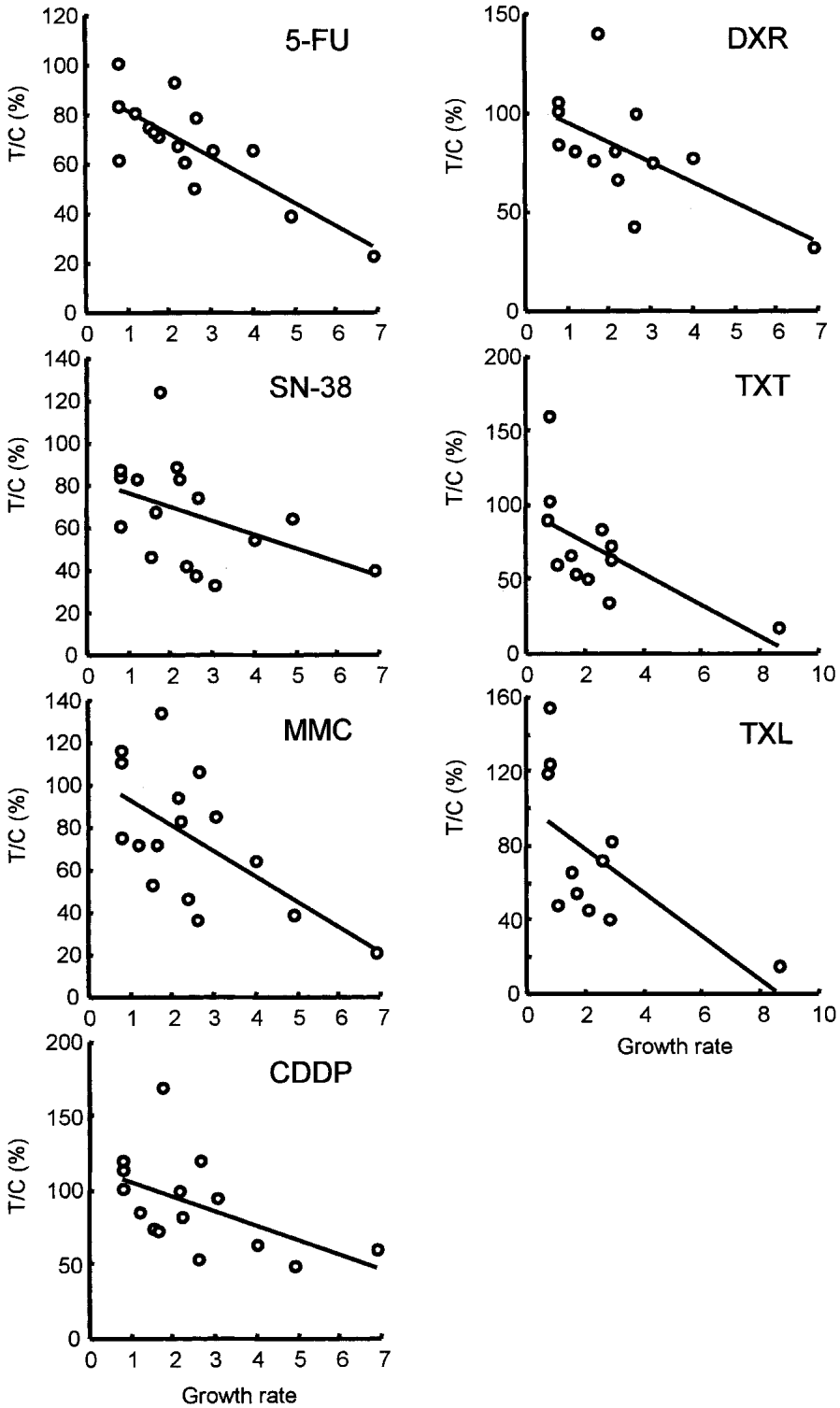

Fig. 1. Relationship between the T/C% values of 5-FU, SN-38, MMC, CDDP, DXR, TXT, and TXL and the growth rate of human colorectal adenocarcinomas. Spearman's correlation coefficients (ρ) [associated probabilities (p) and number of samples (n)] were ρ = –0.657 (p = 0.006, n = 16), ρ = –0.534 (p = 0.033, n = 16), ρ = –0.520 (p = 0.039, n = 16), ρ = –0.551 (p = 0.033, n = 15), ρ = –0.642 (p = 0.018, n = 13), ρ = –0.635 (p = 0.026, n = 12), and ρ = –0.661 (p = 0.027, n = 11), respectively.

about 1% or less of those used in the MTT assay (21). In this study, concentrations of 1.0 μg/ml, 0.03 μg/ml, 0.2 μg/ml, 0.02 μg/ml, 0.01 μg/ml, and 1.0 μg/ml were applied for the evaluation of chemosensitivity for 5-FU, MMC, CDDP, DXR, VDS, and VP-16, respectively. These were concentrations often used for the CD-DST method (13–21), and the average values of T/C% for human colorectal adenocarcinomas were 68.1%, 75.7%, 90.6%, 81.8%, 77.3%, and 84.9%, respectively.

Table II. Spearman's Correlation Coefficients (ρ) and Associated Probabilities (p) for the Correlation of the T/C% Values in the Human Colorectal Adenocarcinomas

		SN-38	MMC	CDDP	DXR	TXT	TXL
5-FU	ρ	0.721	0.747	0.650	0.654	0.706	0.709
	p	0.002	0.001	0.009	0.015	0.010	0.015
		n = 16	n = 16	n = 15	n = 13	n = 12	n = 11
SN-38	ρ		0.744	0.646	0.731	0.601	0.518
	p		0.001	0.009	0.005	0.039	0.102
			n = 16	n = 15	n = 13	n = 12	n = 11
MMC	ρ			0.936	0.819	0.406	0.364
	p			<0.001	0.001	0.191	0.272
				n = 15	n = 13	n = 12	n = 11
CDDP	ρ				0.896	0.517	0.482
	p				<0.001	0.085	0.133
					n = 13	n = 12	n = 11
DXR	ρ					0.727	0.673
	p					0.007	0.023
						n = 12	n = 11
TXT	ρ						0.982
	p						<0.001
							n = 11

5-FU; 5-fluorouracil; SN-38, 7-ethyl-10-hydroxycamptothecin; MMC, mitomycin C; CDDP, cisplatin; DXR, doxorubicin HCl; TXT, docetaxel; TXL, paclitaxel; MTX, methotrexate; CPA, cyclophosphemide monohydrate; GEM, gemcitabine HCl; VDS, vindesine sulfate; VP-16, etoposide.

Herein, six anticancer drugs were also evaluated using a concentration of 0.03 μg/ml for SN-38, 0.1 μg/ml for TXT, 0.1 μg/ml for TXL, 0.3 μg/ml for MTX, 0.2 μg/ml for CPA, and 0.4 μg/ml for GEM. The concentrations of SN-38, TXT, TXL, MTX, CPA, and GEM were determined as the quotient of the area under the concentration–time curve by 24 h. MTX and CPA were ineffective for human colorectal adenocarcinomas with T/C% values of 118.5% and 107.4%, respectively, (Table I), being consistent with the fact that these anticancer drugs are hardly used for colorectal adenocarcinomas in a clinical setting. Among the 12 anticancer drugs tested here, GEM showed the highest efficacy (T/C% = 49.5%), suggesting that it might be a promising anticancer drug for the treatment of human colorectal adenocarcinomas. Generally, more than two anticancer drugs are used in cancer chemotherapy, but the chemosensitivity tests have hardly been used for selection of the combination. The CD-DST can be conducted using a relatively small number of cells and therefore might be useful to find an optimal regimen in a clinical situation. It is noted that growth enhancement was observed in two patients, nos. 9 and 24 (Table I). Anthracyclines have been suggested to enhance tumor growth via the production of reactive oxygen species in a human cervical carcinoma cell line, HeLa (28). However, the stimulation of growth was not restricted to a specific anticancer drug (Table I). The anticancer drugs were effective against the colorectal adenocarcinomas showing a relatively high growth rate (Fig. 1). This finding suggests that the cancer chemotherapy might be effective for those in initial stage; however, little such information is available, as the colorectal adenocarcinomas in initial stage can be excised and the chemotherapy is not performed usually. In addition, the tumors in which an anticancer drug was more effective showed a higher sensitivity to other anticancer drugs (Table II). The latter was observed for gastric cancer for 5-FU,CDDP, MMC, DXR, and VP-16 (20).

In this study, the correlation between the chemosensitivity for 5-FU, SN-38, MMC, CDDP, DXR, TXT, and TXL and the expression levels of mRNAs for MDR1, MRP1, and MRP2 was evaluated. MDR1, MRP1, and MRP2 are responsible for the chemosensitivity of tumor cells through enhanced drug efflux (22–24). DXR, TXT, and TXL are the

Table III. Spearman's Correlation Coefficients (ρ) and Associated Probabilities (p) for the Correlation of the T/C% Values with MDR1, MRP1, and MRP2 mRNA Expression in the Human Colorectal Adenocarcinomas

Anticancer drugs		mRNA expression		
		MDR1	MRP1	MRP2
5-FU	ρ	−0.154	0.115	−0.241
	p	0.616	0.707	0.428
		n = 13	n = 13	n = 13
SN-38	ρ	−0.099	0.396	−0.255
	p	0.748	0.181	0.401
		n = 13	n = 13	n = 13
MMC	ρ	−0.126	0.231	−0.589
	p	0.681	0.448	0.034
		n = 13	n = 13	n = 13
CDDP	ρ	−0.022	0.269	−0.432
	p	0.943	0.374	0.141
		n = 13	n = 13	n = 13
DXR	ρ	0.028	0.329	−0.444
	p	0.931	0.297	0.149
		n = 12	n = 12	n = 12
TXT	ρ	0.345	0.109	−0.023
	p	0.298	0.750	0.947
		n = 11	n = 11	n = 11
TXL	ρ	0.345	0.000	−0.118
	p	0.298	1.000	0.729
		n = 11	n = 11	n = 11

5-FU; 5-fluorouracil; SN-38, 7-ethyl-10-hydroxycamptothecin; MMC, mitomycin C; CDDP, cisplatin; DXR, doxorubicin HCl; TXT, docetaxel; TXL, paclitaxel; MTX, methotrexate; CPA, cyclophosphemide monohydrate; GEM, gemcitabine HCl; VDS, vindesine sulfate; VP-16, etoposide.

substrates for MDR1, however, their T/C% values were not correlated with the levels of MDRI mRNA (Table III). MRP1 expression is suggested to be associated with cellular resistance to TXL (24), but the mRNA level showed no correlation with the T/C% (Table III). Recently, five human MRP superfamily members (MRP2 to MRP6) have been identified as similar transporters to MRP1 (29). The genes encoding MRP1–6 are on different chromosomes and are expressed in various normal tissues (29). MPR2 appears to mediate the transport of SN-38 in liver canalicular membrane (30); however, little is known concerning the effects of its expression on chemosensitivity in human tumors. Hinoshita *et al.* have reported that MRP2 mRNA expression was significantly resistant to CDDP in colorectal carcinomas (31), but they adopted a SDI test to evaluate the chemosensitivity, which required a 10-fold higher concentration of anticancer drugs compared with peak plasma concentrations. The SDI test often fails due to a low success rate of primary culture. The data are not free from contamination by fibroblasts. The extremely high concentration of anticancer drugs applied might result in data with a different mode of action from the clinical situation. Herein, the CD-DST method was applied for the evaluation of chemosensitivity, and it has been demonstrated that the T/C% value of MMC was negatively correlated with MRP2 mRNA expression ($p = 0.034$). To date, there is no experimental evidence that MMC is a substrate for MRP2, thus further study should be conducted on this mechanism.

In conclusion, a recently developed novel chemosensitivity test, CD-DST, has been applied for the evaluation of human colorectal adenocarcinomas obtained from 25 patients. The chemosensitivity of 12 anticancer drugs was successfully evaluated for 16 of 25 patients, and they were effective against the samples showing a relatively high growth rate. It was found that GEM could be more promising than those often prescribed for the treatment of colorectal adenocarcinoma (e.g., 5-FU, CPT-11, MMC, and CDDP). The expression levels of MDR1, MRP1, and MRP2 mRNA in colorectal adenocarcinomas were also evaluated by real-time quantitative RT-PCR, and their correlation with the chemosensitivity suggested no effects of MDR1 and MRP1 mRNA on the chemosensitivity, except that MMC was more effective for the colorectal adenocarcinoma with relatively high expression of MRP2 mRNA.

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