To supplement the limited operative view, the thoracoscope is used throughout the operative procedure, and the port for the thoracoscope could be used for drainage afterward (Fig. 2). Although room-fixed light and the videothoracoscope are simultaneously available as a light source, the use of a head-mounted light is strongly recommended to increase the brightness of the operative field. Although the ribs do not need to be cut, two rib spreaders are used perpendicularly (see Fig. 2). The intercostal space is usually extended gently 3 to 6 cm to avoid fracturing the ribs. Especially for the direction along the rib, the incision can be well extended by another spreader. It is usually much more than expected.

In this incision, the vascular and bronchial structures can be easily accessed under direct vision. However, for the ligation of vascular structures, especially the small branches of pulmonary arteries, instruments such as end staplers, clips, and looped-knot devices (Endoloop) are better than direct ligation, and can save a great deal of time (Fig. 3). Endostaplers are usually used to divide lobar or major segmental branches of pulmonary arteries and veins, while knot devices or direct ligation are used for the ligation of smaller branches of pulmonary arteries. Even in direct ligation with threads, an instrumental knotting fine forceps is often necessary. Surgeons should be familiar with this type of intrathoracic maneuver (Fig. 4).

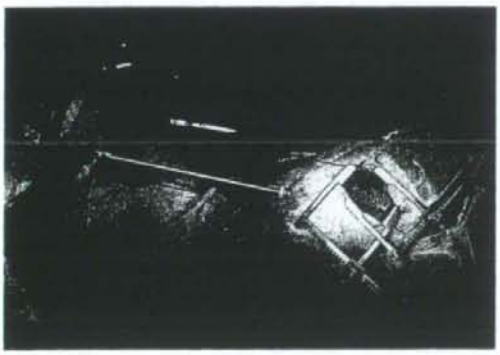

Fig. 2. A port for the thoracoscope and application of two spreaders. Thoracoscopic assistance is maintained throughout the procedure as a supplement to the small operative field and as a light source. Two spreaders maximize the available operative field. The intercostal space is widened to 3 to 5 cm, depending on the flexibility of the chest wall. Another spreader applied along the rib can extend the operative view.

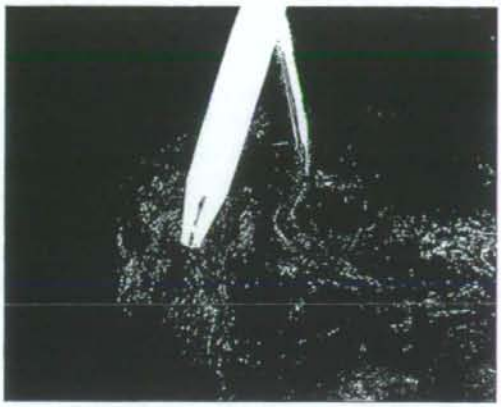

Fig. 3. Looped-knot devices can replace troublesome ligation for the small branches of pulmonary vessels in the thorax. In the MIOS approach, ligation is instrumental for most locations except for just below the incision.

Possible advantages and disadvantages of the minimally invasive open surgery approach over video-assisted thoracic surgery lobectomy

The MIOS approach offers several potential advantages over VATS lobectomy. First, based on direct assessment of the lesion, including manual palpation, the status of the cancer lesion might be evaluated more precisely. The chance of overlooking inoperable factors is minimized. In VATS lobectomy, management of the vascular structure has been a challenge, especially in cases with incomplete fissure or inflammatory change. To ensure the safe maneuvering of vascular structures, direct access to the vascular structure offers a significant advantage. Also, in the case of

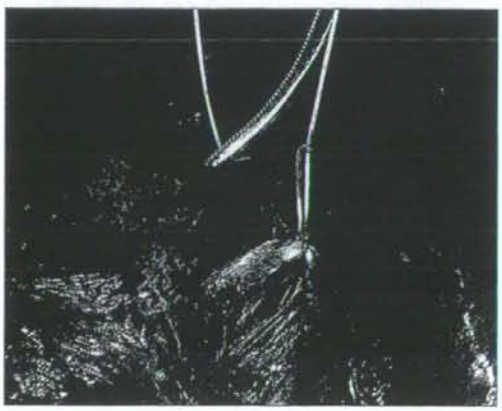

Fig. 4. Instrumental knotting for small vessels. Smaller Kelly-type forceps can be easily used.

unexpected bleeding, the MIOS approach enables a faster recovery. In VATS lobectomy, complete hilar or mediastinal lymph node dissection has been a technical challenge. For example, the subcarinal space on the left side is hardly cleared in a VATS situation, because strong traction of the overlying aorta, esophagus, and left main bronchus is indispensable for exposing the whole area. In the MIOS approach, these lymph node stations can be accessed as in conventional posterolateral thoracotomy (Fig. 5). Thus, the MIOS approach ensures the complete lymph node dissection for lung cancer. As a result of the factors mentioned above, the operative time is greatly reduced. Furthermore, this approach can be applied to procedures that are more complex than lobectomy. Generally, in segmentectomy, more precise, meticulous dissection of the hilum is needed. The hilar structure needs to be dissected and isolated at a more peripheral level than in lobectomy. In the right upper lobectomy, isolation of the upper lobe bronchus is enough, while the anterior segmental bronchus must be isolated in anterior segmentectomy. Recently, smaller and fainter nodules have been found on computed tomography (CT) imaging. This is partly because of the markedly improved quality of CT images and the increased likelihood of CT examinations in screening programs. The trend in pulmonary resection is toward segmentectomy, and this type of incisional approach is expected to be increasingly important.

In comparison with VATS lobectomy, the MIOS approach might be more invasive from the viewpoint of incisional length. With regard to the hospital stay, our anecdotal experience has shown that patients can be dismissed on the same postoperative day (usually postoperative day 4), and the length of the hospital stay is not prolonged by this approach. The degree of postoperative pain is an issue, and this must be assessed by a scientific comparison of the two approaches. Our impression is that, despite a slight increase in postoperative pain, patients can well tolerate this increased pain and achieve a quick postoperative recovery.

The overall advantages and disadvantages of VATS lobectomy and the MIOS approach need to be evaluated fairly, and the surgeonapos;s environment and patientapos;s requests are other important factors that must be determined when approaching this choice.

Future perspectives of the minimally invasive approach

Recently, there has been a growing likelihood of encountering smaller, earlier lung cancers. Some of these lesions are called "ground glass opacity," and are characterized by a mild or moderate focal increase in CT density with or without a solid or cystic or linear component within the nodule (Fig. 6). Pathologically, many of them are atypical adenomatous hyperplasia,

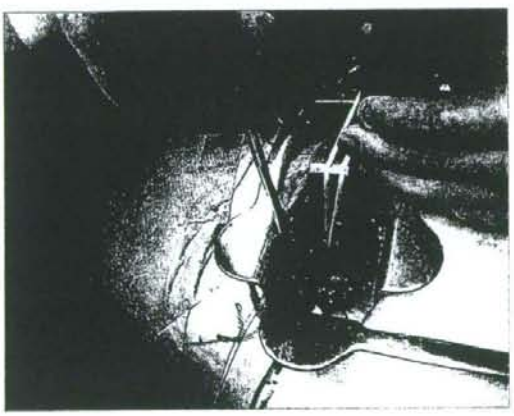

Fig. 5. Subcarinal node dissection in the MIOS approach. With a lever retractor and sponge stick, the subcarinal space is well exposed.

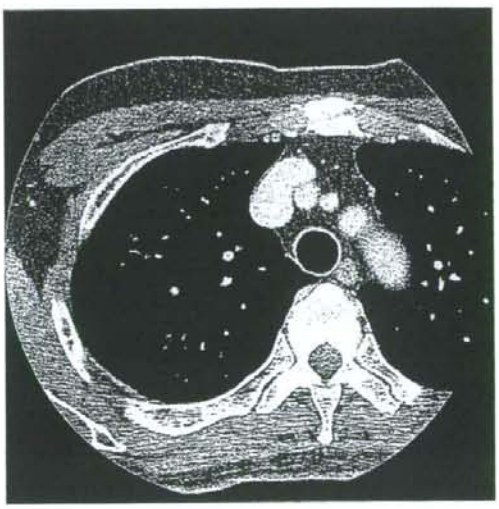

Fig. 6. Typical presentation of a ground glass opacity lesion in the right upper lobe. This needs to be diagnosed on high-resolution (thin-slice) CT with a scanning thickness of less than 1 to 2 mm.

bronchioloalveolar carcinoma (BAC), or minimally invasive BAC. For these tumors, the possibility of sublobar resection seems to be more and more realistic because of the non- or minimally invasive nature of the tumors [8,9], although lobectomy remains the gold standard in surgical resection. Here, limited, sublobar resections include wide wedge resection and segmentectomy. The concern about lobectomy as the gold standard even for tumors smaller than 2 cm in diameter is promoting new studies. The definitive answer to this crucial question can only be obtained by a controlled randomized, phase III trial. Although more than 1,000 patients need to be enrolled in such studies to achieve sufficient statistical power, the results might revise the standard surgical care for tumors without nodal involvement. Such studies are already open in North America (CALGB) and will be launched soon in Japan.

In this trend toward sublobar resection for lung cancer, the VATS approach is being applied to more complex procedures, such as segmentectomy. One issue is the nonpalpable nature of the VATS technique. Surgeons do not directly access the tumor during the VATS procedure, especially for tumors located deep in the lung parenchyma. When the nonpalpable approach by VATS is used for sublobar resections, the new technique may be needed to ensure a safe surgical margin. This is an important technical challenge. However, the MIOS, direct approach can easily enable handling of the surgical margin, and ensures the safety of complicated hilar dissection in segmentectomy. The technical merit of the MIOS approach might be even greater in the era of limited resection.

Another important feature must be addressed. Although VATS lobectomy is respected as a minimally invasive technique, this is only true regarding the incisional approach on the chest wall. Regardless of whether VATS lobectomy or open lobectomy is used, the volume of resected lung parenchyma is essentially the same (lobe). However, in the future, lung parenchyma may matter as an indicator of minimal invasiveness. A comparison of VATS lobectomy and segmentectomy by the MIOS approach is being made in which the incisional approach on the chest and resected lung volume are considered as the total surgical burden (Fig. 7).

While the trend toward a minimally invasive approach will remain, greater flexibility in selecting the appropriate technique based on the nature of the tumor is needed. A dogmatic VATS approach seems to diminish the advantage of direct access to the lung structures.

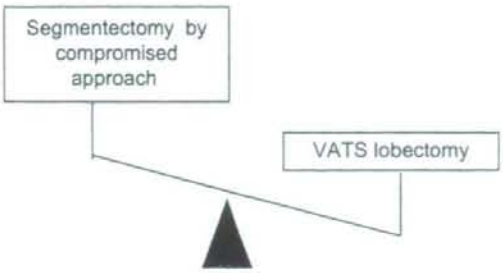

Fig. 7. Overall comparison of the different combinations in minimally invasive settings.

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ORIGINAL ARTICLE

A Japanese Lung Cancer Registry Study
Prognosis of 13,010 Resected Lung Cancers

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Purpose: The validation of tumor, node, metastasis staging system in terms of prognosis is an indispensable part of establishing a better staging system in lung cancer.

Methods: In 2005, 387 Japanese institutions submitted information regarding the prognosis and clinicopathologic profiles of patients who underwent pulmonary resections for primary lung neoplasms in 1999 to the Japanese Joint Committee of Lung Cancer Registry. The data of 13,010 patients with only lung carcinoma histology (97.6%) were analyzed in terms of prognosis and clinicopathologic characteristics.

Results: The 5-year survival rate of the entire group was 61.4%. For the small cell histology (n = 390), the 5-year survival rates according to clinical (c) and pathologic (p) stages were as follows: 58.8% (n = 161) and 58.3% (n = 127) for IA, 58.0% (n = 77) and 60.2% (n = 79) for IB, 47.1% (n = 17) and 40.6% (n = 29) for IIA, 25.3% (n = 38) and 41.1% (n = 29) for IIB, 29.0% (n = 61) and 28.3% (n = 60) for IIIA, 36.3% (n = 19) and 34.6% (n = 40) for IIIB, and 27.8% (n = 12) and 30.8% for IV (n = 13). For the non-small cell histology (n = 12,620), the 5-year survival rates according to c-stage and p-stage were as follows: 77.3% (n = 5642) and 83.9% (n = 4772) for IA, 59.8% (n = 3081) and 66.3% (n = 2629) for IB, 54.1% (n = 205) and 61.0% (n = 361) for IIA, 43.9% (n = 1227) and 47.4% (n = 1330) for IIB, 38.3% (n = 1628) and 32.8% (n = 1862) for IIIA, 32.6% (n = 526) and 29.6% (n = 1108) for IIIB, and 26.5% (n = 198) and 23.1% (n = 375) for IV. Adenocarcinoma, female gender, and age less than 50 years were significant favorable prognostic factors.

Conclusion: This large registry study provides benchmark prognostic statistics for lung cancer. The prognostic difference between stages IB and IIA was small despite different stages. Otherwise, the present tumor, node, metastasis staging system well characterizes the stage-specific prognoses.

Key Words: Lung cancer, Surgery, Prognosis, TNM stage, Resection, Cancer registry.

(*J Thorac Oncol.* 2008;3: 000–000)

The newly revised version of the Union Internationale Contre le Cancer tumor, node, metastasis (TNM) staging system is to be promulgated for general use in 2009. The present TNM staging system for lung cancer has been available worldwide since 1978,[1] and the revision process is underway. To establish a more sophisticated, truly prognostic staging system, the validation of the existing system as well as the simulation of the proposed revision based on a large, updated data set are indispensable.

In Japan, the three major societies that deal with patients with lung neoplasms, the Japan Lung Cancer Society, the Japanese Association for Chest Surgery, and the Japanese Respiratory Society, established a task force committee (The Japanese Joint Committee of Lung Cancer Registry) to perform a nationwide registry study on the prognosis and clinicopathologic profiles of lung neoplasms, both retrospectively and prospectively. The prospective follow-up registry study has been underway for all lung cancer patients who newly visited the hospital in 2002. This prospective registry study includes both resected and nonresected cases. Beside this, the committee has periodically performed three separate retrospective studies focused on cases resected in the years 1989, 1994, and 1999 after a 5-year follow-up period. These studies were planned at 5-year intervals to observe changes and trends in the prognosis, staging, histologic distribution, etc. of resected lung cancer patients in Japan. The results of the second study for patients who were resected in 1994 have already been published elsewhere[2] together with our proposal for possible revisions to the present staging system.[3]

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AQ: 2 AQ: 3 Disclosure: ●●●.

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The current study deals with third retrospective registry for patients who were resected in 1999.

Therefore, the purpose of the present study was to provide the most up-to-date benchmark statistics on the prognosis of resected lung cancer, and to clarify the appropriateness and insufficiencies of the present TNM staging system for lung cancer.

PATIENTS AND METHODS

Registry

In 2005, the Japanese Joint Committee of Lung Cancer Registry performed a nationwide retrospective registry study on the prognosis and clinicopathologic profiles of resected primary lung neoplasms in Japan. Only primary lung neoplasms that had been resected in 1999 at the certified teaching hospitals in Japan were considered for the registry, which had a follow-up period of at least 5 years. The Committee received the registries of 13,344 patients from 387 teaching hospitals. The questionnaire included 32 items such as gender, age, clinical (c)-T, c-N, c-M, c-stage, preoperative treatment, surgical procedure, extent of lymph node dissection, curability, residual tumor, primary site by lobe, tumor diameter, histology, organ invasion, pathologic (p)-T, p-N, p-M, p-stage, pleural involvement, pleural dissemination, intrapulmonary metastasis, pleural cytology, location of nodal metastasis, survival time, recurrence, and cause of death. Recurrent or multiple lung cancers were not included in this registry. The c-stage and p-stage were based on the 6th edition of the Union Internationale Contre le Cancer-TNM staging system published in 1997.[1] The histology of the tumor was described according to the World Health Organization classification.[4]

Patients

Sixty-nine patients (0.5%) with incomplete descriptions of their tumor histology and 265 patients with low-malignant histology or nonepithelial tumor histology (2.0%) were excluded from the study. Therefore, the present study focused on the remaining 13,010 patients with adenocarcinoma, squamous cell carcinoma, small cell carcinoma, large cell carcinoma, or adenosquamous carcinoma. The surgical resections for these patients were various in terms of surgical mode, level of lymph node exploration, and curability. Especially, the resection was either complete in 11,528 patients (88.6%) or incomplete in 1108 patients (8.5%), and the curability was not clearly described in 374 patients (2.9%). Despite these, the TNM staging of each patient was determined on the basis of best available information before, during, and after surgical resections.

Statistical Analysis

The survival time was defined as the time from the date of surgery to the last follow-up date. The survival curves were estimated by the Kaplan-Meier method, and the difference in survival was tested by the log-rank test in which a p value of less than 0.05 was considered significant.

RESULTS

For 13,010 registered patients with lung cancer, the most common histologic type was adenocarcinoma in 8239 patients (63.3%) followed by squamous cell carcinoma in 3700 patients (28.4%), large cell carcinoma in 474 patients (3.6%), small cell carcinoma in 390 patients (3.0%), and adenosquamous carcinoma in 207 patients (1.6%). The survival curve of the entire registry population is shown in Figure 1, in which the 5-year survival rate was 61.4%. The survival curves according to histologic type of all stages are shown in Figure 2. The 5-year survival rates according to the histologic type were as follows: 67.3% for adenocarcinoma,

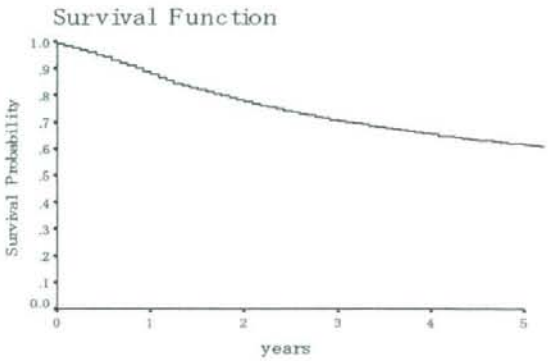

FIGURE 1. A survival curve for all histologic types and all stages (n = 13,010). The 5-year survival rate for the entire group is 61.4%.

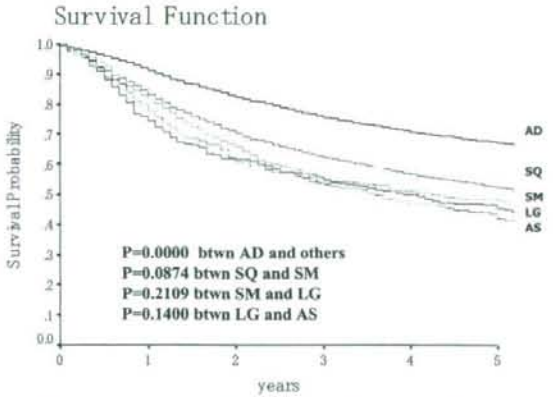

FIGURE 2. Survival curves according to histologic type. The 5-year survival rates according to histologic type are as follows: 67.3% for adenocarcinoma (n = 8239), 52.5% for squamous cell carcinoma (n = 3700), 48.1% for small cell carcinoma (n = 390), 45.5% for large cell carcinoma (n = 474), and 42.1% for adenosquamous carcinoma (n = 207). There is a significant difference in survival between adenocarcinoma and others (p = 0.0000). AD, adenocarcinoma; SQ, squamous cell carcinoma; SM, small cell carcinoma; LG, large cell carcinoma; AS, adenosquamous carcinoma.

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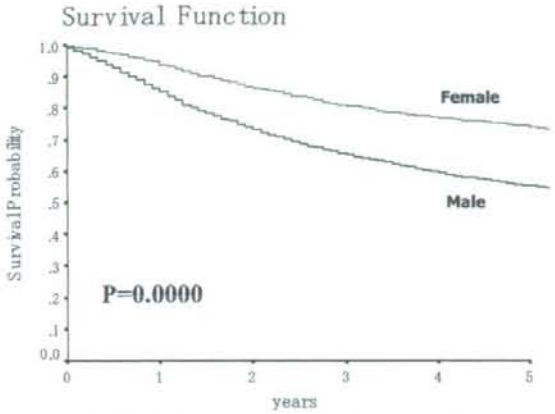

FIGURE 3. Survival curves according to gender. The 5-year survival rates of female (n = 4228) and male (n = 8664) patients are 74.1% and 55.2%, respectively. The survival of female patients is significantly better than that of male patients (p = 0.0000).

52.5% for squamous cell carcinoma, 48.1% for small cell carcinoma, 45.5% for large cell carcinoma, and 42.1% for adenosquamous carcinoma. The adenocarcinoma histology had significantly better survival than other histologic types (p = 0.0000 each). Female patients comprised 32.5% (n = 4228) of the entire registered population, and male patients comprised 66.6% (n = 8664). The 5-year survival rates of the female and male patients were 74.1% and 55.2%, respectively. These survival curves are shown in Figure 3, and the difference in survival between the 2 genders was significant (p = 0.0000). The clinical profiles and stage-specific prognosis were described separately for small cell and non-small cell histologic categories because of the known differences in the pathobiologic nature and response to treatment between these malignancies.

Small Cell Carcinoma

For 390 patients with resected small cell carcinoma of all stages, the 5-year survival rate was 48.6%. The survival curves according to stage are shown in Figure 4. The distribution of c-stage and p-stage, stage-specific 5-year survival rates, and the difference in survival between neighboring stages are presented in Table 1.

Non-small Cell Carcinoma

For 12,620 patients with resected non-small cell histologies of all stages, the 5-year survival rate was 61.8%. The survival curves according to stage are shown in Figure 5. The distribution of c-stage and p-stage, stage-specific 5-year survival rates, and difference in survival between neighboring stages are presented in Table 2. For the c-stage, the difference in survival was significant between all neighboring c-stages except for those between stages IB and IIA and between IIIA and IIIB. For the p-stage, the difference in survival was significant between all neighboring stages, although the difference between p-stages IB and IIA was approaching the marginal significance level.

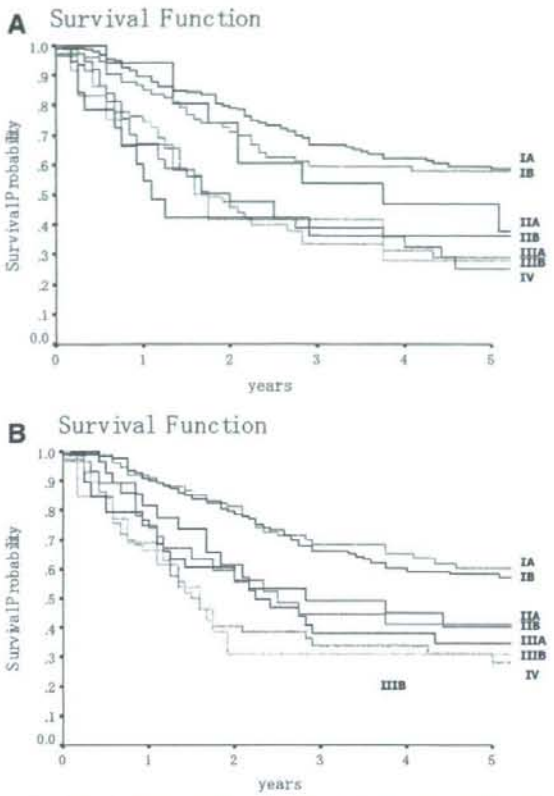

FIGURE 4. Survival curves of small cell lung carcinoma cancers according to c-stage (*A*) and p-stage (*B*) (n = 390). The 5-year survival rates by c-stage are as follows: 58.8% for IA (n = 161), 58.0% for IB (n = 77), 47.1% for IIA (n = 17), 25.3% for IIB (n = 38), 29.0% for IIIA (n = 61), 36.3% for IIIB (n = 19), and 27.8% for IV (n = 12). The 5-year survival rates by p-stage are as follows: 58.3% for IA (n = 127), 60.2% for IB (n = 79), 40.6% for IIA (n = 29), 41.1% for IIB (n = 29), 28.3% for IIIA (n = 60), 34.6% for IIIB (n = 40), and 30.8% for IV (n = 13).

Survival was further analyzed according to patient age. The survival curves according to three age groups, those <50 years (n = 797), those ≥50 years but <70 years (n = 6563), and those ≥70 years (n = 5147) are shown in Figure 6. The 5-year survival rates for the three age groups were 69.9, 66.0, and 54.9%, respectively. The survival of patients aged ≥70 years was significantly worse than those in the other two age groups (p = 0.0000 and p = 0.0000).

Comparison between the 1994 and 1999 Registry Studies

The distribution of histologic types was compared between 1994 and 1999 (Fig. 7). Within the 5-year interval,

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TABLE 1. Stage-Specific 5-Yr Survival Rates for Small Cell Carcinoma According to the Clinical and Pathological Settings (n = 390)

	Stage						
Stage Settings	**IA**	**IB**	**IIA**	**IIB**	**IIIA**	**IIIB**	**IV**
Clinical, n (%)	161 (41.3)	77 (19.7)	17 (4.4)	38 (9.7)	61 (15.6)	19 (4.9)	12 (3.1)
5-Yr survival rate, %	58.8	58.0	47.1	25.3	29.0	36.3	27.8
Difference in survival[a]	0.5627	0.4110	0.1577	0.9807	0.7045	0.7265	—
Pathological, n (%)	127 (32.6)	79 (20.3)	29 (7.4)	29 (7.4)	60 (15.4)	40 (10.3)	13 (3.3)
5-Yr survival rate, %	58.3	60.2	40.6	41.1	28.3	34.6	30.8
Difference in survival[a]	0.9331	0.0415	0.8289	0.2300	0.5217	0.6115	—

[a] Significance of the difference in survival between neighboring (lower and next higher) stages (p value).

incidence of adenocarcinoma increased 7%, from 56 to 63%, whereas that of squamous cell carcinoma decreased 5%, from 33 to 28%. The proportion of other histologic types remained almost unchanged. When the overall survival was compared, an improvement of the 5-year survival rate from 52.0 to 61.4% was achieved for all histologic types, and from 52.6 to 61.8% for non-small cell carcinomas. The gender distribution did not change remarkably between 1994 and 1999: female patients comprised 29.9% of the all the registered patients in 1994, and 32.8% in 1999. Nevertheless, the difference in survival according to gender grew within the 5-year interval: the difference in the 5-year survival rate between women and men was 13.2% in 1994, and 18.9% in 1999.

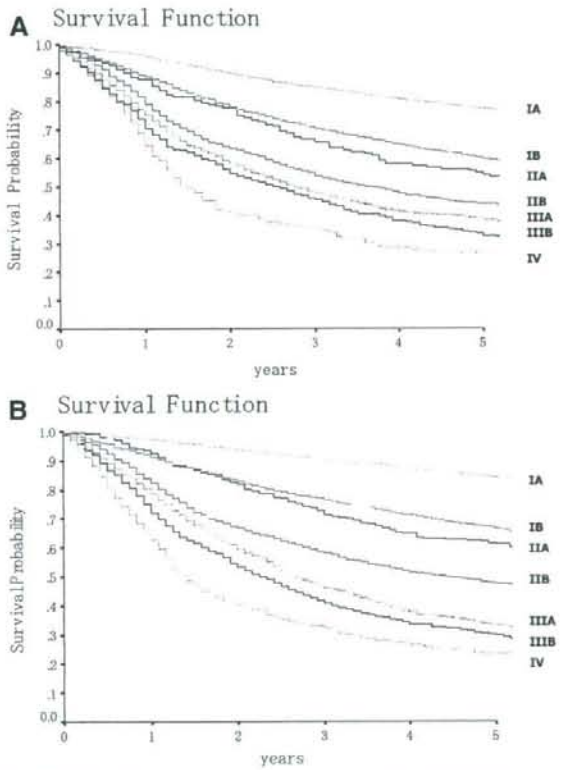

FIGURE 5. Survival curves of non-small cell histologies according to c-stage (A) and p-stage (B) (n = 12,620). The 5-year survival rates by c-stage are as follows: 77.3% for IA (n = 5642), 59.8% for IB (n = 3081), 54.1% for IIA (n = 205), 43.9% for IIB (n = 1227), 38.3% for IIIA (n = 1628), 32.6% for IIIB (n = 526), and 26.5% for IV (n = 198). The 5-year survival rates by p-stage are as follows: 83.9% for IA (n = 4772), 66.3% for IB (n = 2629), 61.0% for IIA (n = 361), 47.4% for IIB (n = 1330), 32.8% for IIIA (n = 1862), 29.6% for IIIB (n = 1108), and 23.1% for IV (n = 375).

The stage distribution was compared in non-small cell lung carcinoma between 1994 and 1999 (Fig. 8). The percentage of stages IA and IB increased 11%, from 59 to 70%, in the c-setting, and 8%, from 51 to 59%, in the p-setting. Stage-specific 5-year survival rates in non-small cell carcinoma were compared between the 1994 and 1999 registry studies for c-stage (Table 3) and for p-stage (Table 4). Although a survival improvement was achieved in all stages, the change in stage IB was remarkable. The 5-year survival rate in stage IB improved from 49.9 to 59.8% in a c-setting, and from 60.1 to 66.3% in a p-setting. Summarizing these, the trends from 1994 to 1999 consisted of an increase in the adenocarcinoma histology and earlier stages, and an improvement in the overall as well as the stage-specific survival.

DISCUSSION

This is a report on the third nationwide registry study conducted by the Japanese Joint Committee of Lung Cancer Registry representing three major Japanese societies that deal with patients with lung cancer, in which the clinicopathologic features and prognosis of the resected lung cancer were studied. Three registry studies have independently and periodically focused on cases that were resected in the years 1989, 1994, and 1999 after a 5-year follow-up period. The details of the second study involving cases resected in 1994 in which 7393 patients with primary lung neoplasms were registered from 307 teaching hospitals in Japan have already been published elsewhere.[2,3] The number of registered patients in the third study (13,344 patients) was almost twice that of the second study (7393 patients) with only a slight increase in the number of participating institutions from 307 to 387. The number of cases registered from each institute ranged from 1 to 212 cases, and 15 institutes registered more than 100 cases. Considering that the total number of lung

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TABLE 2. Stage-Specific 5-Yr Survival Rates for Non-small Cell Carcinoma According to the Clinical and Pathological Settings (n = 12,620)

	Stage						
Stage Settings	**IA**	**IB**	**IIA**	**IIB**	**IIIA**	**IIIB**	**IV**
Clinical, n (%)	5642 (44.7%)	3081 (24.4%)	205 (1.6%)	1227 (9.7%)	1628 (12.9%)	526 (4.2%)	198 (1.6%)
5-Yr survival rate, %	77.3	59.8	54.1	43.9	38.3	32.6	26.5
Difference in survival[a]	0.0000	0.1444	0.0022	0.0013	0.0755	0.0111	—
Pathological, n (%)	4772 (37.8%)	2629 (20.8%)	361 (2.9%)	1330 (10.5%)	1862 (14.8%)	1108 (8.8%)	375 (3.0%)
5-Yr survival rate, %	83.9	66.3	61.0	47.4	32.8	29.6	23.1
Difference in survival[a]	0.0000	0.0367	0.0000	0.0000	0.0054	0.0001	—

[a] Significance of the difference in survival between neighboring (lower and next higher) stages (p value).

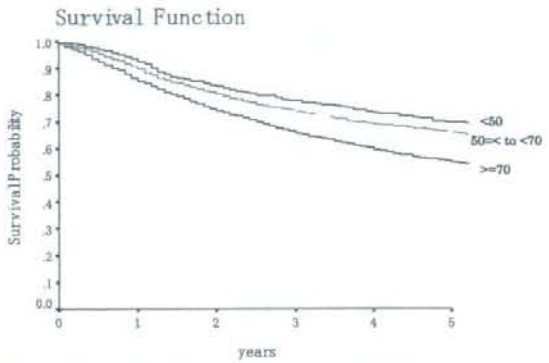

FIGURE 6. Survival curves according age in non-small lung cancer. The 5-year survival rates for the three age groups, <50 years (n = 797), ≥50 years but <70 years (n = 6563), and ≥70 years (n = 5147) are 69.9%, 66.0%, and 54.9%, respectively.

cancer resections in Japan was approximately 30,000, these registered cases are estimated to comprise 30 to 40% of the total. The results of this registry study represent the findings based on the largest series ever published.

There has been remarkable difference in survival between patients resected in 1994 and 1999, where the overall survival rate at 5 years in the registry population improved from 52.6 to 61.4%. The stage-specific survival also improved. Because the survival improvement was achieved not only in all stages but also in the entire population, this improvement should not be interpreted as simply the result of a stage migration phenomenon. The possible reasons for the improvement might be refinements in the evaluation of surgical candidates, advancements and improvement in treatment, and the shift of the registry population toward more curable lung cancer.

Refinement in the preoperative work-up for surgical candidates may better identify patients with distant disease, resulting in a better selection of patients for surgery. Nevertheless, except for an improvement in imaging diagnosis techniques such as computed tomography (CT), the difference in the quality of preoperative work-up between 1994 and 1999 does not seem significant. Even in 1999, positron emission tomography scans were not used as part of a routine preoperative work-up in Japan. Therefore, the difference in preoperative work-up does not seem to account for the difference in survival between the years 1994 and 1999.

When looking at the changes in surgical interventions for lung cancer patients in the 5 years between 1994 and

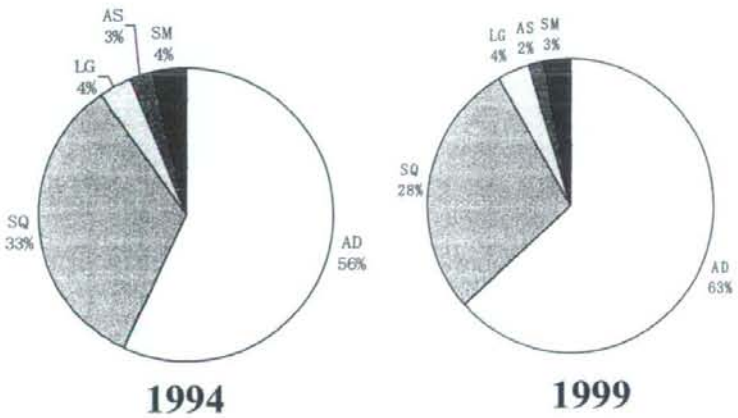

FIGURE 7. Distribution of histologic types in 1994 and 1999. Adenocarcinoma increases 7% (from 56% to 63%) and squamous cell carcinoma decreases 5% (from 33% to 28%).

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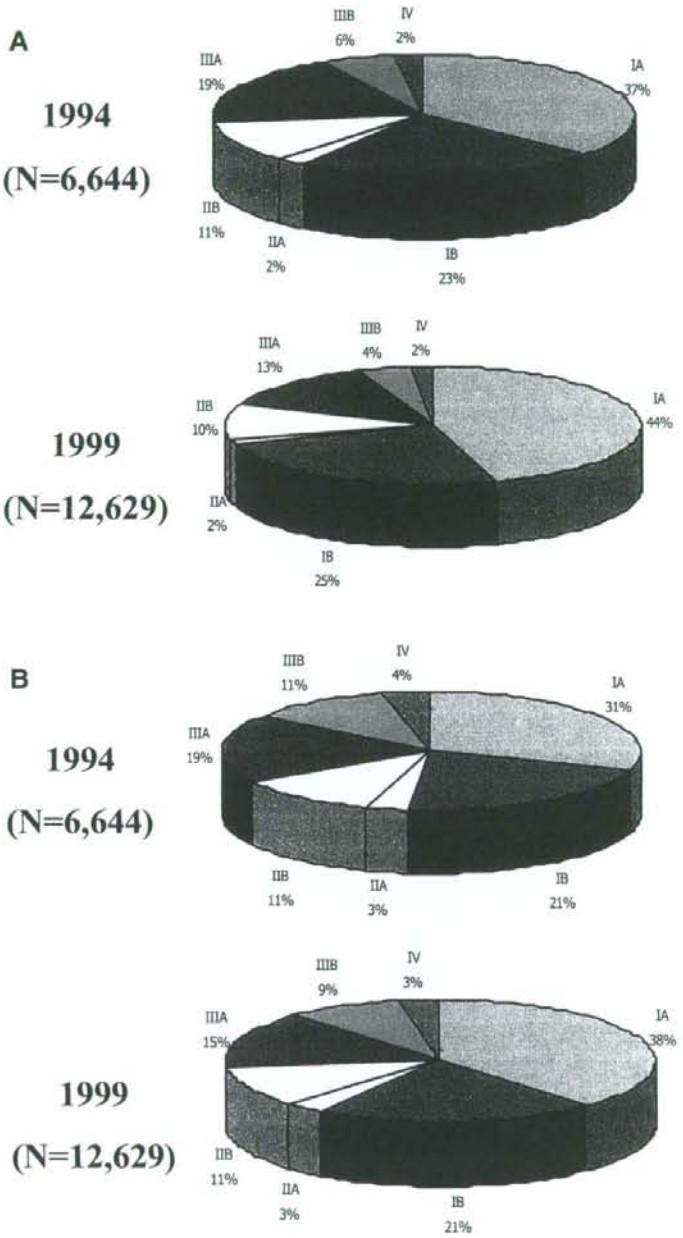

FIGURE 8. Distribution of c-stage (*A*) and p-stage (*B*) for non-small cell lung histologies in 1994 and 1999. The percentage of stage I increased from 60 to 69% (11%) in the c-setting, and from 52 to 59% (7%) in the p-setting.

1999, we recognized that less (or minimally) invasive surgery with or without video assistance had been more generalized.[5] In these minimally invasive techniques, the faster postoperative recovery has been speculated, and this is a present-day trend in oncologic surgery of any sites. Nevertheless, knowing that no one study has ever definitely demonstrated that minimally invasive surgery improves the survival of patients with lung cancer, or the mortality/morbidity, it is unlikely that the improvement in survival of the present registry population was solely because of the advancements in surgical interventions.

Comparing the distribution of histologic types between 1994 and 1999, the 7% increase in adenocarcinomas and the 5% decrease in squamous cell carcinoma were remarkable changes. In this registry study, the noninvasive form of

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TABLE 3. Comparison of Stage-Specific 5-Yr Survival Rate (%) between 1994 and 1999 (c-Stage) in Non-small Cell Histologies

	c-Stage							
Year of Survey	IA	IB	IIA	IIB	IIIA	IIIB	IV	All Stages
1994 (n = 6,644)	72.1	49.9	48.7	40.6	35.8	28.0	20.8	52.6
1999 (n = 12,620)	77.3	59.8	54.1	43.9	38.3	32.6	26.5	61.8

TABLE 4. Comparison of Stage-Specific 5-Yr Survival Rate (%) between 1994 and 1999 (p-Stage) in Non-small Cell Histologies

	p-Stage							
Year of Survey	IA	IB	IIA	IIB	IIIA	IIIB	IV	All Stages
1994 (n = 6,644)	79.5	60.1	59.9	42.2	29.8	19.3	20.0	52.6
1999 (n = 12,620)	83.9	66.3	61.0	47.4	32.8	29.6	23.1	61.8

adenocarcinoma, nonmucinous bronchioloalveolar carcinoma, was included in the adenocarcinoma category. These tumors are well known for their characteristic presentation on high-resolution CT images as ground glass opacity and a superb prognosis without recurrence after intervention. Considering that the evaluation of these faint, small-sized tumors using high-resolution CT was being generalized in Japan in late 1990s, the increase in bronchioloalveolar carcinoma might have resulted in the inclusion of these earlier, less-aggressive tumors into the registry population. The distribution of the stage of the disease at diagnosis also changed remarkably between 1994 and 1999 as can be seen in Figure 8. The earliest disease, stage IA and IB, comprised 60% of the c-stage and 52% of the p-stage in 1994, and 69% and 59% in 1999, respectively. The shift of the patients' diagnosis toward an earlier staged disease at the time of surgery definitely had a significant impact on the improvement in overall survival.

Based on the second registry study of cases resected in 1994, we proposed a revision of the TNM staging system in which the unification of stages IB and IIA and the division of T1 into T1a and T1b by the cutoff length of a diameter of 2 cm were shown to be necessary. In this latest 1999 data set the prognostic difference in survival between stages IB and IIA was small. In the c-setting, the 5-year survival rates for IB and IIA were 59.8% and 54.1%, and the difference in survival was not statistically significant ($p = 0.1444$). In the p-setting, the 5-year survival rates for IB and IIA were 66.3% and 61.0%, and the difference in survival was marginally significant ($p = 0.0367$), probably because of the increase in the overall number of patients. Because the survival improvement in patients with stage IB was so remarkable, the prognostic difference between stages IB and IIA seemed to increase in 1999 compared with that in 1994. Nevertheless, considering the limited number of patients with stage IIA disease, we believe that the unification of stages IB and IIA would well characterize the stage-specific prognosis of both groups.

In the report on the second registry study, the large prognostic difference in survival by gender, age, and histology was addressed. Also, in this third registry study, a significant difference in survival according to these variables was reproduced. Especially, the difference in 5-year survival rate by gender was almost 20% in non-small cell carcinomas, in which the 5-year survival rates for men and women were 55.5% and 74.5%, respectively. It is still unclear what factors account for the large survival difference between men and women. It is necessary to see the relationship between female gender and other significant prognostic variables such as histology and their biologic characteristics. Considering the difference in smoking status between the two genders in Japan, the difference in the biologic nature of cancers in women versus men might have some impact on overall survival.

The present retrospective, nationwide, large-scale registry study provides the most updated benchmark statistics for patients with lung cancer. Further studies to elucidate the factors associated with the improvement of survival and the impact of several prognostic variables is underway.

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Immunohistochemical detection of GLUT-1 can discriminate between reactive mesothelium and malignant mesothelioma

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The separation of benign reactive mesothelium (RM) from malignant mesothelial proliferation can be a major challenge. A number of markers have been proposed, including epithelial membrane antigen, p53 protein, and P-glycoprotein. To date, however, no immunohistochemical marker that allows unequivocal discrimination of RM from malignant pleural mesothelioma (MPM) has been available. A family of glucose transporter isoforms (GLUT), of which GLUT-1 is a member, facilitate the entry of glucose into cells. GLUT-1 is largely undetectable by immunohistochemistry in normal epithelial tissues and benign tumors, but is expressed in a variety of malignancies. Thus, the expression of GLUT-1 appears to be a potential marker of malignant transformation. Recently, in fact, some studies have shown that GLUT-1 expression is useful for distinguishing benign from malignant lesions. The purpose of the present study was to evaluate the diagnostic utility of GLUT-1 expression for diagnostic differentiation between RM and MPM. Immunohistochemical staining for GLUT-1 was performed in 40 cases of RM, 48 cases of MPM, and 58 cases of lung carcinoma. Immunohistochemical GLUT-1 expression was seen in 40 of 40 (100%) MPMs, and in all cases the expression was demonstrated by linear plasma membrane staining, sometimes with cytoplasmic staining in addition. GLUT-1 expression was also observed in 56 out of 58 (96.5%) lung carcinomas. On the other hand, no RM cases were positive for GLUT-1. GLUT-1 is a sensitive and specific immunohistochemical marker enabling differential diagnosis of RM from MPM, whereas it cannot discriminate MPM from lung carcinoma.
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Keywords: Glut-1; reactive methothelium; malignant pleural mesothelioma; immunohistochemistry; lung carcinoma

The separation of benign reactive mesothelium (RM) from malignant mesothelial proliferation can be a major challenge. The common cytomorphological features associated with malignancy, such as high cellularity/proliferation, marked cytonuclear atypia and high mitotic rate are of very limited use in this setting. Thus, it is sometimes very difficult, or almost impossible even for expert pathologists to make a definite diagnosis of malignant mesothelioma, especially in small specimens, unless there is unequivocal invasion of adjacent tissues by tumor cells.[1] On the other hand, early diagnosis of malignant pleural mesothelioma (MPM) in small closed pleural biopsy samples, or by cytology, is crucial for patient management and may facilitate the avoidance of invasive surgical procedures.

A number of immunohistochemical markers have been proposed to assist conventional morphological diagnosis, including epithelial membrane antigen (EMA)[2–5] p53 protein,[2–11] and P-glycoprotein.[2,5,12] Other markers tested have included Bcl-2,[2,3,13] platelet-derived growth factor receptor (PDGF-R) β-chain[2,5,8] and desmin.[2] To date, however, no single immunohistochemical marker that can unequivocally discriminate RM from MPM has been available.

GLUT-1 is one of 14 members of the mammalian facilitative glucose transporter (GLUT) family of passive carriers that function as an energy-independent system for transport of glucose down a concentration gradient.[14] GLUT-1 is not detectable

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in a large proportion of cells from normal tissues and benign lesions, except for erythrocytes, germinal cells of the testis, renal tubules, perineurium of peripheral nerves, endothelial cells in blood–brain barrier vessels, and placenta (trophoblasts and capillaries).[15,16] In contrast, GLUT-1 is expressed in a variety of carcinomas such as those of the breast, head and neck, bladder, renal cells, and lung.[15–24] Previous reports suggest that the expression of GLUT-1 may be a potential marker for malignancy.

Recently, some studies have shown that GLUT-1 expression is useful for resolving the common diagnostic dilemma of distinguishing benign from malignant lesions.[25,26] Although a few studies have demonstrated that GLUT-1 is useful for distinguishing RM from metastatic adenocarcinoma in body cavity effusions,[27–29] the study cohorts did not include MPM. Using immunohistochemistry, Godoy *et al*[16] analyzed coexpression of GLUT-1 and other GLUT isoforms (GLUT-2 to -6 and GLUT-9) in a variety of benign and malignant tumors, and demonstrated that two of four MPMs were positive for GLUT-1. However, they did not analyze reactive and normal mesothelium.

The purpose of the present study was to evaluate the diagnostic utility of GLUT-1 detection for differential diagnosis between RM and MPM.

Materials and methods

Case Selection

The materials for the present study were extracted from cases deposited in the pathology files of the National Cancer Center Hospital, Tokyo, between 1971 and 2005. They comprised 40 cases of RM, 48 cases of MPM (epithelioid, 36 cases; biphasic, 11 cases; sarcomatoid, 1 case), and 58 cases of lung carcinoma (squamous cell carcinoma, 28 cases; adenocarcinoma, 30 cases). All diagnoses had been made on the basis of conventional histopathologic features evident in slide preparations stained with hematoxylin and eosin, some special stains, and immunohistochemical techniques available at that time.[30,31] In the present study, immunohistochemistry for D2-40 and calretinin was added for all cases to confirm the identity of mesothelial cells (see below).

Immunohistochemistry

For immunohistochemical staining, 5-μm-thick sections were deparaffinized and treated with 3% hydrogen peroxide for 30 min to block endogenous peroxidase activity, followed by washing in deionized water for 2–3 min. Heat-induced epitope retrieval with Target Retrieval Solution (DAKO, Carpinteria, CA, USA) was performed for GLUT-1 and calretinin. After the slides had been allowed to cool at room temperature for 40 min, they were rinsed with deionized water and then washed in phosphate-buffered saline for 5 min. The slides were then stained by overnight incubation with primary antibodies against GLUT-1 (1:200, polyclonal, Dako), D2-40 (1:200, clone D2-40, Signet Laboratories, Dedham, MA, USA), and calretinin (1:100, polyclonal, Zymed, San Francisco, CA, USA). Immunoreactions were detected by the labeled streptavidin–biotin method, and visualized with 3, 3′-diaminobenzidine, followed by counterstaining with hematoxylin. Appropriate positive and negative controls (red blood cells for GLUT-1) were used for each antibody. The area of GLUT-1 staining was evaluated on a sliding scale of 0 to 3+ to represent the percentage of positive cells among mesothelial cells (indicated by D2-40 and calretinin immunostain) or tumor cells (0 = <1%, 1+ = 1–25%, 2+ = 26–50%, 3+ = >51%). Immunohistochemical staining was scored independently by two observers (YK and KT).

Results

The results of immunohistochemistry are summarized in Table 1. GLUT-1 expression was demonstrated by distinct linear plasma membrane staining, sometimes with cytoplasmic staining in addition

Table 1 Immunoreactivity of GLUT-1

	n	*GLUT-1 positive (%)*	*Staining area*			
			0	*1+*	*2+*	*3+*
Mesothelioma, all subtypes	48	48 (100)	0	15	15	18
Epithelioid	36	36 (100)	0	9	12	15
Biphasic	11	10 (90.9)[a] 7 (63.6)[b]	1[a] 4[b]	6[a] 3[b]	3[a] 2[b]	1[a] 2[b]
Sarcomatoid	1	1 (100)	0	1	0	0
Reactive mesothelium	40	0 (0)	40	0	0	0
Lung carcinoma	58	56 (96.5)	2	12	9	35
Squamous cell carcinoma	28	28 (100)	0	1	3	24
Adenocarcinoma	30	28 (93.3)	2	11	6	11

[a]Epithelioid areas.
[b]Sarcomatoid areas.

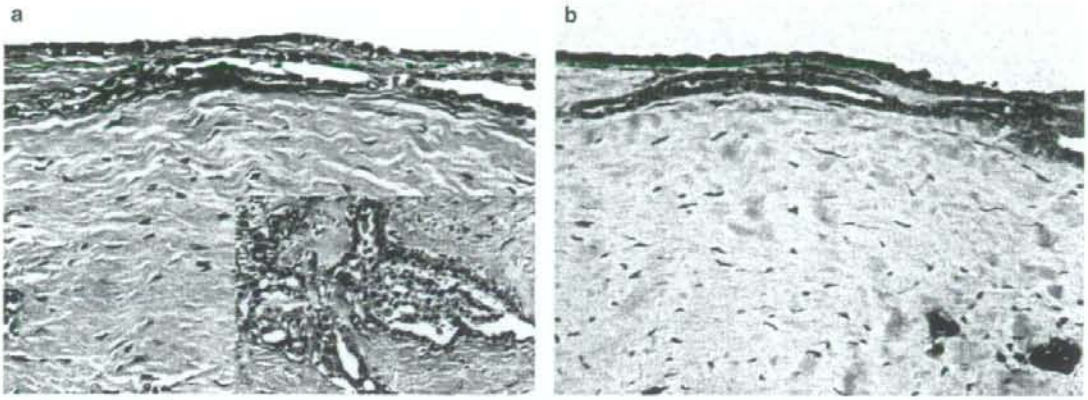

Figure 1 (**a**) In the surface area, the tumor cells showed bland cytologic atypia, nevertheless malignant mesothelioma(HE stain, × 10). Inset: the tumor cells arranged complex branching tubular formation (HE stain, × 10). (**b**) Most of the tumor cells in the epithelioid MPM were positive for GLUT-1 and red blood cells were served as internal positive control (× 10).

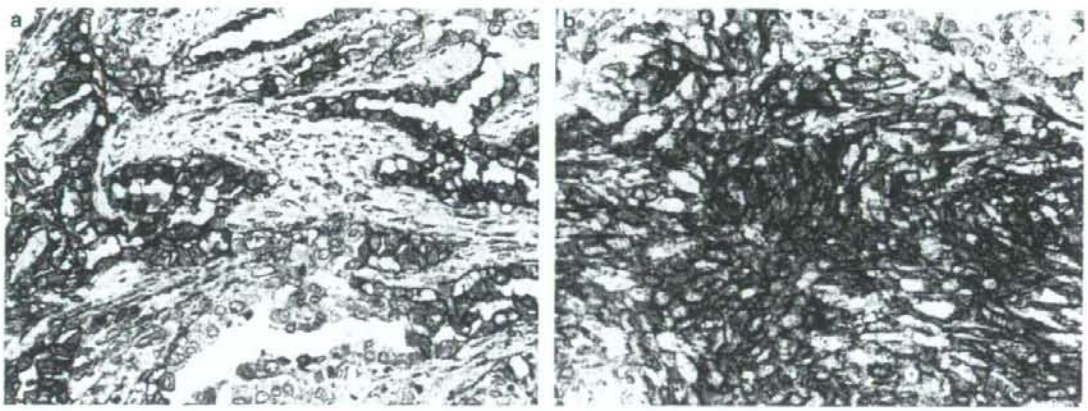

Figure 2 (**a**) More than half of the epithelioid tumor cells were positive for GLUT-1 (× 10). (**b**) Most of the sarcomatoid tumor cells were positive for GLUT-1 (× 10). The immunoreactivity was observed as distinct linear plasma membrane staining, with weak cytoplasmic staining in addition.

Table 2 GLUT-1 immunoreactivity acording to MPM histological subtype

	n	*GLUT-1-positive (%)*	*Staining area*			
			0	*1+*	*2+*	*3+*
Epithelioid area	47	46 (97.8)	1	15	15	16
Sarcomatoid area	12	8 (66.7)	4	4	2	2

(Figure 1a and b). GLUT-1 immunoreactivity was seen in 48 of 48 (100%) MPM cases, whereas no RM cases were positive for GLUT-1.

We also evaluated GLUT-1 immunoreactivity according to histological subtype, as shown in Table 2. Immunoreactivity was observed in 46 of 47 (96.7%) epithelioid mesothelioma (Figure 2a) including epithelioid areas of biphasic mesothelioma, and in seven of 12 (66.7%) sarcomatoid mesothelioma (Figure 2b) including sarcomatoid areas of biphasic mesothelioma. However, immunoreactive cells more than half of tumor cell was only 16 of 47 (34%) of epithelioid mesothelioma including epithelioid areas of biphasic mesothelioma, and two of 12 (14.1%) of sarcomatoid mesothelioma including sarcomatoid areas of biphasic mesothelioma. The GLUT-1-positive cells varied from a few cells to almost all cells in the clusters, but no characteristic staining pattern was observed in MPM.

GLUT-1 immunoreactivity was also seen in 56 of 58 (96.5%) cases of lung carcinoma. According to histological subtype, immunoreactivity was

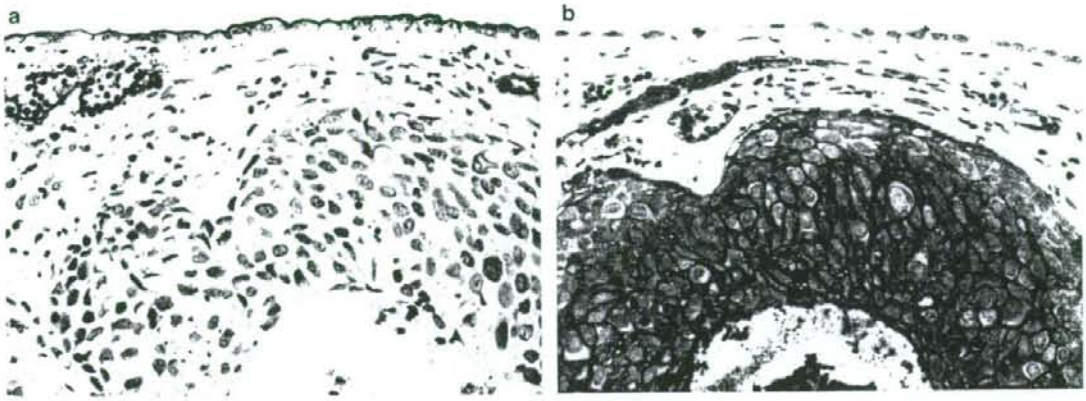

Figure 3 (**a**) D2-40 immunoreactivity was observed in the RM and lymph vessels beneath the pleura, but no immunoreactivity was observed in the poorly differentiated squamous cell carcinoma (× 10). (**b**) Most of the tumor cells without peripheral lesion in of the poorly differentiated squamous cell carcinoma were positive for GLUT-1 (red blood cells were served as internal positive control). On the other hand, RM showed no immunoreactivity for GLUT-1 (× 10).

observed in 28 of 28 (100%) cases of squamous cell carcinoma (Figure 3a and b) and 28 of 30 (93.3%) cases of adenocarcinoma. In squamous cell carcinoma, the area of positive staining was 3 + in 24 of 28 (85.7%) cases, compared with only 11 of 30 (36.7%) in cases of adenocarcinoma. Also in squamous cell carcinoma, a characteristic staining pattern was observed; tumor cells showed more intensely positive staining in the central area of tumor nests than in the peripheral area (Figure 3b).

Discussion

Morphologic differentiation between RM and MPM in small specimens can be a diagnostic challenge. The difficulty is compounded when neoplastic cells demonstrate only slight atypia. In addition, there are currently no reliable markers that allow immunohistochemical discrimination between RM and MPM. In the present study, we clearly demonstrated that GLUT-1 is a sensitive and specific immunohistochemical marker that can differentiate RM from MPM. To our knowledge, this is the first report to describe the usefulness of GLUT-1 immunostaining for discriminating between RM and MPM.

Elevated levels of expression or activation of GLUT-1, or both, have been shown to be associated with transformation of cells and malignancy, and to be modified by changes in the physiological microenvironment in tissues.[32,33] High GLUT-1 expression correlates with increased metabolism and glucose utilization in a number of normal tissues, and this transporter is overexpressed in a variety of human tumors.[15,16] Increased expression of GLUT-1 is also seen in conditions that induce greater dependency on glycolysis as an energy source, such as ischemia, hypoxia, or both.[34] These data suggest that overexpression of GLUT-1 may play an important role in the survival of tumor cells by maintaining an adequate energy supply to support their high metabolism and rapid growth in an often less-than-ideal physiological environment.[35]

GLUT-1 expression has been revealed in a variety of carcinomas, such as those of the breast, head and neck, bladder, and renal cells.[15–19,23] In the lung, about 34.3–100% of lung adenocarcinomas[16,20–22,24] and 100% of lung squamous cell carcinomas[20–22,24] are reported to express GLUT-1 at the primary site. With regard to MPM, only one article has describe that two of four studied cases were positive for GLUT-1.[16] In the present study, GLUT-1 immunoreactivity was observed in all MPMs and 56 out of 58 (96.5%) cases of lung carcinoma. These results indicate that GLUT-1 cannot discriminate between MPM and lung carcinoma. Therefore, additional appropriate positive and negative mesothelial markers are needed in order to differentiate between MPM and lung carcinoma.[31]

The heterogeneity of GLUT-1-positive areas has been reported previously. In squamous cell carcinoma, cells in the center of cancer nests, close to the necrotic area, were stained more strongly than those in peripheral areas. In adenocarcinoma, poorly differentiated areas such as the solid central area were stained more strongly than well differentiated areas such as those showing lepidic growth.[20–22,24] In the present study, more than half of all tumor cells were positive for GLUT-1 in 37.5% of MPMs, 85.7% of lung squamous cell carcinomas, and 36.7% of lung adenocarcinomas. These results indicate that GLUT-1 negativity in small samples such as those obtained by biopsy does not exclude malignancy, and that positive immunoreactivity for GLUT-1 may be an aid to accurate diagnosis of malignancy.

The GLUT-1 positivity rate in RM has been reported to be 0% (present study and Afify *et al*[29]), 3% (Zimmerman *et al*[28]), and 20% (Burstein *et al*[27]).

However, Zimmerman *et al* and Burstein *et al* reported that GLUT-1-positive cells of RM showed equivocal-to-weak staining and were easily distinguishable from unequivocal positivity of other cell types, so that the specificity of GLUT-1 was not diminished. According to them, a number of 'false-positive' cases occurred in patients with cirrhosis. The RM resulting from cirrhosis may be prompted by glucose intake to compensate for the unfavorable environment in effusion. Our cohort of RM consisted of surgically resectable cases within the physiological range or without effusion.

Positron emission tomography (PET) measurements of fluorodeoxyglucose (FDG) accumulation in different animal tumors has shown a correlation between tracer FDG uptake and the GLUT-1 mRNA content. GLUT-1 has been found to be overexpressed in tumor cells and to promote glucose metabolism and FDG accumulation in humans.[22,24] In MPM, Carretta *et al*[16] have reported that FDG-PET can differentiate RM from MPM. These findings are consistent with the present immunohistochemical results.

In summary, GLUT-1 appears to be a sensitive and specific marker for differentiating between RM and MPM, although it is unable to discriminate between MPM and lung carcinoma.

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Original Article

Immunohistochemical expression of TTF-1 in various cytological subtypes of primary lung adenocarcinoma, with special reference to intratumoral heterogeneity

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The immunohistochemical expression of thyroid transcription factor-1 (TTF-1) was investigated in various cytological subtypes of primary lung adenocarcinoma, with special reference to intratumoral heterogeneity. Three groups were categorized according to cytological subtype: group A, adenocarcinomas with either a Clara cell and/or type II epithelial cell component (Clara/type II) or a mixed Clara/type II and bronchial surface epithelial cell component (BSE) (mCB), in addition to other components; group B, adenocarcinomas with components including either BSE, a goblet cell component (GOB) or a mixed BSE and GOB component (mBG), and lacking Clara/type II or mCB; group C, adenocarcinomas with only a poorly differentiated component (POR). In group A all Clara/type II, mCB, BSE and the majority of POR were TTF-1 positive. In group B the majority of BSE, POR and all GOB were TTF-1 negative. BSE and POR in both groups had a different phenotype, possibly reflecting their different natural history. In group C 80% of cases were TTF-1 positive, suggesting that the majority were derived from group A tumors.

Key words: adenocarcinoma, immunohistochemistry, intratumoral heterogeneity, lung, TTF-1

Thyroid transcription factor (TTF) is a 38 kDa DNA-binding protein containing a homeodomain. It was originally identified in thyroid follicular cells as a regulator of thyroid-specific genes, such as those responsible for the production of thyroglobulin,[1,2] thyroperoxidase,[2,3] and thyrotropin receptor.[4] In addition to thyroid epithelial cells, TTF-1 was subsequently found in epithelial cells of the respiratory tract, and in areas of the developing brain.[5,6] In adult lung, TTF-1 is expressed in type II pneumocytes, non-ciliated bronchiolar epithelial cells (Clara cells) and bronchiolar basal cells,[7] and is responsible for transcriptional activation of surfactant proteins A, B, and C, as well as Clara cell secretory proteins by direct binding to the promoters of these molecules.[8–11]

Neoplasms of pulmonary origin retain expression of TTF-1, although immunoreactivity varies according to histological tumor type. It has been reported that TTF-1 is expressed in 75–80% of adenocarcinomas[12–23] and >90% of small cell carcinomas,[24–26] whereas it is found at very low frequency in squamous cell carcinomas[14–16,20,21] and large cell carcinomas.[13,14,16] Previous reports have indicated that its expression does not seem to be closely related to the histological pattern of adenocarcinoma growth, because it has been found in tumors with acinar, papillary, and bronchioloalveolar morphology.[13,14,16,17,19] However, there is growing evidence that adenocarcinomas with a predominantly mucinous component tend to be TTF-1 negative.[13,27–29] Particularly, mucinous-type bronchioloalveolar carcinomas, in contrast to non-mucinous bronchioloalveolar carcinomas, tend to be uniformly negative.[13,27,28] Yatabe *et al.* reported that adenocarcinomas with terminal respiratory unit (TRU) morphology, resembling type II pneumocytes, Clara cells, and/or bronchioles, were frequently (88%) TTF-1 positive, whereas only 25% of adenocarcinomas without TRU morphology expressed TTF-1.[22] In contrast, it is noteworthy that there is significant morphological variation in lung adenocarcinoma, not only among tumors, but also within a single tumor. Thus, the relationship between cytological characteristics or histological patterns and TTF-1 expression in adenocarcinoma should be studied in more detail.

In the World Health Organization (WHO) classification, adenocarcinomas are subdivided into bronchioloalveolar carcinoma (non-mucinous type and mucinous type), papillary adenocarcinoma, acinar adenocarcinoma and solid

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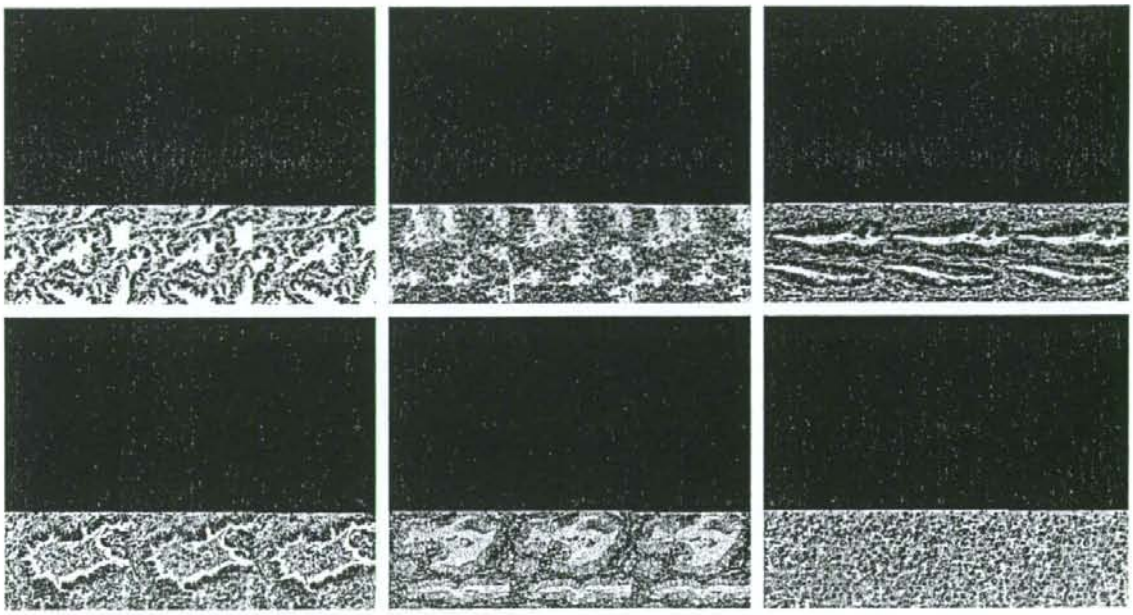

Figure 1 (a) Clara; (b) Type II; (c) bronchial surface epithelial component (BSE); (d) mixed Clara/type II and BSE component (mCB); (e) goblet cell component (GOB); (f) poorly differentiated component (POR) (HE).

adenocarcinoma with mucin, according to structural pattern.[30] For cytological types, Shimosato *et al.* followed the original proposal by Kimula[31] and subclassified adenocarcinoma into six cytological types:[32] Clara cell type, type II alveolar epithelial cell type, bronchial surface cell type with little or no mucus production, goblet cell type, bronchial gland cell type, and mixed cell type or intermediate cell type.

In the present study we subdivided adenocarcinomas into the following cytological components by modifying the cytological classification of Shimosato: Clara cell and/or type II alveolar epithelial component, bronchial surface epithelial component, goblet cell component, poorly differentiated component, and mixed types. We examined in more detail the relationship between various cytological subtypes of adenocarcinomas and TTF-1 expression, with special reference to intratumoral heterogeneity, in order to better understand the carcinogenesis and natural history of pulmonary adenocarcinoma.

MATERIALS AND METHODS

Patients and histological evaluation

A total of 779 patients with primary lung adenocarcinomas, who underwent lobectomy between 1998 and 2002 at the National Cancer Center Hospital, Tokyo, Japan, were analyzed. Resected lung tissues were fixed with 10% formalin for 1 or 2 days, embedded in paraffin and sliced into 4 μm-thick sections. Histological findings in each case were reviewed independently by at least two pulmonary pathologists (AMM, KT, YM).

Tumor cell morphology

Adding some modification to Kimula's and Shimosato's descriptions, cytological components in each adenocarcinoma were subdivided according to the morphological features of routinely prepared HE-stained paraffin sections as follows: Clara cell component and/or type II alveolar epithelial component (Clara/type II), bronchial surface epithelial component (BSE), goblet cell component (GOB), poorly differentiated component (POR) and mixed types. The Clara cell component was defined by the presence of hobnail-shaped or cuboidal cells with eosinophilic cytoplasm, or columnar cells with an apical snout (Fig. 1a). The type II alveolar epithelial component was defined by cuboidal or low-columnar cells with clear or vacuolated cytoplasm (Fig. 1b). Because these two components often appeared admixed, and could not be distinguished from each other, a single category of Clara/type II was established. BSE was

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Table 1 Definition of three groups of lung adenocarcinomas

Group A: Adenocarcinomas with either Clara/type II or mCB, in addition to other components
Group B: Adenocarcinomas with components including either BSE, GOB or mBG, and lacking Clara/type II or mCB
Group C: Adenocarcinomas with only POR

BSE, bronchial surface epithelial component; GOB, goblet cell component; mCB, mixed Clara/type II and BSE component; mBG, mixed BSE and GOB component; POR, poorly differentiated component.

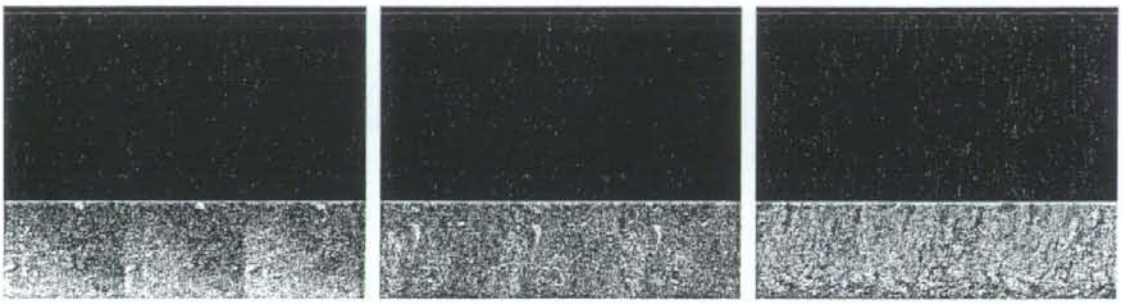

Figure 2 (**a**) Clara (bottom), bronchial surface epithelial component (BSE) (left) and poorly differentiated component (POR) (right) in group A tumor. (**b**) BSE (left), GOB (middle) and POR (right) in group B tumor. (**c**) Group C tumor (HE).

defined by tall columnar cells with eosinophilic cytoplasm and a smooth luminal surface (Fig. 1c). When a tumor contained areas showing an intimate admixture of Clara/type II- and BSE-type cells, and also admixed with columnar cells indeterminate for Clara/type II- or BSE-type cells, it was judged as having a mixed Clara/type II and BSE component (mCB, Fig. 1d). GOB was defined by tall columnar cells with plump mucinous cytoplasm, a smooth surface and a basally displaced nucleus (Fig. 1e). A component showing a close admixture of BSE- and GOB-type cells, and/or showing cells indistinguishable from either BSE- or GOB-type cells, was described as a mixed BSE and GOB component (mBG). A cytological component composed of poorly differentiated polygonal cells with mucin was defined as POR (Fig. 1f). This component usually had a solid or scirrhous growth pattern.

In each tumor the presence of areas containing each component was described regardless of predominance. Although many adenocarcinomas contained an extensive variety of these components, adenocarcinomas were further categorized into three groups according to their similarities or shared components: group A, adenocarcinomas showing various proportions of areas of either Clara/type II or mCB, in addition to other components (representing tumors with Clara/type II morphology); group B, adenocarcinomas with components including either BSE, GOB or mBG, and lacking Clara/type II or mCB (representing differentiated tumors without Clara/type II morphology); group C, adenocarcinomas with only POR (Table 1, Fig. 2).

Immunohistochemistry

Representative tissue sections from each case were used for immunohistochemistry. TTF-1 antigen was retrieved by autoclave treatment (121°C, 10 min) with pH 6.0 citrate buffer. The standard avidin–biotin–peroxidase complex method was used for immunohistochemical detection after incubation with a monoclonal antibody against TTF-1 (8G7G3/1, NeoMarkers, Fremont, CA, USA). Immunostaining was performed within 1 week of preparation of the tissue slides. Positive nuclear staining of non-neoplastic type II pneumocytes served as an internal control for antigen preservation. The extent of tumor cells with positive nuclear staining was graded using five categories: 0, 0% (negative); 1+, 1–10%; 2+, 11–50%; 3+, 51–90%; 4+, 91–100%.

RESULTS

According to the categorization, the 779 cases analyzed comprised 689 group A adenocarcinomas, 80 group B adenocarcinomas and 10 group C adenocarcinomas. Among them, a sequential series of 147 cases consisting of 104 group A, 33 group B and 10 group C were used for further study. According to the WHO tumor classification,[30] 101 of the tumors were diagnosed as adenocarcinomas of mixed subtypes, 31 as bronchioloalveolar carcinomas, 10 as solid adenocarcinomas with mucin, and five as acinar adenocar-

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Table 2 Extent of immunohistochemical TTF-1 expression in adenocarcinomas of the lung

Positive degree	0 (0%: negative)	1+ (1–10%)	2+ (11–50%)	3+ (51–90%)	4+ (91–100%)
Group A, 104 tumors	0	1 (1%)	10 (10%)	29 (27%)	65 (62%)
Components					
Clara/type II ($n = 87$)	0	1	5	4	77
mCB ($n = 39$)	0	1	7	9	22
BSE ($n = 5$)	0	1	2	1	1
GOB ($n = 1$)	1	0	0	0	0
POR ($n = 44$)	3	6	9	14	12
Group B, 33 tumors	26 (79%)	4 (12%)	2 (6%)	0	1 (3%)
Components					
BSE ($n = 15$)	9	3	2	0	1
mBG ($n = 10$)	9	1	0	0	0
GOB ($n = 8$)	8	0	0	0	0
POR ($n = 15$)	11	2	1	0	1
Group C, 10 tumors	2 (20%)	1 (10%)	2 (20%)	3 (30%)	2 (20%)
POR ($n = 10$)	2	1	2	3	2

BSE, bronchial surface epithelial component; GOB, goblet cell component; mCB, mixed Clara/type II and BSE component; mBG, mixed BSE and GOB; POR, poorly differentiated component; TTF-1, thyroid transcription factor-1.

cinomas. The patients consisted of 80 men and 67 women, ranging in age from 26 to 81 years with a mean age of 62 years. Maximum tumor diameter ranged from 0.7 cm to 13.0 cm with a mean diameter of 3.1 cm.

In group A 104 tumors (100%) contained areas with Clara/type II or mCB as a component by definition. Among them, 87 (84%) had Clara/type II, 39 (38%) had mCB. As for other components identified in tumors in this group, five (4%) had BSE, one (1%) had GOB and 44 (42%) had POR. In group B, 15 (45%) had BSE, 10 (30%) had mBG, eight (24%) had GOB and 15 (45%) had POR, as cytological components. Group C consisted of only POR by definition.

The extent of TTF-1 immunohistochemical staining of tumor cell nuclei is summarized in Table 2, and examples of staining patterns are shown in Fig. 3. Immunostaining extent and intensity were usually parallel. Tumors with 3+ or 4+ TTF-1 staining mostly had a strong staining pattern. A total of 81% (119/147 tumors) of all adenocarcinomas were positive for TTF-1 in at least a few percent of the tumor cells.

In group A all the tumors were TTF-1 positive. Specifically, all of the Clara/type II, mCB and BSE components were TTF-1 positive, showing strong immunoreactivity in most cases. A proportion of group A tumors with marked nuclear atypia showed a low extent and weak intensity of TTF-1 expression. GOB, found in only one case in group A, was TTF-1 negative; 93% (41/44 lesions) of POR were TTF-1 positive. Thus, most of the group A tumors had TTF-1 expression even in BSE or POR.

In group B 21% (7/33) of tumors were TTF-1 positive. Specifically, 40% (6/15 lesions) of BSE, 10% (1/10 lesions) of mBG, 0% (0/8 lesions) of GOB and 27% (4/15 lesions) of POR were TTF-1 positive. In group C 80% (8/10) of tumors were TTF-1 positive.

Table 3 highlights the difference of TTF-1 positivity among the same morphological components BSE and POR found in different groups. Although they are categorized as an identical cytomorphological component, TTF-1 expression was more frequent in components found in group A tumors.

DISCUSSION

We examined the immunohistochemical expression of TTF-1 in various cytological subtypes of primary lung adenocarcinoma, with special reference to intratumoral heterogeneity, in order to better understand the natural history of lung adenocarcinoma.

In group A all Clara/type II and mCB were TTF-1 positive. Moreover, TTF-1 expression was maintained in all BSE and the majority of POR. These findings suggest two important facts. First, in lung adenocarcinomas with a hobnail or cuboidal morphology, TTF-1 will always be positive if a lung adenocarcinoma is primary. If TTF-1 is negative, then metastatic adenocarcinoma should be considered by priority even if the morphology is similar. Second, the natural history of group A tumors, suggested on the basis of morphology, is that they are derived from peripheral alveolar epithelium, and acquire heterogeneity and undergo dedifferentiation during progression according to cytological and structural morphology, which is consistent with retention of TTF-1 expression through Clara/type II, mCB, BSE and POR. The latter hypothesis is compatible with the description of Yatabe *et al.*,[22] that is, adenocarcinomas with TRU morphology, resembling type II pneumocytes, Clara cells, and/or bronchioles, were frequently (88%) TTF-1 positive, and TTF-1-positive adenocarcinomas frequently maintained TTF-1 immunoreactivity at

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