of patients was collected, and histology-specific characteristics were extensively analyzed.

PATIENTS AND METHODS

Patients

A total of 383 patients with a histologic diagnosis of primary pulmonary NE tumor at each institution were enrolled onto this retrospective study. Intermediate- and high-grade NE tumors were a focus for enrollment. Samples were obtained from 10 institutions in the Japanese Multicenter Study Group of NE Tumors (Appendix). To ensure that there would be enough specimens for pathologic examination, only surgical cases were considered. Patients who were diagnosed only by biopsy sample and treated by some modality other than surgery were excluded. Histopathologic and clinicopathologic studies were performed. The final histologic diagnosis was established by an expert central review, as described later in detail. Extensive clinical information was also collected and included demographic data, surgical information, preoperative serum tumor marker levels, pathologic data, endocrine syndromes (Cushing's syndrome, acromegaly, and so on), tumor recurrence, and survival. For serum tumor markers, three markers, carcinoembryonic antigen (CEA; normal range, < 5 ng/mL), neuron-specific enolase (NSE; normal range, < 15 ng/mL), and progastrin-releasing peptide (proGRP; normal range, < 46 ng/mL), were studied. All patients were staged postsurgically according to the International Union Against Cancer TNM classification system.[13]

Pathologic Diagnosis: Central Review

To ensure an accurate histologic diagnosis as NE tumor, the histology of all of the enrolled patients was reviewed by a pathology panel consisting of six experts (T.K., Y.M., T.I., Y.I., M.N., and T.Y.). Paraffin-embedded blocks or unstained slide glasses were obtained in all cases and processed by routine hematoxylin and eosin staining and immunohistochemical studies solely at one institution (T.K. and S.-X.J.). To demonstrate the NE phenotype, at least three antibodies to chromogranin-A, CD56 (neural adhesion molecule), and synaptophysin were used. Immunohistochemically, the tumor was considered as positive if the tumor cells exhibited focal, patchy, or diffuse staining in the intracellular locations for each antigen. The classification criteria were based on the revised WHO classification of lung carcinoma (1999),[14] in which TC, AC, LCNEC, and SCLC are strictly differentiated. The process of central review was as follows. First, the pathology panel members performed a pathology review independently, and their respective reports were sent directly to the central office. After the individual reviews were completed, a review meeting was held to establish a final consensus on the histologic type in each case. The evaluation of immunohistochemical staining was also documented.

Statistics

The Kaplan-Meier product limit estimator was used to graphically display the survival curves, and the log-rank test was used to compare survival between different groups. The Cox proportional hazard model was used to examine the effects of variables that may have affected the prognosis of patients with NE tumors. $P \leq .05$ was considered significant.

RESULTS

Among the 383 patients enrolled, 18 were excluded from the study. In 17 patients, the specimens were judged to be inappropriate because either the tumors were of nonpulmonary origin or no specimens were available from the primary site. In one patient, the eligibility criteria were not met because this was an autopsy case. The remaining 365 tumors were considered for further central pathology review.

Central Pathology Review

Of the 365 tumors, as a final agreement of the review meetings, a total of 318 (87.1%) were diagnosed as pulmonary NE tumors, whereas a histology of non-NE tumor was confirmed in 47 tumors (12.9%; Table 1). Actually, the pathology panel could not reach a consensus with regard to the histologic type of 14 high-grade NE tumors at the initial session of panel meetings. Therefore, after enough intervals, the panel meetings were held again, and the final consensus as either LCNEC or SCLC was established. Of the NE tumors, a diagnosis of TC, AC, LCNEC, and SCLC was made in 55, nine, 141, and 113 patients, respectively. In the non-NE tumors, large-cell carcinoma (LCC) was most commonly seen (33 patients), followed by poorly differentiated squamous cell carcinoma (seven patients), poorly differentiated adenocarcinoma (three patients), pulmonary blastoma (two patients), and indeterminate histology by treatment (two patients). When looking at the histologic subtypes of 74 LCCs, 141 were diagnosed as LCNEC because of the coexistence of NE morphology and phenotype. However, the NE phenotype was not demonstrated despite the presence of NE morphology in 11 patients (LCC with NE morphology), and the NE morphology was not demonstrated despite the presence of NE phenotype in 12 patients (LCC with NE phenotype). In the remaining 10 patients, neither NE phenotype nor NE morphology was demonstrated (LCC). Among 141 LCNECs, 15 tumors (10.6%) were combined with other histologic types, and 126 tumors (89.14%) were not combined (Table 2). Also, among 113 SCLCs, 30 tumors (26.6%) were combined with other histologic types, and 83 tumors (73.4%) were not combined (Table 3). Despite the various combinations of high-grade NE tumors with other histologic types, neither TC nor AC was seen as the combined histology for LCNEC and SCLC.

Clinicopathologic Profiles

The clinical background and profiles were studied according to the histologic type (Table 4). Aggressive tumors tended to affect older patients. In particular, patients with TC were significantly younger than patients with other tumor histologies. A remarkable difference in sex distribution was seen between carcinoid tumors (TC and AC) and other high-grade NE carcinomas (LCNEC and SCLC). Compared with carcinoid tumors, the high-grade NE tumors affected men significantly more often than women, with males accounting for more than 80% to 90% of the tumors. Also, 95% to 100% of the patients with high-grade NE carcinomas had a smoking history, whereas only half of the patients with carcinoid tumors were smokers. Only four patients (1.3%) in the entire group of patients with NE tumors showed

Table 1. Histologic Diagnosis

Histologic Type	No. of Patients	%
NE tumors	318	87.1
TC	55	15.1
AC	9	2.5
LCNEC	141	38.6
SCLC	113	31.0
Non-NE tumors	47	12.9
LCC	33	9.0
Others	14	3.8
Total	365	100

Abbreviations: NE, neuroendocrine; TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma; LCC, large-cell carcinoma.

Table 2. Details of Histologic Diagnosis of LCNEC

Histologic Type	No. of Patients	%
LCNEC, not combined	126	89.4
LCNEC, combined	15	10.6
With AD	5	3.5
With SQ	8	5.7
With others	2	1.4
Total	141	100

Abbreviations: LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma; AD, adenocarcinoma; SQ, squamous-cell carcinoma.

symptoms related to the paraneoplastic syndromes. The following syndromes were seen: Eaton-Lambert's syndrome in two patients with SCLC, syndrome of inappropriate antidiuretic hormone secretion in one patient with SCLC, and carcinoid syndrome in one patient with TC. In AC and LCNEC, paraneoplastic syndrome was not seen. The serum tumor markers of CEA, NSE, and proGRP were measured before surgery in 298 (93.7%), 240 (75.5%), and 79 (24.8%) of 318 patients, respectively (Table 5). The serum CEA level was elevated in half of the patients with LCNEC or SCLC. Although proGRP was a good marker of high-grade NE tumors, the elevation of NSE level was limited in these patients, probably because of the relatively early stage for the tumors. The pathologic profiles of resected tumors are listed in Table 6. The average size of LCNEC (41 mm) was the largest among NE tumors; other types averaged approximately 30 mm in diameter. In TC, nodal involvement was seen in only two patients (3.6%), whereas approximately half of the patients with other histologic types had lymph node involvement in both the pulmonary hilum and mediastinum. Accordingly, the postsurgical stage of TC was stage I in more than 90% of the patients. However, approximately half of the patients with the other types of tumors were categorized as stage I, and there was no remarkable difference in the stage distribution between the different histologic types.

Prognosis

The follow-up for the patients in this study ranged from 2 to 197 months. The median follow-up time was 60 months. There were 124 tumor recurrences (39.0%) among all of the patients with NE tumors (Table 7). Compared with carcinoid tumors, high-grade NE tumors had a higher recurrence rate, at appproximately 50%. The survival curves for the 318 patients with NE tumors according to the histologic type are shown in Figure 1. The 5-year survival rates for patients with TC, AC, LCNEC, and SCLC were 96.2%, 77.8%, 40.3%, and 35.7%, respectively. The histologic type as NE tumor significantly affected the prognosis of the patients ($P = .0001$). The prognosis of AC was significantly better than the prognosis of both LCNEC and SCLC ($P = .0406$), which means that intermediate-grade malignancy (AC) could be differentiated from high-grade malignancy (LCNEC and SCLC). The survival curves of LCNEC and SCLC were superimposed, and there was no difference in survival ($P = .9147$). Survival was further analyzed within the same stage category, and a range of prognoses was seen. The relative grade of malignancy was reproduced within each stage category; in stage I patients (n = 175), the 5-year survival rates for TC, AC, LCNEC, and SCLC were 98.0%, 75.0%, 57.8%, and 42.2%, respectively (Fig 2). Again, there was no survival difference between LCNEC and SCLC ($P = .1851$), although the 5-year survival rate was numerically better for LCNEC. In stage II patients (n = 46), the 5-year survival rates for TC, AC, LCNEC, and SCLC were 75.0%, 100%, 31.9%, and 38.9%, respectively. In the multivariate analyses, the following variables were entered based on the results of univariate analyses: histologic type, symptoms, completeness of resection, nodal status, pathologic stage, and age. Among these variables, a histologic type of high-grade NE tumor was the most significant prognostic factor, with risk ratios (RRs) for SCLC and LCNEC of 17.40 and 17.69, respectively. Other significant prognostic factors included incomplete resection (RR = 3.13), symptoms (RR = 1.69), nodal involvement (RR = 2.23), and old age (RR = 1.53).

Table 3. Details of Histologic Diagnosis of SCLC

Histologic Type	No. of Patients	%
SCLC, not combined	83	73.4
SCLC, combined	30	26.6
With LCNEC	15	13.3
With AD	9	8.0
With SQ	5	4.4
With AD + SQ	1	0.9
Total	113	100

Abbreviations: LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma; AD, adenocarcinoma; SQ, squamous-cell carcinoma.

Table 4. Clinicopathologic Profiles According to the Histologic Type

Profile	Histologic Type TC (n = 55)	AC (n = 9)	LCNEC (n = 141)	SCLC (n = 113)	Total (N = 318)
Age, years					
Median	52	63	66	67	65
Range	17-83	38-73	38-88	40-84	17-88
Sex					
Female, No.	23	5	15	23	66
Male					
No.	32	4	126	90	252
%	58.2	44.4	89.4	79.7	79.3
Paraneoplastic syndrome					
No.	1	0	0	3	4
%	1.8	0	0	2.7	1.3
Present and past smokers					
No.	30	5	139	106	280
%	54.6	55.6	98.6	93.8	88.1

Abbreviations: TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma.

DISCUSSION

A population of NE cells can be recognized in the normal bronchoalveolar structures in the lung, where NE defines specific cellular characteristics and the ability to uptake and decarboxylate amine precursors.[1] These features are reflected by the morphology, such as

Table 5. Percentage of Abnormal Elevations of the Tumor Markers CEA, NSE, and proGRP

	Histologic Type											
	TC (n = 55)			AC (n = 9)			LCNEC (n = 141)			SCLC (n = 113)		
Tumor Marker	%	No. of Patients With Abnormally Elevated Serum Level	No. of Patients Measured	%	No. of Patients With Abnormally Elevated Serum Level	No. of Patients Measured	%	No. of Patients With Abnormally Elevated Serum Level	No. of Patients Measured	%	No. of Patients With Abnormally Elevated Serum Level	No. of Patients Measured
CEA	5.9	3	51	11.1	1	9	48.5	63	130	40.7	44	108
NSE	0	0	42	0	0	5	12.4	13	105	2.3	2	88
proGRP	7.1	1	14	100	1	1	25.8	8	29	48.5	16	31

Abbreviations: TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma; LCC, large-cell carcinoma; CEA, carcinoembryonic antigen; NSE, neuron-specific enolase; proGRP, progastrin-releasing peptide.

secretory granules and dense core granules by electron microscopy. However, the clinical implications of these NE characteristics (NE phenotype and NE morphology) in lung tumors have not yet been defined, especially in relation to the proper choice of treatment strategy. For SCLC, which shows a chemosensitive and aggressive nature, a standard therapeutic strategy has been established apart from other histologies. However, other NE tumors require the further refinement of histology-specific treatment.

NE lung tumors exhibit a spectrum of histologies, clinical profiles, and biologic behaviors ranging from relatively indolent TC to histologically high-grade, biologically aggressive tumors.[2-5] The grading was proposed in the 1999 WHO classification, with rigorous criteria for each subtype, even though LCNEC is still considered a variant form of large-cell carcinoma.[14] According to the WHO classification, AC can be differentiated from TC by a higher mitotic activity and/or the presence of necrosis. Although LCNEC is characterized by the NE morphology (nesting, palisading, and rosettes), a high mitotic rate, necrosis, cytologic features similar to non–small-cell lung cancer, and positive immunohistochemical staining for NE markers, it can sometimes be difficult to differentiate between LCNEC and SCLC. Even for an expert pathologist, the cytologic features falling between LCNEC and SCLC can make it difficult to define the histology as either SCLC or LCNEC, as seen in 14 tumors in the present series. One of the issues in the present WHO classification is that, despite the morphologic and clinical close relationship between SCLC and LCNEC, these tumors are placed in different categories. Specifically, LCNEC is recognized as a part of non–small-cell carcinoma, and the present therapeutic strategy is being planned in a histology-specific basis as SCLC or non-SCLC. Further assessment of therapeutic response is a high-priority issue, which will also justify the distinction between LCNEC and SCLC.

The most significant clinical and pathologic implication of the present study is the determination of the relative grade of malignancy of each histologic type among NE tumors. In particular, for the three

Table 6. Pathologic Profiles According to the Histologic Type

	Histologic Type									
	TC (n = 55)		AC (n = 9)		LCNEC* (n = 141)		SCLC (n = 113)		Total (N = 318)	
Profile	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Tumor diameter, mm										
Mean	26		26		41		29		34	
Range	9-70		13-44		7-140		7-75		7-140	
Postsurgical stage										
I	50	90.9	4	44.4	63	45.3	58	51.3	175	55.4
II	4	7.3	2	22.2	22	15.9	18	16.0	46	14.6
IIIA	1	1.8	2	22.2	32	23.0	24	21.2	59	18.7
IIIB	0	0	0	0	13	9.4	12	10.6	25	7.9
IV	0	0	1	11.1	9	6.5	1	0.9	11	3.5
Nodal involvement										
N0	53	96.4	5	55.6	76	55.1	65	57.5	199	63.2
N1	1	1.8	2	22.2	26	18.8	23	20.4	52	16.5
N2	1	1.8	2	22.2	33	23.9	24	21.2	60	19.1
N3	0	0.0	0	0.0	3	2.2	1	0.9	4	1.3

Abbreviations: TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma.
*Data on the stage and nodal status were not available in two and three patients with LCNEC, respectively.

Table 7. Outcome of Patients With NE Tumors

Outcome	Histologic Type: TC (n = 55) No. of Patients	%	AC (n = 9) No. of Patients	%	LCNEC (n = 141) No. of Patients	%	SCLC (n = 113) No. of Patients	%	Total (N = 318) No. of Patients	%
Tumor recurrence	2	3.6	3	33.3	68	48.2	54	47.8	124	39.0
Locoregional	1		1		17		10		30	
Distant	1		2		34		18		55	
Both	0		0		16		16		36	
Unknown	0		0		1		1		4	
All deaths	3	5.5	2	22.2	84	59.6	69	61.1	158	49.7
Cancer death	1	33.3	0	0.0	62	73.8	43	63.2	106	67.5

Abbreviations: TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small-cell lung carcinoma; NE, neuroendocrine.

histologic types that are considered intermediate- or high-grade malignancy (AC, LCNEC, and SCLC), the present findings clearly revealed their relative prognoses. There have been several previous reports on the prognosis of NE tumors of the lung. However, relatively few cases of high-grade NE tumors have been included. On the basis of their own diagnostic criteria, Travis et al[5] reported that the 5-year survival rates for TC, AC, LCNEC, and SCLC were 87%, 56%, 27%, and 9%, respectively. Garcia-Yuste et al[4] reported that the 5-year survival rates for TC, AC, LCNEC, and SCLC were 96%, 72%, 21%, and 14%, respectively. Neither report described a significant difference in survival between LCNEC and SCLC. As for LCNEC, the reported 5-year survival rates have ranged from 13% to 47%.[4,-6,9,11,12] The 5-year survival rate of LCNEC in our present series was 41.3%, which is within the range of the rates reported previously. Even for stage I disease, the reported 5-year survival rates have been approximately 10% to 30%.[4,6,9,12] In the present series, however, the 5-year survival rate of stage I LCNEC was 60%, which was higher than the rates in previous reports. However, considering the 5-year survival rate of stage I non–small-cell lung cancer, LCNEC is the histology with the worst prognosis among non–small-cell histologies.[15] Also, we confirmed that LCNEC shows almost the same prognosis as SCLC. These two histologies also shared similar clinicopathologic backgrounds, such as smoking history and sex.

In high-grade NE tumors, the existence of borderline cases between LCNEC and SCLC has been noted. In the process of central pathologic review of the present study, there were 14 borderline cases between LCNEC and SCLC, which required another session of panel meetings to reach the consensus regarding the histology as either LCNEC or SCLC. There might be three factors that are closely related to the difficulties in the diagnosis; these are technical issues in the preparation of specimens, diagnostic reproducibility issues, and diagnostic criteria issues. There are several technical issues that make the diagnosis difficult. One is the poor histology as a result of poor fixation, extensive tumor necrosis, and sections that are cut too thick or poorly staining, although the preparation of the slides was completely centralized in the present study to minimize these issues. The histologic heterogeneity with the different cellular sizes and different proportions also affects the diagnosis.[16] The fact that the cell size in SCLC tends to be larger in the large, well-fixed specimens should be well recognized.[17]

It has been well known for SCLC that expert lung cancer pathologists disagree about the diagnosis in approximately 5% to 6% of the

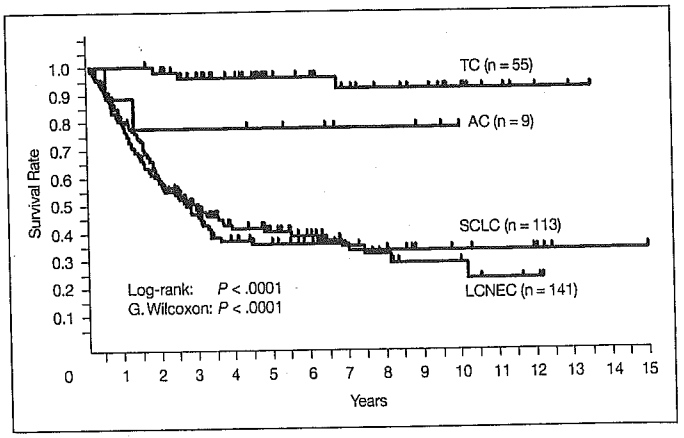

Fig 1. Overall survival curves in neuroendocrine tumors of all stages (N = 318) according to the following histologic types: TC, typical carcinoid (n = 55); AC, atypical carcinoid (n = 9); LCNEC, large-cell neuroendocrine carcinoma (n = 141); and SCLC, small-cell lung carcinoma (n = 113). The histologic type significantly affected the survival ($P < .0001$, log-rank test).

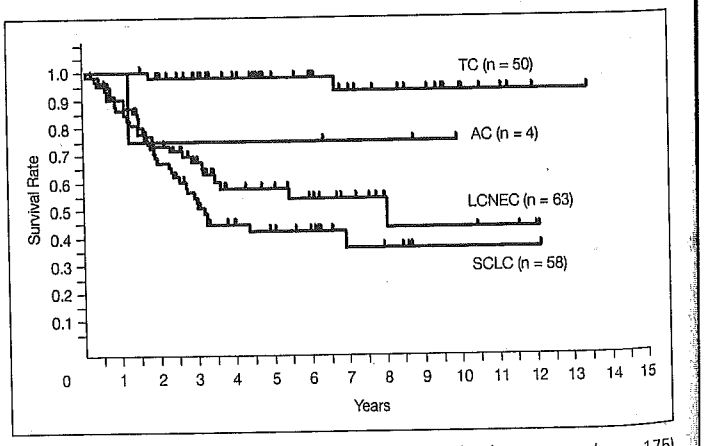

Fig 2. Overall survival curves in stage I neuroendocrine tumors (n = 175) according to the following histologic types: TC, typical carcinoid (n = 50); AC, atypical carcinoid (n = 4); LCNEC, large-cell neuroendocrine carcinoma (n = 63); and SCLC, small-cell lung carcinoma (n = 58). The histologic type significantly affected the survival ($P < .0001$, log-rank test).

cases.[18] In the present study, there was difficulty in the diagnosis of 14 tumors, which composed 5.5% of the 254 high-grade NE tumors; this percentage is quite similar to those previously reported. As part of the diagnostic criteria, the cellular and nuclear size is an important part in the differentiation between LCNEC and SCLC. According to the morphometric analysis by Marchevsky et al,[19] a considerable overlap of nuclear size was shown between LCNEC and SCLC, and the authors addressed that these two histologies should be merged as a single group of high-grade NE carcinoma. However, it is not clear how they could reach the definitive diagnosis as LCNEC or SCLC despite the overlapping cellular and nuclear size. These data, as well as our own, demonstrate that the cell size alone is insufficient as a criterion for establishing the diagnosis of high-grade NE tumors, and a constellation of criteria needs to be used. We still need more pathobiologic characteristics (and perhaps, they are more likely to be molecular rather than morphologic) to make the differentiation between SCLC and LCNEC clearer.

The limitations of this study must also be addressed. In this study, only surgical cases were collected to ensure that a thorough investigation of histopathologic features would be possible, and advanced, unresectable tumors were excluded. As is well known, most patients with SCLC are not candidates for resection because of local/systemic spread of the tumor. It is speculated that typical SCLC arises in the hilum and metastasizes to remote organs at a relatively early stage of the disease. In this sense, the resected SCLC in the present series may not represent typical SCLC, which might have a more aggressive nature. Although it will still be difficult to obtain enough specimens or to perform an immunohistochemical study using only biopsy samples in nonsurgical patients, future studies should include advanced diseases.

In conclusion, the present, large-scale, multi-institutional study defined the prognostic spectrum of pulmonary NE tumors as TC, AC, LCNEC, and SCLC, where LCNEC and SCLC were similarly aggressive. Future studies should clarify the histology-specific sensitivity to treatment, especially with regard to chemoradiotherapy. If similar responses are found, the histologic distinction at least has little significance in the planning of treatment strategy.

REFERENCES

1. Pearse AGE: The cytochemistry and ultrastructure of polypeptide hormone producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J Histochem Cytochem 17:303-313, 1969
2. Travis WD, Linnoila RI, Tsokos MG, et al: Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma: An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. Am J Surg Pathol 15:529-553, 1991
3. Cooper WA, Thourani VH, Gal AA, et al: The surgical spectrum of pulmonary neuroendocrine neoplasms. Chest 119:14-18, 2001
4. Garcia-Yuste M, Matilla JM, Alvarez-Gago T, et al: Prognostic factors in neuroendocrine lung tumors: A Spanish multicenter study. Ann Thorac Surg 70:258-263, 2000
5. Travis WD, Rush W, Flieder DB, et al: Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. Am J Surg Pathol 22:934-944, 1998
6. Dresler CM, Ritter JH, Patterson GA, et al: Clinical-pathologic analysis of 40 patients with large cell neuroendocrine carcinoma of the lung. Ann Thorac Surg 63:180-185, 1997
7. Jiang SX, Kameya T, Shoji M, et al: Large cell neuroendocrine carcinoma of the lung: A histological and immunohistochemical study of 22 cases. Am J Surg Pathol 22:526-537, 1998
8. Iyoda A, Hiroshima K, Toyozaki T, et al: Clinical characterization of pulmonary large cell neuroendocrine carcinoma and large cell carcinoma with neuroendocrine morphology. Cancer 91:1992-2000, 2001
9. Takei H, Asamura H, Maeshima A, et al: Large cell neuroendocrine carcinoma of the lung: A clinicopathologic study of eighty-seven cases. J Thorac Cardiovasc Surg 124:285-292, 2002
10. Mazieres J, Daste G, Molinier L, et al: Large cell neuroendocrine carcinoma of the lung: Pathological study and clinical outcome of 18 resected cases. Lung Cancer 37:287-292, 2002
11. Zacharias J, Nicholson AG, Ladas GO, et al: Large cell neuroendocrine carcinoma and large cell carcinomas with neuroendocrine morphology of the lung: Prognosis after complete resection and systematic nodal dissection. Ann Thorac Surg 75:348-352, 2003
12. Paci M, Cavazza A, Annessi V, et al: Large cell neuroendocrine carcinoma of the lung: A 10-year clinicopathologic retrospective study. Ann Thorac Surg 77:1163-1167, 2004
13. Sobin LH, Wittekind C: International Union Against Cancer: TNM Classification of Malignant Tumors (ed 5). New York, NY, Wiley-Liss, 1997
14. Travis WD, Colby TV, Corrin B, et al: Histological typing of lung and pleural tumors, in World Health Organization International Histological Classification of Tumors. Berlin, Germany, Springer, 1999, pp 7-12
15. Naruke T, Kondo H, Tsuchiya R, et al: Prognosis and survival after resection for bronchogenic carcinoma based on the 1997 TNM-staging classification: The Japanese experience. Ann Thorac Surg 71:439-442, 2001
16. Vollmer RT: The effect of cell size on the pathologic diagnosis of small and large cell carcinomas of the lung. Cancer 50:1380-1383, 1982
17. Nicholson S, Beasley MB, Brambilla E, et al: Small cell lung carcinoma (SCLC): A clinicopathologic study of 100 cases with surgical specimens. Am J Surg Pathol 26:1184-1197, 2002
18. Roggli VL, Vollmer RT, Greenberg SD, et al: Lung cancer heterogeneity: A blinded and randomized study of 100 consecutive cases. Hum Pathol 16:569-579, 1985
19. Marchevsky AM, Gal AA, Shah S, et al: Morphometry confirms the presence of considerable nuclear size overlap between "small cells" and "large cells" in high-grade pulmonary neuroendocrine neoplasms. Am J Clin Pathol 116:466-472, 2001

Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF (via Adobe® Acrobat Reader®) version.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

Author Contributions

Conception and design: Hisao Asamura, Toru Kameya, Yoshihiro Matsuno, Masayuki Noguchi, Hirohito Tada, Yuichi Ishikawa, Tomoyuki Yokose, Shi-Xu Jiang, Takeshi Inoue, Ken Nakagawa, Kanji Nagai
Financial support: Hisao Asamura
Administrative support: Hisao Asamura
Provision of study materials or patients: Hisao Asamura, Toru Kameya, Yoshihiro Matsuno, Masayuki Noguchi, Hirohito Tada, Yuichi Ishikawa, Tomoyuki Yokose, Shi-Xu Jiang, Takeshi Inoue, Ken Nakagawa, Kanji Nagai
Collection and assembly of data: Hisao Asamura, Toru Kameya, Yoshihiro Matsuno, Masayuki Noguchi, Hirohito Tada, Yuichi Ishikawa, Tomoyuki Yokose, Shi-Xu Jiang, Takeshi Inoue, Ken Nakagawa, Kinuko Tajima, Kanji Nagai
Data analysis and interpretation: Hisao Asamura, Toru Kameya, Yoshihiro Matsuno, Masayuki Noguchi, Hirohito Tada, Yuichi Ishikawa, Tomoyuki Yokose, Shi-Xu Jiang, Takeshi Inoue, Ken Nakagawa, Kinuko Tajima, Kanji Nagai
Manuscript writing: Hisao Asamura, Toru Kameya, Yoshihiro Matsuno, Masayuki Noguchi, Hirohito Tada, Yuichi Ishikawa, Tomoyuki Yokose, Shi-Xu Jiang, Takeshi Inoue, Ken Nakagawa, Kanji Nagai
Final approval of manuscript: Hisao Asamura

Hepatoma-derived growth factor as a prognostic marker in completely resected non-small-cell lung cancer

TERUO IWASAKI[1], KATSUHIRO NAKAGAWA[1], HIDEJI NAKAMURA[4], YOSHIAKI TAKADA[2], KAORU MATSUI[3] and KUNIMITSU KAWAHARA[2]

Departments of [1]Respiratory Surgery, [2]Pathology and [3]Thoracic Malignancy, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases (formerly Osaka Prefectural Habikino Hospital); [4]Division of Hepatobiliary and Pancreatic Medicine, Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan

Received January 4, 2005; Accepted February 22, 2005

Abstract. Hepatoma-derived growth factor (HDGF), unrelated to hepatocyte growth factor, is a heparin-binding protein originally purified from human hepatoma HuH-7 cells. HDGF exhibits mitogenic activities for certain hepatoma cells, fibroblasts and vascular smooth muscle cells, and angiogenic activities through nuclear targeting. Recently, HDGF was found to be a mitogen for lung epithelial cells *in vitro* and *in vivo*. This suggests that HDGF may play a critical role in the development and progression of lung cancer. We investigated, immunohistochemically, the relationship between HDGF expression and clinicopathological variables, and the prognostic significance of HDGF in 102 patients with completely resected non-small-cell lung cancer (NSCLC: 70 adenocarcinomas and 32 squamous cell carcinomas). To address the mechanism of action of HDGF, we evaluated the contribution of HDGF to tumor cell proliferation and intratumor angiogenesis using anti-Ki-67 and anti-CD31 antibodies, respectively. HDGF expression was strongly detected in the nucleus of cancer cells; the HDGF-labeling index (LI) was 20-95% (median 64.5%). There was no significant association between HDGF-expression level and clinicopathological variables. Patients with NSCLC showing a high HDGF-LI (≥65%) had significantly worse overall and disease-free survivals than those with NSCLC showing a low HDGF-LI. Multivariate analysis revealed that HDGF is a significant independent prognostic factor, more powerful than pathological stage. Moreover, HDGF expression correlated with Ki-67-LI and intratumor microvessel density. We consider HDGF as a useful prognostic marker for patients with completely resected NSCLC and it may play a critical role in the pathobiology of lung cancer through its mitogenic and angiogenic activities.

Correspondence to: Dr Teruo Iwasaki, Department of Respiratory Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3-7-1 Habikino, Habikino-City, Osaka 583-8588, Japan
E-mail: teruteruah@m4.dion.ne.jp

Key words: hepatoma-derived growth factor (HDGF), non-small-cell lung cancer, prognostic factor, immunohistochemical study, microvessel density

Introduction

Hepatoma-derived growth factor (HDGF), unrelated to hepatocyte growth factor (HGF) produced by non-parenchymal cells, is a secretory heparin-binding protein that was purified from the conditioned medium of human hepatoma HuH-7 cells, and its cDNA was cloned from HuH-7 cells (1,2). HDGF represents a new family of growth factors called HDGF-related proteins (HRPs), including HRP1, HRP2, HRP3, HRP4 and p52/p75/lens epithelium-derived growth factor (LEDGF) (3). These proteins have in common the following characteristics: i) homology in the N-terminal amino acids [termed homologous to the amino terminus of HDGF (hath) region] containing a PWWP domain, which is suspected to play a role in cell growth and differentiation possibly by DNA binding, ii) bipartite nuclear localization signals, and iii) lack of signal peptides (3-5). Recent studies have shown that HDGF is an exogenous mitogen for HuH-7, Swiss 3T3 fibroblasts (2), endothelial cells (6-8), and vascular smooth muscle cells (9,10), and that nuclear targeting of HDGF is essential for its mitogenic activity (10,11).

As for roles of HDGF in tumor pathobiology, HDGF stimulates *in vitro* proliferation of hepatoma cells such as HuH-7, and antisense oligonucleotides of HDGF can suppress it (12). *In vivo*, HDGF induces tumorigenesis of NIH3T3 cells in nude mice through its angiogenic activity (7) and may also play an important role in the development and progression of hepatocellular carcinoma in humans and rodents on the basis that HDGF expression is higher in hepatoma cells than in the adjacent non-cancerous tissues (13).

Although HDGF was originally identified in hepatoma cells, HDGF and its mRNA are expressed in various normal adult tissues, including lung tissue (2,6,14). HDGF may be involved in fetal lung development (15). Recently, Mori *et al* (14) reported that HDGF is also a mitogen for lung epithelial cells *in vitro* and *in vivo*. Taken together, these findings suggest that HDGF may play a critical role in the development and progression of lung cancer.

The most common cancer in Japan today is lung cancer. Lung cancer was the leading indication for general thoracic

surgery (~43%) and more than 20,000 patients were operated on at Japanese institutions in 2002 (16). Non-small-cell lung carcinomas (NSCLC) represent 98% of all operable cases of lung cancer, and they are still associated with a poor prognosis, even when operable. Many molecular markers of prognosis have been studied, although the critical cause for the poor prognosis of patients with NSCLC remains to be determined.

In the present study, we investigate immunohistochemically the relationship between HDGF expression and clinicopathological variables and the prognostic significance of HDGF in NSCLC patients who underwent complete resection. Additionally, to address the mechanism of action of HDGF on lung cancer biology, we evaluated the contribution of HDGF to tumor cell proliferation and intratumor angiogenesis.

Materials and methods

Patients and tumors. Among patients with primary lung carcinoma who were operated on at the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases (Osaka, Japan) from 1994 through 1997, one hundred and two patients underwent complete resection for adenocarcinoma (n=70) or squamous cell carcinoma (n=32) without previous chemotherapy or radiotherapy, and adequate paraffin-embedded tissue sections were available. These patients had no other form of malignancy. Tumor specimens were fixed in 10% formaldehyde solution, embedded in paraffin and microscopically examined after hematoxylin and eosin (HE) staining. Histological classification of tumors was based on the World Health Organization criteria. Visceral pleural involvement was classified according to the Japan Lung Cancer Society (17) as follows: P0, the tumor does not penetrate the elastic layer of the visceral pleura; P1, the tumor penetrates the elastic layer but is not exposed on the pleural surface; P2, the tumor is exposed on the pleural surface but does not involve adjacent anatomic structures; and P3, the tumor involves adjacent anatomic structures (18). A tumor larger than 3 cm in diameter or a P2 tumor of any size was defined as T2 classification. All tumors were staged according to the TNM pathological classification of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) (19): 40 stage I (23 cases in stage IA and 17 cases in stage IB), 21 stage II (3 cases in stage IIA and 18 cases in stage IIB), 35 stage III (26 cases in stage IIIA and 9 cases in stage IIIB) and 6 stage IV (patients with a metastatic nodule in the ipsilateral non-primary-tumor lobe of the lung). The patients (69 men and 33 women) were between 40 and 80 years of age (mean 64 years) and grouped according to age as being either <70 or ≥70 years old. Smoking status was 0-232 (median 44.5) pack-year, and patients were divided into 2 groups: those who smoked <40 pack-year and those who smoked ≥40 pack-year. Survival was calculated from the day of surgery, and follow-up of the 102 patients ranged from 4.1 to 108.9 (median 61.3) months; 54 patients (52.9%), without exception, died of recurrence or metastasis of lung cancer during follow-up. Our study was carried out with the approval of the ethical committee of the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases.

Immunohistochemical examination. Immunohistochemical staining for HDGF was performed essentially as previously described (7,12,14,20). The paraffin sections (4 μm thick) were deparaffinized, microwaved in 10 mmol/l citrate buffer (pH 6.0) and then immersed in methanol containing 0.3% hydrogen peroxide. Slides were blocked with normal goat serum and incubated with a 1:5,000 dilution of rabbit polyclonal IgG raised against C-terminus (231-240) of the human HDGF sequence for 30 min at room temperature. After washing the sections twice with phosphate-buffered saline, they were incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin (Envision; Dako, Glostrup, Denmark) for 30 min at room temperature. After washing, diaminobenzidine tetrahydrochloride (DAB) solution was applied. The sections were then counterstained in hematoxylin. Specificity of the anti-HDGF antibody (Ab) had been previously demonstrated by Western blot analysis using recombinant human HDGF (14). Weak staining of smooth muscle cells and endothelial cells of blood vessels was used as the internal positive control. Negative controls were treated in the same way, but anti-HDGF Ab was replaced by non-immune rabbit serum. HDGF was detected mainly in the nucleus of cancer cells more strongly than in that of smooth muscle cells, and weakly in the cytoplasm of some cancer cells. HDGF immunoreactivity was judged positive when HDGF staining in the nucleus of tumor cells was equivalent to or stronger than that in the nucleus of smooth muscle cells. HDGF-labeling index (LI) was expressed as the proportion of cancer cells with positive HDGF nuclear reactivity.

Immunohistochemical staining for Ki-67 nuclear antigen was performed using a mouse monoclonal anti-human Ki-67 antigen Ab (MIB-1, DAKO) according to the manufacturer's instructions. Ki-67-LI was expressed as the proportion of Ki-67-positive cancer cells. For evaluation of HDGF- and Ki-67-LI, more than 1,000 cancer cells were counted in at least 5 representative areas without necrosis in each section. Intratumor angiogenesis was assessed by counting the microvessels detected with CD31 staining using a mouse monoclonal anti-human CD31 Ab (JC/70A, DAKO) according to the manufacturer's instructions. Intratumoral microvessel density (MVD) was calculated as the average value of microvessels/mm^2 using the criteria previously described elsewhere (21,22). After the area of highest vascularization was identified by scanning sections at low power, individual microvessel counts were determined at magnification x200 (0.95 mm^2 area) in 3 different fields under an Olympus microscope (Tokyo, Japan). All values determined by slide examination were presented by the median of scores evaluated by 3 investigators (Teruo Iwasaki, Yoshiaki Takada and Kunimitsu Kawahara).

Statistical analysis. The relationship between HDGF expression and clinicopathological variables [age, sex, smoking, tumor size, pathological stage, T-factor (classification), N-factor (classification), pleural involvement, vascular involvement, lymphatic involvement, histological type and degree of differentiation] was analyzed by the χ^2-test. The significance of differences in Ki-67-LI and MVD was tested by Student's t-test. The Kaplan-Meier method was used to estimate overall and disease-free survival as a function of time, and survival differences were analyzed by the log-rank test. Factors potentially related to overall and disease-free survival were analyzed by the Cox proportional-hazards model. For all

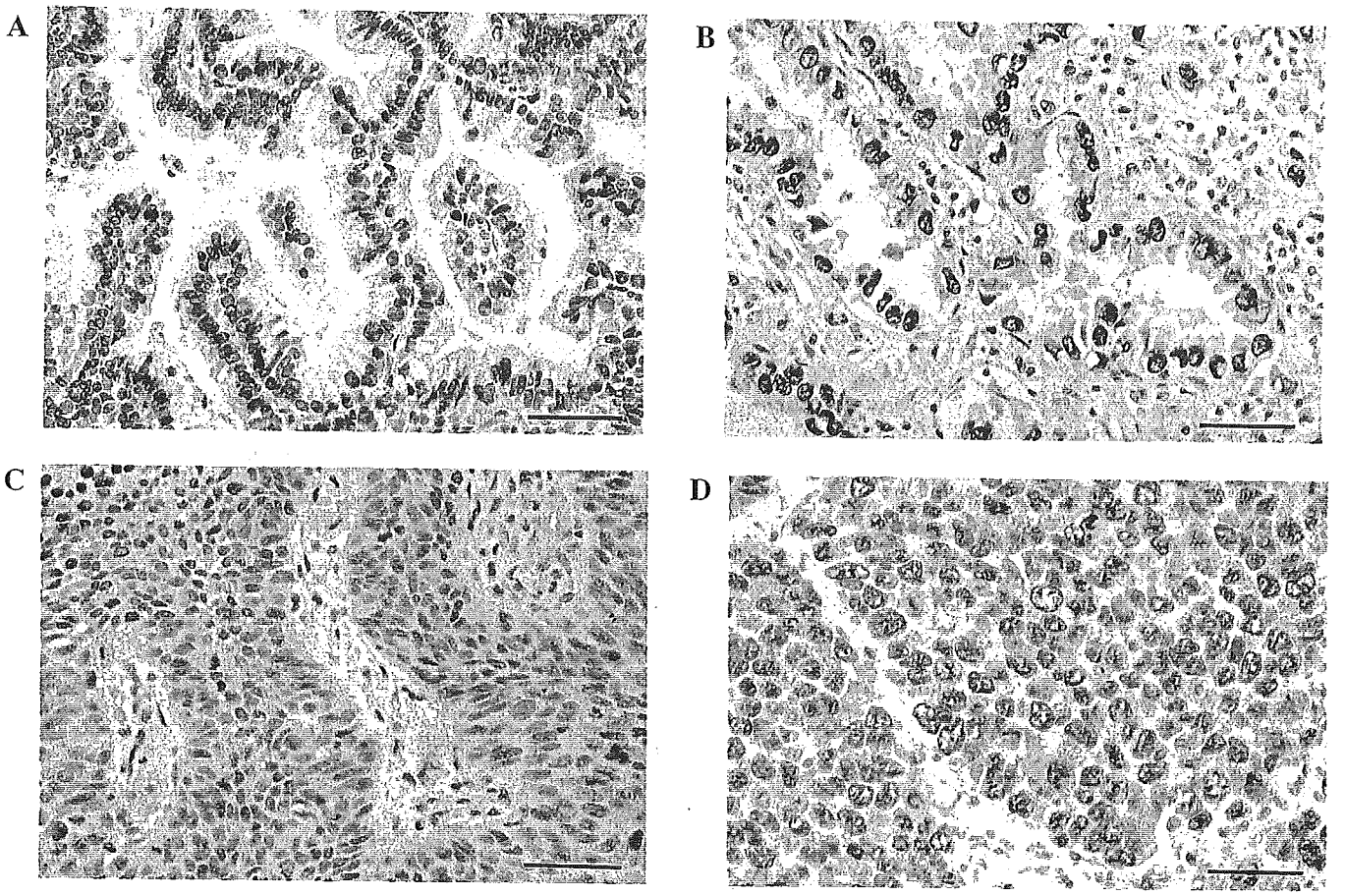

Figure 1. Representative photomicrographs of immunohistochemical staining of HDGF in adenocarcinoma (A and B) and squamous cell carcinoma (C and D) cases. HDGF is expressed weakly in the nucleus of <65% of tumor cells in A and C (defined as low HDGF-expression). To the contrary, HDGF staining was more intense in the nucleus and weak in the cytoplasm of ≥65% of tumor cells in B and D (defined as high HDGF-expression). Scale bars, 50 μm.

statistical analyses, the criterion of significance was defined as $P<0.05$.

Results

HDGF expression was detected in all tumor sections in various proportions. Many cancer cells exhibited strong HDGF-staining, mainly in the nucleus, and some cancer cells presented weak staining in the cytoplasm. Representative cases of adenocarcinoma and squamous-cell carcinoma are shown in Fig. 1. The median score of HDGF-LI in all cases was 64.5% (20-95%), and therefore we defined 65% as a cut-off for low (<65%, n=51) and high (≥65%, n=51) expression. Weak staining in the endothelial cells and smooth muscle cells in the vessels was used as the internal control as mentioned above. HDGF was also detected weakly in some of the non-cancerous type II pneumocytes and ciliated columnar epithelial cells (data not shown). These findings were consistent with those of a previous report on idiopathic pulmonary fibrosis (14).

The relationship between HDGF expression and clinicopathological variables (age, sex, smoking, tumor size, stage, T-factor, N-factor, pleural involvement, vascular involvement, lymphatic involvement, histology and differentiation) in all cases is summarized in Table I. There was no significant relationship between HDGF expression and any clinicopathological variable.

Kaplan-Meier overall and disease-free curves for HDGF expression dichotomized by the median level are shown in Figs. 2 and 3, respectively. Patients with lung cancer expressing high HDGF had a significantly worse overall and disease-free survival than those with lung cancer expressing low HDGF ($P=0.0004$ and $P=0.0005$ by the log-rank test, respectively). Among 102 patients, 25 received adjuvant therapy: radiotherapy was given to 6 patients and systemic chemotherapy including cisplatin or a combination of uracil and tegafur (UFT) was given to 19 patients. There was no significant difference in the proportion of patients who received adjuvant therapy between the 2 groups (13/51 in the low-HDGF group and 12/51 in the high-HDGF group, $P>0.99$).

In the univariate analysis of correlations between prognosis and potential prognostic factors evaluated (HDGF expression, adjuvant therapy and the 12 clinicopathological variables shown in Table I), vascular involvement, smoking, N-factor, tumor size, sex, pathological stage and HDGF were significant prognostic factors ($P<0.05$) for overall and disease-free survival. Adjuvant therapy was not a significant factor for overall ($P=0.580$) or disease-free ($P=0.536$) survival in this study. These 7 significant variables were entered into the Cox proportional-hazards model and multivariate analysis was performed (Table II). Pathological stage and HDGF-expression level were significant independent prognostic factors for overall and disease-free survival, and moreover HDGF had

Table I. Association between HDGF-expression and clinicopathological variables in all cases.

Variables	High-HDGF	(%)	Low-HDGF	P-value[a]
Age				
<70 years	37	(50.0)	37	
≥70 years	14	(50.0)	14	>0.999
Sex				
Male	38	(55.1)	31	
Female	13	(39.4)	20	0.204
Smoking				
<40 pack-year	28	(54.9)	23	
≥40 pack-year	23	(45.1)	28	0.428
Tumor size				
≤30 mm	17	(40.5)	25	
>30 mm	34	(59.5)	26	0.159
pStage[b]				
Stage I + II	30	(49.2)	31	
Stage III + IV	21	(51.2)	20	>0.999
pT-factor[b]				
T1 + T2	42	(49.4)	43	
T3 + T4	9	(52.9)	8	>0.999
pN-factor[b]				
N0	25	(51.0)	24	
N1 + N2	26	(49.0)	27	>0.999
Pleural involvement[c]				
P0 + P1	40	(49.4)	41	
P2 + P3	11	(50.6)	10	>0.999
Vascular involvement				
v (-)	19	(48.7)	20	
v (+)	32	(50.8)	31	>0.999
Lymphatic involvement				
ly (-)	16	(50.0)	16	
ly (+)	35	(50.0)	35	>0.999
Histology[d]				
Ad	38	(54.3)	32	
Sq	13	(40.6)	19	0.286
Differentiation				
Well	25	(45.5)	30	
Moderate/poor	26	(55.3)	21	0.427

[a]χ^2-test. [b]According to the AJCC/UICC TNM pathological classification. pStage, pathological stage; pT, pathological tumor; pN, pathological lymph node; [c]According to the general rules for clinical and pathological record of lung cancer established by the Japan Lung Cancer Sosiety. [d]Ad, adenocarcinoma; Sq, squamous cell carcinoma.

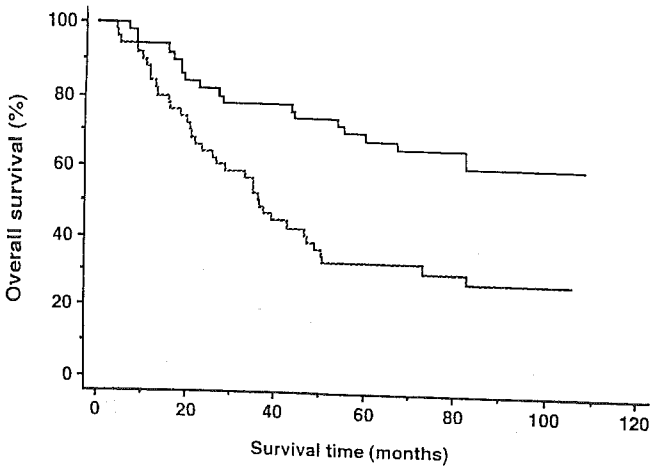

Figure 2. Kaplan-Meier analysis of the overall survival of NSCLC patients with low (a solid line) and high (a dotted line) HDGF-expression. P=0.0004 by the log-rank test.

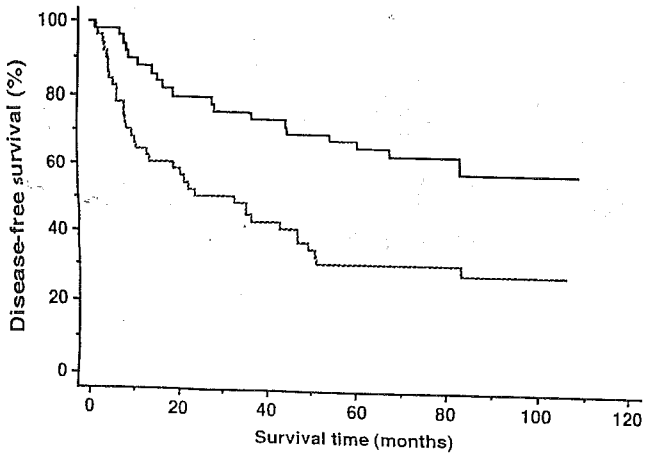

Figure 3. Kaplan-Meier analysis of the disease-free survival of NSCLC patients with low (a solid line) and high (a dotted line) HDGF-expression. P=0.0005 by the log-rank test.

higher risk-ratios than pathological stage for overall (2.976 versus 1.964) and disease-free survivals (2.970 versus 1.848).

The role of HDGF in the biological behavior of NSCLC remains to be fully elucidated. HDGF was very recently reported to be not only a mitogenic factor for lung epithelial cells but also an angiogenic factor (6,9,10,14). We, therefore, examined the relationship between HDGF-expression level and Ki-67-LI or MVD in serial sections. Ki-67-LI values for low and high HDGF-expressing cases were 19.3±14.3% and 34.1±17.7%, respectively (mean ± SD, P<0.00005 by Student's t-test). MVD values for low and high HDGF-expressing cases were 49.8±23.6 and 68.5±20.7 vessels/mm^2, respectively (mean ± SD, P<0.00005 by Student's t-test). These findings suggested that HDGF may promote the proliferation of tumor cells and intratumor angiogenesis in lung cancer.

Discussion

We demonstrated that HDGF is mainly expressed in the nucleus of NSCLC cells and that high expression of HDGF

Table II. Multivariate analysis of prognostic factors.

Variables	Unfav./Fav.[a]	Overall survival Risk ratio (95% CI[b])	P-value	Disease-free survival Risk ratio (95% CI)	P-value
Vascular involvement	v (+)/v (-)	1.016 (0.512-2.014)	0.9644	1.044 (0.532-2.048)	0.9009
Smoking	≥40/<40 pack-year	1.398 (0.700-2.792)	0.3427	1.632 (0.815-3.270)	0.1667
pN-factor[c]	N1 + N2/N0	1.462 (0.760-2.811)	0.2548	1.246 (0.649-2.392)	0.5083
Tumor size	>30/≤30 mm	1.572 (0.853-2.895)	0.1468	1.443 (0.782-2.663)	0.2404
Sex	Male/female	1.957 (0.856-4.472)	0.1114	1.956 (0.860-4.450)	0.1096
pStage[c]	III + IV/I + II	1.964 (1.084-3.556)	0.0259	1.848 (1.018-3.355)	0.0436
HDGF	High/low	2.976 (1.641-5.398)	0.0003	2.970 (1.651-5.344)	0.0003

[a]Unfavorable vs. favorable characteristics. [b]CI, confidence interval. [c]According to the AJCC/UICC TNM pathological classification. pStage, pathological stage; pN, pathological lymph node.

is an independent significant factor for worse overall and disease-free survival of patients with completely resected NSCLC. We also showed that HDGF-expression level was associated with both a high Ki-67-LI and a high intratumor MVD.

Recently, Ren *et al* (23) reported that overexpression of HDGF was a marker of poor prognosis only in patients with curatively resected stage I NSCLC. They found no association between HDGF expression and Ki-67-LI of cancer cells, which is inconsistent with our results. This difference may be because our study included stage I-IV cases whereas theirs only included stage I cases: HDGF-expression correlated with Ki-67-LI in stage IB-IV but not in stage IA (data not shown). Thus, we demonstrated that HDGF-expression level is a prognostic factor independent of and more powerful than the pathological stage of NSCLC by the multivariate analysis.

Exogenous HDGF promotes *in vitro* DNA synthesis and cell proliferation in rat and human lung epithelial cells. Endogenous HDGF overexpressed via transient gene transfer was translocated into the nucleus and promoted the proliferation of human lung epithelial A549 cells. Mori *et al* (14) confirmed, using short interfering RNA technique, that endogenously produced HDGF has a mitogenic effect on A549 cells. Collectively, HDGF probably stimulates the proliferation of lung epithelial cells, at least partially, in an autocrine manner. These findings support our result that high expression of HDGF correlates with a high Ki-67-LI of cancer cells. To date it is unknown if the exogenous mitogenic effect of HDGF is mediated by a cell surface receptor or uptake of the protein (4). Further exploration of this mechanism will contribute to the precise understanding of the biological functions of HDGF.

HDGF induced tumorigenesis of NIH3T3 cells in nude mice via direct angiogenic activity and induction of VEGF (7). Moreover, HDGF is a highly expressed vascular endothelial cell protein *in vivo* and is a potent endothelial mitogen and regulator of endothelial cell migration that acts through mechanisms distinct from those of VEGF (6). Since we could not observe a remarkable enhancement of HDGF expression in endothelial cells or vascular smooth muscle cells in NSCLC sections (data not shown), overproduction of HDGF by cancer cells might induce a high intratumor MVD possibly in a paracrine manner.

HDGF shows a homology to high mobility group-1 (HMG-1), a DNA binding protein (24), but lacks the characteristics of an HMG-1 protein, especially of the HMG box responsible for DNA bindings (2). HMG-1 enhances the activity of several transcription factors, including the glucocorticoid receptor, as well as the activity of RAG recombinase (24,25). The molecules controlled by HDGF in the nucleus and subsequent functions of HDGF have not been identified. Therefore, HDGF may display other tumorigenic behavior besides tumor-cell proliferation or angiogenesis.

Bernard *et al* (26) detected HDGF expression mainly in the nucleus and much more strongly in melanoma cell lines than in melanocytes. They showed by immunohistochemical analysis of clinical samples that 54% of benign nevoid cells reacted positively, whereas 78-90% of melanoma cells were positive in all stages of melanoma. We found that HDGF was expressed in ~30-40% of non-cancerous alveolar or bronchial epithelial cells (data not shown) and 20-95% (median 64.5%) of NSCLC cells. The proportion of HDGF-positive cells seems slightly smaller in NSCLC than in melanoma, but our results were in general compatible with those of Bernard *et al* (26). With regard to other malignancies, a recent study using differential display revealed that HDGF expression was associated with radiosensitivity in esophageal cancer (27). Expression profiling of gastric adenocarcinoma using cDNA array revealed that HDGF was one of the overexpressed genes in gastric cancer as compared with normal gastric mucosa (28). Thus, HDGF probably plays a critical role in the development and progression of various malignancies.

Based on the above findings, we consider HDGF is a useful marker of poor prognosis in patients with completely resected NSCLC, and high HDGF-expression might be a potential indicator of the need for adjuvant therapy. Although further investigations need to be done on the molecular characteristics and biological functions of HDGF, this factor may be a target molecule for the treatment of NSCLC.

References

1. Nakamura H, Kambe H, Egawa T, *et al*: Partial purification and characterization of human hepatoma-derived growth factor. Clin Chim Acta 183: 273-284, 1989.

2. Nakamura H, Izumoto Y, Kambe H, *et al*: Molecular cloning of complementary DNA for a novel human hepatoma-derived growth factor. J Biol Chem 269: 25143-25149, 1994.
3. Dietz F, Franken S, Yoshida K, Nakamura H, Kappler J and Gieselmann V: The family of hepatoma-derived growth factor proteins: characterization of a new member HRP-4 and classification of its subfamilies. Biochem J 366: 491-500, 2002.
4. Everett AD and Bushweller J: Hepatoma derived growth factor is a nuclear targeted mitogen. Curr Drug Targets 4: 367-371, 2003.
5. Izumoto Y, Kuroda T, Harada H, Kishimoto T and Nakamura H: Hepatoma-derived growth factor belongs to a gene family in mice showing significant homology in the amino terminus. Biochem Biophys Res Commun 238: 26-32, 1997.
6. Everett AD, Narron JV, Stoops T, Nakamura H and Tucker A: Hepatoma-derived growth factor is a pulmonary endothelial cell-expressed angiogenic factor. Am J Physiol Lung Cell Mol Physiol 286: L1194-L1201, 2004.
7. Okuda Y, Nakamura H, Yoshida K, *et al*: Hepatoma-derived growth factor induces tumorigenesis *in vivo* through both direct angiogenic activity and induction of vascular endothelial growth factor. Cancer Sci 94: 1034-1041, 2003.
8. Oliver JA and Al-Awqati Q: An endothelial growth factor involved in rat renal development. J Clin Invest 102: 1208-1219, 1998.
9. Everett AD, Lobe DR, Matsumura ME, Nakamura H and McNamara CA: Hepatoma-derived growth factor stimulates smooth muscle cell growth and is expressed in vascular development. J Clin Invest 105: 567-575, 2000.
10. Everett AD, Stoops T and McNamara CA: Nuclear targeting is required for hepatoma-derived growth factor-stimulated mitogenesis in vascular smooth muscle cells. J Biol Chem 276: 37564-37568, 2001.
11. Kishima Y, Yamamoto H, Izumoto Y, *et al*: Hepatoma-derived growth factor stimulates cell growth after translocation to the nucleus by nuclear localization signals. J Biol Chem 277: 10315-10322, 2002.
12. Kishima Y, Yoshida K, Enomoto H, *et al*: Antisense oligonucleotides of hepatoma-derived growth factor (HDGF) suppress the proliferation of hepatoma cells. Hepatogastroenterology 49: 1639-1644, 2002.
13. Yoshida K, Nakamura H, Okuda Y, *et al*: Expression of hepatoma-derived growth factor in hepatocarcinogenesis. J Gastroenterol Hepatol 18: 1293-1301, 2003.
14. Mori M, Morishita H, Nakamura H, *et al*: Hepatoma-derived growth factor is involved in lung remodeling by stimulating epithelial growth. Am J Respir Cell Mol Biol 30: 459-469, 2004.
15. Cilley RE, Zgleszewski SE and Chinoy MR: Fetal lung development: airway pressure enhances the expression of developmental genes. J Pediatr Surg 35: 113-119, 2000.
16 Yada I, Wada H and Fujita H: Thoracic and cardiovascular surgery in Japan during 2002: annual report by the Japanese Association for Thoracic Surgery. Jpn J Thorac Cardiovasc Surg 52: 491-508, 2004.
17. The Japan Lung Cancer Society: General rule for clinical and pathological record of lung cancer. (In Japanese). 6th edition. Kanehara Publ. Corp., Tokyo, 2003.
18. Shimizu K, Yoshida J, Nagai K, Nishimura M, Yokose T, Ishii G and Nishiwaki Y: Visceral pleural invasion classification in non-small cell lung cancer: a proposal on the basis of outcome. J Thorac Cardiovasc Surg 127: 1574-1578, 2004.
19. Mountain CF: Revisions in the International System for Staging Lung Cancer. Chest 111: 1710-1717, 1997.
20. Enomoto H, Yoshida K, Kishima Y, *et al*: Hepatoma-derived growth factor is highly expressed in developing liver and promotes fetal hepatocyte proliferation. Hepatology 36: 1519-1527, 2002.
21. Bosari S, Lee AKC, De Lellis RA, Wiley BD, Heatley GJ and Silverman ML: Microvessel quantitation and prognosis in invasive breast carcinoma. Hum Pathol 23: 755-761, 1992.
22. Weidner N: Intratumor microvessel density as a prognostic factor in cancer. Am J Pathol 147: 9-19, 1995.
23. Ren H, Tang X, Lee JJ, *et al*: Expression of hepatoma-derived growth factor is a strong prognostic predictor for patients with early-stage non-small-cell lung cancer. J Clin Oncol 22: 3230-3237, 2004.
24. Bianchi ME and Beltrame M: Upwardly mobile proteins: the role of HMG proteins in chromatin structure, gene expression and neoplasia. EMBO Rep 1: 109-119, 2000.
25. Bustin M: Regulation of DNA-dependent activities by the functional motifs of the high-mobility-group chromosomal proteins. Mol Cel Biol 19: 5237-5246, 1999.
26. Bernard K, Litman E, Fitzpatrick JL, *et al*: Functional proteomic analysis of melanoma progression. Cancer Res 63: 6716-6725, 2003.
27. Matsuyama A, Inoue H, Shibuta K, *et al*: Hepatoma-derived growth factor is associated with reduced sensitivity to irradiation in esophageal cancer. Cancer Res 61: 5714-5717, 2001.
28. El-Rifai W, Frierson HF Jr, Harper JC, Powell SM and Knuutila S: Expression profiling of gastric adenocarcinoma using cDNA array. Int J Cancer 92: 832-838, 2001.

CASE REPORT

Ectopic Cervico-mediastinal Thymoma Confirmed by Flow Cytometric Analysis of Tumor-derived Lymphocytes

Ectopic cervical or cervico-mediastinal thymomas are very rare and most of them are asymptomatic, except for the presence of a cervical mass. We present the case of a 71-year-old man with an ectopic cervico-mediastinal thymoma threatening superior vena cava syndrome. He had a slight headache and presented with venous dilatation on the chest wall. A computed tomographic scan and magnetic resonance imaging of the chest demonstrated a mass extending from the right neck to the hilum, that indented the trachea and compressed and displaced the brachiocephalic veins anteriorly. Under a right hemicollar incision and median sternotomy, the mass was resected *en bloc* together with the thymus. The resected specimen was an encapsulated mass measuring 11×7×4 cm. The pathological diagnosis was type AB, non-invasive thymoma, confirmed by 3-color flow cytometry of tumor-derived lymphocytes. Flow cytometry using biopsy material may contribute to the preoperative diagnosis of ectopic thymoma.
(Jpn J Thorac Cardiovasc Surg 2006; 54: 35–39)

Key words: ectopic thymoma, superior vena cava syndrome, flow cytometry, magnetic resonance imaging, surgical resection

Teruo Iwasaki, MD, Katsuhiro Nakagawa, MD, Motoaki Yasukawa, MD, Hiroyuki Shiono, MD,* Teruaki Nagano, MD,** and Kunimitsu Kawahara, MD.**

Ectopic cervical thymoma is a rare tumor that arises from unusual thymic tissue anywhere along the path of thymic descent from the neck to the mediastinum during development.[1,2] We report the 39th case of ectopic cervical or cervico-mediastinal thymoma in the English-language literature; this is the first case of ectopic cervico-mediastinal thymoma threatening superior vena cava (SVC) syndrome. Moreover, the pathological diagnosis was supported by 3-color flow cytometry of tumor-derived lymphocytes.

From Department of Respiratory Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, *Department of General Thoracic Surgery, E1, Osaka University Graduate School of Medicine, Suita, and **Department of Pathology, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Osaka, Japan.
Received for publication July 8, 2005.
Accepted for publication October 27, 2005.
Address for reprints: Teruo Iwasaki, MD, Department of Respiratory Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3-7-1 Habikino, Habikino, Osaka 583-8588, Japan.

Case

A 71-year-old otherwise healthy man was admitted for further evaluation of an abnormal shadow found on a chest X-ray. On admission, he reported he had been having slight headaches during the previous month, and he did not have myasthenia gravis or any other autoimmune diseases. On physical examination, facial edema and dilated veins on the anterior chest wall were noted. A prominent firm mass, 5 cm in diameter with unclear caudal margin, was palpable in the right supraclavicular fossa. Laboratory data, including tumor markers (AFP, CA19-9, CEA, CYFRA, HCG-β, NSE, Pro-GRP, SCC and thyroglobulin) and thyroid function tests were all within normal limits. Serum anti-acetylcholine-receptor antibodies were negative. A chest X-ray showed an oval shadow in the right upper mediastinum with a shift of the trachea to the left (Fig. 1). A computed tomographic scan of the chest demonstrated that the right lobe of the thyroid gland was mostly replaced by a mass, which indented the trachea and compressed and displaced the brachiocephalic veins anteriorly. Magnetic resonance imaging (MRI) showed that the mass extended from

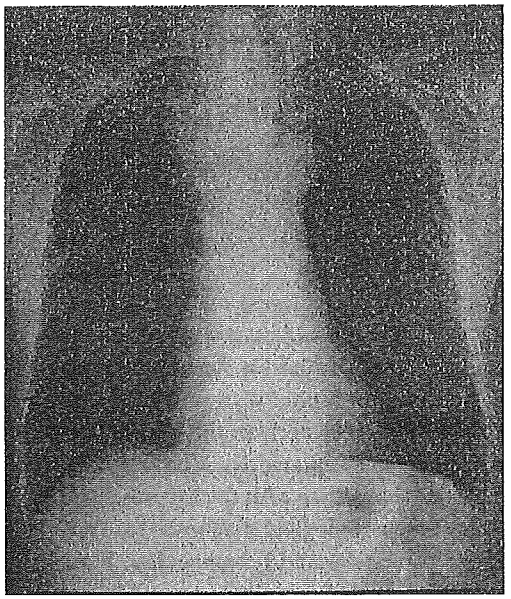

Fig. 1. Chest X-ray showing a right upper mediastinal mass with a shift of the trachea to the left.

the right neck to the right hilum along the trachea (Fig. 2A). On T1-weighted imaging, the mass was isointense to skeletal muscle. On T2-weighted imaging, it had a slightly lower signal intensity than muscle, accompanied by some high signal intensity suggestive of hemorrage or degenerative necrosis, and a lobulated internal architecture separated by linear areas of low signal intensity (Fig. 2A). No calcification was detected. Venography performed by bilaterally transcubital catheterization revealed no visualization of the brachiocephalic veins or SVC but many collateral vessels from the subclavian veins (Fig. 2B). No invasion of the tracheal wall by the mass was observed under bronchoscopic examination. This location of the mass was suggestive of intrathoracic goiter. Ultrasound-guided needle (14-gauge) biopsy of the right supraclavicular part of the mass revealed spindle-shaped epithelial cells arranged in a swirling pattern and accompanied by lymphocytes. These findings suggested a cervico-mediastinal thymoma.

A right hemicollar incision confirmed that the right lobe of the thyroid gland had been replaced by an encapsulated mass extending to the right paravertebral region along the trachea and that the mass could not be removed via a transcervical approach. Additional median sternotomy revealed that the thymus was located orthotopically in front of the mediastinal part of the mass across the brachiocephalic trunk and veins and that the right upper pole of the thymus adhered to a cervical part of the mass via the thyrothymic ligament. Thymectomy was carried out, and the mass could be bluntly or sharply

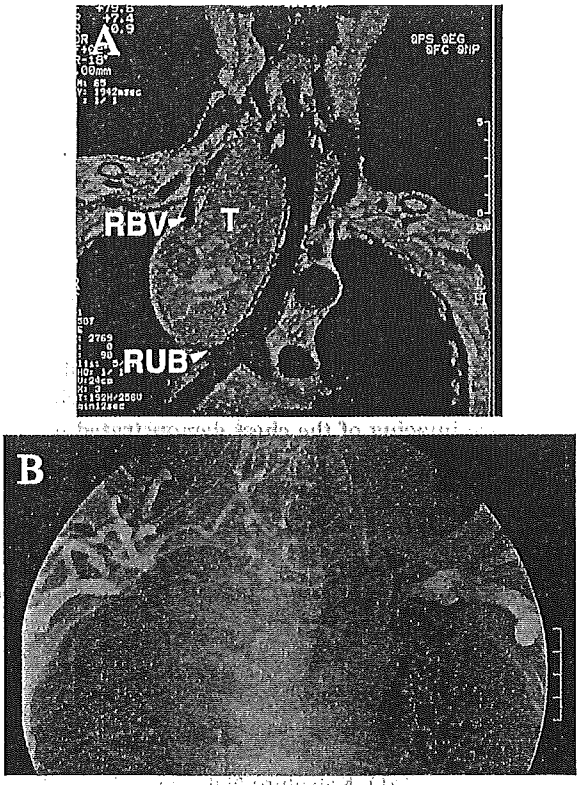

Fig. 2.

A: Coronal MRI (T2-weighted) along the trachea showing a well-defined, large oval mass (T).

B: Venography performed under manual pressure revealing no visualization of the brachiocephalic veins or SVC but many collateral vessels.

RBV, Right brachiocephalic vein; *RUB*, right upper bronchus.

dissected from the trachea, vertebrae, SVC and brachiocephalic trunk and veins. Right thyroidectomy was also performed to remove the mass *en bloc* together with the orthotopic thymus, including the fatty tissue around.

The resected specimen was an encapsulated mass measuring 11×7×4 cm (255 g, Fig. 3A). The cut surface showed multiple tan colored nodules of various sizes separated by fibrous bands, including a small necrotic and hemorrhagic area. Macroscopically, the thyroid gland was not observed in the mass. The microscopic examination revealed the mass had a nodular growth pattern and was formed by a lymphocyte-poor component (right half in Fig. 3B) and a lymphocyte-rich component (left half in Fig. 3B), indicating a type AB thymoma. The thyroid gland was observed to be atrophic and was separated from the tumor by a fibrous capsule. Additionally, the tumor was found to be completely separated from the orthotopic thymus by a fibrous capsule. The orthotopic thymus was also atrophic and free of tumor cells. These findings led us

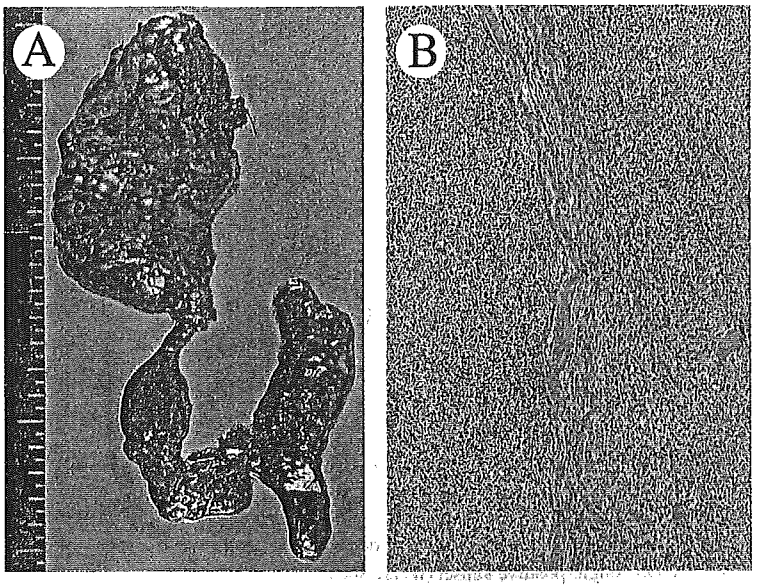

Fig. 3.
A: Macroscopic findings of the resected specimen. The mass was encapsulated and connected with the right upper pole of the orthotopic thymus via the thyrothymic ligament.
B: Microscopic findings of the resected specimen. HE staining. Original magnification: ×40.

to diagnose this tumor as an ectopic cervico-mediastinal thymoma, not as a cervical extension of a mediastinal thymoma derived from the orthotopic thymus.

To confirm the pathological diagnosis, 3-color flow cytometry of tumor-derived lymphocytes was performed with anti-CD3, anti-CD4 and anti-CD8 antibodies.[3] Identification of the $CD4^+CD8^+$ double-positive lymphocytes (29%) by flow cytometric analysis suggested that this tumor derived from the thymus (Fig. 4A). Moreover, the limited expression level (36%) of CD3 in the $CD4^+CD8^-$ single-positive cells indicated that some cells in the population of $CD4^+CD8^-$ single-positive cells were immature (Fig. 4B). On the basis of flow cytometry, this tumor was diagnosed as a thymoma.[3] The patient has now been followed up for 24 months postoperatively and has shown no signs of recurrence.

Discussion

The thymus derives from the endoderm of the third and probably the fourth pair of branchial pouches. The two thymic primordia migrate caudally and medially along the neck, until they fuse to form a bilobed organ in the anterior mediastinum. Incomplete descent or persistence of the cervical portion of the thymus results in the so-called ectopic thymus.[1] Chan and Rosai proposed 4 categories of tumors arising from ectopic thymic tissues: ectopic hamartomatous thymoma, ectopic cervical thymoma, spindle epithelial tumor with thymus-like differentiation (SETTLE), and carcinoma showing thymus-like differentiation (CASTLE).[2] Among them, ectopic thymomas, with or without connection to the orthotopic mediastinal thymus, have been described in submandibular, paratracheal and intratracheal locations, within the thyroid gland, and in several other locations.

We screened the English-language literature based on the PubMed data base and, to the best of our knowledge, 38 cases of ectopic cervical or cervico-mediastinal thymoma (or thymic carcinoma) have been reported. The characteristics of previously reported cases of ectopic cervical thymomas, including our case, are summarized as follows. They are more common in women (82%; 32 cases) than in men (18%; 7 cases). Patients' ages ranged from 11 to 71 years (mean: 48 years). Tumors were located on the left (54%; 21 cases), right (33%; 13 cases) or anterior neck (13%; 5 cases). Tumors measured 1.2–11 (median: 5.5) cm in the major dimension and the tumor in our patient was the largest. Such thymomas are more commonly non-invasive (69%; 27 cases) than invasive (18%; 7 cases) or carcinomatous (13%; 5 cases). Most cases of cervical thymomas were asymptomatic, except for the presence of a cervical mass. The present case is the only one threatening SVC syndrome. Moreover, it is noteworthy that association with myasthenia gravis is very rare (5%; 2 cases).[4,5]

Preoperative diagnosis is difficult because of the unusual location and rarity of a cervical thymoma. When situated in the vicinity of the thyroid gland, a

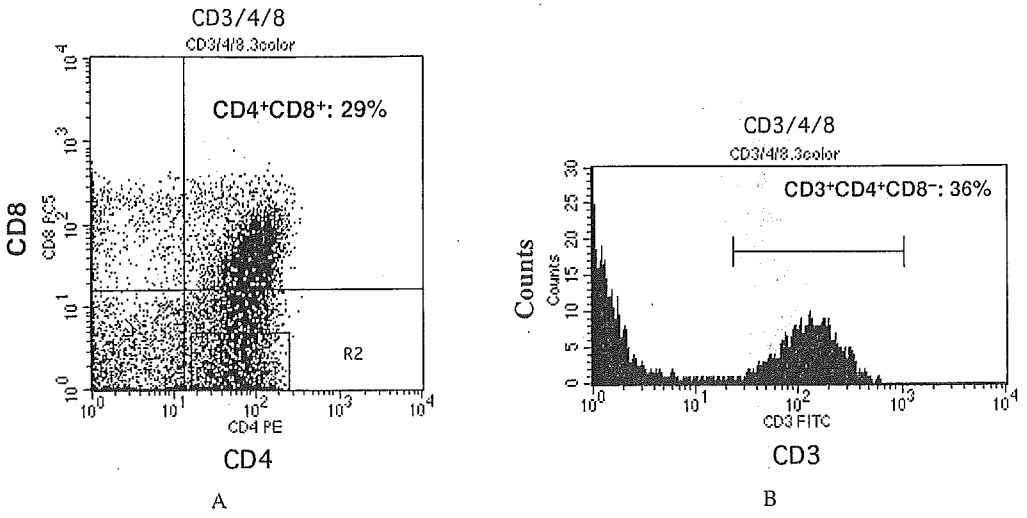

Fig. 4. Three-color flow cytometric analysis of lymphocytes derived from the tumor. Expression of CD4 and CD8 (A) and expression of CD3 in $CD4^+CD8^-$ single-positive subset (B) are shown.

cervical thymoma can mimic a thyroid nodule. Only 5 (13%) of 39 cases could be preoperatively diagnosed as ectopic cervical thymomas: one case was diagnosed by MRI; another case by incisional biopsy; and 3 cases by needle biopsy. As in our case, MRI may facilitate the diagnosis of cervical thymoma by revealing the internal fibrous septa of the tumor.[6] Ectopic thymomas have often been misdiagnosed as chronic thyroiditis or malignant lymphoma by fine needle biopsy. Since our patient had a type AB thymoma, the combination of epithelial tumor cells and lymphocytes and the swirling arrangement of tumor cells were helpful for the differential diagnosis of the tumor. To our knowledge, this is the 2nd case of ectopic cervical thymoma in which the presence of immature T-cells was confirmed by flow cytometry of tumor-derived lymphocytes. In the other case, preoperative flow-cytometry was performed using material obtained by fine needle aspiration biopsy.[7] Flow cytometry may contribute to the preoperative diagnosis of ectopic thymoma.

There was no case of recurrence among the completely resected cases of ectopic cervical non-invasive thymomas. Whereas, recurrence was reported in one of 7 cases (14%) of invasive thymomas and 3 of 5 cases (60%) of thymic carcinomas.[8-10] Complete surgical resection of the lesion, if possible, appears to be the treatment of choice, and adjuvant irradiation or chemotherapy had better be performed in invasive or carcinomatous cases; however, it remains to be elucidated whether ectopic thymic tumors exhibit the same biological behavior as orthotopic mediastinal ones. There was one case of an ectopic thymoma in the anterior neck accompanied by a simultaneous mediastinal thymoma arising from the orthotopic thymus.[2] Therefore, additional thymectomy should be considered when an orthotopic thymoma is simultaneously suspected.

Conclusion

We report the first case of ectopic cervico-mediastinal thymoma threatening SVC syndrome. MRI and fine needle biopsy were useful to establish the preoperative diagnosis, and after complete resection, the pathological diagnosis of type AB thymoma was supported by postoperative flow-cytometry of tumor-derived lymphocytes. If a cervical mass is encountered even in a patient without myasthenia gravis, the possibility of an ectopic thymoma should be considered. Flow cytometry using biopsy material may contribute to the preoperative diagnosis of ectopic thymoma.

REFERENCES

1. Suster S, Rosai J. Histology of the normal thymus. Am J Surg Pathol 1990; 14: 284–303.
2. Chan JK, Rosai J. Tumors of the neck showing thymic or related branchial pouch differentiation: A unifying concept. Hum Pathol 1991; 22: 349–67.
3. Okumura M, Fujii Y, Miyoshi S, Shiono H, Inoue M, Kadota Y, et al. Three-color flow cytometric study on lymphocytes derived from thymic diseases. J Surg Res 2001; 101: 130–7.
4. MacLean G, Guberman A, Giulivi A. Late pseudo-exacerbation of myasthenia gravis due to ectopic

thymoma invading lower cranial nerves. Can J Neurol Sci 1990; 17: 46–8.

5. Mineo TC, Biancari F, D'Andrea V. Myasthenia gravis, psychiatric disturbances, idiopathic thrombocytopenic purpura, and lichen planus associated with cervical thymoma. J Thorac Cardiovasc Surg 1996; 111: 486–7.
6. Nagasawa K, Takahashi K, Hayashi T, Aburano T. Ectopic cervical thymoma: MRI findings. AJR Am J Roentgenol 2004; 182: 262–3.
7. Ponder TB, Collins BT, Bee CS, Silverberg AB, Grosso LE, Dunphy CH. Diagnosis of cervical thymoma by fine needle aspiration biopsy with flow cytometry. A case report. Acta Cytol 2002; 46: 1129–32.
8. Watanabe I, Tezuka F, Yamaguchi M, Sagawa J, Kaise N. Thymic carcinoma of the thyroid. Pathol Int 1996; 46: 450–6.
9. Dorfman DM, Shahsafaei A, Miyauchi A. Immunohistochemical staining for bcl-2 and mcl-1 in intrathyroidal epithelial thymoma (ITET)/carcinoma showing thymus-like differentiation (CASTLE) and cervical thymic carcinoma. Mod Pathol 1998; 11: 989–94.
10. Jung JI, Kim HH, Park SH, Lee YS. Malignant ectopic thymoma in the neck: A case report. AJNR Am J Neuroradiol 1999; 20: 1747–9.

病理学的に肺気腫を合併していた肺癌切除例における術後予測肺機能の検討
—肺容量減少術（LVRS）効果について—

安川元章[1]・中川勝裕[1]・桂　浩[1]・
岩崎輝夫[1]・大瀬尚子[1]

要旨——目的．従来の機能的肺切除基準では切除適応外と判断されていた肺気腫合併低肺機能肺癌症例に肺容量減少術（LVRS）効果により手術を施行し得たとする報告が散見されるようになった．そこで，どのような症例でLVRS効果が期待できるかを検討した．対象と方法．肺癌切除術後の残存肺機能（FVC，$FEV_{1.0}$）について，病理学的に正常肺組織群（61例）と肺気腫合併群（43例）にわけ，予測残存率と実測残存率をpaired t-testにて検定した．結果．正常肺組織群ではFVC，$FEV_{1.0}$の予測残存率と実測残存率に有意差を認めなかったが，肺気腫群で$FEV_{1.0}$の実測残存率は予測残存率より高値を示した($p=0.0072$)．肺気腫群で血流シンチグラム上，切除部位が血流の低下部位と一致した23例で，FVC，$FEV_{1.0}$の実測残存率はともに予測残存率より高値を示した(FVC: $p=0.0042$，$FEV_{1.0}$: $p=0.00014$)．結語．肺気腫合併例で術後残存$FEV_{1.0}$が予測より高くなり，特に切除部位に血流低下を認める症例でLVRS効果が得られると推察された．(肺癌．2005;45:705-710)

索引用語——肺癌，術後予測肺機能，肺血流シンチグラム，肺気腫，肺容量減少術

Assessment of Predicted Postoperative Pulmonary Function After Pulmonary Resection for Lung Cancer Pathologically Associated With Emphysema
—Effects of Lung Volume Reduction Surgery（LVRS）—

Motoaki Yasukawa[1]; *Katsuhiro Nakagawa*[1]; *Hiroshi Katsura*[1]; *Teruo Iwasaki*[1]; *Naoko Ohse*[1]

ABSTRACT —— ***Objective.*** Lung volume reduction surgery（LVRS）reportedly enables surgeons to perform pulmonary resection in case of lung cancer associated with emphysema and low pulmonary function, which do not comply with the conventional criteria for functional pulmonary resection. This study was performed to predict cases in which LVRS effects are attainable. ***Method.*** To assess predicted and measured residual pulmonary function after resection for lung cancer, the percentage of predicted residual pulmonary function and the percentage of measured residual pulmonary function（FVC and $FEV_{1.0}$）were compared by Student's paired t-test between the group of patients with pathologically normal lung tissue（61 patients）and the group of patients with concomitant emphysema（43 patients）. ***Results.*** In the patients with normal lung tissue, the percentage of measured residual pulmonary function was not significantly dif-

[1]大阪府立呼吸器・アレルギー医療センター呼吸器外科．
別刷請求先：安川元章，大阪府立呼吸器・アレルギー医療センター呼吸器外科，〒583-8588　大阪府羽曳野市はびきの3-7-1．
[1]Department of Thoracic Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Japan.
Reprints: Motoaki Yasukawa, Department of Thoracic Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3-7-1 Habikino, Habikino-shi, Osaka 583-8588, Japan.
Received April 18, 2005; accepted July 7, 2005.

ferent from the percentage of predicted residual pulmonary function (FVC and $FEV_{1.0}$). On the other hand, in the group of patients with emphysema, the percentage of measured residual pulmonary function was higher than the percentage of predicted residual pulmonary function ($FEV_{1.0}$, $p = 0.0072$). In the group of patients with emphysema, the percentage of measured residual pulmonary function was higher than the percentage of predicated residual pulmonary function (FVC and $FEV_{1.0}$) in 23 patients with matching resection site and area of low blood flow in the blood flow scintigram (FVC: $p = 0.0042$, $FEV_{1.0}$: $p = 0.00014$). ***Conclusion.*** Measured postoperative residual $FEV_{1.0}$ tends to be higher than predicted in patients with emphysema and it is assumed that LVRS effects are attained particularly in patients with lower blood flow at the resection site. (*JJLC.* 2005;45:705-710)

KEY WORDS —— Lung cancer, Predicted postoperative pulmonary function, Lung perfusion scanning, Pulumonary emphysema, Lung volume reduction surgery

はじめに

肺気腫合併肺癌症例に対する手術療法は術後低肺機能のため術後合併症が高頻度に発生すると報告されている.[1,2] 一方 Cooper ら[3] が重度の肺気腫患者に対して，肺容量減少術（lung volume redaction surgery; LVRS）による肺機能の改善を報告して以来，LVRS が世界的に広まった．最近では症例を慎重に選択することで，少なくとも 5 年間は LVRS の効果が得られると報告されている.[4]

菊地ら[5] や Korst ら[6] は慢性閉塞性肺疾患を合併する肺癌症例に肺葉切除術を行ったところ，術後 $FEV_{1.0}$ の改善がみられ，LVRS としての効果が得られたと報告をしている．

今回，我々はどのような症例において LVRS 効果が期待できるのかを明らかにするために，肺癌切除術後の残存肺機能について正常肺組織症例と肺気腫合併症例にわけ以下の検討を行った．

対象と方法

1997 年から 2001 年の全肺癌切除術例 467 例中，区域切除術以上を施行し，術後合併症がなく術後 1 年目に肺機能検査を施行できた症例を対象とした．この期間の機能的肺切除基準は残存予測%肺活量≧40%，残存予測 1 秒量≧800 ml を原則とした．全摘例，胸壁合併切除例を除外した．切除肺における肺実質は摘出肺を浸漬保存後，HE 染色にて切除標本を作成し，腫瘍病変部以外を背景肺組織とし，病理学的に正常肺または肺気腫と判断した．切除標本は厚さ 1 cm に全割しマクロ所見にて背景肺組織の切片が特異的な部分とならないことを確認した．切除肺に有意の病理学的異常所見を認めなかった正常肺組織例（N 群）は 61 例，肺気腫合併例は 43 例（E 群）であった．

手術前 3 週間以内にスパイロメトリーで努力肺活量（FVC），1 秒量（$FEV_{1.0}$）を測定した．また ^{99m}Tc-MAA 肺血流シンチグラムにて肺血流左右分布を測定し，術後残存予測 FVC および $FEV_{1.0}$ を肺血流分布比と手術後に残存する亜区域数を用いて算出し，術後予測残存率（＝術後予測値/術前実測値）とした．また術後 1 年目にスパイロメトリーで FVC と $FEV_{1.0}$ を実測し，術前値を基準とし術後実測残存率（＝術後実測値/術前実測値）を算出した．Paired *t*-test により両群間の比較を行った．統計学的有意差の検定には Student's *t*-test または Welch's *t*-test または χ^2 検定を用いた．

結　果

N 群と E 群に左右別，腫瘍存在部位，組織型，腫瘍径，術式，術前 FVC に有意差は認めなかった．E 群で年齢，男性の比率，喫煙箱数が N 群より有意に高値であった．術前%1 秒量（$\%FEV_{1.0}$）は N 群で 89.47±13.42%，E 群で 75.51±19.56%，また術前 $FEV_{1.0\%}$ は N 群で 74.15±10.43%，E 群で 64.71±12.85% とそれぞれ E 群が有意に低値を示し（$p<0.001$），肺機能検査上も E 群は N 群と比し閉塞性障害を認め，気腫性変化が強いと考えられた（Table 1）．

術後予測残存率と実測残存率に差を paired *t*-test で検討すると，N 群では，FVC は術後予測残存率が 0.8270±0.0819，術後実測残存率が 0.8005±0.1169 で p 値は 0.0956，$FEV_{1.0}$ は術後予測残存率が 0.8270±0.0819，術後実測残存率が 0.8171±0.1176 で p 値は 0.5015 でそれぞれ有意差を認めなかった（Figure 1A）．

一方，E 群では FVC が術後予測残存率は 0.8016±0.0803，術後実測残存率は 0.8303±0.1843 で両値の間の p 値は 0.2817，$FEV_{1.0}$ が術後予測残存率は 0.8016±0.0803，術後実測残存率は 0.8793±0.1680 で p 値は 0.0072 と，$FEV_{1.0}$ において術後実測残存率が術後予測残存率より有意に高値を示した（Figure 1B）．以上から N 群では術後予測残存率と術後実測残存率に差を認めないが，E 群では $FEV_{1.0}$ において術後実測残存率は術後予測残存率より有意に高値を示した．

Table 1. Characteristics of Patients

	No respiratory disease (Group N)	Emphysema (Group E)	P values
Number	61	43	
Age	63.6 ± 8.7	67.4 ± 9.9	0.043
Gender (M/F)	33/28	37/6	< 0.001
Side (Lt/Rt)	25/36	15/28	0.529
Area (upper/lower)	36/25	26/17	0.882
His (ad/sq/others)	46/13/2	28/16/0	0.125
Smoking (pack-years)	30.8 ± 35.5	57.5 ± 35.6	< 0.001
Tumor size (mm)	2.7 ± 1.4	3.2 ± 1.7	0.099
Operation			0.927
Lobectomy	49	34	
Segmentectomy	12	9	
Preoperative respiratory function			
FVC (*l*)	2.89 ± 0.69	3.15 ± 0.86	0.102
%FVC (%)	100.96 ± 14.69	99.67 ± 20.84	0.726
$FEV_{1.0}$ (*l*)	2.13 ± 0.57	2.05 ± 0.72	0.546
$\%FEV_{1.0}$ (%)	89.47 ± 13.42	75.51 ± 19.56	< 0.001
$FEV_{1.0\%}$ (%)	74.15 ± 10.43	64.71 ± 12.85	< 0.001

Lt: left; Rt: right; His: histology; ad: adenocarcinoma; sq: squamous cell carcinoma

E群で血流シンチグラム上，切除部位が血流の低下部位と一致した症例は23例存在しており，この23例においても同様に術後の予測残存率と実測残存率にpaired *t*-testを行い，差を検討した．術後予測残存率は0.8110±0.5752で，FVCの術後実測残存率は0.9036±0.6480，$FEV_{1.0}$の術後実測残存率は0.9415±0.6750で，ともに術後予測残存率より術後実測残存率が有意に高値を示した（FVC: $p = 0.0042$，$FEV_{1.0}$: $p = 0.00014$）(Figure 2)．

E群で血流シンチグラム上，切除部位が血流の低下部位と一致した症例の23例を除く20例を同様に術後の予測残存率と実測残存率にpaired *t*-testで差を検討した．術後予測残存率は0.7909±0.5463で，FVCの術後実測残存率は0.7460±0.5240，$FEV_{1.0}$の術後実測残存率は0.8078±0.5645で，ともに術後予測残存率と術後実測残存率に有意差を認めなかった（FVC: $p = 0.2838$，$FEV_{1.0}$: $p = 0.7175$）(Figure 3)．

術前$FEV_{1.0}$と比較し，術後$FEV_{1.0}$が増加もしくは不変であった例（実測残存率≧1.0）はE群で43例中11例，N群では61例中2例であった．このE群の11例において，肺葉切除術を5例に，区域切除術を6例に行い，いずれも完全切除を施行できた．これら11例はすべて，肺癌切除部位が血流シンチグラム上の血流の低下部位と一致していた．つまり，肺癌切除部位が，LVRSにおけるtarget areaにあたる部位と一致した症例であった．

考　察

高齢化に伴い，肺気腫を合併している肺癌症例が増加している．肺切除術の適応を決定する上で，術前の$FEV_{1.0}$が一つの因子とされており，肺癌の手術適応として術後予測$FEV_{1.0}$ 800 ml以上という基準が提唱されている．[7-9]

LVRSは慢性肺気腫に対して呼吸困難感，肺機能の改善を目的とした術式であり，[3,4] 肺気腫合併肺癌に対して肺切除術を行ったところ，LVRS効果により肺機能の改善を得たとする報告例が散見される．[5,6,10-12] 肺気腫は元来，病理組織学的診断名であり，繊維化を伴わずに肺胞壁の断裂により終末細気管支より末梢の気腔が異常にかつ恒常的に拡張した病態と定義されている．[13] 今回，我々は病理学的に肺気腫を合併している肺癌症例と背景肺が正常肺組織の肺癌症例を対象とした．なお，今回の検討期間中の全肺癌切除術例467例のうち，104例(22.27%)が対象であるが，脱落のほとんどは術後の肺機能検査の記録が存在しなかったことにより，術後の肺機能検査をルーチン化していなかったためで，これは無作為である．

肺癌手術の機能的適応を決定する手段として肺血流シンチグラムによる残存肺機能予測が多く用いられている．この方法による予測肺機能と術後実測値は強く相関するとされている．[14-18] 今回はこの方法で求めた術後予測肺機能を用いて，術後の予測残存率と実測残存率について差があるのかを検討した(paired *t*-test)．なお，今回の検討で正常肺組織群の術後実測値と術後予測値の相関関係は，FVCにおいて相関係数0.8648，寄与率0.7479，また$FEV_{1.0}$では相関係数0.8896，寄与率0.7914であった．

正常肺組織群(N群)では術後予測残存率と術後実測残存率に差を認めないが，肺気腫群（E群）では$FEV_{1.0}$