Expression of Dysadherin and Cytokeratin as Prognostic Indicators of Disease-free Survival in Patients with Stage I NSCLC

KENJI ONO, HIDETAKA URAMOTO and TAKESHI HANAGIRI

Second Department of Surgery,
School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan

Abstract. *Background: Adjuvant chemotherapy is required following the resection of aggressive NSCLC. It is therefore necessary to identify factors that accurately predict prognosis. Materials and Methods: Tumor specimens were collected from 107 patients who underwent a complete resection for NSCLC from 1994-2000 in this Department. The expression of E-cadherin, dysadherin, and cytokeratin in stage I NSCLC specimens was analyzed by immunohistochemistry. Results: Seventeen percent of tumors showed reduced E-cadherin immunostaining. Twenty-nine per cent of tumors showed dysadherin expression in over 50% of the cancer cells. Positive expression of cytokeratin was identified in 30 (28.0%) patients. The incidence of positive expression of dysadherin in females and elderly patients was higher than that in other patients. Cytokeratin immunoreactive tumor cells in lymph nodes were identified in 34 (28.0%) out of 107 patients. The incidence of positive expression of cytokeratin in T1 tumors was higher than that in T2 tumors. There was a significant inverse correlation between the expression of E-cadherin and dysadherin. The increased expression of cytokeratin was significantly associated with recurrence. Logistic regression models indicated that cytokeratin expression was an independent predictor of recurrence. The increased expression of dysadherin and cytokeratin had a significant impact on patient survival. Furthermore, tumors with an increased expression of dysadherin and a reduced expression of E-cadherin showed the worst prognosis. Conclusion: The detection of dysadherin in tumors and cytokeratin in the lymph nodes may be a potential significant indicator of a poor prognosis for patients who undergo complete resection of stage I NSCLC.*

Correspondence to: Hidetaka Uramoto, Second Department of Surgery, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan. Tel: +81 936917442, Fax: +81 936924004, e-mail: hidetaka@med.uoeh-u.ac.jp

Key Words: E-Cadherin, dysadherin, cytokeratin recurrence, NSCLC, prognosis.

Although surgery remains the only curative treatment for non-small cell lung cancer (NSCLC), approximately 30% of all patients with pathological stage I have tumor recurrence and eventually succumb to the cancer, despite a complete surgical resection (1). Adjuvant chemotherapy is considered necessary following a resection for such a biologically aggressive NSCLC (2). It is therefore essential to identify factors that can accurately predict prognosis. However, there are no carefully useful markers for selecting appropriate candidates for adjuvant chemotherapy.

Metastasis is the most frequent cause of treatment failure (3) and occult metastases are present in a significant proportion of patients at the time of surgical intervention (4). The disintegration of cell–cell interactions in cancerous tissues leads to invasive and metastatic growth (5). Decreased E-cadherin function is associated with tumor progression in many cancers, and E-cadherin acts as a suppressor of invasive ability (5). The loss of E-cadherin is considered to be a defining characteristic of the epithelial-mesenchymal transition and metastasis (6). In contrast, dysadherin is a cancer-associated cell membrane glycoprotein that down-regulates E-cadherin expression and promotes metastasis (5). In addition, an antibody against cytokeratin, which is widely used as a marker of epithelial cells, can detect occult metastases from different kinds of cancer (7).

Therefore, E-cadherin, dysadherin, and cytokeratin may also be useful predictive indicators of tumor recurrence in patients following lung resection. The present study is the first molecular analysis of E-cadherin, dysadherin, and cytokeratin expression, which attempted to elucidate their clinical significance in patients with resected stage I NSCLC.

Materials and Methods

Patients, clinical features, and follow-up. The Institutional Review Board approved this study and informed consent for the use of the tumor specimens was obtained either from all the patients or their legal guardians. Tumor samples were obtained

from 408 patients with NSCLC who had undergone a surgical resection in this Department between 1994 and 2000. Twenty-four, 59, and 101 of these patients were stage II, III, IV, respectively, and 38 underwent an incomplete resection. The tumor samples from 79 patients were too small to evaluate by immunohistochemical (IHC) staining for the three molecules. A total of 301 patients were excluded from further analyses. Therefore, a total of 107 tumor specimens were evaluated. All of the patients in this series were Japanese, including 71 males and 36 females, with a mean age of 67.8 years (range 47-85 years). There were 34 non-smokers (never smokers), 18 former, and 55 current smokers. Former smokers were defined as those who quit smoking at least 3 years before the time of surgery. The tumor stage was classified according to the Revisions in the International System for Staging Lung Cancer (8). A pathological analysis revealed that 51 patients were at stage IA and 36 at IB. The histological types included 74 adenocarcinoma (69.2%), 22 SQ (21.4%), 6 large cell carcinoma (5.6%), and 3 others. The surgical procedures included a lobectomy in 105 patients, and a pneumonectomy and a segmentectomy in each of the remaining two patients. None of the patients had received either chemotherapy or radiotherapy prior to the resection.

The patients were followed up every month within the first postoperative year and at approximately 2- to 4-month intervals thereafter. The evaluations included a physical examination, chest roentgenography, an analysis of blood chemistry, and measurements of tumor markers. Chest and abdominal computed tomography, brain magnetic resonance imaging, and a bone scintiscan were performed every 6 months for 3 years after surgery. Additional examinations were performed if any symptoms or signs of recurrence were detected. A follow-up was conducted in all patients. The median follow-up period was 55.8 months. Sixty-five patients were alive and free of cancer at the last follow-up, while 19 patients had died of other causes without evidence of cancer, 17 patients had died of cancer, 5 patients were alive with recurrent cancer, and 1 patient had died of other causes with recurrence of cancer. Twenty-two (20.6%) out of the 107 patients demonstrated disease recurrence after surgery.

Detection of E-cadherin and dysadherin expression in tumors. IHC staining was conducted using serial sections from the same paraffin-embedded blocks by previously described methods (4, 9). Briefly, all tissue specimens were formalin-fixed and processed similarly, according to the standard histological methods. A 3 μm-thick formalin-fixed, paraffin-embedded tissue section was treated with 0.3% hydrogen peroxidase for 15 minutes to quench the endogenous peroxidase activity. The sections were then incubated with an E-cadherin mouse monoclonal antibody (C20820; BD Bioscience, San Diego, CA, USA) diluted 1:200, or dysadherin (NCC-M53, kindly provided by Dr Hirohashi, National Cancer Center Research institute Tokyo, Japan) diluted 1:500 (10), respectively, in PBS and incubated at 4˚C for at 18 h. The labeled streptavidin-biotin kit (Dako Corporation, Carpinteria, CA, USA) was used as the secondary antibody (11-13). The diaminobenzidine method was used to visualize the peroxidase with hematoxylin as a counterstain. The percentage of immunoreactive tumor cells in five ×400 fields selected randomly on one slide was recorded, and then the final value of positive tumor cells was determined as the average of the number of positively immunostained cells. The stained specimens were scored as previously reported: positive E-cadherin expression (≥70% of tumor cell membranes stained) or negative (<70% of tumor cells stained) (4), and positive dysadherin expression (≥50% of tumor cell membranes stained) or negative (<50% of tumor cells stained) (10). Necrotic areas were excluded from the evaluation. Positive staining of normal epithelial cells adjacent to the tumor and lymphocytes in the primary tumor sections was used as an internal positive control, for E-cadherin and dysadherin staining, respectively. The negative controls were sections stained with the exclusion of the primary antibody. Two observers (K.O. and H.U.), who had no previous knowledge of the clinical parameters and outcomes for each patient, independently reviewed the immunohistochemically stained sections; all discrepancies were resolved by a joint review of the slides in question.

Detection of micrometastasis of cytokeratin-positive cells in the lymph nodes. The lymph nodes were removed from these 107 patients during surgery and they were thereafter analyzed for micrometastasis using cytokeratin IHC staining by previously described methods (4). Briefly, five 4-μm slices, representing every other slice from 10 slices of each paraffin-embedded lymph nodes section, were attached to glass slides. The slides were then stained with primary antibodies against cytokeratin using the labeled streptavidin-biotin method (Dako LSAB kit; Dako Corp). The primary antibodies were mouse biclonal antibody AE1/AE3 (Progen Biotechnik GmbH, Heidelberg, Germany) to cytokeratin. The staining procedures were: (i) deparaffinization with xylene and ethanol, (ii) incubation with the primary antibodies (dilutions: AE1/AE3, 1/200), (iii) incubation with the secondary antibody, (iv) developing with peroxidase-labeled streptavidin and diaminobenzidine-H_2O_2, and (v) counterstaining with hematoxylin. The presence of cytokeratin-positive cells within the whole-body section of the lymph nodes was accepted as evidence of micrometastatic tumor cells, even if only a single cytokeratin-positive cell was detected. Ninety-eight (91.6%) out of 107 patients had undergone dissection of the hilar and mediastinal lymph nodes (systematic nodal dissection) and 9 patients had only the hilar lymph nodes dissected.

Statistical analyses. Statistical significance was evaluated using the Fisher's exact test. A multivariate logistic regression analysis was used to evaluate independent associations. The Kaplan-Meier method was used to estimate the probability of survival, and survival differences were analyzed by the log-rank test. A multivariate analysis was then performed according to Cox's proportional hazards model. The odds ratio and 95% confidence interval were calculated for each variable. Differences with p-values <0.05 were considered to be statistically significant. The data were analyzed using the Stat View software package (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Expression of E-cadherin. The majority of the tumors showed a heterogeneous intratumoral expression pattern. Seventeen per cent (18 out of 107) of tumors showed reduced immunostaining for E-cadherin. There was no significant association between E-cadherin expression and the clinicopathological factors (Table I).

Table I. *Relationships between the molecular parameters and the clinicopathological characteristics.*

Variable	Category	No. of patients n=107	E-Cadherin P (%) 89 (83.2)	E-Cadherin N 18	Dysadherin P (%) 31 (29.0)	Dysadherin N 76	Cytokeratin P (%) 30 (28.0)	Cytokeratin N 77
Gender	Male	71	59 (83.1)	12	15 (21.1) $^{\$1}$	56	19 (26.8)	52
	Female	36	30 (83.3)	6	16 (44.4)	20	11 (30.6)	25
Age (years)	<68	45	38 (84.4)	7	7 (15.6) $^{\$2}$	38	15 (24.2)	30
	≥68	62	51 (82.3)	11	24 (38.7)	38	15 (33.3)	47
Smoking$^{\#}$	C/E	71	58 (81.7)	13	19 (26.8)	52	18 (25.4)	53
	N	34	29 (85.3)	5	11 (32.4)	23	12 (35.3)	22
T Status	T1	51	41 (80.4)	10	15 (29.4)	36	9 (17.6) $^{\$3}$	42
	T2	56	48 (85.7)	8	16 (28.6)	40	21 (37.5)	35
Histology$^{\#}$	Adenocarcinoma	74	62 (83.8)	12	25 (33.8)	49	23 (31.1)	51
	All other	32	26 (81.3)	6	6 (18.8)	26	7 (21.9)	25
Differentiation$^{\#}$	Well	39	34 (87.2)	5	12 (30.8)	27	11 (28.2)	28
	All other	60	51 (85.0)	9	17 (28.3)	43	16 (26.7)	44

P: Positive, N: negative, C/E: current/ever smoker, N: never smoker, $^{\#}$unclassified patients were excluded; $^{\$}$statistically significant *p*-value: $^{\$1}$0.012, $^{\$2}$0.009, $^{\$3}$0.022.

Table II. *Associations between the molecular parameters.*

Variable	Category	No. of patients n=107	E-Cadherin P (%) 89 (83.2)	E-Cadherin N 18	Dysadherin P (%) 31 (29.0)	Dysadherin N 76	Cytokeratin P (%) 30 (28.0)	Cytokeratin N 77
E-Cadherin	P	89	-	-	21 (23.6) $^{\$}$	68	25 (28.1)	64
	N	18	-	-	10 (55.6)	8	5 (27.8)	13
Dysadherin	P	31	-	-	-	-	10 (32.3)	21
	N	76	-	-	-	-	20 (26.3)	56

P: Positive, N: Negative; $^{\$}$Statistically significant p-value:<0.01.

Expression of dysadherin. Dysadherin immunostaining was primarily observed in the membranes of the neoplastic cells and was homogenous throughout the neoplasm. Twenty-nine percent (31 out of 107) of tumors showed dysadherin expression in over 50% of the cancer cells. There was an increased incidence of positive expression of dysadherin in specimens resected from females and elderly patients. There was no significant correlation between the increased expression of dysadherin and the other clinicopathological factors such as smoking, T status, histology, and differentiation (Table I). The incidence of a positive expression of dysadherin was 23.6% (21/89) and 55.6% (10/18) in the specimens with positive and negative expression of E-cadherin, respectively (p <0.01; Table II).

Table III. *Recurrent sites of the tumors (n=22).*

	Site	Number*
Hematogeneous (n=18$^{\$}$)	Lung	10
	Bone	3
	Brain	2
	Skin	1
	Liver	1
	Adrenal	1
Locoregional (n=10$^{\$}$)	Pleural dissemination	7
	Lymph node	3

*The numbers of the recurrent sites overlap. $^{\$}$Six patients had recurrent tumors in both hematogeneous and locoregional lesions.

Expression of cytokeratin. Micrometastatic tumor cells in were detected in pathological lymph nodes in 34 (28.0%) out of 107 patients. The incidence of positive expression of cytokeratin was 17.6% (9/51) and 37.5% (21/56) in the T1 and T2 patients, respectively (p=0.022). There was no significant correlation between the increased expression of

Table IV. *Associations between the molecular parameters and recurrence.*

	E-Cadherin		Dysadherin		Cytokeratin	
Variable	P (%)	N	P (%)	N	P (%)	N
With recurrence	18 (81.8)	4	10 (45.5)	12	14 (63.6) $	8
Without recurrence	71 (83.5)	14	21 (24.7)	64	16 (18.8)	69

P: Positive, N: Negative; $Statistically significant: *p*-value<0.0001.

cytokeratin and other clinicopathological factors such as gender, age, smoking, histology, and differentiation (Table I). No significant association was observed between E-cadherin or dysadherin and cytokeratin (Table II).

Influence of E-cadherin, dysadherin, and cytokeratin expression and the clinicopathological factors on recurrence. The majority of the sites of tumor recurrence were hematogenous metastases (Table III). No significant association was observed between E-cadherin or dysadherin and recurrence. Cytokeratin expression was identified in 14 (63.6%) out of 22 patients with recurrence and 16 (18.8%) out of 85 patients in patients without recurrence ($p<0.0001$; Table IV). The logistic regression models indicated cytokeratin expression, but not E-cadherin or dysadherin expression, as being an independent predictor for recurrence (Table V and IV).

Influence of E-cadherin, dysadherin, and cytokeratin expression and clinicopathological factors on disease-free survival. Four groups were defined by the expression of E-cadherin and dysadherin; positive expression of both E-cadherin and dysadherin (+/+), positive and negative (+/–), negative and positive: (–/+), and both negative (–/–). The 2-year DFS rate in the (+/+) group was 72.2%, 85.9% in the (+/–) group, 60.0% in the (–/+), and 100% in the (–/–) group. The (–/+) group had longer progression-free survival than the others ($p=0.0392$). The (–/+) group also had longer overall survival (OS) than the others ($p=0.0119$). Dysadherin and cytokeratin expression was associated with a poorer disease-free survival (DFS) according to the univariate survival analysis was as the pathological T status (Table VII). A multivariate survival analysis demonstrated that dysadherin and cytokeratin expression were independently associated with an increased risk for a poor DFS (Table VIII).

Table V. *Univariate analysis of the factors contributing to recurrence.*

Variables	OR	95% CI	*p*-Value
Gender: male	0.860	0.323-2.288	0.763
Age: ≤69 years	1.349	0.512-3.554	0.542
pT: 2	4.016	1.269-11.90	<0.01
E-Cadherin: negative	1.127	0.331-3.839	0.850
Dysadherin: positive	2.538	0.960-6.711	0.063
Cytokeratin: positive	7519	2.710-20.833	<0.0001

OR: Odds ratio, 95% CI: 95% confidence interval.

Table VI. *Multivariate analysis of the factors contributing to recurrence.*

Variables	OR	95% CI	*p*-Value
Gender: male	0.916	0.277-3.030	0.886
Age: ≤69 years	1.373	0.424-4.453	0.597
pT: 2	2.986	0.897-9.900	0.075
E-Cadherin: negative	0.885	0.197-3.981	0.873
Dysadherin: positive	2.544	0.724-8.929	0.145
Cytokeratin: positive	6.711	2.217-20.408	<0.001

Discussion

The present study demonstrated three prominent facts. First, the expression of dysadherin had a significant impact on patient survival. Furthermore, patients with tumors with increased expression of dysadherin and reduced expression of E-cadherin showed the worst prognosis. These results suggest that dysadherin expression might be an excellent indicator for the patients with NSCLC and the combined immunohistochemical analysis of dysadherin and E-cadherin expression could provide further prognostic information to identify those candidates who would benefit most from adjuvant chemotherapy following complete resection of NSCLC. The present study also suggests dysadherin expression was associated with age, which is consistent with previous findings in patients with gastric cancer (14). The association with aging might be the result of multiple acquired genetic and/or epigenetic events on carcinogenesis. On the other hand, the incidence of positive expression of dysadherin in females was higher than that in males. These data seem to be inconsistent with previous finding of the female gender being correlated with a favorable prognosis (15). In fact, data mining of the results from large-scale clinical microarray studies revealed that dysadherin is much more

Table VII. *Univariate analysis using a proportional hazards model for disease-free survival.*

	Characteristic		HR	95% CI	*p*-Value
Variable	Unfavorable	Favorable			
Gender	Male	Female	1.295	0.497-3.378	0.600
Age (years)	≤69	>69	1.210	0.501-2.922	0.672
T Status	T2-4	T1	4.717	1588-14.084	<0.01
E-Cadherin	Negative	Positive	1.179	0.394-3.525	0.769
Dysadherin	Positive	Negative	2.618	1.083-6.329	0.032
Cytokeratin	Positive	Negative	6.25	2.519-15.625	<0.0001

HR: Hazard ratio.

Table VIII. *Multivariate analysis using a proportional hazards model for disease-free survival.*

	Characteristic		HR	95% CI	*p*-Value
Variable	Unfavorable	Favorable			
Gender	Male	Female	1.689	0.596-4.854	0.324
Age (years)	≤69	>69	0.895	0.337-2.378	0.824
T Status	T2-4	T1	3.268	0.975-10.989	0.055
E-Cadherin	Negative	Positive	1.318	0.417-4.169	0.638
Dysadherin	Positive	Negative	2.950	1.098-7.937	0.032
Cytokeratin	Positive	Negative	4.444	1.661-11.905	<0.01

highly expressed in estrogen receptor-negative rather than in estrogen receptor-positive breast cancer (16). Epidermal growth factor receptor (*EGFR*) mutations also occur more frequently in adenocarcinomas of the lung with strong expression of the estrogen receptor beta subtype (15). Therefore, the cause of high incidence of dysadherin in females observed here remains to be elucidated.

The current study also revealed that an increased expression of cytokeratin was significantly correlated with postoperative recurrence. Furthermore, the increased expression of cytokeratin had a significant impact on patient survival, which was consistent with a previous prospective study (17). Dysadherin was not a predictive postoperative recurrence despite showing a significant impact on survival. This inconsistency may result from the association between recurrence and survival because (i) increased expression of dysadherin alone might be insufficient to cause recurrence after surgery, or (ii) that the current study had insufficient statistical power. In fact, the positive expression of dysadherin was identified in 10 (45.5%) out of 22 and 21 (24.7%) out of 85 patients with and without recurrence, respectively, with marginal significance (p=0.060).

The current study also found an inverse correlation between E-cadherin and dysadherin, which is consistent with a previous report (18). However, data presented here failed to show that the expression of E-cadherin expression was a prognostic factor. There has been only one report investigating the expression of dysadherin in patients with NSCLC (19). Tamura *et al*. reported that patients with reduced positive staining for E-cadherin show a significantly shorter survival time than those with a normal E-cadherin expression and that the OS of patients with dysadherin-positive tumors is significantly worse than that of those with dysadherin-negative tumors (19). Furthermore, dysadherin expression was not found to be correlated with E-cadherin expression. This discrepancy could be related to the stage of NSCLC, a difference in the method used for IHC, the duration of the follow-up, calculation of survival (OS or DFS). Although the present study found a significantly inverse correlation between E-cadherin and dysadherin, the overexpression of dysadherin, not E-cadherin, was significantly correlated with a worse prognosis, thereore suggesting the E-cadherin-independent activity of dysadherin as a factor in the regulation of cancer invasiveness (20). Many other factors may also be involved in the function of E-cadherin, such as methylation of the promoter region (9), and catenin abnormalities (4). Nam *et al*. recently suggested that dysadherin might affect E-cadherin function rather than its expression (5).

In conclusion, the current results indicate that the expression of dysadherin and cytokeratin is useful for predicting postoperative recurrence, as well as for selecting the optimal candidates with lung adenocarcinoma that need to undergo postoperative adjuvant chemotherapy following surgery. Further investigations are necessary in order to elucidate whether dysadherin and cytokeratin expression levels are associated with the efficacy of adjuvant chemotherapy.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

References

1 Asamura H, Goya T, Koshiishi Y, Sohara Y, Eguchi K, Mori M, Nakanishi Y, Tsuchiya R, Shimokata K, Inoue H, Nukiwa T, and Miyaoka E: A Japanese Lung Cancer Registry study: prognosis of 13,010 resected lung cancers. J Thorac Oncol *3*: 46-52, 2008.

2 Arriagada R, Auperin A, Burdett S, Higgins JP, Johnson DH, Le Chevalier T, Le Pechoux C, Parmar MK, Pignon JP, Souhami RL, Stephens RJ, Stewart LA, Tierney JF, Tribodet H, and van Meerbeeck J: Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small cell lung cancer: two meta-analyses of individual patient data. Lancet *375*: 1267-1277, 2010.

3 Christofori G: New signals from the invasive front. Nature *441*: 444-450, 2006.
4 Yamashita T, Uramoto H, Onitsuka T, Ono K, Baba T, So T, So T, Takenoyama M, Hanagiri T, Oyama T, and Yasumoto K: Association between lymphangiogenesis-/micrometastasis- and adhesion-related molecules in resected stage I NSCLC. Lung Cancer in press.
5 Nam JS, Hirohashi S, and Wakefield LM. Dysadherin: a new player in cancer progression. Cancer Lett 255: 161-169, 2007.
6 Uramoto H, Iwata T, Onitsuka T, Shimokawa H, Hanagiri T, Oyama T, and Yasumoto K: Epithelial-mesenchymal transition in EGFR-TKI-acquired resistant lung adenocarcinoma. Anticancer Res in press.
7 Braun S, Pantel K, Müller P, Janni W, Hepp F, Kentenich CR, Gastroph S, Wischnik A, Dimpfl T, Kindermann G, Riethmüller G, and Schlimok G: Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med *342*: 525-533, 2000.
8 Mountain CF: Revisions in the International System for Staging Lung Cancer. Chest *111*: 1710-1717, 1997.
9 Nakata S, Sugio K, Uramoto H, Oyama T, Hanagiri T, Morita M and Yasumoto K: The methylation status and protein expression of CDH1, p16(INK4A), and fragile histidine triad in nonsmall cell lung carcinoma: epigenetic silencing, clinical features, and prognostic significance. Cancer *106*: 2190-2199, 2006.
10 Aoki S, Shimamura T, Shibata T, Nakanishi Y, Moriya Y, Sato Y, Kitajima M, Sakamoto M, and Hirohashi S: Prognostic significance of dysadherin expression in advanced colorectal carcinoma. Br J Cancer *88*: 726-732, 2003.
11 Ono K, Sugio K, Uramoto H, Baba T, Ichiki Y, Takenoyama M, Hanagiri T, Oyama T and Yasumoto K: Discrimination of multiple primary lung cancers from intrapulmonary metastasis based on the expression of four cancer-related proteins. Cancer *115*: 3489-500, 2009.
12 Onitsuka T, Uramoto H, Nose N, Takenoyama M, Hanagiri T, Sugio K and Yasumoto K: Acquired resistance to gefitinib: The contribution of mechanisms other than the T790M, MET, and HGF status. Lung Cancer *68*: 198-203, 2010.
13 Onitsuka T, Uramoto H, Ono K, Takenoyama M, Hanagiri T, Oyama T, Izumi H, Kohno K and Yasumoto K: Comprehensive molecular analyses of lung adenocarcinoma with regard to the epidermal growth factor receptor, K-ras, MET, and hepatocyte growth factor status. J Thorac Oncol *5*: 591-596, 2010.
14 Shimada Y, Yamasaki S, Hashimoto Y, Ito T, Kawamura J, Soma T, Ino Y, Nakanishi Y, Sakamoto M, Hirohashi S and Imamura M: Clinical significance of dysadherin expression in gastric cancer patients. Clin Cancer Res *10*: 2818-2823, 2004
15 Nose N, Sugio K, Oyama T, Nozoe T, Uramoto H, Iwata T, Onitsuka T and Yasumoto K: Association between estrogen receptor-beta expression and epidermal growth factor receptor mutation in the postoperative prognosis of adenocarcinoma of the lung. J Clin Oncol *27*: 411-417, 2009.
16 Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EM, Atkins D and Foekens JA: Gene-expression profiles to predict distant metastasis of lymph node-negative primary breast cancer. Lancet *365*: 671-679, 2005.
17 Yasumoto K, Osaki T, Watanabe Y, Kato H and Yoshimura T: Prognostic value of cytokeratin-positive cells in the bone marrow and lymph nodes of patients with resected non-small cell lung cancer: a multicenter prospective study. Ann Thorac Surg *76*: 194-201, 2003.
18 Kyzas PA, Stefanou D, Batistatou A, Agnantis NJ, Nakanishi Y, Hirohashi S and Charalabopoulos K: Dysadherin expression in head and neck squamous cell carcinoma: association with lymphangiogenesis and prognostic significance. Am J Surg Pathol *30*: 185-193, 2006.
19 Tamura M, Ohta Y, Tsunezuka Y, Matsumoto I, Kawakami K, Oda M and Watanabe G: Prognostic significance of dysadherin expression in patients with non-small cell lung cancer. J Thorac Cardiovasc Surg *130*: 740-745, 2005.
20 Batistatou A, Peschos D, Tsanou H, Charalabopoulos A, Nakanishi Y, Hirohashi S, Agnantis NJ and Charalabopoulos K: In breast carcinoma dysadherin expression is correlated with invasiveness but not with E-cadherin. Br J Cancer *96*: 1404-1408, 2007.

Received June 3, 2010
Revised July 12, 2010
Accepted July 15, 2010