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

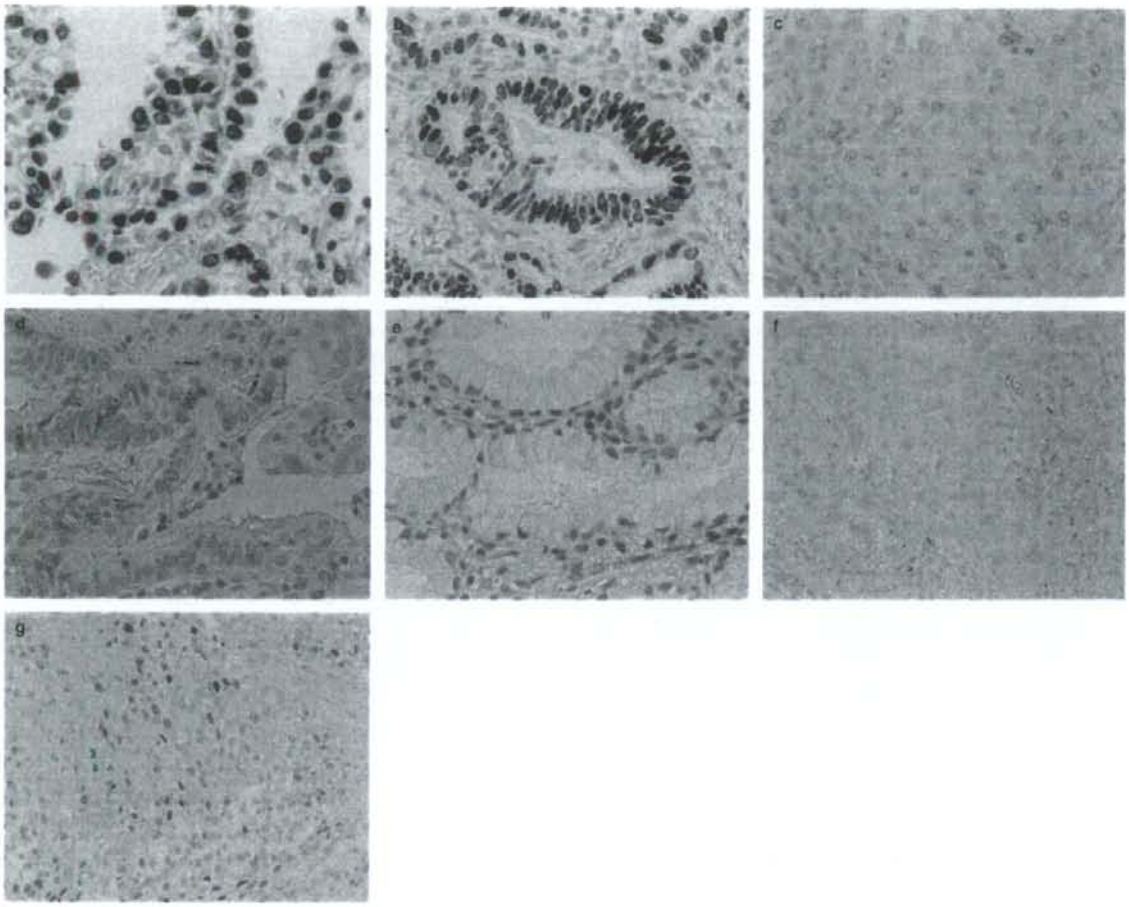

Figure 3 Immunohistochemical thyroid transcription factor-1 expression. Strongly positive in (**a**) Clara and (**b**) bronchial surface epithelial component (BSE), and weakly positive in (**c**) poorly differentiated component (POR) in group A tumor. Weakly positive in (**d**) BSE, and negative in (**e**) GOB and (**f**) POR in group B tumor. (**g**) Positive in group C tumor. (HE).

Table 3 Immunohistochemical TTF-1 expression in adenocarcinomas

Positive degree	0 (0%: negative)	1+ (1–10%)	2+ (11–50%)	3+ (51–90%)	4+ (91–100%)
BSE (no. cases)					
Group A ($n = 5$)	0	1	2	1	1
Group B ($n = 15$)	9	3	2	0	1
POR					
Group A ($n = 44$)	3	6	9	14	12
Group B ($n = 15$)	11	2	1	0	1
Group C ($n = 10$)	2	1	2	3	2

BSE, bronchial surface epithelial component; POR, poorly differentiated component; TTF-1, thyroid transcription factor-1.

metastatic sites even if they had dedifferentiated to poorly differentiated adenocarcinoma.

BSE in group B (lacking Clara/type II or mCB) were frequently TTF-1 negative, in contrast to constant TTF-1 expression of BSE in group A. Although it is impossible to distinguish a BSE component of these two groups solely on the basis of cytological and structural morphology, the natural histories through which they acquire their morphologies may

be different. Group B tumors are suspected to be derived from bronchial epithelium or bronchial metaplastic epithelium, and not from peripheral alveolar epithelium such as group A tumors. Although the possibility remains that group B tumors are derived from peripheral alveolar epithelium and lose their TTF-1 expression completely during progression, this seems unlikely when considering the difference in cytological constituents between these two groups, as well as the conserved expression of TTF-1 in group A. A similar hypothesis can be suggested for POR. Although POR in group A and POR in group B show similar morphology, they are suggested to have different characters (history and biological nature), which reflect the presumed natural history of each group.

All GOB lesions were TTF-1 negative, both in group A and in group B. Although most goblet cell adenocarcinomas are of the pure form, small numbers of mixed goblet cell and non-goblet cell adenocarcinoma also exist.[27,33] Lau *et al.* reviewed seven cases of mixed non-mucinous and mucinous BAC, and reported that 6/7 of the mucinous component (almost identical to BAC comprising only the goblet component) were TTF-1 negative, whereas 5/7 of the non-mucinous component were TTF-1 positive.[27] Also in the present study, the non-goblet cell component in mixed goblet cell and non-goblet cell adenocarcinomas in both groups A and B expressed TTF-1 in some cases. On the basis of previous reports and the present data, we hypothesize that TTF-1 expression is completely lost when adenocarcinomas differentiate to a goblet cell morphology in mixed goblet cell and non-goblet cell adenocarcinoma. However, in pure goblet cell adenocarcinoma it is difficult to explain the lack of TTF-1 expression, and therefore the mechanism of controlling TTF-1 expression should be clarified from the viewpoint of the physiological function of TTF-1 and mucin production.

Eight of 10 group C tumors were TTF-1 positive and some of them expressed TTF-1 strongly. Although their number was small, it is suggested that the majority of group C tumors were derived from group A tumors.

In summary, group A and group B tumors are suggested to have different natural histories, from the viewpoint of immunohistochemical expression of TTF-1. Group A tumors might be derived from peripheral alveolar epithelial cells and retain their TTF-1 expression even if they differentiate to BSE or dedifferentiate to POR. Conversely, the majority of BSE and POR in group B tumors were TTF-1 negative. Although BSE and POR in both groups had a similar morphology, their TTF-1 expression patterns were different, thus reflecting their different natural histories. From the viewpoint of TTF-1 expression, the majority of group C tumors are suggested to be derived from group A tumors.

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Minimally Invasive Open Surgery Approach for the Surgical Resection of Thoracic Malignancies

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For resection of intrapulmonary malignancies, lobectomy is still respected as the mainstay, the most appropriate surgical mode. Especially for lung cancer, the gold standard for surgical resection has been lobectomy with lymph node sampling or dissection ever since the landmark study by the Lung Cancer Study Group of North America [1]. This has been the only randomized phase III trial to compare lobectomy and sublobar resection for lung cancer. All other studies have been based on retrospective case series.

Because of the increasing frequency of early-stage lung cancer, in the past decades the attempted surgical resection is becoming increasingly common in a minimally invasive setting. In 1992, Lewis and colleagues [2] described the first video-assisted thoracic surgery (VATS) lobectomy for 40 patients. Although their technique, which involved a simultaneous hilar stapling technique, has not been commonly used thereafter, it was actually a landmark in thoracic surgery. Many studies have been reported subsequently, and new studies emerged that have evaluated the use of VATS procedure and clinical outcomes. Large case series with more than 1,000 cases have been published [3], and the indications, advantages, disadvantages, morbidity or mortality, and socioeconomic aspects are becoming increasingly clear.

The definition of VATS lobectomy is somewhat ambiguous. This technique varies with regard to the number of incisions (2 to 5), length of utility incisions (4 to 10 cm), degree of rib spreading, if any, and individual hilar ligation versus tourniquet lobectomy. Thoracic surgeons might use their own combinations of these parameters in their technique. Thus, there might be considerably wide variation in VATS lobectomies performed today. In contrast to the enthusiasm with which VATS lobectomy is promoted, there are still many "conventional" lobectomies with open thoracotomy, in which the type of thoracotomy might vary among standard posterolateral thoracotomy, complete muscle-sparing thoracotomy, thoracotomy sparing only the serratus muscle, anterolateral thoracotomy, and sternotomy.

This article describes minimally invasive open surgery (MIOS). With this approach, which lies between conventional and VATS lobectomy, the surgery remains minimally invasive, but the weak points of VATS lobectomy are compensated for.

Present status of video-assisted thoracic surgery lobectomy

Since the early 1990s, there have been many reports on VATS lobectomy. These have included a few small randomized trials, case-control series, and case series, and have focused on the feasibility and advantage of the VATS procedure over the conventional open procedure. Flores and Alam [4] reviewed the literature from 1996 in a systematic manner, and summarized the results. They write that few randomized trials have compared VATS lobectomy and open lobectomy, whereas there have been numerous case-control studies and case series. Most of the procedures in the literature involved the use of mini-thoracotomy (utility thoracotomy) and different numbers of ports, with or without rib spreading. Many of the reports mentioned that stage I peripheral

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lung cancer was an indication for VATS lobectomy. There have been many comparisons in case-control series between VATS lobectomy and open lobectomy with regard to postoperative pain, changes in pulmonary function tests, nocturnal hypoxemia, and various markers of inflammation. While they generally favored VATS approaches, the inadequacy of the control group was noted. As a conclusion of this systematic review, the investigators stated that VATS lobectomy can be safely performed and is an adequate operative procedure for early-stage non-small cell lung cancer. However, they also addressed the need for adequately powered well-balanced studies.

The report from the Cancer and Leukemia Group B is one of the few prospective, multi-institutional studies on VATS lobectomy for early non-small cell lung cancer [5]. In this study, VATS lobectomy was technically defined as a procedure that involved one 4- to 8-cm access thoracotomy and two 0.5 cm port incisions without rib spreading. They mandated videoscopic guidance and a traditional hilar dissection. VATS lobectomy was successfully performed on 86.5% of 127 patients. The operative indicators were a median operative time of 130 minutes, a median chest tube duration of three days, and a perioperative mortality rate of 2.7%. Data regarding the hospital stay were not given. The investigators concluded that a standardized approach to VATS lobectomy as specifically defined with the avoidance of rib spreading is feasible. The data and the definition of VATS lobectomy should be respected as the reference for this approach.

Overall, VATS lobectomy is respected as a technically feasible alternative to conventional lobectomy by way of open thoracotomy with an acceptable range of morbidity or mortality. However, as a precaution, the following points should be carefully noted.

1. Indicators, such as operative time and bleeding volume, are usually taken from successfully accomplished surgeries. In contrast, the data for open thoracotomy have always included those indicators with conversion from VATS or those that are technically difficult because of inflammatory adhesion and fusion. Therefore, the comparison of VATS and open cases is sometimes unfair even in case-control series.
2. Although the definition of VATS lobectomy is being standardized, there are still considerable technical variations. Some of the techniques categorized as VATS lobectomy might be close to a small-thoracotomy lobectomy.
3. In many studies, rib spreading is considered to be an important factor which characterizes the minimally invasive nature of VATS lobectomy, and it has been reported that "invasive" VATS lobectomy can limit favorable effects on perioperative pain and recovery [6,7]. However, the degree to which rib spreading affects postoperative recovery has not been determined. In particular, the advantage of rib spreading for instrumental freedom has never been demonstrated.

The overall balance between downsized and standard surgical approaches is important.

The minimally invasive open surgery technique

The concept of the MIOS approach can be summarized as light-assisted lobectomy with direct vision through a small thoracotomy. The ribs are usually spread to enable direct access and an adequate operative field. The goal of this approach is to ensure direct manipulation for the whole procedure and consequently less time for the procedure.

An incision of 8 to 12 cm in length, depending on the size of the body, is made near the auscultatory triangle just 1 to 2 cm below the scapula (posterolateral thoracotomy, Fig. 1). Because of the need for complete hilar or mediastinal lymph node dissection, the posterolateral incisional location is usually preferred, since it is more likely to provide easier access to the subcarinal area than an anterolateral incisional location.

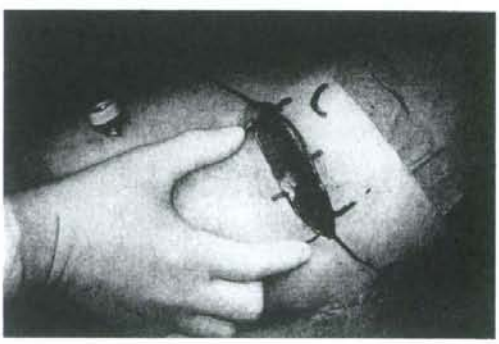

Fig. 1. Skin incision in the MIOS approach. An 8- to 12-cm incision is made approximately on the auscultatory triangle, 1 to 2 cm below the lower tip of the scapula.

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).

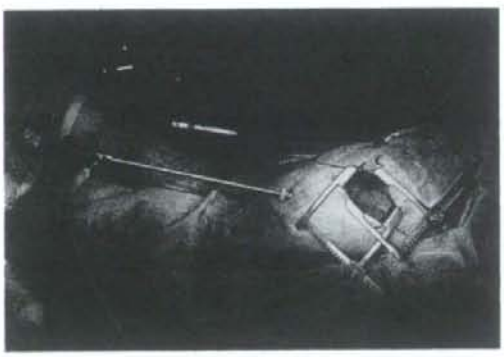

Fig. 2. A port for the thoracoscope and application of two spreaders. Thoracoscopic assistance is maintained throughout the procedure as a supplement to the small operative field and as a light source. Two spreaders maximize the available operative field. The intercostal space is widened to 3 to 5 cm,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

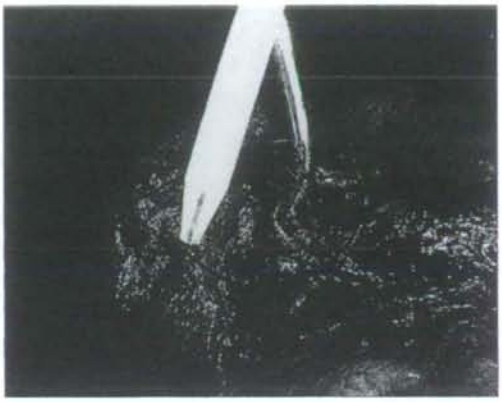

Fig. 3. Looped-knot devices can replace troublesome ligation for the small branches of pulmonary vessels in the thorax. In the MIOS approach, ligation is instrumental for most locations except for just below the incision.

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

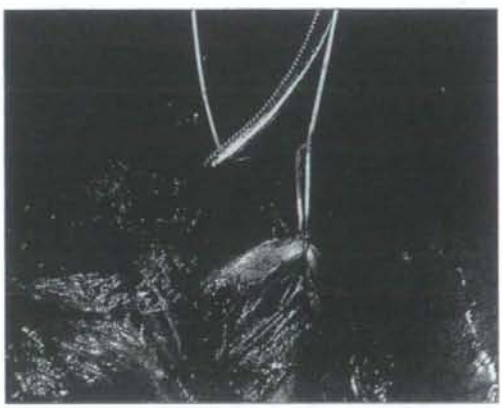

Fig. 4. Instrumental knotting for small vessels. Smaller Kelly-type forceps can be easily used.

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,

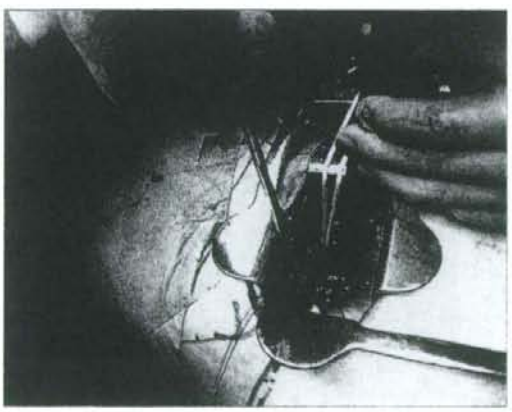

Fig. 5. Subcarinal node dissection in the MIOS approach. With a lever retractor and sponge stick, the subcarinal space is well exposed.

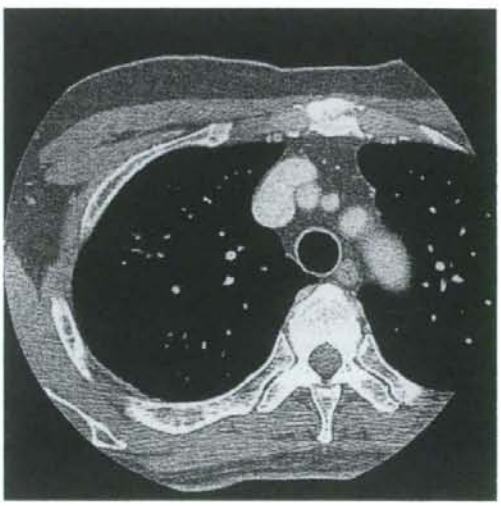

Fig. 6. Typical presentation of a ground glass opacity lesion in the right upper lobe. This needs to be diagnosed on high-resolution (thin-slice) CT with a scanning thickness of less than 1 to 2 mm.

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.

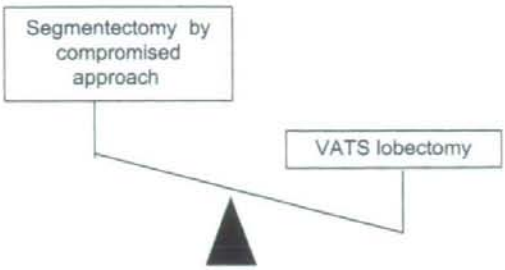

Fig. 7. Overall comparison of the different combinations in minimally invasive settings.

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Histological Evaluation of the Effect of Smoking on Peripheral Small Adenocarcinomas of the Lung

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Introduction: As there is little information on the histologic characteristics of adenocarcinoma in smokers, we histologically examined the effect of smoking on the carcinogenesis and progression of peripheral small lung adenocarcinomas.

Methods: Two hundred thirty-six consecutive patients with peripheral adenocarcinoma of the lung 30 mm or less in diameter were studied. Prognosis, histology, and location of the adenocarcinoma were compared among patients with a Brinkman index (B.I.) of 0, 1 to 500, and more than 500.

Results: The ratio of smokers to nonsmokers was 1.4:1. The rate of carcinogenesis in the upper region of the lung (S1-3) was 1.4 times as high that in the lower region (S4-10) in smokers, but almost equal in the two regions in nonsmokers. Outcome tended to be worse in patients with a B.I. of more than 500 than in those with a B.I. of less than or equal to 500 for adenocarcinomas 30 mm or less in diameter (p 0.0855), and was significantly worse for adenocarcinomas 20 mm or less in diameter (p 0.0359). Patients with a high B.I. tended to have invasive adenocarcinoma (IAC) without a bronchioloalveolar carcinoma (BAC) component (IAC BAC) or IAC with a BAC component (IAC BAC) rather than noninvasive adenocarcinoma. For adenocarcinomas as a whole, B.I. was correlated with several pathologic prognostic factors, including pathologic stage, lymphatic permeation, vascular invasion, presence of a solid component, necrosis, and modified scar grade, particularly in the upper region. Specifically, in IAC BAC, B.I. was correlated with modified scar grade and the presence of a solid component. In IAC BAC, B.I. was correlated with the presence of a solid component and necrosis.

Conclusions: Small adenocarcinoma in smokers seems to occur frequently in the upper region of the lung, shows invasive features more frequently, and shows greater progression and dedifferentiation than that in nonsmokers. Tobacco-smoking may have an effect on the carcinogenesis and multistep progression of peripheral lung adenocarcinoma 30 mm or less in diameter.

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Among several histologic types of lung carcinoma, a gradual increase in the incidence of peripheral adenocarcinoma has been reported,[1,2] although the effect of smoking on the carcinogenesis and progression of peripheral adenocarcinoma is unclear. The relative risk for lung cancer among smokers over nonsmokers is reported to be 2 to 10 for adenocarcinoma, and more than 10 for squamous cell carcinoma and small cell carcinoma.[3] Squamous cell carcinoma and small cell carcinoma tend to occur in the upper lobe,[4–8] but there have been few reported studies on the location of adenocarcinoma.[8,9]

Several studies have indicated that the prognosis of adenocarcinoma[10–12] or nonsmall nonsquamous cell carcinoma[13] in smokers is worse than that in nonsmokers. Suzuki et al.[14] and Morita and Urano[15] reported that the incidence of moderately to poorly differentiated adenocarcinoma was higher in smokers than in nonsmokers, but no detailed histologic study has yet examined the relationship between smoking status and histologic changes in these cancers.

With regard to the progression of lung adenocarcinoma, Shimosato et al.[16] and Clayton[17] indicated that bronchioloalveolar carcinoma might progress to mixed subtypes with a BAC component (sclerosing bronchioloalveolar carcinoma). On the other hand, papillary, acinar, solid adenocarcinoma, and mixed subtypes without a BAC component were considered to be de novo carcinoma,[18] or a progressed form of mixed subtypes with a BAC component.

In the present study, we pathologically examined the effect of smoking on the carcinogenesis and progression of peripheral small lung adenocarcinoma, with reference to tumor location.

PATIENTS AND METHODS

We reviewed 236 consecutive patients with peripheral lung adenocarcinomas 30 mm or less in diameter, who underwent lobectomy at the National Cancer Center Hospital between 1984 and 1990. Peripheral lung adenocarcinoma was defined as a tumor located in a fourth branching bronchus or more peripheral region. Recently, now that the prevalence of smoking is decreasing, the incidence of adenocarcinoma with a predominantly bronchioloalveolar carcinoma (BAC) com-

ponent has been increasing and that of invasive adenocarcinoma (IAC without a BAC component (IAC BAC) has been decreasing. Therefore, to examine the relationship of smoking to invasiveness of adenocarcinoma, we selected the period when the prevalence of smoking and the incidence of overt IAC were relatively high. Clinical information was extracted from medical records. Tumor, node, metastasis staging was determined in accordance with the Union Internationale Contre le Cancer (International Union Against Cancer) staging system.[19] Tumor location was classified as the upper region (lung segments 1–3) or lower region (segments 4–10). Noninvasive adenocarcinoma (NAC) was defined as pure BAC without a desmoplastic reaction.

Survival rates of patients divided into three Brinkman index (B.I.) groups (B.I. 0, 1–500, and 500) were compared, and the distribution of NAC, IAC with a BAC component (IAC BAC) and IAC BAC in each B.I. group was analyzed. Correlations between B.I. and several pathologic prognostic factors (pathologic (p) stage, pT, pN, pleural invasion, lymphatic permeation (ly), vascular invasion (v), presence of papillary, acinar or solid component, necrosis, and modified scar grade (MSG)[20]) were examined: MSG is one of the prognostic factors for small lung adenocarcinoma, evaluated according to the structure and size of the central scar of adenocarcinoma, being a modification of the scar grade proposed by Shimosato et al.[16] When the p value was less than 0.10, the same examination was performed for tumors in the upper region and lower region, respectively.

Survival rates of each B.I. group were compared for adenocarcinoma with a BAC component (NAC and IAC BAC), IAC BAC, and IAC BAC, respectively. In IAC BAC and IAC BAC, prognostic factors that were correlated with B.I. were extracted.

The patients were followed up extensively, and the follow-up period ranged from 1 to 204 months, with a median of 84 months. Patients who died of causes other than lung adenocarcinoma were censored at the last follow-up. The 5- and 10-year survival rates were calculated by the Kaplan-Meier method. Correlations of B.I. with several pathologic factors were examined by the Mann-Whitney U test or Spearman coefficient test. When the p value was less than 0.05, differences were considered significant, and when between 0.05 and 0.10, we considered that there was a tendency for a difference.

TABLE 1. Relationship Between B.I. and Presence of a BAC Component and Invasion

Smoking Habit	B.I. 0 (n 97)	B.I. 1–500 (n 30)	B.I. > 500 (n 109)	Spearman Coefficient (p)
Histology				
NAC (n 28)	17 (61%)	4 (14%)	7 (25%)	0.0001
IAC BAC (n 139)	63 (46%)	20 (14%)	56 (40%)	
IAC BAC (n 69)	17 (25%)	6 (9%)	46 (66%)	

B.I., Brinkman index; BAC, bronchioloalveolar carcinoma; NAC, noninvasive adenocarcinoma; IAC BAC, invasive adenocarcinoma with a BAC component; IAC BAC, invasive adenocarcinoma without a BAC component.

RESULTS

The patients comprised 144 men and 92 women, ranging in age from 26 to 84 years with a median of 60 years. Among the 236 patients, 142 were classified as p stage IA, 8 as stage IB, 13 as stage IIA, 7 as stage IIB, 39 as stage IIIA, 20 as stage IIIB, and 7 as stage IV. Tumors were smaller than 20 mm in 112 cases and 20 to 30 mm in 124 cases. Lymph node metastasis was present in 77 cases (33%), ly in 117 cases (50%), v in 120 cases (51%), and pleural involvement (p2–3) in 49 cases (23%). Pleural involvement was defined as p0: no invasion of the visceral pleura, p1: invasion beyond the elastic framework of the visceral pleura, p2: exposure to the thoracic cavity, and p3: invasion to the parietal pleura, mediastinum, or diaphragm.

The ratio of smokers to nonsmokers was 1.4:1 (139:97 patients). Correlations between B.I. and presence of a BAC component and invasion are shown in Table 1. As the B.I. increased, NAC decreased and IAC BAC and IAC BAC increased (Table 1). The BAC component was

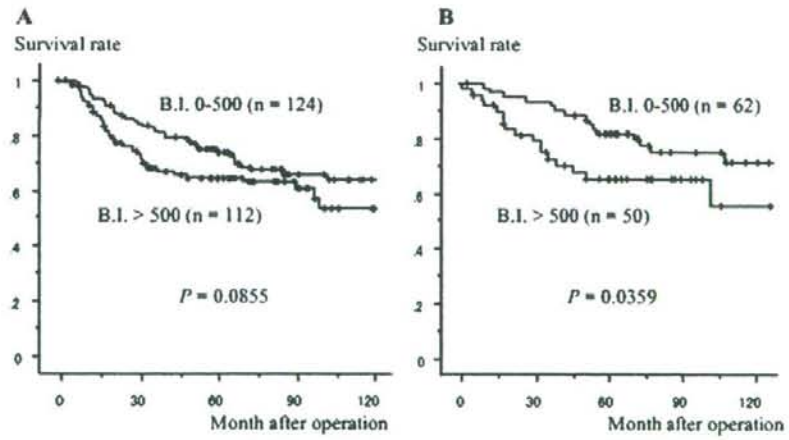

FIGURE 1. Survival curves for each B.I. group in patients with lung adenocarcinoma. *A*, Tumors 30 mm or less in diameter. *B*, Tumors 20 mm or less in diameter.

the nonmucinous type (Clara/type II) in most cases (165/167) and mucinous type in two. The ratio of the number of tumors in the upper region to that in the lower region was 1.2:1 (129:107 tumors). A higher rate of carcinogenesis in the upper region to lower region was detected in smokers (1.4 times, 81:58 tumors), but almost equal in the two regions in nonsmokers (48:49 tumors).

The group with B.I. of more than 500 tended to have a worse outcome than the group with B.I. of less than or equal to 500 for adenocarcinomas 30 mm or less in diameter (p 0.0855, Figure 1 *A*), and had a significantly worse outcome for adenocarcinomas 20 mm or less in diameter (p 0.0359, Figure 1 *B*). The respective 5- and 10-year survival rates were 77.6 and 66.0% in the group with B.I. of 500 or less, and 66.3 and 59.6% in the group with B.I. of more than 500 for adenocarcinomas 30 mm or less in diameter. The respective 5- and 10-year survival rates were 81.5 and 73.6% in the group with B.I. of 500 or less, and 63.0 and 59.8% in the group with B.I. of more than 500 for adenocarcinomas 20 mm or less in diameter. Outcomes did not differ between men and women for adenocarcinomas 30 mm or less in diameter and 20 mm or less in diameter.

For adenocarcinoma as a whole, the correlations of B.I. with several pathologic prognostic factors we have reported

TABLE 2. Relationship Between B.I. and Clinicopathologic Prognostic Factors in Adenocarcinoma 30 mm or Less in Diameter

	No. of cases B.I. (0/1–500/>500)	5-yr Survival (%)	Mann-Whitney *U* Test *p**	*p*† for Upper (U) and Lower (L) Region
Total	97/30/109	72.7		
p Stage				
Stage I	67/19/64	91.8	0.0761	U: ns
Stage II–IV	30/11/45	35.5		L: ns
pT				
T1	76/22/89	83.6	ns	
T2–4	21/8/20	33.3		
pN				
N0	71/19/69	79.6	ns	
N1–3	26/11/40	25.8		
Pleural invasion				
p 0–1	76/22/89	74.6	ns	
p 2–3	21/8/20	21.6		
Lymphatic permeation				
Negative	56/14/49	82.9	0.0486	U: 0.0848
Positive	34/17/66	41.1		L: ns
Vascular invasion				
Negative	53/13/50	75.6	0.0376	U: 0.0858
Positive	39/19/62	48.3		L: ns
Papillary component				
Absent	21/8/32	84.0	ns	
Present	76/22/77	68.7		
Acinar component				
Absent	49/15/48	79.5	ns	
Present	48/15/61	65.5		
Solid component				
Absent	73/18/52	78.5	0.0001	U: 0.0002
Present	24/12/57	62.7		L: 0.0692
Necrosis				
Absent	76/22/73	76.2	0.0544	U: ns
Present	21/8/36	61.9		L: ns
Modified scar grade				
Grade 1	17/4/8	100		
Grade 2	31/8/21	91.9	0.0002	U: 0.0001
Grade 3	28/10/39	65.4		L: ns
Grade 4	21/8/41	51.9		

*Mann-Whitney *U* test *p* value for relationship between B.I. and pathologic factors.
†Mann-Whitney *U* test *p* value for upper (U) region and lower (L) region.
ns: not significant or no tendency (p 0.1).
B.I.: Brinkman index.

previously[20] are shown in Table 2. In our previous study, a papillary, acinar or solid component, necrosis, and MSG were prognostic factors, and some conventional prognostic factors including p stage, pT, pN, pleural invasion, ly, and v. In the present study, correlations of B.I. with some of these factors including p stage, ly, v, presence of a solid component, necrosis, and MSG were detected. The correlations with ly, v, presence of a solid component, and MSG were strong for tumors in the upper region.

As we considered that carcinogenesis and progression might differ in each B.I. group, a further study was performed. Adenocarcinoma with a BAC component (NAC and IAC BAC) was slightly dominant in males and smokers; the male:female ratio was 1.7:1 (89:77 cases), and the numbers of tumors in the B.I. 0, 1 to 500, and more than 500 groups were 80, 24, and 63, respectively. However, when limited to NAC, the incidence of females and nonsmokers was high; the male:female ratio was 1:1.6 (11:17 cases) and the numbers of tumors in the respective B.I. groups were 17, 4, and 7, respectively. For adenocarcinomas with a BAC component (NAC and IAC BAC) 30 mm or less in diameter, the group with B.I. of more than 500 tended to have a worse outcome than the group with B.I. of less than or equal to 500 (p 0.0729, Figure 2*A*), and this was also the case for adenocarcinomas 20 mm or less in diameter (p 0.0661, Figure 2*B*). When limited to IAC BAC, no significant difference in outcome was detected between the group with B.I. of 500 or less and the group with B.I. of more than 500, but B.I. was correlated with the presence of a solid component and MSG, particularly in the upper region (Table 3).

IAC BAC was dominant in males and heavy smokers; the male:female ratio was 1:2.9 (18:51 cases) and the numbers of tumors in the B.I. 0, 1 to 500, and more than 500 groups were 17, 6, and 46, respectively. Although the survival rate did not differ significantly between the groups with B.I. of less than or equal to 500 and more than 500, B.I. and the presence of a solid component and necrosis were positively correlated, and B.I. and the presence of an acinar component showed a negative correlation, particularly in the upper region.

DISCUSSION

We reviewed 236 cases of peripheral lung adenocarcinoma 30 mm or less in diameter and examined some aspects of their carcinogenesis and progression pathologically. The relative risk for lung cancer among smokers compared with nonsmokers is reported to be 2 to 10 for adenocarcinoma.[3] Our results were similar, as smokers were dominant; nonsmoker:smoker ratio was 1:1.4. It is well known that lung cancer, especially squamous cell carcinoma and small cell carcinoma, occurs frequently in the upper region of the lung among smokers or individuals with asbestos exposure, because transairway carcinogens persist longer in the upper lobe owing to the lower ventilation rate and less efficient lymphatic clearance.[4–9] Our study showed that the carcinogenesis rate was higher in the upper region than in the lower region in smoker, suggesting that tobacco-smoking affected carcinogenesis of small adenocarcinoma in the upper region.

As the B.I. increased, NAC decreased and IAC BAC and IAC BAC increased, suggesting that NAC might progress to IAC BAC, and thereafter to IAC BAC, as a result of smoking.

The group with B.I. of more than 500 tended to have a worse outcome than the group with B.I. of less than or equal to 500. It has been reported that smoking is a poor prognostic factor in patients with lung adenocarcinoma[11] or stage I lung adenocarcinoma.[12] Shiba et al. reported that in patients with nonsmall, nonsquamous cell carcinoma, heavy smokers (30 pack-years) had a significantly worse prognosis than lighter smokers (0–30 pack-years) for overall stage and also stage I.[13] We only examined cases of definite adenocarcinoma, and found that the survival rate tended to be different between the group with B.I. of more than 500 and group with B.I. of 0 to 500 in adenocarcinomas 30 mm or less in diameter (p 0.0855), whereas it was significantly different for adenocarcinomas 20 mm or less in diameter (p 0.0359), suggesting that the degree of smoking affects the prognosis of lung adenocarcinoma. Several reasons why p values differed between adenocarcinomas 30 mm or less in diameter and those 20 mm or less in diameter were speculated that a prognostic

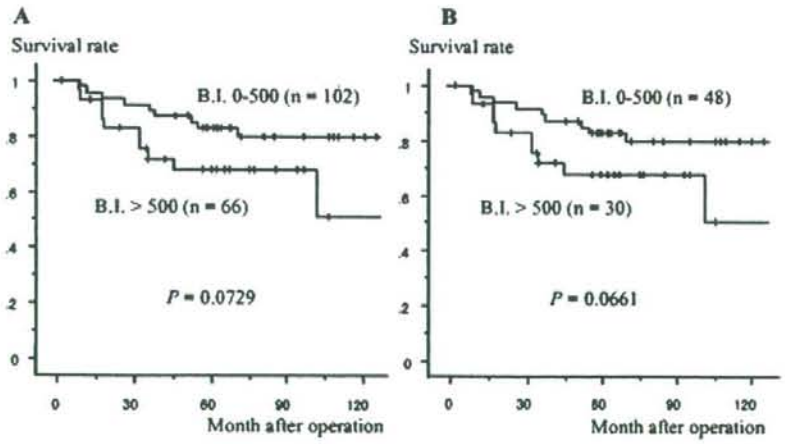

FIGURE 2. Survival curves for each B.I. group in patients with adenocarcinoma with a BAC component. *A*, Tumors 30 mm or less in diameter. *B*, Tumors 20 mm or less in diameter.

TABLE 3. Relationship Between B.I. and Clinicopathologic Prognostic Factors in IAC BAC and IAC BAC

	IAC BAC No. of Case B.I. (0/1–500/>500)	Mann-Whitney *U* Test *p**	*p*† for U and L Region	IAC BAC No. of Case B.I. (0/1–500/>500)	Mann-Whitney *U* Test *p**	*p*† for U and L Region
Total (139)						
p Stage						
Stage I	40/12/32	ns		10/3/2	ns	
Stage II–IV	23/8/24			7/3/21		
pT						
T1	44/14/43	ns		15/4/39	ns	
T2–4	19/6/13			2/2/7		
pN						
N0	43/12/34	ns		11/3/28	ns	
N1–3	20/8/22			6/3/18		
Pleural invasion						
p 0–1	44/14/43	ns		15/4/39	ns	
p 2–3	19/6/13			2/2/7		
Lymphatic permeation						
Negative	34/8/21	ns		10/3/18	ns	
Positive	29/12/35			7/3/28		
Vascular invasion						
Negative	33/7/26	ns		6/1/16	ns	
Positive	30/13/30			11/5/30		
Papillary component						
Absent	8/4/10	ns		4/4/16	ns	
Present	55/16/46			13/2/30		
Acinar component						
Absent	31/11/22	ns		2/0/19	0.0011	U: 0.0032‡
Present	32/9/34			15/6/27		L: ns
Solid component						
Absent	47/12/30	0.0135	U: 0.0144	9/2/15	0.0964	U: ns
Present	16/8/26		L: ns	8/4/31		L: ns
Necrosis						
Absent	44/15/44	ns		15/3/22	0.0217	U: 0.0842
Present	19/5/12			2/3/24		L: ns
Modified scar grade						
Grade 1	—			0/0/1		
Grade 2	29/7/17	0.0518	U: ns	2/1/4	ns	
Grade 3	18/7/21		L: ns	10/3/18		
Grade 4	16/6/18			5/2/23		

*Mann-Whitney *U* test *p* value for relationship between B.I. and pathologic factors.
†Mann-Whitney *U* test *p* value for upper (U) region and lower (L) region.
‡Acinar component present frequently in low B.I. patients, particularly in upper region.
ns, not significant or no tendency (*p* 0.1); B.I., Brinkman index; BAC, bronchioloalveolar carcinoma; NAC, noninvasive adenocarcinoma; IAC BAC, invasive adenocarcinoma with a BAC component; IAC BAC, invasive adenocarcinoma without a BAC component.

difference was only detected in adenocarcinomas smaller than 20 mm, but was not significant for large and more progressed tumors, or that smoking was a mildly influential prognostic factor so that the *p* value did not constantly indicate significance.

We examined the correlations of B.I. with several pathologic prognostic factors for adenocarcinoma as a whole, and found that ly, v, presence of a solid component, necrosis, and MSG were correlated. Because these correlations were strong in the upper region of the lung, adenocarcinoma might progress and dedifferentiate predominantly in the upper region as a result of smoking. In adenocarcinoma with a BAC component, the group with B.I. of more than 500 tended to have a poorer outcome than the group with B.I. of less than or equal to 500, and in IAC BAC, B.I. correlated with MSG and the presence of a solid component, suggesting that the degree of smoking affects tumor progression in adenocarcinoma with a BAC component. In IAC BAC, which is considered to be de novo carcinoma or a progressed form of adenocarcinoma with a BAC component, outcome did not differ between the group with B.I. of 500 or less and the group with B.I. of more than 500. Nevertheless, a detailed examination revealed that an acinar component was predominant in the group with B.I. of 500 or less, whereas a solid

component and necrosis was predominant in the group with B.I. of more than 500. We considered that the mechanism of progression might differ according to the degree of smoking, and that smokers tended to have a dedifferentiated component. Previously, it has been suggested that lung adenocarcinoma in smokers is progressed or has special characteristics on the basis of immunohistochemical or molecular biological examinations, exhibiting a high Ki-67 labeling index,[13] a high frequency of K-*ras*[21–24] or p53 mutation,[23,24] a low frequency of epidermal growth factor receptor (EGFR) mutation,[22] a high incidence of loss of heterozygosity,[25] and a high frequency of p53 mutation with transversion pattern.[26]

In conclusion, small adenocarcinoma in smokers seems to occur frequently in upper region, have more frequent invasive features, and be much more progressed and dedifferentiated than that in nonsmokers, predominantly in the upper region of the lung. Tobacco-smoking may have an effect on the carcinogenesis and multistep progression of peripheral lung adenocarcinoma 30 mm or less in diameter, particularly in the upper region.

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Analysis of Expression Patterns of Breast Cancer–Specific Markers (Mammaglobin and Gross Cystic Disease Fluid Protein 15) in Lung and Pleural Tumors

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• *Context.*—The lung is the most common site of metastasis during the natural history of malignant tumors. Breast carcinoma has a propensity for distant metastasis, and the lung and pleura are among the most common metastatic sites. Although it is often difficult to make a clear-cut differential diagnosis between the two, distinguishing primary lung carcinoma from breast carcinoma metastatic to the lung is important because the treatment modalities are different.

Objective.—To elucidate the utility of mammaglobin and gross cystic disease fluid protein 15 (GCDFP-15), which are known to be breast-specific antigens, in distinguishing various primary lung and pleural tumors from breast carcinoma metastasizing to the lung.

Design.—A total of 20 cases of breast carcinoma metastatic to the lung and 263 tumors of nonbreast origin located in the lung and pleura were analyzed.

Results.—Of the 20 cases of breast carcinoma metastatic to the lung, 10 (50.0%) were immunoreactive for mammaglobin and 9 (45.0%) for GCDFP-15, the frequency of positivity being slightly higher for the former than for the latter. The area immunopositive for mammaglobin showed more diffuse staining than the area immunopositive for GCDFP-15. Furthermore, the specificity of mammaglobin for breast carcinoma metastatic to the lung was superior (98.9%) to that of GCDFP-15 (91.8%).

Conclusion.—The sensitivity of mammaglobin is equal or superior to that of GCDFP-15 for investigation of breast carcinoma. Immunopositivity for mammaglobin is more diffuse than that for GCDFP-15. In terms of practical diagnosis, mammaglobin immunohistochemistry can serve as a differential marker of breast carcinoma and should be added to the immunohistochemical panel.

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The lung is the most common site of metastasis during the natural history of malignant tumors.[1] In particular, breast carcinoma has a propensity for distant metastasis, and the lung and pleura are the second most common metastatic sites following bone.[2] It is also well known that breast carcinoma metastatic to the lung may be found even after a postoperative disease-free interval up to 20 years after resection of the primary lesion. Distinction of primary lung carcinoma from breast carcinoma metastatic to the lung is important under certain clinical situations, such as when a solitary solid nodule is found in the peripheral lung of a patient with a history of resected breast carcinoma, because the treatment modalities for these 2 lesions are different. Furthermore, histologic information regarding hormone receptor status and HER2 status provides a target for therapy in breast carcinoma.

Differentiating between primary lung carcinoma and breast carcinoma metastatic to the lung is often problematic when only a small amount of material is available, as the histologic features may not be sufficient to permit unequivocal distinction. Therefore, reliable immunohistochemical markers are required to facilitate the differentiation of these malignancies.

In breast cancer, the estrogen receptor (ER)/progesterone receptor (PgR) status of the tumor is useful for both prognosis and therapy, with more chemotherapeutic options being available for patients with hormone receptor–positive tumors.[3,4] Breast adenocarcinoma has been shown to be positive for ER in 24% to 63% of cases and positive for PgR in 9% to 37% of cases.[5,6] Breast adenocarcinoma may demonstrate immunophenotypic variability in its expression of ER and PgR, with differences that are dependent on the histologic grade, histologic subtype, antibody clone applied, and immunohistochemical techniques used. These factors limit the sensitivity of these markers for excluding metastatic breast adenocarcinoma in cases of unknown primary site.

When primary unknown metastatic tumor is suspected

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Table 1. Immunoreactivity of Mammaglobin

	No. of Cases Examined	% Positive	Staining Area			
			0	1+	2+	3+
Breast carcinoma, metastasis to lung	20	50	10	4	6	0
Primary lung carcinoma	250	1.2	247	3	0	0
Adenocarcinoma	100	0	100	0	0	0
Squamous cell carcinoma	60	1.6	59	1	0	0
Pleomorphic carcinoma	20	0	20	0	0	0
Carcinoid tumor	19	5.2	18	1	0	0
Large-cell neuroendocrine carcinoma	20	0	20	0	0	0
Small-cell carcinoma	15	0	15	0	0	0
Adenoid cystic carcinoma	11	9.1	10	1	0	0
Mucoepidermoid carcinoma	5	0	5	0	0	0
Malignant mesothelioma	13	0	20	0	0	0

to have originated from the breast, ER, PgR, and gross cystic disease fluid protein 15 (GCDFP-15) have been shown to be useful immunohistochemical markers.[7–9] However, ER and PgR have also been documented in many neoplasms from various organs. In lung tumors, ER and PgR expressions are reported to range from 0% to 96.7% and from 0% to 46.5%, respectively.[10–12] A panel consisting of anticytokeratin 7 and anticytokeratin 20 (CK7/CK20) antibodies is useful for determining the origin of an unknown primary tumor. However, numerous tumors exhibit an identical $CK7^+/CK20^-$ immunophenotype, including nearly all breast carcinomas and adenocarcinomas of the ovary, lung, endometrial, thyroid, and salivary gland.[13]

Additionally, GCDFP-15 has also been documented in many neoplasms from various locations. In lung tumors, GCDFP-15 expression has been reported to range from 0% to 3.3%[14,15]

The mammaglobin gene sequence fragments were first isolated in 1994 by Watson and Fleming.[16] The mammaglobin gene encodes a 10-kDa molecule, which is related to a family of secretory proteins, including rat prostatic steroid-binding protein subunit C3, human Clara cell 10-kDa protein, and rabbit uteroglobin. Mammaglobin is expressed specifically in breast tissue.[16–18] Recently, an anti-mammaglobin antibody that can be applied to formalin-fixed, paraffin-embedded sections has become commercially available.

In the present study, we elucidated the expression of mammaglobin and GCDFP-15 in order to distinguish various primary lung and pleural tumors from breast carcinoma metastatic to the lung.

MATERIALS AND METHODS

Histologic Analysis

Materials for the present study were extracted from the pathology files of the National Cancer Center Hospital (Tokyo, Japan). The specimens comprised 20 cases of breast carcinoma metastatic to the lung and 263 lung and pleural tumors other than metastatic breast carcinomas: 100 adenocarcinomas, 60 squamous cell carcinomas, 20 pleomorphic carcinomas, 20 large-cell neuroendocrine carcinomas, 15 small-cell carcinomas, 19 carcinoids (14 cases typical and 5 cases atypical), 16 salivary gland–type tumors of the bronchus and/or trachea (11 cases of adenoid cystic carcinoma and 5 cases of mucoepidermoid carcinoma), and 13 malignant pleural mesotheliomas.

The 100 cases of adenocarcinoma were divided into 2 subtypes according to the growth pattern: 60 cases showing lepidic growth and 40 cases without lepidic growth. The 60 cases showing lepidic growth were further divided into 20 cases of the nonmucinous type (tumor cells resembling Clara cells or type II pneumocytes) and 40 cases of the mucinous type (tumor cells resembling goblet cells and/or bronchial surface epithelial cells). Furthermore, the 40 cases without lepidic growth were divided into 20 cases of the acinar-cribriform type (tumor showing an acinar and/or cribriform growth pattern with some degree of cytoplasmic mucin) and 20 cases of the solid type (tumor showing solid growth with some degree of cytoplasmic mucin formation, such as intracytoplasmic lumina).

The 60 cases of squamous cell carcinoma were divided into the well-differentiated type (tumor cells showing a stratified pattern and abundant keratinization), moderately differentiated type (cells showing a lower degree of stratification than that of the well-differentiated type), and poorly differentiated type (the tumor composed of more atypical cells that show only focal squamous cell differentiation).

Immunohistochemistry

For immunohistochemical staining of mammaglobin (clone 304-1A5, 1:200; DAKO, Carpinteria, Calif) and GCDFP-15 (clone D6, 1:200; Signet, Dedham, Mass), 5-μm-thick formalin-fixed sections from each paraffin block were routinely deparaffinized. The sections were exposed to 3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity, and then washed in deionized water for 2 to 3 minutes. Then, for heat-induced epitope retrieval, the sections stained for mammaglobin were subjected to a 0.02M concentration of citrate buffer (pH 6.0) in a steamer at 120°C for 20 minutes. The sections were allowed to cool at room temperature for 40 minutes, and after rinsing with deionized water and washing in phosphate-buffered saline for 5 minutes, the slides were incubated with primary antibody for 1 hour at room temperature. Then the slides were washed in phosphate-buffered saline 3 times for 5 minutes each time. Subsequently, the slides were labeled with EnVision+/HRP system (DAKO). Diaminobenzidine was used as the chromogen, and Meyer hematoxylin as the counterstain.

Grading the intensity of immunostaining was performed using a sliding scale of 0 to 3+ according to the percentage of reactive cells (0 = <1%; 1+ = 1%–10%; 2+ = 26%–50%; 3+ = 51%–100%).

RESULTS

The results of the immunostains of mammaglobin and GCDFP-15 on each tumor are listed in Tables 1 and 2, respectively.

Breast Carcinoma Metastatic to the Lung

Of the 20 cases of breast carcinoma metastatic to the lung, mammaglobin (Figure 1, A and B) and GCDFP-15 (Figure 1, C and D) stained 10 cases (50.0%) and 9 cases (45.0%), respectively, with mammaglobin showing a

Table 2. Immunoreactivity of Gross Cystic Disease Fluid Protein 15						
	No. of Cases Examined	% Positive	Staining Area			
			0	1+	2+	3+
Breast carcinoma, metastasis to lung	20	45	11	5	4	0
Primary lung carcinoma	250	8.2	231	19		
Adenocarcinoma	100	15	85	15	0	0
Squamous cell carcinoma	60	0	60	0	0	0
Pleomorphic carcinoma	20	5	19	1	0	0
Carcinoid tumor	19	5.2	18	1	0	0
Large-cell neuroendocrine carcinoma	20	0	20	0	0	0
Small-cell carcinoma	15	0	15	0	0	0
Adenoid cystic carcinoma	11	0	11	0	0	0
Mucoepidermoid carcinoma	5	40	3	2	0	0
Malignant mesothelioma	13	0	13	0	0	0

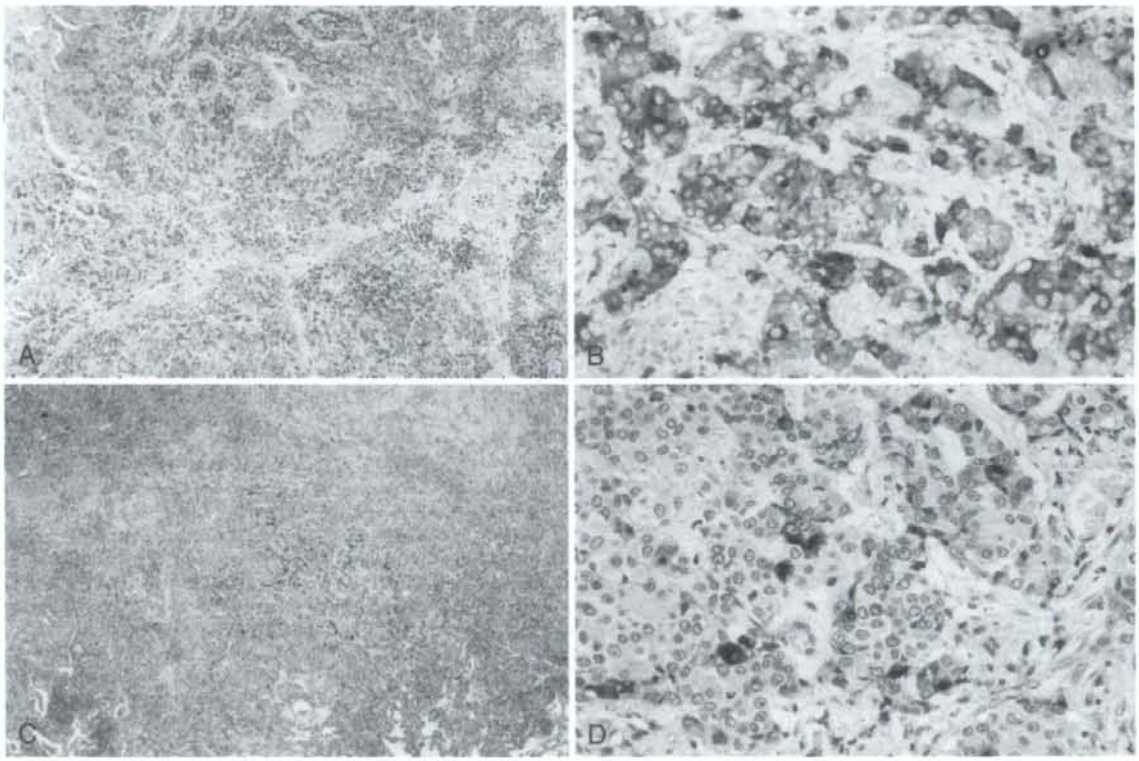

Figure 1. *Breast carcinoma metastatic to the lung, demonstrating solid growth. A to D represent the same case. A and B, Diffuse and strong staining for mammaglobin (original magnifications ×2 [A] and ×20 [B]). C and D, Focal but strong staining for gross cystic disease fluid protein 15 (original magnifications ×2 [C] and ×20 [D]).*

slightly higher frequency of immunostaining than GCDFP-15. Furthermore, mammaglobin showed diffuse immunoreactivity in 60% of immunopositive cases, whereas diffuse immunoreactivity of GCDFP-15 was observed in 44% of all immunopositive cases of breast carcinoma metastatic to the lung. As positivity for either marker was identified in 13 cases, a combination of mammaglobin and GCDFP-15 as a panel raised the sensitivity for identifying breast carcinoma metastatic to the lung to 65%.

The specificity of mammaglobin for detecting breast carcinoma metastatic to the lung (3/263 cases; 98.9%) was higher than that of GCDFP-15 (19/263 cases; 92.8%).

Primary Lung and Pleural Tumors

Primary Lung Adenocarcinomas.—All primary lung adenocarcinomas were immunonegative for mammaglobin. However, GCDFP-15 expression was observed in 15 (15%) of 100 cases of primary lung adenocarcinoma. The lepidic growth pattern was positive in 12 (20%) of 60 cases, and the nonlepidic growth type was positive in 3 (7.5%) of 40 cases. In the lepidic growth type, the non-

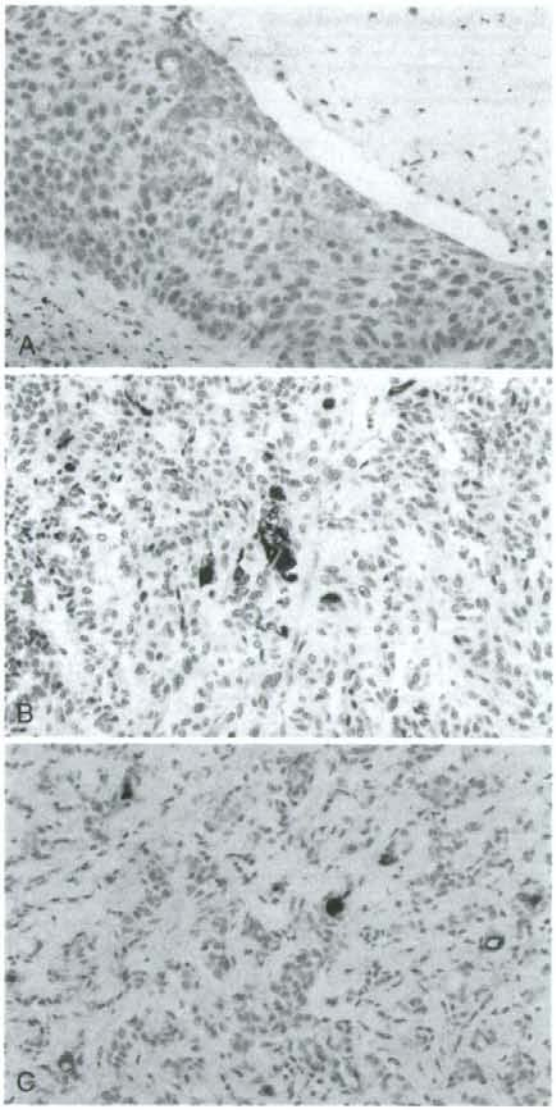

Figure 2. *A, Focal but strong staining for mammaglobin was evident in moderately differentiated squamous cell carcinoma of the lung (original magnification ×20). B, Focal but strong staining for mammaglobin was evident in carcinoid of the lung (original magnification ×20). C, Focal but strong staining for mammaglobin was evident in adenoid cystic carcinoma. Positivity was observed on the luminal side of the cytoplasm of true ductal cells (original magnification ×20).*

mucinous type was positive in 2 (10.0%) of 20 cases, and the mucinous type was positive in 10 (25%) of 40 cases. In the nonlepidic growth type, the acinar-cribriform type was positive in 3 (15.0%) of 20 cases, and the solid type was negative in all cases.

Squamous Cell Carcinomas.—Mammaglobin expression was observed in only one case of moderately differentiated squamous cell carcinoma, but this positive area was restricted to a small part of the tumor (Figure 2, A). All other squamous cell carcinomas were negative for mammaglobin. All squamous cell carcinomas were immunonegative for GCDFP-15.

Pleomorphic Carcinoma.—In pleomorphic carcinomas, expression of GCDFP-15 was observed in 1 (5.0%) of 20 cases, whereas all cases were immunonegative for mammaglobin.

Neuroendocrine Tumors.—Expression of mammaglobin was observed in 1 (5.2%) of the 19 carcinoid tumors, but positivity was limited to a small area (Figure 2, B). Furthermore, expression of GCDFP-15 was observed in 1 different case (5.2%), but the positivity again was limited to a small area (Figure 2, C).

All high-grade neuroendocrine tumors (large-cell neuroendocrine carcinoma and small-cell carcinoma) were immunonegative for both mammaglobin and GCDFP-15.

Salivary Gland–Type Tumors (Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma) of the Bronchus and/or Trachea

In mucoepidermoid carcinomas, expression of GCDFP-15 was observed in 2 (40.0%) of 5 cases, whereas all cases were immunonegative for mammaglobin. In adenoid cystic carcinoma, 1 (9.1%) of 11 cases was immunopositive for mammaglobin, whereas all cases were immunonegative for GCDFP-15. Mammaglobin (Figure 2, C) and GCDFP-15 showed focal staining.

Malignant Mesotheliomas

All malignant mesotheliomas were immunonegative for both mammaglobin and GCDFP-15.

COMMENT

In the present study, we demonstrated 3 advantages of using mammaglobin over GCDFP-15 to identify breast carcinoma metastatic to the lung. The first advantage is that the sensitivity of mammaglobin is slightly higher than that of GCDFP-15. The mammaglobin positivity rate for primary breast cancer is reported to be between 47.9%[19] and 71%.[20] Although we focused on analyzing breast carcinoma metastatic to the lung, the overall prevalence of mammaglobin expression in our series (50%) is almost accordant with the findings of previous reports[19,20] on primary breast cancer. The expression status of some molecules may be altered between primary and metastatic lesions. For example, the expression of surfactant apoprotein in lung cancer is frequently reduced in metastatic sites.[21] However, a previous report has demonstrated that the concordance rate of mammaglobin expression between the primary site and lymph node metastases was 93%.[20] These findings indicate that mammaglobin expression is not altered in the metastatic lesion.

Several studies have analyzed the mammaglobin expression pattern in breast carcinoma using immunohistochemical methods. Two of three studies used a noncommercial antibody[15] or a cocktail of antibodies[20] to identify mammaglobin, and the positivity rate in primary breast carcinoma was around 70%. However, Sasaki et al[19] reported that the positivity rate for mammaglobin in primary breast carcinoma analyzed by commercially available monoclonal antibody (clone 304-1A5) was lower than that using a noncommercial antibody or a cocktail of antibodies. These findings suggest that commercially available monoclonal antibody has a lower sensitivity and that there might be differences in the patient population. According to analyses by histologic type, mammaglobin ex-

pression was reported to be higher in lobular carcinoma than in ductal carcinoma.[19,20] Another study has suggested that mammaglobin expression is evident mainly in well-differentiated hormone receptor–positive breast carcinomas.

The second advantage of using mammaglobin is that the immunopositive area shows more diffuse staining than that of GCDFP-15. Similar findings have also been reported by Bhargava et al.[20] These findings indicate that examination of mammaglobin expression would be advantageous when the diagnosis is based on a limited sample, such as biopsy material. However, as cases that are immunopositive for mammaglobin and GCDFP-15 are partly exclusive, the combined use of both markers is important.

The third advantage of using mammaglobin is that its expression was found in only 1.1% of nonbreast tumors of the lung and pleura. One carcinoid tumor, 1 squamous cell carcinoma, 1 adenoid cystic carcinoma of the trachea, and none of the primary lung adenocarcinomas were positive. In primary lung adenocarcinoma, about 30% of cases demonstrated a nonlepidic growth pattern.[22] In particular, the cribriform and/or acinar and solid type might be confused with a lung metastasis from breast cancer. In the present study, we demonstrated that mammaglobin was negative in the cribriform and/or acinar and solid types of primary lung adenocarcinoma. The mammaglobin expression rate in lung tumors is reported to range from 0% to 16.7%.[15,19,20,23] Mammaglobin expression was reported to have been found in 20% of salivary gland tumors.[15,20] Therefore, the finding that adenoid cystic carcinoma of the trachea showed immunoreactivity for mammaglobin is not surprising but does require attention.

The differential diagnosis of malignant effusion involving the serosal membrane may be difficult. In the present study, all malignant mesotheliomas were immunonegative for mammaglobin. These findings indicated that mammaglobin should be added as one of the mesothelioma-negative markers, especially in female patients and/or cases of peritoneal mesothelioma.

Thyroid transcription factor 1 (TTF-1) has been shown to play a crucial role in the morphogenesis and function of the lung by regulating gene expression of surfactant proteins.[24] Most studies have reported finding TTF-1 expression in more than 70% of primary adenocarcinomas of the lung.[25] Therefore, TTF-1 has been considered a reliable marker to distinguish between primary lung adenocarcinoma and metastatic adenocarcinoma. It is reasonable that TTF-1 should be added to the antibody panel as a negative marker for metastatic tumors.

In conclusion, we demonstrated that the sensitivity of mammaglobin is equal or superior to that of GCDFP-15 for investigation of breast carcinoma metastatic to the lung. Immunopositivity for mammaglobin is more diffuse than that for GCDFP-15. In terms of practical diagnosis, mammaglobin immunopositivity can serve as a differential marker of breast carcinoma and should be added to immunohistochemical panels.

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