Data acquisition for the histoculture drug response assay in lung cancer

Tatsuya Yoshimasu, MD, PhD,[a] Shoji Oura, MD, PhD,[a] Issei Hirai, MD, PhD,[a] Takeshi Tamaki, MD,[a] Yozo Kokawa, MD, PhD,[a] Kazuhito Hata, MD,[a] Fuminori Ohta, MD,[a] Rie Nakamura, MD,[a] Mitsumasa Kawago, MD,[a] Hirokazu Tanino, MD, PhD,[a] Yoshitaka Okamura, MD, PhD,[a] and Tomoko Furukawa, MD, PhD[b]

Objective: Application of the histoculture drug response assay for lung cancer was investigated by using data acquired from lung cancer specimens.

Methods: From May 1994 through February 2005, histoculture drug response assay data were obtained from 359 lung cancer specimens held in our institute. We examined chemosensitivities of the tissues to cisplatin, doxorubicin, mitomycin C, 5-fluorouracil, docetaxel, paclitaxel, etoposide, irinotecan, and gemcitabine. Cutoff inhibition rates were determined with each drug for non–small cell lung cancer and were used to calculate predictabilities for chemotherapy responses.

Results: The evaluability of the histoculture drug response assay was high at 97.4%. Good predictability, including true-positive and true-negative rates of 73.2% and 100%, respectively, with an accuracy of 83.0%, was observed.

Conclusion: The histoculture drug response assay appears to be applicable to non–small cell lung cancer for the prediction of responses to chemotherapy.

From the Department of Thoracic and Cardiovascular Surgery,[a] Wakayama Medical University, Wakayama, Japan, and the Analytical Center for Medical Science,[b] SRL, Inc, Tokyo, Japan.

Received for publication Dec 18, 2005; revisions received May 17, 2006; accepted for publication June 15, 2006.

Address for reprints: Tatsuya Yoshimasu, MD, PhD, Department of Thoracic and Cardiovascular Surgery, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8509, Japan (E-mail: yositatu@mail.wakayama-med.ac.jp).

J Thorac Cardiovasc Surg 2007;133:303-8

0022-5223/$32.00

doi:10.1016/j.jtcvs.2006.06.030

Chemotherapy is not highly effective for the treatment of non–small cell lung cancer (NSCLC). Sensitive drugs need to be identified and used for each patient to improve responses to chemotherapy. In vitro drug response assays[1-6] have been used for identification of such drugs. The histoculture drug response assay (HDRA)[4-6] is a representative in vitro drug response assay method used for anticancer agents. Several clinical studies involving colorectal and gastric cancers revealed that inhibition rates obtained with the HDRA can predict clinical responses to chemotherapy.[4,5]

Because the biologic characteristics of lung cancer are different from those of colorectal and gastric cancers, it would be better to identify, before routine clinical use, whether the HDRA is applicable and useful for lung cancer.

From May 1994, we instituted the HDRA for lung cancers, mainly using resected surgical specimens obtained from patients with operable NSCLC.[7-11] After data acquisition, we investigated drug concentrations for the HDRA, cutoff inhibition rates, and correlations among inhibition rates and clinical responses.

Patients and Methods

Patients

From May 1994 through February 2005, 359 specimens obtained from patients with lung cancer (257 male and 95 female patients ranging from 25-82 years old [average, 66 ± 9

GTS

Abbreviation and Acronyms
HDRA = histoculture drug response assay
NSCLC = non–small cell lung cancer
SCLC = small cell lung cancer

years]) were used for the HDRA in our institute. The HDRA was performed on 352 of the specimens because 7 were too small. There were 183 adenocarcinomas, 119 squamous cell carcinomas, 13 large cell carcinomas, 2 pleomorphic carcinomas, 2 adenosquamous carcinomas, 18 small cell carcinomas, and 4 carcinomas of unknown histology (NSCLC). Histologic data were not available for 8 cases. Specimens were derived from primary lesions in 294 cases, metastatic lymph nodes in 42 cases, distant metastases in 15 cases, and pleural dissemination in 1 case. Chemotherapy had been performed before the HDRA in 7 cases. This study was approved by our institutional review board for clinical practice, and written informed consent was obtained from the patients.

HDRA

Methods for the HDRA were as reported by Furukawa and colleagues.[5] Collagen sponge gels manufactured from pig skin were purchased from Sumitomo Medical Inc. Cancerous portions of specimens were minced into pieces to approximately 10 mg, which were then placed on prepared collagen surfaces in 24-well microplates. Plates were incubated for 7 days at 37°C in the presence of drugs dissolved with RPMI 1640 medium containing 20% fetal calf serum and left in a humidified atmosphere containing 95% air–5% CO_2. Concentrations of drugs were 20 μg/mL for cisplatin (CDDP), 300 μg/mL for 5-fluorouracil (FU), 15 μg/mL for adriamycin (ADM), 2 μg/mL for mitomycin C (MMC), 500 μg/mL for etoposide (VP-16), 0.2 μg/mL for irinotecan (SN38), 100 μg/mL for docetaxel (DOC), 40 μg/mL for paclitaxel (PAC), and 1000 μg/mL for gemcitabine (GEM).

After histoculture, 100 μL of Hank's balanced salt solution containing 0.1 mg/mL type I collagenase (Sigma) and 100 μL of 3-(4,5-Dimethyl-2-thiazotyl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) solution and dissolved in 5 mg/mL phosphate buffer solution were added to each culture well and incubated for another 16 hours. After extraction with dimethyl sulfoxide, absorbance of the solution in each well was read at 540 nm. Absorbance per gram of cultured tumor tissue was calculated from the mean absorbance of tissue from 4 culture wells, and the tumor-tissue weight was determined before culture.

The inhibition rate was calculated by using the following formula:

$$\textit{Inhibition rate}\ (\%) = (1 - \textit{Mean absorbance of treated tumor/Weight}/\textit{Mean absorbance of control tumor/Weight}) \times 100$$

The HDRA was regarded as evaluable when the mean absorbance of extracted formazan at 540 nm of the control tumor was 15 or more per gram. When the inhibition rate of the drug was a negative value, it was regarded as zero, which meant absolutely no chemosensitivity.

Data acquisitions of CDDP, FU, ADM, and MMC were started in May 1994. That of VP-16 was started in August 1994, with SN38 started in December 1994, DOC in April 1996, GEM in July 2000, and PAC in September 2000.

Statistical Analysis

All values were reported as means ± standard deviation (minimum-maximum). The χ^2 test and analysis of variance were used to evaluate the significance of differences between groups.

Results

Evaluability of the HDRA

Of the 352 cases, the assay failed in 3 cases because of bacterial contamination and in 2 cases because of insufficient cell viability. An average of 6.0 ± 1.9 (min-max, 1-9) drugs were tested in 347 cases. Among them, data were judged as not reliable in 4 cases because control optical density/weight was less than 15. Therefore there were 343 evaluable specimens, and the evaluability of the HDRA was 97.4% (343/352).

Determination of Cutoff Inhibition Rates

Assay results from 315 NSCLC cases without prior chemotherapy were used to determine cutoff inhibition rates for each drug. Figure 1 shows the distribution of inhibition rates for each drug. Cutoff levels of drugs were determined according to previously reported clinical response rates of each drug. Cutoff levels were determined a bit lower to avoid false-negative cases. This resulted in a larger rate of HDRA-sensitive patients than reported clinical response rates. Determined cutoff levels, average inhibition rates, and rates of sensitive patients for each drug are summarized in Table 1.

By using these cutoff levels, an average of 2.2 ± 2.0 (min-max, 0-8) HDRA-positive drugs were obtained in 340 patients with available HDRA data. Distributions of positive drugs are shown in Figure 2. The modest population included 88 (25.9%) cases with no positive drug.

Clinical Correlations

Consistency between HDRA results and clinical responses was evaluated in 57 patients: 21 concurrent chemoradiation (CCRT) cases and 36 chemotherapy cases. The total number of chemotherapy agents was 88, including 33 CCRTs and 55 chemotherapies. Numbers of uses of each drug were as follows: CDDP in 36 cases, MMC in 13 cases, DOC in 12 cases, VP-16 in 8 cases, PAC in 7 cases, ADM in 5 cases, SN38 in 3 cases, GEM in 2 cases, and FU in 2 cases.

Clinical responses were regarded as effective when a complete response or partial response was obtained and not effective when stable disease or progressive disease was obtained. In most cases treatment protocols contained more than 1 drug. Therefore consistency with the HDRA was judged according to the rules summarized in Table 2. These rules might result in overestimations in diagnostic accuracies.

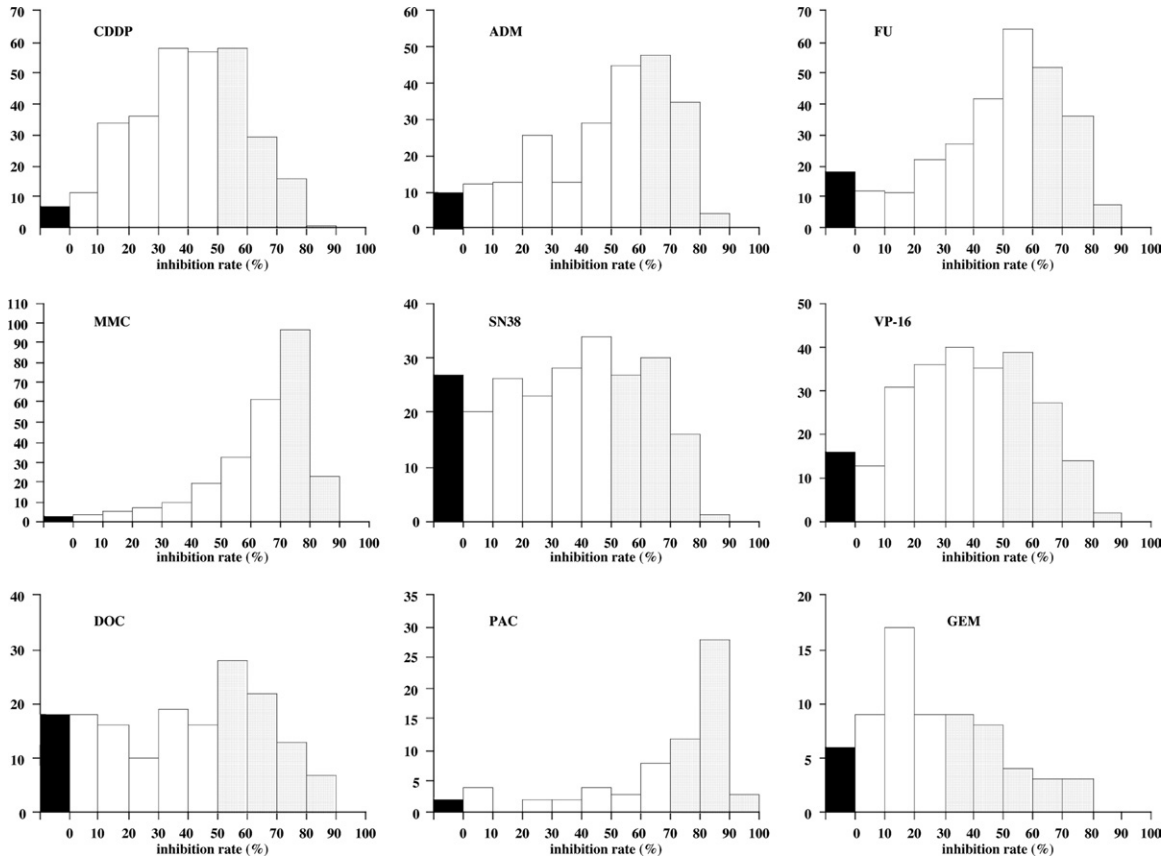

Figure 1. Distribution of inhibition rates for cisplatin *(CDDP)*, adriamycin *(ADM)*, 5-fluorouracil *(FU)*, mitomycin C *(MMC)*, irinotecan *(SN38)*, etoposide *(VP-16)*, docetaxel *(DOC)*, paclitaxel *(PAC)*, and gemcitabine *(GEM)*.

Clinical correlations are summarized in Table 3. There were 11 true-positive cases, 29 true-negative cases, 15 false-positive cases, and no false-negative cases in 55 chemotherapy cases. There were 30 true-positive cases and 3 true-negative cases in 33 CCRT cases. Neither false-positive cases nor false-negative cases were observed. The yield was an accuracy of 72.3% in chemotherapy and 100% in CCRT. Good predictabilities were observed for both chemotherapy and CCRT.

The doctors could not avoid using negative agents for these 32 true-negative cases because there was no suitable protocol according to the HDRA results. Therefore a chemotherapy protocol including negative agents was used under the informed consent of these patients.

Table 1. Inhibition rates of drugs

Drug	n	IR ≤ 0	IR	Cutoff	> Cutoff	Positive rate
CDDP	307	7	41.5 ± 17.7 (2.7-83.0)	50	104	33.9
FU	292	18	50.7 ± 19.3 (0.5-84.3)	60	97	33.2
ADM	235	10	49.8 ± 20.6 (0.7-82.9)	60	87	37
MMC	260	2	61.1 ± 16.8 (1.6-88.1)	70	121	46.5
VP-16	253	16	40.1 ± 19.5 (0.3-82.0)	50	82	32.4
SN38	232	27	40.6 ± 21.4 (1.0-81.0)	50	74	31.9
DOC	167	18	43.9 ± 24.1 (1.0-89.1)	50	70	41.9
PAC	68	2	69.3 ± 22.8 (2.6-91.2)	70	43	63.2
GEM	68	6	29.6 ± 19.2 (1.7-78.9)	30	28	41.2

IR, inhibition rates; *CDDP*, cisplatin; *FU*, 5-fluorouracil; *ADM*, adriamycin; *MMC*, mitomycin C; *VP-16*, etoposide; *SN38*, innotecan; *DOC*, docetaxel; *PAC*, paclitaxel; *GEM*, gemcitabine.

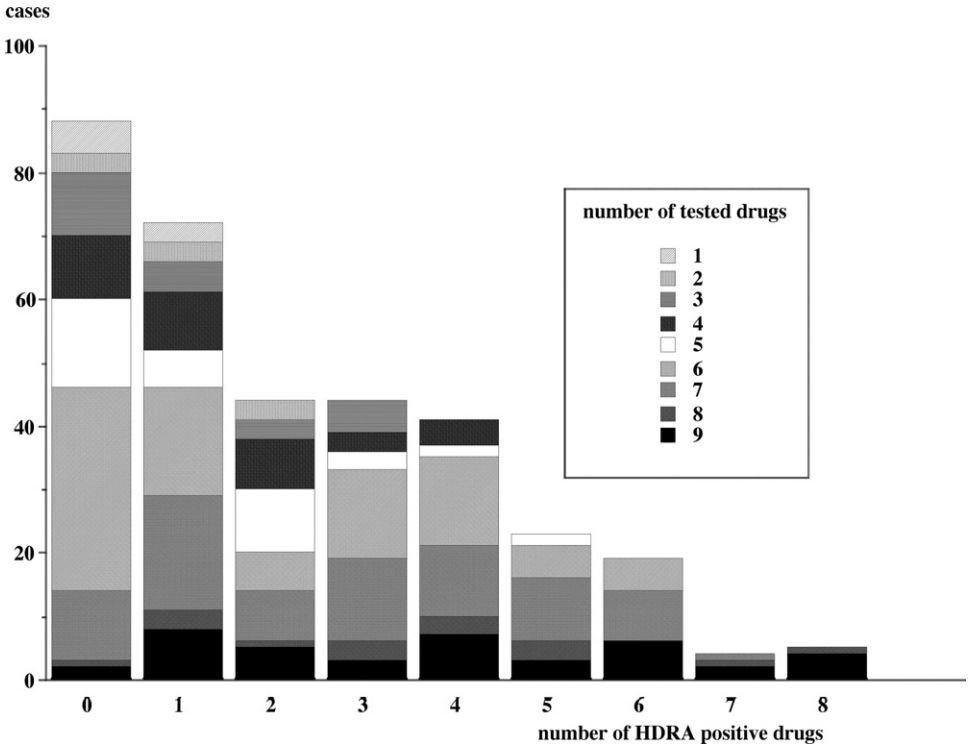

Figure 2. Distributions of numbers of positive drugs.

As for CDDP, for which the largest amount of data was available, there were 17 true-positive cases, 5 false-positive cases, and 14 true-negative cases. Yields of diagnostic properties were 77.2% (17/22) for positive predictive value, 100% (14/14) for negative predictive value, 100% (17/17) for sensitivity, 73.7% (14/19) for specificity, and 79.5% (31/36) for accuracy.

Discussion

Response rates for chemotherapy are usually lower for NSCLC than for small cell lung cancer (SCLC). The new chemotherapy agents developed since 1994 (ie, taxanes, SN38, and GEM) have improved response rates for chemotherapy for NSCLC. However, a highly effective standard chemotherapy protocol for NSCLC has not been determined, and therefore the HDRA should contribute to improvement of response rates for chemotherapy in patients with NSCLC.

Several negative studies concerning chemosensitivity tests for lung cancer were reported previously.[12,13] The culture methods of these negative studies were different from our system. Their evaluabilities were quite inferior to our results. It seemed that this inferiority in culture technique might lead to their negative results.

The HDRA is one of the chemosensitivity tests for anticancer agents.[4-6] It is characterized by its high evaluability compared with other assay methods. A high evaluability rate (97.4%) in lung cancer specimens was also demonstrated in this study. This is thought to be due to advantages of the histoculture method over other methods using single-cell suspensions. Histoculture methods maintain cell-to-cell contacts, resulting in good cell viability. The high evaluability of the HDRA is also thought to result from its good predictability for clinical responses.

We used the HDRA for NSCLC in 1994[7-11] because of its reported high evaluability.[4,5] The first step in the use of the HDRA for NSCLC was to adjust concentrations and to determine cutoff inhibition rates for each drug to NSCLC. We identified these parameters by using surgical specimens from patients with resectable lung cancer.

For CDDP, FU, ADM, MMC, and PAC, concentrations to be used had already been decided from data obtained from

Table 2. Rules for judgment of clinical correlations

		Clinical response	
Drug for judgment	**Combined drug**	**Responder**	**Nonresponder**
Sensitive	Sensitive	TP	FP
	Resistant	TP	FP
	Unknown	TP	FP
Resistant	Sensitive	NE	TN
	Resistant	FN	TN
	Unknown	NE	TN

TP, True positive; *FP,* false positive; *NE,* not evaluable; *TN,* true negative; *FN,* false negative.

Table 3. Clinical correlations

HDRA results	Response	Chemotherapy cases	CCRT cases	Total
Sensitive	Responder			
	CR	1	1	2
	PR	10	29	39
	Total	11	30	41
	Nonresponder			
	SD	11	0	11
	PD	4	0	4
	Total	15	0	15
Resistant	Responder			
	CR	0	0	0
	PR	0	0	0
	Total	0	0	0
	Nonresponder			
	SD	15	3	18
	PD	14	0	14
	Total	29	3	32
Diagnostic accuracies				
Positive predictive value		42.3%	100%	73.2%
Negative predictive value		100%	100%	100%
Sensitivity		100%	100%	100%
Specificity		65.9%	100%	68.1%
Accuracy		72.7%	100%	83.0%

HDRA, Histoculture drug response assay; *CCRT*, concurrent chemoradiation; *CR*, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease.

other kinds of cancer, such as gastrointestinal cancers and ovarian cancers. We checked whether the same concentrations could be used for NSCLC. Concentrations were initially decided for NSCLC with DOC, VP-16, SN38, and GEM. As shown in Figure 1, inhibition rates showed a broad distribution, including nonresponding cases, and we then decided on cutoff inhibition rates in NSCLC for all drugs.

In this article cutoff levels for NSCLC were decided by using data acquired from our institute. Cutoff inhibition rates of several anticancer agents (ie, CDDP, DOC, PAC, CPT-11, and GEM) were decided according to response rates of single-agent chemotherapy in NSCLC,[14-22] and those for FU, ADM, MMC, and VP-16 were decided according to those from combined chemotherapy. In this retrospective analysis a good clinical correlation was observed by using these cutoff inhibition rates. In particular, no false-negative cases were observed, as was reported in past studies on malignancies in the gastrointestinal tract. From the results, it could be concluded that the HDRA was applicable to NSCLC.

In CCRT cases an accuracy of 100% was observed. This result was probably due to the small sample size of patients who were treated with HDRA-negative agents. Most CCRT cases consisted of locally advanced diseases in this study. In these cases surgical resection of metastatic lymph nodes was undergone only to obtain specimens for the HDRA, and then HDRA-positive agents were intentionally used for CCRT.[9,10] This treatment strategy gave rise to the smaller sample size of patients using HDRA-negative agents. Moreover, cutoff inhibition rates in HDRA in this article were determined not for CCRT but for chemotherapy. Response rates of CCRT are usually superior to those of chemotherapy in NSCLC, and thus we believe that false-negative cases can occur in future studies using larger sample sizes.

The inhibition rate of SCLC was significantly lower than that of NSCLC with CDDP ($P = .02$), FU ($P = .04$), ADM ($P = .0003$), and MMC ($P = .004$). This result indicated that NSCLC was more highly sensitive to chemotherapy compared with SCLC. This result was not consistent with clinical observations. Therefore we speculate that the cutoff inhibition rates for NSCLC determined in this article could not be applied for SCLC, and we could not determine cutoff inhibition rates for SCLC in this article because of the insufficient number of samples.

For high evaluability, the HDRA requires large amounts of biopsy specimens. An average of 100 mg of specimen with good cell viability is needed to evaluate the sensitivity of 1 drug, whereas more than 1 drug requires another 40 mg of specimen. This large requirement limits applications of the HDRA. To obtain sufficient specimens for the HDRA, some surgical procedures must be used even for inoperable cases because bronchoscopic biopsy specimens or needle biopsy specimens are not applicable for the HDRA.

Some recent articles revealed that gene expression microarray analysis data might predict the chemosensitivity in lung cancer.[23,24] This can be accomplished on much smaller amounts than needed for the HDRA and therefore might become a good option for chemosensitivity testing in lung cancer.

It has already been reported that the HDRA improves the prognosis of patients with gastrointestinal cancer.[4,5] Our results demonstrated that the HDRA was applicable to NSCLC and that it might contribute to the improvement of responses to chemotherapy. However, it remains unclear whether HDRA-orientated chemotherapy will improve the prognosis of patients with NSCLC. Further data acquisition in respect to the clinical results of chemotherapy is needed to better evaluate this.

References

1. Park JG, Kramer BS, Steinberg SM, Carmichael J, Collins JM, Minna JD, et al. Chemosensitivity testing of human colorectal carcinoma cell lines using tetrazolium-based colorimetric assay. *Cancer Res.* 1987; 47:5875-9.
2. Kobayashi H. Collagen gel droplet culture method to examine in vitro chemosensitivity. *Methods Mol Med.* 2005;110:59-67.
3. Kobayashi H. Development of a new in vitro chemosensitivity test using collagen gel droplet embedded culture and image analysis for clinical usefulness. *Recent Results Cancer Res.* 2003;161:48-61.
4. Kubota T, Sasano N, Abe O, Nakao I, Kawamura E, Saito T, et al. The potential of the histoculture drug response assay to contribute to cancer patient survival. *Clin Cancer Res.* 1995;1:1537-43.
5. Furukawa T, Kubota T, Hoffman RM. Clinical applications of the histoculture drug response assay. *Clin Cancer Res.* 1995;1:305-11.
6. Ohie S, Udagawa Y, Aoki D, Nozawa S. Histoculture drug response assay to monitor chemoresponse. *Methods Mol Med.* 2005;110:79-86.
7. Yoshimasu T, Oura S, Maebeya S, Tanino H, Bessho T, Arimoto J, et al. Histoculture drug response assay on non-small cell lung cancer [in Japanese]. *Gan To Kagaku Ryoho.* 2000;27:717-22.
8. Yoshimasu T, Oura S, Hirai I, Kokawa Y, Okamura Y, Furukawa T. Dose response curve of paclitaxel measured by histoculture drug response assay [in Japanese]. *Gan To Kagaku Ryoho.* 2005;32:497-500.
9. Yoshimasu T, Oura S, Hirai I, Kokawa Y, Sasaki R, Honda K, et al. Histoculture drug response assay (HDRA) guided induction concurrent chemoradiotherapy for mediastinal node-positive non-small cell lung cancer [in Japanese]. *Gan To Kagaku Ryoho.* 2003;30:231-5.
10. Yoshimasu T, Oura S, Hirai I, Kokawa Y, Tanino H, Okamura Y. Chemosensitivity test for lung cancer using biopsy specimens obtained by endoscopic surgery [in Japanese]. *Jpn J Chest Surg.* 2005;19:82-6.
11. Yoshimasu T, Oura S, Hirai I, Kokawa Y, Hata K, Kawago M, et al. Cutoff level of docetaxel, paclitaxel and gemcitabine in histoculture drug response assay for non-small cell lung cancer [in Japanese]. *Gan To Kagaku Ryoho.* 2005;32:1013-6.
12. Gazdar AF, Steinberg SM, Russell EK, Linnoila RI, Oie HK, Ghosh BC, et al. Correlation of in vitro drug-sensitivity testing results with response to chemotherapy and survival in extensive-stage small cell lung cancer: a prospective clinical trial. *J Natl Cancer Inst.* 1990;82:117-24.
13. Shaw GL, Gazdar AF, Phelps R, Steinberg SM, Linnoila RI, Johnson BE, et al. Correlation of in vitro drug sensitivity testing results with response to chemotherapy and survival: comparison of non-small cell lung cancer and small cell lung cancer. *J Cell Biochem Suppl.* 1996; 24:173-85.
14. Francis PA, Rigas JR, Kris MG, Pisters KM, Orazem JP, Woolley KJ, et al. Phase II trial of docetaxel in patients with stage III and IV non-small cell lung cancer. *J Clin Oncol.* 1994;12:1232-7.
15. Fossella FV, Lee JS, Murphy WK, Lippman SM, Calayag M, Pang A, et al. Phase II study of docetaxel for recurrent or metastatic non-small cell lung cancer. *J Clin Oncol.* 1994;12:1238-44.
16. Yasuda K, Igishi T, Kawasaki Y, Yamamoto M, Kato K, Matsumoto S, et al. Phase II trial of weekly paclitaxel in previously untreated advanced non-small-cell lung cancer. *Oncology.* 2003;65:224-8.
17. Feliu J, Martin G, Lizon J, Chacon JI, Dorta J, de Castro J, et al. Sequential therapy in advanced non-small-cell lung cancer with weekly paclitaxel followed by cisplatin-gemcitabine-vinorelbine. A phase II study. *Ann Oncol.* 2001;12:1369-74.
18. Abratt RP, Bezwoda WR, Falkson G, Goedhals L, Hacking D, Rugg TA, et al. Efficacy and safety profile of gemcitabine in non-small cell lung cancer; a phase II study. *J Clin Oncol.* 1994;12:1535-40.
19. LeChevalier T, Gottfried M, Gatzemier U, Shepherd FA, Weynants P, Cottier B, et al. Confirmatory activity of gemcitabine in non-small cell lung cancer. *Eur J Cancer.* 1993;29a(suppl 6):160.
20. Negoro S, Fukuoka M, Kurita Y, Niitani H. Results of phase II studies of gemcitabine in non-small cell lung cancer. *Proc Am Soc Clin Oncol.* 1994;13:367.
21. Crino L, Mosconi AM, Scagliotti G, De Marinis F, Darwish S, Calandri C, et al. Salvage therapy worth gemcitabine in pretreated advanced non-small cell lung cancer. *Proc Am Soc Clin Oncol.* 1997; 16:446.
22. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, et al. A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small-cell lung cancer. *J Clin Oncol.* 1992;10:16-20.
23. Oshita F, Ikehara M, Sekiyama A, Hamanaka N, Saito H, Yamada K, et al. Genomic-wide cDNA microarray screening to correlate gene expression profile with chemoresistance in patients with advanced lung cancer. *J Exp Ther Oncol.* 2004;4:155-60.
24. Kikuchi T, Daigo Y, Katagiri T, Tsunoda T, Okada K, Kakiuchi S, et al. Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs. *Oncogene.* 2003;10: 2192-205.