Evaluation of 5-fluorouracil applicability by the collagen gel droplet embedded drug sensitivity test with area under the curve analysis

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We have evaluated the 5-fluorouracil sensitivity of cancer cells from colorectal cancer patients using the collagen gel droplet embedded drug sensitivity test under multiple drug concentrations and contact durations. After converting drug concentration and contact time to the area under the curve (AUC) and plotting against the growth inhibition rate, the correlation between AUC and the growth inhibition rates was approximated to the logarithmic regression curve. In this study, to further validate the reliability of the regression curve, the growth inhibition rate was calculated from the regression curve and the actual growth inhibition rate was compared at AUC of 48 μg h/ml. No significant difference was observed in the growth inhibition rates between the two groups by paired *t*-test ($P=0.590$). A strong positive correlation was found between the two groups by regression analysis ($y=0.7555x+10.514$, $R^2=0.8236$). This result strongly suggests that in-vitro antitumor effect of 5-fluorouracil depends on the AUC in colorectal cancer and the AUC–inhibition rate curve is reliable. We can obtain the inhibition rate from AUC and vice versa using the AUC–inhibition rate curve. We can also calculate the individualized AUC_{IR50}, AUC value that gives 50% growth inhibition, using the AUC–inhibition rate curve. This could be useful to establish individualized chemotherapy using the collagen gel droplet embedded drug sensitivity test. *Anti-Cancer Drugs* 18:17–21 © 2007 Lippincott Williams & Wilkins.

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Introduction

In recent years, combination chemotherapy regimens including irinotecan and oxaliplatin (FU/LV, FOLFOX, FOLFIRI, etc.) have markedly improved the response rate and prolonged median survival time. FOLFOX/FOLFIRI is regarded as a global standard for colorectal cancer chemotherapy [1–3]. Moreover, improvement of the response rate and the median survival time with molecular targeting drugs has been shown [4–8]. Combination therapy, however, is associated with greater toxicity than is observed with single agents administered alone. Furthermore, it is true that there are some nonresponders. On the other hand, individualized therapy, which applies individualized doses of chemotherapy on the basis of the biological characteristics of the tumor, has been proposed to improve patients' clinical outcome while maintaining the quality of life.

In the case of a gastrointestinal tumor, 5-fluorouracil (5-FU) is the most frequently used drug and assessing its antitumor effect is a very important issue. One of these methods is an anticancer drug sensitivity test [9–12]. We have evaluated the 5-FU sensitivity of cancer cells from colorectal cancer patients using the collagen gel droplet embedded drug sensitivity test (CD-DST) under multiple drug concentrations and contact durations. After converting drug concentration and contact time to the area under the curve (AUC), and plotting against the growth inhibition rate, the correlation between AUC and the growth inhibition rates was approximated to the logarithmic regression curve. Moreover, there was no significant difference in the growth inhibition rate for 1.0 μg/ml for 24 h and 0.2 μg/ml for 120 h, which gives the same AUC of 24 μg h/ml [13].

To further validate the reliability of the regression curve, the growth inhibition rate calculated from the regression curve and the actual growth inhibition rate were compared at AUC of 48 μg h/ml in this study.

Patients and methods

Patients

Surgical specimens were obtained from 21 colorectal cancer patients who have resected without preoperative

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chemotherapy between January 2004 and March 2006. Informed consent for measuring drug sensitivity was obtained from all patients.

Methods

The tumor tissue was excised from the primary surgical specimen and applied to the CD-DST. The specimen was washed out with saline (50 ml) 5 times. Thereafter, the specimen was washed out 5 times with antibiotic fluid (50 ml) containing 1.0 mg/ml piperacillin and 0.5 mg/ml kanamycine. The transport bottle contained 1.0 mg/ml piperacillin, 0.5 mg/ml kanamycine and 2.5 μg/ml amphotericin B. The CD-DST is used to evaluate 5-FU sensitivity of tumors and was performed as described by Kobayashi *et al.* [14]. One gram of tissue was treated with dispersion enzyme cocktail containing 1.0% collagenase for 2 h. Dispersed cell suspensions were inoculated in preculture media on collagen-coated flasks overnight and then viable tumor cells were recovered by 0.05% collagenase treatment. Recovered cells were embedded in 30-μl collagen gel droplets. Embedded cells were cultivated in culture media containing 5-FU at 0.2 μg/ml for 24 h, 1.0 μg/ml for 24 h, 0.2 μg/ml for 120 h, 10 μg/ml for 3 h, 1 μg/ml for 120 h and 10 μg/ml for 24 h. After removal of 5-FU-containing media, cells were further cultured for 7 days in serum-free culture media to prevent the growth of fibroblasts. Viable cells were stained by neutral red solution and were counted by the imaging colorimetric quantification method. The surviving cell number ratio between drug-treated group and control group was calculated. All patients were measured in these six conditions.

After converting the drug concentrations and contact time to AUC, and plotting against the growth inhibition rate, the correlation between AUC and the growth inhibition rates was evaluated. In all the patients, the growth inhibition rates (calculated value) of AUC 48 were obtained from the AUC–inhibition rate (IR) curve. In addition, the growth inhibition rates of all patients (measured value) were measured at 0.2 μg/ml for 120 h after 1.0 μg/ml for 24 h. The total AUC of this condition was 48. The growth inhibition rates of AUC 48 in each method were also evaluated.

A growth rate above 0.8 was regarded as a successful case.

Statistics

After converting the drug concentrations and contact time to AUC, and plotting against the growth inhibition rate, the correlation between AUC and the growth inhibition rates was evaluated. In all the patients, the growth inhibition rates (calculated value) of AUC_{48} were obtained from the AUC–IR curve.

Correlation between different contact conditions was analyzed by paired *t*-test. Correlation between the calculated value and the measured value was analyzed by regression analysis. P values < 0.05 were regarded as statistically significant.

Result

The backgrounds of 21 patients are shown in Table 1. The growth inhibition rates for six conditions in all patients are shown in Table 2. AUC and the growth inhibition rate were highly approximate to the logarithmic curve in all of the patients ($R^2 = 0.6551$–0.9943) (Table 2).

The data of a representative patient are shown in Fig. 1. The approximate expression and correlation coefficients were $y = 15.497\mathrm{Ln}(x) - 10.992$ ($R^2 = 0.9286$) for AUC.

The growth inhibition rates (calculated value) from all patients in the AUC–IR curve and the measured value at 0.2 μg/ml for 120 h after 1.0 μg/ml for 24 h, which corresponds to the same AUC of 48, are also shown in Table 2.

The correlation of the growth inhibition rate between the two groups is shown in Figs 2 and 3. No significant difference was seen in the growth inhibition rates between the two groups by paired *t*-test ($P = 0.590$). A strong positive correlation exists between the two groups by regression analysis ($y = 0.7555x + 10.514$, $R^2 = 0.8236$).

Discussion

From the late 1950s until very recently, 5-FU was the only drug approved for the treatment of colorectal cancer. At present, 5-FU is still the most important key drug in combination chemotherapy for colorectal cancer. Thus, it is very important to evaluate the efficacy and toxicity of 5-FU before starting chemotherapy. The drug sensitivity of tumor cells is one of the key issues to assess the antitumor effect of 5-FU. One of the methods is in-vitro anticancer drug sensitivity test, which is usually performed by one drug concentration and contact time

Table 1 Patient characteristics

No. of patients	21
Median age (years)	65.6 (48–78)
Sex	
male	12
female	9
Histological type	
well-differentiated adenocarcinoma	2
moderately differentiated adenocarcinoma	17
poorly differentiated adenocarcinoma	1
mucinous carcinoma	1
Dukes	
A	1
B	10
C	7
D	3

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Table 2 Measured and calculated growth inhibition rates of all patients

Patient no.	Concentration/contact times						Correlation coefficient		AUC 48	AUC 48
	0.2 μg/ml 24 h	1.0 μg/ml 24 h	0.2 μg/ml 120 h	10 μg/ml 3 h	1 μg/ml 120 h	10 μg/ml 24 h	$y=a\,\mathrm{Ln}(x)+b$	R^2	calculated value	measured value
1	−2.5	11.5	22.2	17.5	37.6	32.2	$y=9.7902\mathrm{Ln}(x)-15.485$	0.8662	22.4	22.8
2	14.4	45.0	27.8	44.6	60.8	76.1	$y=15.497\mathrm{Ln}(x)-10.992$	0.9286	49.0	47.8
3	0.9	42.8	15.7	42.1	62.2	78.5	$y=19.733\mathrm{Ln}(x)-30.652$	0.898	45.7	38.8
4	19.0	30.1	13.9	22.3	33.1	48.9	$y=7.3756\mathrm{Ln}(x)+1.3385$	0.6609	29.9	25.7
5	9.1	12.2	31.1	39.9	53.8	47.9	$y=11.561\mathrm{Ln}(x)-9.2756$	0.7414	35.5	20.8
6	14.3	17.3	19.0	13.4	41.7	34.2	$y=7.0699\mathrm{Ln}(x)-2.1281$	0.6851	25.2	36.6
7	16.4	29.1	PVC	PVC	PVC	59.4	$y=11.141\mathrm{Ln}(x)-3.0138$	0.9832	40.1	37.1
8	12.8	18.8	24.3	36.1	53.8	69.0	$y=15.049\mathrm{Ln}(x)-18.361$	0.9023	39.9	37.4
9	−1.8	30.3	28.8	30.3	58.0	66.7	$y=17.66\mathrm{Ln}(x)-28.174$	0.9943	40.2	43.4
10	4.1	16.1	27.2	20.6	44.1	49.6	$y=12.157\mathrm{Ln}(x)-16.803$	0.9409	30.3	26.1
11	0.1	16.3	7.5	28.7	49.0	54.3	$y=15.261\mathrm{Ln}(x)-28.942$	0.9022	30.1	30.7
12	3.1	27.8	−4.1	34.9	40.7	50.4	$y=12.676\mathrm{Ln}(x)-20.155$	0.6551	28.9	16.0
13	10.3	53.4	56.0	58.2	85.2	89.8	$y=20.25\mathrm{Ln}(x)-14.064$	0.9593	64.3	67.2
14	32.0	32.9	34.1	PVC	PVC	59.9	$y=7.5768\mathrm{Ln}(x)+14.333$	0.8188	43.7	41.3
15	2.9	37.8	PVC	25.9	53.2	70.8	$y=16.581\mathrm{Ln}(x)-22.949$	0.9477	41.2	42.2
16	−4.8	0.9	8.3	4.1	5.8	9.3	$y=3.1106\mathrm{Ln}(x)-7.2617$	0.6695	4.8	1.4
17	2.6	16.7	22.8	24.5	42.9	46.1	$y=11.72\mathrm{Ln}(x)-16.247$	0.9734	29.1	34.3
18	−6.5	9.5	10.2	11.0	48.9	55.1	$y=17.375\mathrm{Ln}(x)-41.166$	0.9386	26.1	10.5
19	13.2	45.1	40.4	47.1	76.0	81.9	$y=18.198\mathrm{Ln}(x)-14.879$	0.9892	55.6	60.1
20	1.1	14.7	9.2	21.0	39.4	53.4	$y=13.819\mathrm{Ln}(x)-26.603$	0.9341	26.9	24.1
21	12.3	25.7	39.0	26.5	54.9	62.3	$y=13.203\mathrm{Ln}(x)-10.735$	0.9108	40.4	35.3

PVC, poor viable cells; 10^3–10^5 cells; AUC, area under curve.

Fig. 1

The growth inhibition by 5-fluorouracil AUC. Representative data. Diamonds: the measured values; open circle: the calculated value. AUC, area under the curve; IR, inhibition rate.

Fig. 2

Comparison of the measured growth inhibition rate (IR) and calculated growth IR.

[15–17]. In this study, 5-FU sensitivity of cancer cells from colorectal cancer patients was evaluated by the CD-DST under multiple drug concentrations and contact durations. The CD-DST is a method to evaluate drug sensitivity using isolated, three-dimensionally cultured tumor cells in a small collagen gel droplet [18]. The CD-DST has the following features: (1) it has a high success rate of testing owing to the micro-three-dimensional culture, (2) the test works with a small quantity of specimen, (3) the antitumor effect of drugs can be evaluated in clinically equivalent doses and (4) the anticancer effect can be accurately evaluated using an image analysis device when the percentage of fibroblast contamination is less than 67% [14]. It is, however, very

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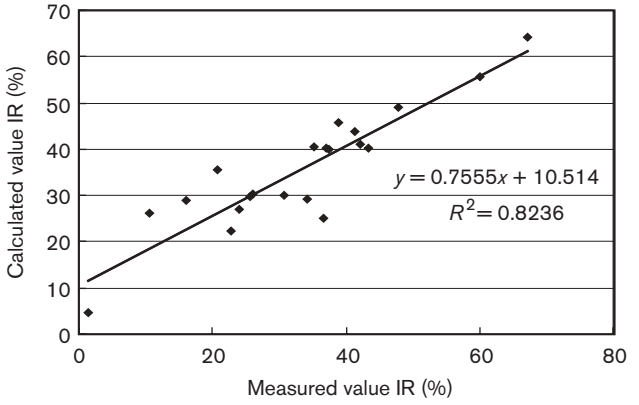
Fig. 3



Correlation between the measured and the calculated growth inhibition rate (IR).

important that the object being examined is obtained from the soft part of the tumor tissue to prevent possible contamination of the fibroblast component.

Bacterial contamination is a serious event in in-vitro chemosensitivity tests. To prevent bacterial contamination, several artifices were applied. Whitehouse *et al.* [19] reported that the addition of amphotericin B and metronidazole did not alter the sensitivity of 5-FU in colorectal adenocarcinoma. On the other hand, we recognized that the mechanical washing out was very important. Thus, we performed many mechanical washing outs with/without antibiotic. The total period of the washing out needs to be about 40 min. After we adopted this method, the success rate was over 90%.

In anticancer drug sensitivity tests there are some reports using drug concentration analysis (e.g. 50% inhibitory concentration: IC_{50}) [19–21]. Those reports did not describe an exposure condition to existing various administration methods for one anticancer drug. Several commonly used modes of 5-FU administration exist, however, including intermittent intravenous infusion (i.v.), bolus i.v., continuous i.v. and oral. In addition to the mode of administration, to determine treatment strategy, several additional parameters, i.e. concentration and schedule, have to be determined. We analyzed in-vitro AUC to determine these parameters rather than IC_{50} analysis. Reports of other anticancer drugs using AUC analysis are also available [22,23]. In general, several drugs, e.g. mitomycin C, cisplatin and doxorubicin, are known as AUC-dependent anticancer drugs [24,25]. On the other hand, two mechanisms of action for 5-FU, i.e. concentration-dependent RNA dysfunction and time-dependent DNA synthesis inhibition, are known [26]. Possibly, owing to this complexity, only limited numbers of reports that discuss whether the antitumor effect of 5-FU depends on AUC are available [27]. Hanatani *et al.* [28] reported that no significant difference of 5-FU antitumor effect was observed in esophageal cancer between 10 μg/ml for 2.4 h, 1.0 μg/ml for 24 h and 0.2 μg/ml for 120 h.

In our previous report using the CD-DST, the correlation between 5-FU AUC and the antitumor effect in colorectal cancer was evaluated, and *in vitro* AUC values and inhibition rates were quite approximate to the logarithmic curve. Moreover, there was no significant difference in the inhibition rate between 1.0 μg/ml for 24 h and 0.2 μg/ml for 120 h. These data suggest that the in-vitro antitumor effect of 5-FU depends on AUC in colorectal tumors.

In the present study, we evaluated the reliability of the AUC–IR curve. In all the patients, the growth inhibition rates (calculated value) of AUC 48 could be calculated from the AUC–IR curve. On the other hand, the growth inhibition rates of all patients (measured value) were continuously measured at 0.2 μg/ml for 120 h after 1.0 μg/ml for 24 h. The total AUC of these conditions was the same as AUC 48. We recognized the conditions of 1.0 μg/ml for 24 h (short-term high dose) as a continuous i.v. mode and 0.2 μg/ml for 120 h (long-term low dose) as an oral mode [29]. In clinical practice in Japan, we often treat with oral 5-FU tablets after 5-FU continuous i.v. therapy. We evaluated the correlation between the calculated values and the measured values. No significant difference was observed in the growth inhibition rates between the two groups. This result suggests that the in vitro antitumor effect of 5-FU depends on the AUC in colorectal cancer and the AUC–IR curve is reliable. We can obtain the inhibition rate from AUC and vice versa using the AUC–IR curve. We can also calculate the individualized AUC_{IR50}, the AUC value that gives 50% growth inhibition, using the AUC–IR curve.

On the other hand, the analysis of correlation between 5-FU metabolic enzymes and antitumor effect has been widely investigated to predict the effect of 5-FU. Thymidylate synthase, dihydropyrimidine dehydrogenase and orotate phosphoribosyl transferase are such enzymes, and the association to 5-FU efficacy has been frequently reported in the clinical literature. We have also reported that the tumor orotate phosphoribosyl transferase activity is useful to distinguish the prognosis of resectable colorectal cancer patients [30]. These pharmacokinetic parameters cannot, however, show quantitative anticancer agent susceptibility. This report suggests that the new evaluating method may show quantitative anticancer agent sensitivity and what kind of administration schedule of 5-FU might raise an individual anticancer effect on colon cancer patients.

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Several clinical reports using the chemosensitivity assay in ovarian cancer, breast cancer and leukemia exist [20–23]. These reports referred to the predictions of the clinical outcome. This is, however, a report from a preclinical study. Therefore, a study based on the individual AUC_{IR50} is currently in progress to establish the individualized chemotherapy using the CD-DST. We intend to report the data of the clinical outcome in colorectal cancer using the individual AUC_{IR50} when it becomes available.

References

1 Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, *et al.* FOLFIRI followed by FOLFOX 6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**:229–237.

2 Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, *et al.* A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J Clin Oncol* 2004; **22**:23–30.

3 de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, *et al.* Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; **18**:2938–2947.

4 Rosenberg AH, Loehrer PJ, Needle MN, Waksal H, Hollywood E, Ramos L, *et al.* Erbitux (IMC-C225) plus weekly irinotecan (CPT-11), fluorouracil (5FU) and leucovorin (LV) in colorectal cancer (CRC) that expresses the epidermal growth factor receptor (EGFR) [Meeting Abstracts]. *J Clin Oncol* 2002; **20**:536.

5 Daiz Rubio E, Tabernero J, van Cutsem E, Cervantes A, Andre T, Humblet Y, *et al.* Cetuximab in combination with oxaliplatin/5-fluorouracil (5-FU)/folinic acid (FA) (FOLFOX-4) in the first-line treatment of patients with epidermal growth factor receptor (EGFR)-expressing metastatic colorectal cancer: an international phase II study [Meeting Abstracts]. *J Clin Oncol* 2005; **23**:3535.

6 Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, *et al.* Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; **21**:60.

7 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, *et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**:2235.

8 Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, *et al.* High dose bevacizumab improves survival when combined with FOLFOX4 in previously treated advanced colorectal cancer: results from the Eastern Cooperative Oncology Group (ECOG) study E3200 [Meeting Abstracts]. *J Clin Oncol* 2005; **23**:2.

9 Kobayashi H, Tanisaka K, Doi O, Kodama K, Higashiyama M, Nakagawa H, *et al.* An *in vitro* chemosensitivity test for solid human tumors using collagen gel droplet embedded cultures. *Int J Oncol* 1997; **11**:449–455.

10 Yasuda H, Takada T, Wada K, Amano H, Isaka T, Yoshida M, *et al.* A new in-vitro drug sensitivity test (collagen-gel droplet embedded-culture drug sensitivity test) in carcinomas of pancreas and biliary tract: possible clinical utility. *J Hepatobiliary Pancreas Surg* 1998; **5**:261–268.

11 Higashiyama M, Kodama K, Yokouchi H, Takami K, Doi O, Kobayashi H, *et al.* Immunohistochemical p53 protein status in nonsmall cell lung cancer is a promising indicator in determining *in vitro* chemosensitivity to some anticancer drugs. *J Surg Oncol* 1998; **68**:19–24.

12 Inaba M, Tashiro T, Sato S, Ohnishi Y, Tanisaka K, Kobayashi H, *et al.* *In vitro–in vivo* correlation in anticancer drug sensitivity test using AUC-based concentrations and collagen gel droplet-embedded culture. *Oncology* 1996; **53**:250–257.

13 Ochiai T, Nishimura K, Noguchi H, Kitajima M, Tsuruoka Y, Takahashi Y. Evaluation of 5-fluorouracil applicability by multi-point collagen gel droplet embedded drug sensitivity test. *Oncol Rep* 2005; **14**:201–205.

14 Kobayashi H, Higashiyama M, Minamigawa K, Tanisaka K, Takano T, Yokouchi H, *et al.* Examination of *in vitro* chemosensitivity test using collagen droplet culture method with colorimetric endpoint quantification. *Jpn J Cancer Res* 2001; **92**:203–210.

15 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**:55–63.

16 Hoffman RM, Connors KM, Meerson-Monosov AZ, Herrera H, Price JH. A general native-state method for determination of proliferation capacity of human normal and tumor tissues *in vitro*. *Proc Natl Acad Sci U S A* 1989; **836**:2013–2017.

17 Kubota T, Sasano N, Abe O, Nakao I, Kawamura E, Saito T, *et al.* Potential of the histoculture drug-response assay to contribute to cancer patient survival. *Clin Cancer Res* 1995; **1**:1537–1543.

18 Matsuo A, Watanabe A, Takahashi T, Futamura M, Mori S, Sugiyama Y, *et al.* A simple method for classification of cell death by use of thin layer collagen gel for the detection of apoptosis and/or necrosis after cancer chemotherapy. *Jpn J Cancer Res* 2001; **92**:813–819.

19 Whitehouse PA, Louise AK, Di Nicolantonio F, Mercer SJ, Sharma S, Cree IA. Heterogeneity of chemosensitivity of colorectal adenocarcinoma determined by a modified *ex vivo* ATP-tumor chemosensitivity assay (ATP-TCA). *Anticancer Drugs* 2003; **14**:369–375.

20 Cree IA, Kurbacher CM, Untch M, Sutherland LA, Hunter EM, Subedi AMC, *et al.* Correlation of the clinical response to chemotherapy in breast cancer with *ex vivo* chemosensitivity. *Anticancer Drugs* 1996; **7**:630–635.

21 Kurbacher CM, Bruckner HW, Cree IA, Kurbacher JA, Willhelm L, Poch G, *et al.* Mitoxantrone combined with paclitaxel as salvage therapy for platinum-refractory ovarian cancer; laboratory study and clinical pilot trial. *Clin Cancer Res* 1997; **3**:1527–1533.

22 Andreotti PE, Cree IA, Kurbacher CM, Hartmann DM, Linder D, Harel G, *et al.* Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res* 1995; **55**:5276–5282.

23 Staib P, Staltmeier E, Ncurohr K, Cornely O, Reiser M, Schinkothe T. Prediction of individual response to chemotherapy in patients with acute myeloid leukaemia using the chemosensitivity index C_i. *Br J Haematol* 2005; **128**:783–791.

24 Ozawa S, Sugiyama Y, Mitsuhashi Y, Kobayashi T, Inaba M. Cell killing action of cell cycle phase-non-specific antitumor agents is dependent on concentration–time product. *Cancer Chemother Pharmacol* 1988; **21**: 185–190.

25 Ozawa S, Sugiyama Y, Mitsuhashi J, Inaba M. Kinetic analysis of cell killing effect induced by cytosine arabinoside and cisplatin in relation to cell cycle phase specificity in human colon cancer and Chinese hamster cells. *Cancer Res* 1989; **49**:3823–3828.

26 Inaba M, Mitsuhashi J. Flow cytometric analysis of cell-killing actions of 5-fluorouracil in human colorectal cancer cells. *Oncol Res* 1994; **6**: 303–309.

27 Hanatani Y, Kobayashi H, Kodaira S, Takami H, Asagoe T, Kaneshiro E. An *in vitro* chemosensitivity test for gastric cancer using collagen gel droplet embedded culture. *Oncol Rep* 2000; **7**:1027–1033.

28 Hanatani Y, Kobayshi H, Kodaira S, Gibo J, Fujita M, Toeda H. A clinical application of collagen embedded culture drug sensitivity test to esophageal cancer. *Jpn J Chemother* 2000; **48**:908–912.

29 Kobayashi H. Development of a new *in vitro* chemosensitivity test using collagen gel embedded culture and image analysis for clinical usefulness. *Recent Results Cancer Res* 2003; **161**:48–61.

30 Ochiai T, Nishimura K, Noguchi H, Kitajima M, Tsukada A, Watanabe E. Prognostic impact of orotate phosphoribosyl transferase among 5-fluorouracil metabolic enzymes in resectable colorectal cancers treated by oral 5-fluorouracil-based adjuvant chemotherapy. *Int J Cancer* 2006; **118**:3084–3088.

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