Outcome of Surgery for Small Cell Lung Cancer – Response to Induction Chemotherapy Predicts Survival

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Abstract

Background: The role of surgery for local control of small cell lung cancer (SCLC) is controversial. **Methods:** Sixty-nine consecutive patients who underwent complete resection of SCLC in our hospital were reviewed. The patients included 62 men and 7 women. Clinical stage at the time of diagnosis was c-stages IA and B in 29, c-stages IIA and B in 12, c-stage IIIA in 21, and c-stage IIIB in 7. **Results:** Thirty-two patients received induction chemotherapy, and 37 patients underwent initial surgery. The overall response rate to induction chemotherapy was 71.9%. The survival rate stratified by clinical stage at the time of diagnosis was 48.9% for c-stage I, 33.3% for c-stage II, 20.2% for c-stage IIIA, and 0% for c-stage IIIB. Downstaging after induction chemotherapy conferred a survival benefit. Survival after lobectomy or bilobectomy was better than after pneumonectomy. Patients who received adjuvant chemotherapy survived longer than patients who did not. **Conclusions:** Surgery combined with chemotherapy is a therapeutic option in selected patients with SCLC. Pathologic nodal status and response to induction chemotherapy are predictors of survival.

Key words
Chemotherapy · lung cancer · surgery · survival · small cell lung cancer

Introduction

Small cell lung cancer (SCLC) is considered a systemic disease, because the potential for hematogenous and lymphogenic metastases is high. At present, concurrent chemoradiotherapy for limited disease (LD) and chemotherapy for extensive disease (ED) are standard practice. About 30 years ago, a randomized study by the British Medical Research Council [1] concluded that radiotherapy alone for LD was superior to surgery. However, the local recurrence rate after radiation therapy alone subsequently was reported to be 18% to 69% [2]. The Veteran's Administration Surgery Oncology Group [3] reviewed data on 148 resected SCLCs to evaluate the role of adjuvant chemotherapy in non-small cell lung cancers (NSCLCs) and reported a 59.5% 5-year survival rate for stage IA disease. Since then, several series looking at the role of surgery for SCLC have been reported from different institutions. The University of Toronto Lung Oncology Group [4] treated 119 SCLCs with surgery and multi-modality therapy. The overall 5-year survival rate in that study was 39%, and the rates stratified by pathologic stage were 51% in stage I, 28% in stage II, and 19% in stage III. These survival rates were relatively good and represent an acceptable outcome.

To define the role of surgery for SCLC, the Lung Cancer Study Group [5] randomized cases of LD excluding stage I, to undergo resection or not after 5 cycles of chemotherapy with CAV (cyclophosphamide [CPA] + adriamycin [ADR] + vincristine [VCR]) followed by radiation. In that study, surgery did not improve survival.

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At present, the role of surgery combined with chemotherapy or radiotherapy for local control of SCLC is still controversial. Even when a radiographic complete response is obtained, up to 75% of patients have residual viable cancer cells in the surgical specimen [6]. Also, residual chemoresistant NSCLC coexist with SCLC in 10% to 25% of specimens resected after administration of chemotherapy [7]. Therefore we believe that complete resection of the primary tumor is indicated in some circumstances. In Germany, a phase II multicenter trial [8] to treat patients with advanced SCLC, stages IIB/IIIA, using combined modality therapy including surgery, proved effective in achieving local control and in increasing survival after complete resection. This is an encouraging outcome and validates the role of surgery for SCLC in combination with chemotherapy or radiotherapy. We retrospectively analyzed consecutive patients who underwent surgery for SCLC in our hospital to better define the role of surgery in this disease.

Patients and Methods

From January 1977 through December 2002, 79 patients underwent resection of an SCLC in our hospital. The 69 patients in whom complete resection was achieved were the subjects of this study. Table **1** shows the clinicopathologic characteristics of the study group. The patients included 62 men and 7 women, age range 39 to 79 years (mean, 62.2). Disease stage was determined based on the American Joint Committee on Cancer criteria [9]. Clinical stages at the time of diagnosis were c-stages IA and B in 29, c-stages IIA and B in 12, c-stage IIIA in 21, and c-stage IIIB in 7. Thirty-two patients received induction chemotherapy followed by surgery, and 37 patients underwent initial surgery. Forty-eight patients received adjuvant chemotherapy. In the induction chemotherapy group, 62.5% (20/32) patients had c-stage IIIA disease or higher stages. Conversely, only 22.6% (8/37) patients in the initial surgery group had c-stage IIIA disease or higher. Median follow-up of patients alive was 65 months.

The survival rate was calculated by the Kaplan-Meier method. Significance of the survival differences between groups was evaluated by the log rank test. A multivariate analysis was carried out according to the Cox proportional hazards model to identify independent risk factors. $p < 0.05$ was considered significant.

Results

Table **2** shows the therapy administered to the patients in this study. Most patients (59/69, 85.5%) received chemotherapy before and/or after surgery. We used CPA-based chemotherapy (CPA 800 mg/m^2 on day 1, ADR 50 mg/m^2 on day 1, and VCR 1.4 mg/m^2 on day 1) until the mid-1980s, and platinum-analog-based chemotherapy (cisplatin [CDDP] 80 mg/m^2 on day 1 and etoposide [VP-16] 100 mg/m^2 on day 1, 3 and 5, or carboplatin [CBDCA] 400 mg/m^2 and VP-16 100 mg/m^2 on day 1, 3 and 5) after the mid-1980s as the standard regimen. The numbers of cycles ranged from 1 to 6.

The overall radiographic response rate to induction chemotherapy was 71.9% (23/32): there was complete response in 4 (12.5%), partial response in 19 (59.4%), and stable disease in 9 (28.1%). Pathologic complete response was obtained in 3 cases (9.4%). The surgical specimens contained small cell carcinoma and another type of cancer, so-called combined small cell carcinoma [10], in 7.2% (5/69); combined small cell and adenocarcinomas were found in 3 and combined small cell and squamous cell carcinomas in 2 cases.

Table **1** Demographics and clinical characteristics of patients who underwent surgery for small cell lung cancer

	Total (n = 69)	*Induction chemotherapy (n = 32)*	*Initial surgery (n = 37)*
Gender			
Male	62	28	34
Female	7	4	3
Age			
Mean ± SD	62.2 ± 9.1	59.5 ± 7.8	64.5 ± 9.5
Clinical stage			
IA	15	1	14
IB	14	4	10
IIA	1	1	0
IIB	11	6	5
IIIA	21	15	6
IIIB	7	5	2
IV	0	0	0
Pathologic stage			
IA	21	9	12
IB	9	4	5
IIA	4	2	2
IIB	8	3	5
IIIA	16	9	7
IIIB	10	4	6
IV	1	1	0

Table **2** Combination chemotherapy regimens and surgery for small cell lung cancer

	Induction therapy (n = 32)		*Adjuvant therapy (n = 48)*[a]	
	Chemo.	*Chemo. + Rad.*	*Chemo.*	*Chemo. + Rad.*
CDDP or CBDCA based				
CDDP + VP-16	20	1	17	1
CBDCA + VP-16	3	1	13	0
CPA based				
CAV	5	2	11	6
Total	28	4	41	7

Chemo. = chemotherapy; Rad. = radiotherapy
CDDP = cisplatin; CBDCA = carboplatin; VP-16 = etoposide;
CPA = cyclophosphamide; CAV = CPA + ADR (adriamycin) + VCR (vincristine)
[a] Both induction and adjuvant therapy were performed in 21 patients.

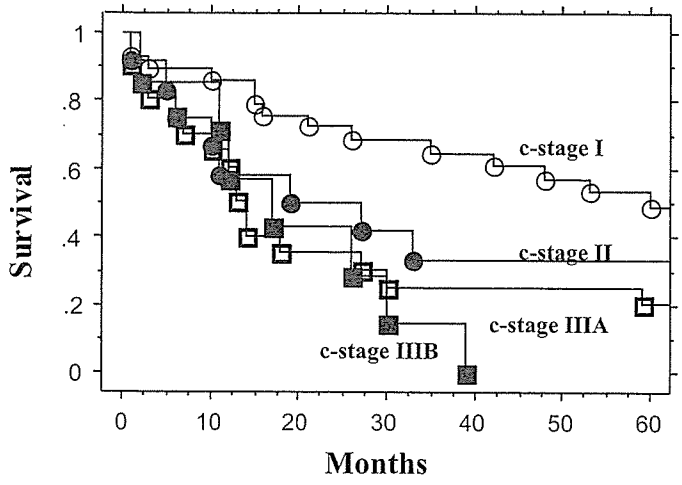

Fig. 1 Comparison of Kaplan-Meier survival curves of patients with small cell lung cancer stratified by clinical stage. The projected 5-year survival rates were 48.9% for c-stage I (n = 29, open circle), 33.3% for c-stage II (n = 12, closed circle), 20.2% for c-stage IIIA (n = 21, open square), and 0% for c-stage IIIB (n = 7, closed square). Survival difference between c-stage I and c-stage IIIA was significant ($p = 0.0349$).

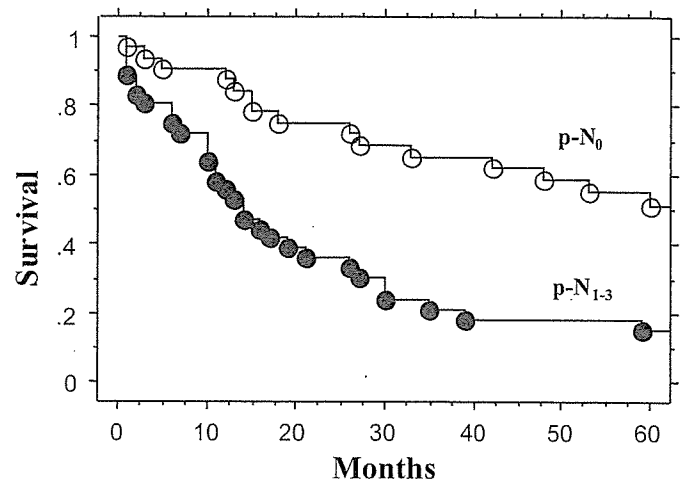

Fig. 2 Comparison of Kaplan-Meier survival curves of patients with small cell lung cancer with and without pathologically proven lymph node metastases. Survival of p-N0 patients (n = 36, open circle) was significantly better than node-positive (p-N1–3) patients (n = 33, closed circle; $p = 0.0001$).

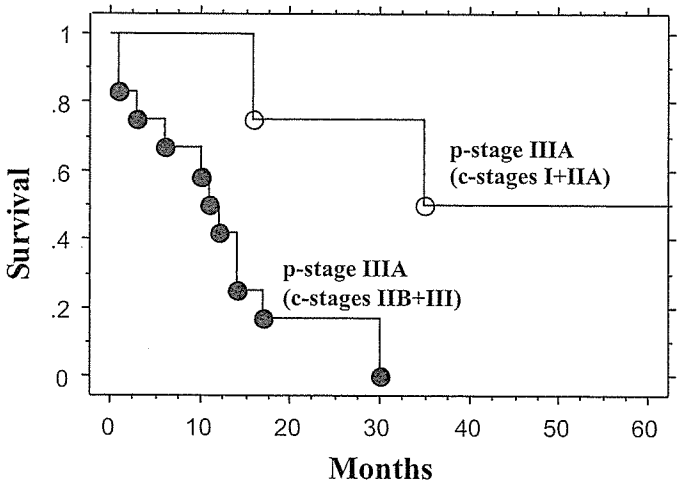

Fig. 3 Comparison of Kaplan-Meier survival curves of patients with p-stage IIIA small cell lung cancer stratified by clinical stage. Survival of patients whose stage was underestimated preoperatively (c-stage I and IIA, n = 5; open circle) was better than the rest of patients with p-stage IIIA disease (c-stage IIB or higher, n = 11; closed circle; $p = 0.0087$).

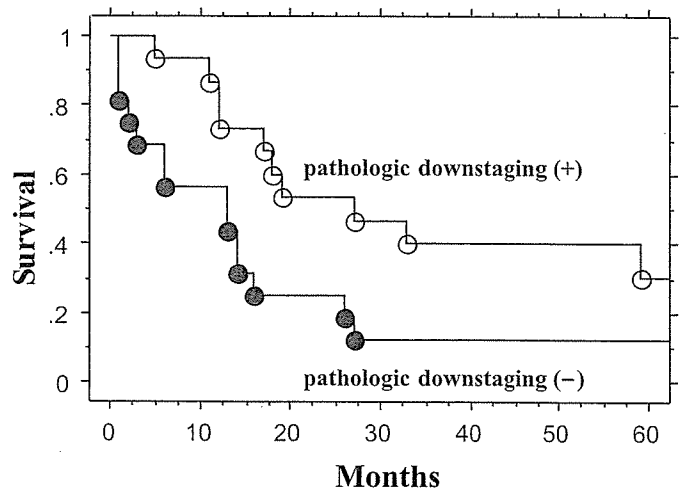

Fig. 4 Comparison of Kaplan-Meier survival curves of patients with small cell lung cancer who did and did not achieve pathologic downstaging with induction chemotherapy. Survival with downstaging (n = 16, open circle) was better than without it (n = 16, closed circle; $p = 0.0312$).

The surgical procedure was a lobectomy in 49 cases (71.0%), bilobectomy in 9 cases (13.0%), and pneumonectomy in 11 cases (15.9%). The overall 5-year survival rate was 32.2%. The 5-year survival rate stratified by clinical stage at the time of diagnosis was 48.9% in c-stage I, 33.3% in c-stage II, 20.2% in c-stage IIIA, and 0% in c-stage IIIB. Survival differences existed between c-stage I and c-stage IIIA, and between c-stage I and c-stage IIIB ($p = 0.0349$ and $p = 0.0018$, respectively; Fig. **1**). The overall 5-year survival rate was 49.5% in p-stage I, 40.0% in p-stage II, 12.5% in p-stage IIIA, 10.0% in p-stage IIIB, and 0% in p-stage IV. A survival difference existed between p-stage I and p-stage IIIA, and between p-stage I and p-stage IIIB ($p = 0.0004$ and $p = 0.0007$, respectively).

Survival of patients with postsurgical pathologic node-negative (p-N0) disease (n = 36) was significantly better than of patients with node-positive (p-N1–3) disease (n = 33, $p = 0.0001$; Fig. **2**). Also survival of patients with clinical node-negative (c-N0) disease (n = 32) was better than of patients with clinical node-positive (c-N1–3) disease (n = 37, $p = 0.0261$). Survival of patients with p-stage IIIA disease whose mediastinal lymph node metastases were underestimated preoperatively (c-stage I and IIA, n = 5) was better than that of the other patients with p-stage IIIA disease (c-stage IIB or higher, n = 11) ($p = 0.0087$) (Fig. **3**).

Pathologic downstaging occurred in 50% (16/32) of patients who underwent induction chemotherapy, and a survival benefit was observed in the downstaging group ($p = 0.0312$; Fig. **4**). Survival after lobectomy or bilobectomy (n = 58) was significantly better

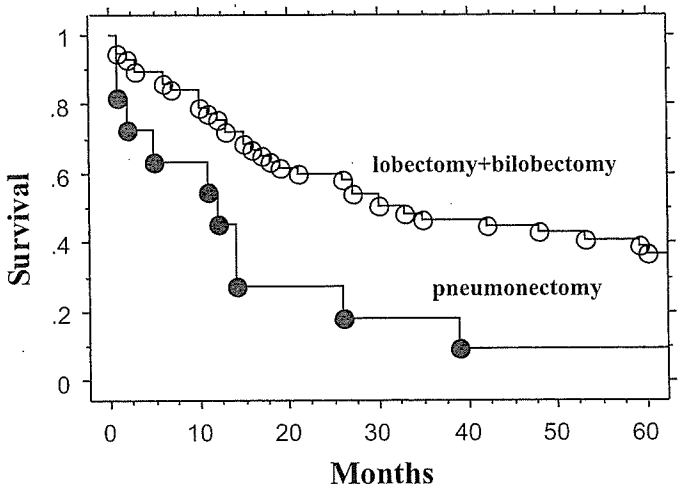

Fig. 5 Comparison of Kaplan-Meier survival curves of patients with small cell lung cancer stratified by the surgical procedure. Survival after lobectomy or bilobectomy (n = 58, open circle) was significantly better than after pneumonectomy (n = 11, closed circle; $p = 0.0163$).

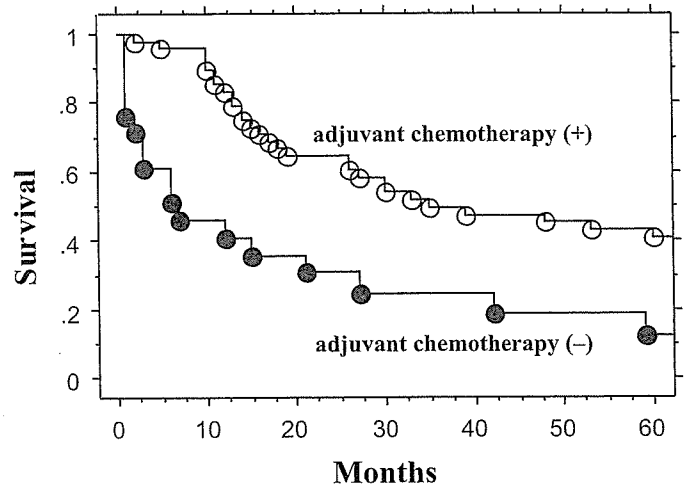

Fig. 6 Comparison of Kaplan-Meier survival curves of patients with small cell lung cancer who did (n = 48, open circle) and did not (n = 21, closed circle) receive adjuvant chemotherapy ($p = 0.0025$).

Table 3 Site of first relapse after surgery for small cell lung cancer as a function of pathologic disease stage

	Pathologic stage							
	IA (n = 21)	*IB* (n = 9)	*IIA* (n = 4)	*IIB* (n = 8)	*IIIA* (n = 16)	*IIIB* (n = 10)	*IV* (n = 1)	*Total*
Brain	1	1	1	2	5	2	1	13
Intrathoracic	1	0	1	1	6	3	0	12
Bone	1	1	0	2	1	0	0	5
Liver	1	2	0	0	0	0	0	3
Axillary lymph node	0	0	0	0	1	0	0	1
Total	4	4	2	5	13	5	1	34

than after pneumonectomy (n = 11, $p = 0.0163$; Fig. **5**). Survival of patients who received adjuvant chemotherapy (n = 48) was better than of patients who did not receive adjuvant chemotherapy (n = 21, $p = 0.0025$, Fig. **6**).

The surgical mortality was 5.8% (4/69), with 2 deaths due to bronchogenic fistula and 2 due to pneumonia.

The first relapse site is shown in Table **3**. In patients with p-stage I disease, relapse after surgery occurred in 8/30 patients (26.7%). The first relapse site was liver in 3, brain in 2, bone in 2, and intrathoracic in 1. The frequency of intrathoracic relapse was 3.3% (1/30). In more advanced p-stages, II to IV, relapse occurred in 27/39 patients (69.2%). The first relapse site in these patients was brain in 12, intrathoracic in 11, bone in 3, and axillary lymph node in 1. Thus, intrathoracic relapses were frequent (11/39, 28.2%) in advanced stages.

Multivariate analysis of prognostic factors revealed that pathologic nodal status ($p = 0.0102$), administration of adjuvant chemotherapy ($p = 0.0039$), and surgical procedure ($p = 0.0432$) were significant predictors of survival (Table **4**).

Discussion

Evaluating the role of surgery for SCLC is difficult for a number of reasons. First, only a small number of patients present in relatively early stages that can be treated by surgery. Second, a comparison between surgery and nonsurgical treatment in the same disease stage is difficult because staging for most patients treated without surgery is based only on the LD/ED classification. LD usually includes a very heterogeneous group of patients, stages IA to IIIB. Third, it is difficult to conduct prospective studies because a multi-institutional randomized controlled study would take a long time to enroll an adequate number of surgical candidates to achieve statistical significance. Thus, retrospective analyses are still essential to advance our understanding of the role of surgery in SCLC.

The main advantage of surgery for SCLC is complete local control of the disease [11]. Even when a complete response is obtained by chemoradiotherapy for LD, the local relapse rate is still 20% to 70% [12–14]. In our study, local relapse after surgery depended on the postsurgical p-stage. In p-stage I, we found that the incidence of intrathoracic recurrence was only 3.3%, whereas it was 28.2% in higher stages. Thus, lymphogenic spread in ad-

Table 4 Multivariate analysis of prognostic factors in patients with small cell lung cancer

Prognostic factors	*P value*	*Hazard ratio*	*95% CI*
Gender (male vs. female)	0.94	1.855	0.620–5.556
Age (≥ 62 vs. < 62)	0.1104	1.741	0.881–3.438
Pathologic N factor (N1–3 vs. N0)	0.0102	2.409	1.232–4.711
Adjuvant chemotherapy (done vs. not done)	0.0039	0.404	0.218–0.748
Surgical procedure (pneumonectomy vs. lobectomy or bilobectomy)	0.0432	2.528	1.028–6.215

CI = confidence interval

vanced stages makes complete local elimination of cancer cells by surgery unlikely. In addition, survival after pneumonectomy was significantly worse than after lobectomy or bilobectomy, and survival of patients with clinical or pathologic lymph node involvement was significantly worse than without lymph node involvement.

The 5-year survival rate after surgery for p-stage I disease ranges from 22% to 67%, and that for p-stage II ranges from 17% to 50% [15–17]. Reported survival in p-stage IIIA or higher varies greatly, from 0% to 55.5% [15,18–20]. The randomized study by the Lung Cancer Study Group [5] showed that surgery does not prolong survival in c-stage IIIA SCLC even in patients who undergo induction therapy. Although 19% of resected tumors showed complete pathologic response, this good response to chemotherapy did not improve the survival. However, in our study, pathologic downstaging did predict improved survival. Thus, we believe pathologic downstaging may be a selection criterion for identifying surgical candidates. Evaluation of the residual tumor cells by positron emission tomography (PET) or by lymph node sampling by mediastinoscopy after induction chemotherapy are alternate strategies.

In conclusion, a 32% overall 5-year survival was obtained in selected patients with SCLC who underwent surgery. Survival after surgery clearly depended on disease stage. Nodal status and pathologic downstaging after induction therapy predict survival. A randomized study is needed to identify surgical candidates.

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Quantitative Detection of Lung Cancer Cells by Fluorescence In Situ Hybridization: Comparison With Conventional Cytology

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Quantitative Detection of Lung Cancer Cells by Fluorescence *In Situ* Hybridization*

Comparison With Conventional Cytology

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Study objective: **The aim of this study was to clarify whether fluorescence *in situ* hybridization (FISH) can diagnose lung cancer in various clinical specimens in comparison with conventional cytology.**
Design: **Prospective study.**
Setting: **University hospital in a metropolitan area.**
Patients: **Fifty consecutive patients with abnormal chest radiography or CT scan findings were enrolled. The patients included 32 men and 18 women, with an average age of 64 years. The final definitive diagnosis was made by histologic examination, as follows: 38 primary lung cancers (24 adenocarcinomas, 8 squamous cell carcinomas, 2 large cell carcinomas, and 4 small cell carcinomas); 1 metastatic renal cell carcinoma; and 11 benign lesions.**
Methods: **Four types of clinical specimens were analyzed. Cells obtained by transbronchial brushing and transbronchial fine-needle aspiration using a fiberoptic bronchoscope under fluoroscopy, CT scan-guided percutaneous needle biopsy, and bronchial washings. On every examination, duplicate slides were made for analyses of conventional cytology and FISH.**
Results: **Classifications according to conventional cytology were as follows: class I, 4 patients; class II, 15 patients; class IIIa, 3 patients; class IIIb, 5 patients; and class V, 23 patients. A classification higher than class IIIb was considered to be positive for cancer. For cytology, we found no false-positive cases and 11 false-negative cases. The specificity was 100%, and the sensitivity was 71.8%. By FISH, 34 cases showed aberrant copy numbers in either chromosome 3 or 17. We found no false-positive cases and five false-negative cases. The specificity was 100%, and the sensitivity was 87.1%.**
Conclusion: **The ability of FISH to detect aneusomic lung cancer cells is superior to conventional cytology for the diagnosis of lung cancer.** ***(CHEST 2005; 128:906–911)***

Key words: aneuploidy; aneusomy; cytology; fluorescence *in situ* hybridization; lung cancer

Abbreviations: BW = bronchial washing; FISH = fluorescence *in situ* hybridization; PN = percutaneous needle biopsy; SSC = standard saline citrate; TB = transbronchial brushing

Conventional cytology plays an important role for the diagnosis of lung cancer, especially in the examination of sputum and pleural effusions. In addition, cell specimens obtained by transbronchial brushing (TB)[1] and needle aspiration under fluoroscopy,[2] percutaneous needle biopsy (PN) under CT scanning,[3] and bronchial washings (BWs)[4] provide important information for the differential diagnosis between benign and malignant disease. Cytologic diagnoses are made by experienced cytologists who can properly evaluate the morphologic features of malignant cells. However, this judgment is some-

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times difficult when the morphologic changes associated with malignancy are mild. Such cells are usually classified as class III, using the classification of Papanicolaou,[5] which is suggestive of, but not conclusive for, malignancy. This is an ambiguous judgment for clinical decision making. In addition, when one obtains a small number of cells from the lesion, the definitive diagnosis is even more difficult. These limitations of morphology-based conventional cytology have stimulated the search for more objective and quantitative methods for an accurate cytologic diagnosis of cancer.

Aneuploidy is the most common feature of many solid tumors, including lung cancer.[6] Solid tumors are characterized by complicated karyotypes by classic cytogenetics.[7,8] Chromosomal instability[9,10] may cause the uneven distribution of chromosomes during cell division.[11,12] Thus, malignant tumors can be diagnosed by detecting aneuploid, usually hyperdiploid, cells. A rapid and sensitive method for detecting aneusomy of a specific chromosome in an individual cell is fluorescence *in situ* hybridization (FISH). For this purpose, specific centromeric DNA probes enumerated the chromosomes. FISH was originally developed as a method to detect chromosomal aberrations,[13] and is now widely used for gene mapping,[14] the diagnosis of congenital diseases,[15] and detecting specific gene copy number changes in malignant cells.[16–18]

One advantage of FISH in detecting malignant cells is its objective and quantitative evaluation. However, the specificity and sensitivity of FISH in the diagnosis of lung cancer is unclear. We report the results of a prospective study comparing FISH with conventional cytology to detect lung cancer cells.

Materials and Methods

Patients

Fifty consecutive patients who underwent cytologic examination for abnormal chest radiography or CT scan findings at Tokyo Medical University Hospital from July 2003 to January 2004 were enrolled in this prospective study. The patients included 32 men and 18 women, with an average age of 64 years. The final definitive diagnosis was made by histologic examination, as follows: 38 primary lung cancers (24 adenocarcinomas, 8 squamous cell carcinomas, 2 large cell carcinomas, and 4 small cell carcinomas); 1 metastatic renal cell carcinoma; and 11 benign lesions. All patients with lung cancer were staged according to the latest Union Internationale Centre le Cancer criteria.[19] Cases included 10 tumors in stage IA, 5 in stage IB, 1 in stage IIA, 3 in stage IIB, 10 in stage IIIA, 6 in stage IIIB, and 3 in stage IV (Table 1).

Cells gathered from lung lesions were independently analyzed by conventional cytology and FISH. Informed consent for the cytologic examinations and genetic analyses of the specimens were obtained from all patients.

Table 1—*Histology and Stage of Lung Cancer in This Series of Patients

Case	Age	Gender	Specimen	Histology	Stage
1	59	M	TB	Sq	cIIIA
2	42	F	PN	B	NA
3	43	M	TB	Ad	PIIIA
4	70	M	TB	Ad	CIV
5	77	M	TB	Ad	PIA
6	73	M	PN	Ad	PIA
7	58	M	TN	Ad	PIA
8	71	M	BW	Ad	pIIIA
9	71	M	BW	La	pIB
10	66	F	TN	RCC	NA
11	65	F	TB	Sm	cIIIB
12	68	F	PN	Ad	pIA
13	69	F	BW	Ad	pIV
14	52	F	TB	Ad	pIIA
15	75	F	TN	Sq	pIA
16	37	M	PN	B	NA
17	64	M	PN	Ad	pIIB
18	58	F	TB	B	NA
19	73	F	PN	Ad	pIA
20	62	M	PN	B	NA
21	69	M	TB	B	NA
22	76	M	BW	La	cIIIB
23	76	M	PN	Ad	cIIIB
24	75	M	PN	Ad	pIB
25	65	M	BW	Sm	cIIIA
26	23	M	PN	B	NA
27	74	M	BW	Ad	cIV
28	72	F	PN	B	NA
29	80	M	TB	B	NA
30	56	M	BW	Ad	pIB
31	64	M	TB	B	NA
32	58	M	PN	Ad	cIIIA
33	72	F	PN	Ad	pIA
34	72	M	PN	Ad	cIIIA
35	66	F	TB	Sm	cIIIB
36	61	M	TB	Ad	pIIB
37	72	F	TB	Ad	pIB
38	52	M	PN	B	NA
39	64	M	TB	Ad	pIA
40	79	F	TB	Sq	cIB
41	39	M	TB	B	NA
42	78	M	BW	Sq	cIIIB
43	62	M	TB	Sq	cIIIA
44	57	M	TB	Sq	pIIIA
45	70	M	PN	Ad	pIA
46	55	F	PN	Ad	pIA
47	58	F	TN	Sq	cIIIB
48	66	F	TN	Ad	pIIB
49	62	M	BW	Sm	cIIIA
50	72	F	BW	Sq	cIIIA

*M = male; F = female; TN = transbronchial needle biopsy; Ad = adenocarcinoma; Sq = squamous cell carcinoma; La = large cell carcinoma; Sm = small cell carcinoma; RCC = renal cell carcinoma; B = benign lesion; c = clinical stage; p = pathologic stage; NA = not applicable.

Cell Samples

In this study, the following four types of cell specimens were analyzed: cells obtained by TB (n = 18) and transbronchial fine-needle aspiration (n = 5) using a fiberoptic bronchoscope

under fluoroscopy, CT scan-guided PN using the 19-gauge Tokyo Medical University Needle[3] (n = 17), and BWs (n = 10). On every examination, duplicate specimens were made for simultaneous analyses of conventional cytology and FISH.

For conventional cytology, cells were stained by the Papanicolaou method. [5] Diagnosis was made by cytologists in the Department of Pathology at Tokyo Medical University Hospital. The various classes in conventional cytology are defined as follows: class I, absence of atypical or abnormal cells; class II, atypical cytology but no evidence of malignancy; class III, cytology suggestive of, but not conclusive for, malignancy (IIIa, mild dysplasia; IIIb, advanced dysplasia); class IV, cytology strongly suggestive of malignancy; and class V, cytology conclusive for malignancy.[5]

FISH

For FISH, cells on glass slides were air-dried overnight and stored at −80°C until they were used. Direct fluorochrome-labeled centromeric probes were used for the enumeration of different chromosomes. Spectrum-orange-labeled or Spectrum-green-labeled probes for the respective centromeric regions of chromosomes 3 and 17 were purchased (Vysis Inc; Downers Grove, IL), and dual-color FISH was performed. Slides were denatured by incubation with 70% formamide (two times the standard saline citrate [SSC] solution) at 74°C for 2 min in a water bath. Then, slides were dehydrated through a graded ethanol system (70% for 2 min, 85% for 2 min, and 100% for 2 min). A hybridization solution (10 μL) was applied to each slide, which was coverslipped and sealed with rubber cement. The hybridization solution contained 1 μL each DNA probe in 70% formamide (two times the SSC solution), and 10% dextran sulfate solution (cot I DNA). After incubation for 16 h at 37°C in a humidified chamber, slides were washed (two times SSC solution) for 3 min at 74°C. A di-amidinophenylindole antifade solution (8 μL) was applied to each spot and coverslipped. The slides were observed under a fluorescence microscope that was connected to a cooled charge-coupled device camera and an image analyzer system (CytoVision; Applied Imaging, Ltd; Newcastle, UK).

FISH signal analysis was performed as follows. All cells in a fluorescence microscopy field, except for those with damaged or overlapped nuclei, were evaluated. One hundred cells were counted, and the numbers of each centromeric signal were recorded. If there were < 100 cells on the slide, as many cells as possible were counted. When the percentages of hyperdisomic cells (*ie*, more than three copies for at least one chromosome) were > 10%, we judged the lesion to be malignant.

Comparison of Conventional Cytology and FISH

FISH diagnoses were made without clinical information or the results of conventional cytology. The results of FISH analysis were not shown to the cytologists. Thus, both diagnoses were independently made in a blind fashion.

Statistical Analysis

Differences in the number of countable cells according to the histology of the lung lesions or the cell-gathering methods used were analyzed by the Kruskal-Wallis test. A p value of < 0.05 was considered to be significant.

Results

Cells countable for FISH analyses ranged from 5 to 100 (maximum). Cell counts according to the cell-gathering method did not differ significantly, but the fewest cells were obtained by PN (Fig 1). Although the fewest cells were obtained from small cell carcinomas, no significant difference was seen according to the histologic type of lung cancer (Fig 2).

The results of conventional cytology according to the Papanicolau classification were as follows: class I, 4 cases; class II, 15 cases; class IIIa, 3 cases; class IIIb, 5 cases; and class V, 23 cases (Table 2). Twenty-eight cases showing a higher grade than class IIIb were considered to be positive for lung cancer. By cytology, we found no false-positive cases and 11

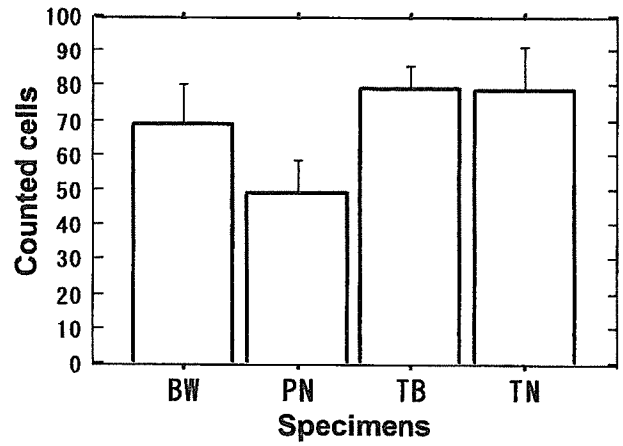


FIGURE 1. Countable cells according to the type of clinical specimen. Although cell counts obtained by PN were the lowest, no statistical significance was obtained by the Kruskal-Wallis test (p = 0.1117). Error bars indicate standard error. TN = transbronchial fine-needle aspiration.

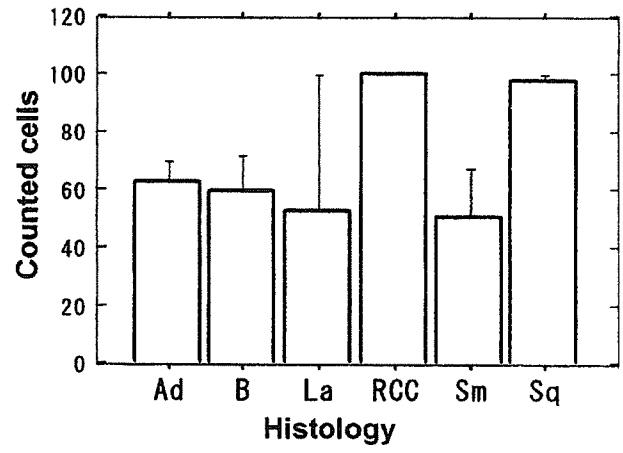


FIGURE 2. Countable cells according to histology. Although cell counts obtained from small cell lung cancer (Sm) were the lowest, no statistical significance was obtained by the Kruskal-Wallis test (p = 0.2369). Error bars indicate SE. Ad = adenocarcinoma; B = benign lesion; La = large cell carcinoma; RCC = renal cell carcinoma; Sq = squamous cell carcinoma.

Table 2—*Results of FISH and Conventional Cytology**

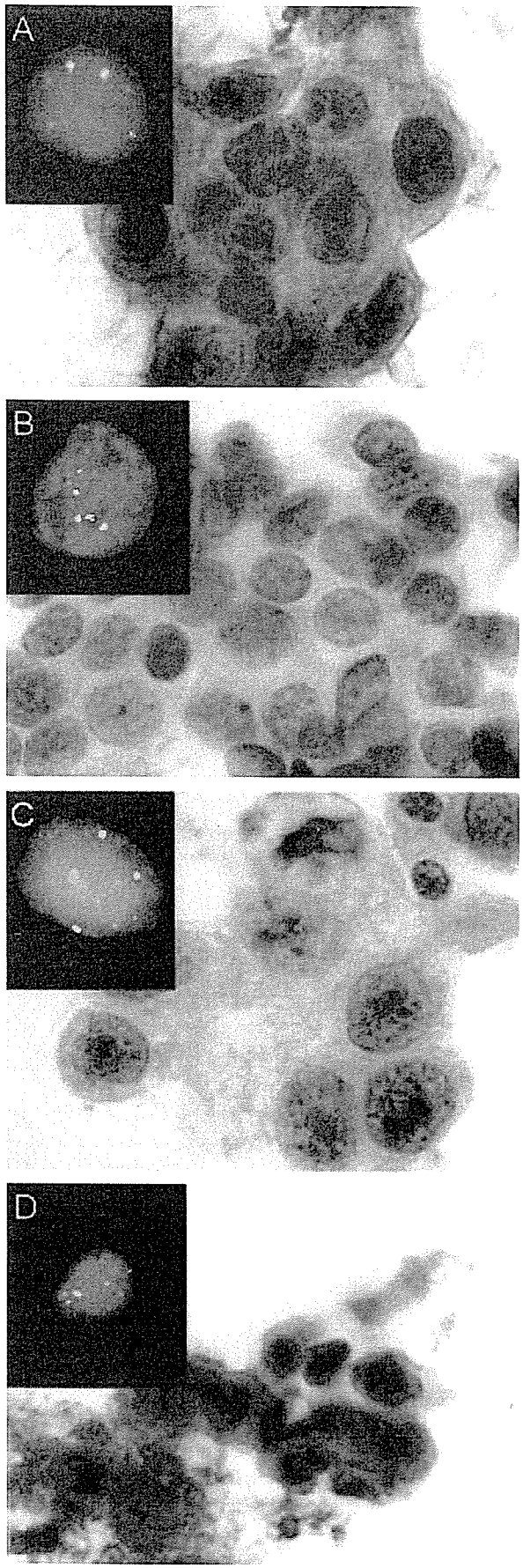
Case	Countable Cells	Three Copies C3/C17	Four Copies C3/C17	Five Copies C3/Ch17	Six Copies or More C3/C17	Hyperdisomy, % C3/C17	FISH	Cytology Stage
1	100	28/18	10/2	2/2	0/0	40/22	Positive	V
2	8	0/0	0/0	0/0	0/0	0/0	Negative	II
3	100	14/9	4/4	1/1	0/0	18/13	Positive	V
4	100	16/8	0/1	1/0	0/0	17/9	Positive	II†
5	52	2/0	1/0	0/0	0/0	3/0	Negative†	II†
6	100	9/0	1/3	1/0	0/0	10/3	Positive	IIIa†
7	44	7/3	0/0	1/0	0/0	18/7	Positive	II†
8	100	6/6	0/2	2/2	0/0	8/10	Positive	V
9	5	0/0	0/0	0/0	0/0	0/0	Negative†	I†
10	100	17/26	2/5	1/2	0/0	20/33	Positive	II†
11	100	38/37	9/2	3/0	1/0	51/39	Positive	V
12	17	2/3	2/1	1/0	0/0	29/29	Positive	V
13	79	5/11	5/3	4/1	0/0	18/19	Positive	IIIb
14	100	7/3	0/0	0/0	1/0	8/3	Negative†	II†
15	100	9/0	1/3	1/0	0/0	11/3	Positive	II†
16	42	0/1	0/0	0/0	0/0	0/2	Negative	II
17	100	22/16	2/11	3/5	0/1	27/33	Positive	V
18	100	2/0	0/0	0/0	0/0	2/0	Negative	I
19	26	5/8	0/0	0/0	0/0	19/31	Positive	IIIb
20	100	1/1	0/0	0/0	0/0	1/1	Negative	II
21	100	0/0	0/0	0/0	0/0	0/0	Negative	II
22	100	17/14	3/1	2/2	1/1	23/18	Positive	IIIb
23	5	1/1	0/0	0/0	0/0	20/20	Positive	IIIb
24	100	22/23	5/7	1/3	2/1	30/34	Positive	V
25	37	13/10	1/7	1/0	0/0	41/46	Positive	V
26	8	0/0	0/0	0/0	0/0	0/0	Negative	IIIa
27	43	1/1	0/0	0/0	0/0	2/2	Negative†	I†
28	100	0/0	0/0	0/0	0/0	0/0	Negative	I
29	44	0/0	0/1	0/0	0/0	0/2	Negative	II
30	100	8/6	1/1	0/1	0/0	9/8	Negative†	IIIb
31	100	2/0	0/0	0/0	0/0	2/0	Negative	II
32	8	2/3	1/1	1/0	0/0	50/50	Positive	V
33	21	4/5	1/0	0/0	0/0	24/24	Positive	V
34	57	27/31	5/2	2/1	0/1	60/61	Positive	V
35	40	10/9	2/3	4/0	0/2	40/35	Positive	V
36	100	15/15	1/4	1/1	0/0	17/20	Positive	V
37	38	2/4	0/0	0/0	0/0	5/11	Positive	V
38	23	0/1	0/0	0/0	0/0	0/4	Negative	II
39	45	9/9	1/4	2/1	0/0	27/31	Positive	V
40	79	14/12	1/1	1/0	0/0	20/16	Positive	V
41	30	0/1	0/0	0/0	0/0	0/3	Negative	II
42	100	17/6	3/1	0/1	0/0	20/8	Positive	V
43	100	19/18	3/6	4/1	2/0	28/25	Positive	II†
44	100	18/15	1/5	0/1	0/0	19/21	Positive	V
45	100	11/7	0/1	0/0	0/0	11/8	Positive	V
46	14	4/3	0/0	0/0	0/0	29/21	Positive	V
47	100	15/15	0/8	0/1	0/0	15/24	Positive	V
48	48	8/6	0/1	0/0	0/0	17/15	Positive	V
49	25	4/6	1/0	0/0	0/0	20/24	Positive	V
50	100	21/28	5/5	1/1	0/0	27/34	Positive	IIIa†

*C3 = chromosome 3; C17 = chromosome 17.
†False-negative result.

false-negative cases. Thus, by conventional cytology, specificity was 100%, and sensitivity was 71.8%.

In FISH analyses, 34 cases showed aberrant copy numbers in either chromosome 3 or 17. Representative findings of conventional cytology and FISH are shown in Figure 3. We found no false-positive cases and five false-negative cases. For FISH analyses, specificity was 100%, and sensitivity was 87.1%.

Seven cases, including one with metastatic renal cell carcinoma, had negative cytology findings and



positive FISH findings. In these cases, the cytologic classifications were as follows: class II, five cases; and class IIIa, two cases. One case was cytology-positive and FISH-negative. In this case, the portion of aneusomic cells were observed to be 8 to 9%, which is just below the predetermined cutoff value.

Discussion

We demonstrated here the usefulness of FISH analyses in diagnosing lung cancer using various clinical specimens. Similar results about the effectiveness of FISH analyses have been reported by several authors. Schenk et al[20] examined 23 patients with lung cancer by FISH with probes specific for chromosomes 3, 8, 11, 12, 17, and 18 for malignant effusions and primary tumors. In that study, chromosomal alterations always consisted of gains in chromosomal signal numbers, and all chromosomes were found to be aneusomic to a similar extent. According to this observation, we used only two probes in the present study, which were specific for chromosomes 3 and 17.

Recently, Sokolova et al[21] analyzed BW specimens from 48 patients with lung cancer by FISH using four probes (*ie*, centromeric region of chromosome 1, 5p15, 8q24 (c-myc), and 7p12 [epidermal growth factor receptor]). In that report, FISH detected 15 of 18 specimens that were falsely negative by cytology. The sensitivity of FISH for the detection of lung cancer was 82% compared with 54% sensitivity by conventional cytology. The same group[22] used a similar FISH probe set to show that significantly higher frequencies of abnormal cells were found in each of the 20 surgical specimens of non-small cell carcinoma (100%) and in the 3 sputum specimens (100%) from lung cancer patients. These probes detected a 4.8 to 7.3% rate of abnormal copy numbers in normal control specimens. In these retrospective studies, FISH detected lung cancer cells in touch preparations of resected tumors and BWs. Thus, we planned a prospective study to compare conventional cytology with FISH using various specimens from lung lesions.

In our study, we determined the cutoff value for the percentage ofhyperdisomic cells to be 10%, be-

FIGURE 3. Hyperdisomic cells detected by FISH. Red signals are the centromeric region of chromosome 3, and green signals are the centromeric region of chromosome 17.Representative findings of conventional cytology (Papanicolaou stain, original ×400) and FISH in the same cases, as follows: *top left*, *A*: adenocarcinoma (case 39); *top right*, *B*: squamous cell carcinoma (case 1); *bottom left*, *C*: large cell carcinoma (case 24); and *bottom right*, *D*: small cell carcinoma (case 11).

cause we often count ≦6% hyperdisomic cells in normal cell specimens, probably due to counting sister chromatids as two copies. When we set the cutoff value at 10%, a specificity of 100% and a sensitivity of 87.1% were obtained by FISH, whereas the sensitivity of cytology was 71.8%. As a result, we successfully detected seven lung cancer cases that were cytology-negative. Among these cases, two were class IIIa that we could not diagnose as malignant based on morphologic features. FISH may provide decisive information for the detection of malignancies, especially cases with IIIa classification.

Although the sensitivity of FISH is superior to that of conventional cytology, there are some disadvantages to FISH analyses. First, we do not generate information about the histologic type of lung cancer since we cannot observe morphologic features. Second, FISH is expensive. Third, FISH signal counting under fluorescence microscopy is time-consuming. Thus, the present FISH assay system probably can play a complementary role to that of conventional cytology.

We had five cases that we could not correctly diagnose by FISH. There are two possible reasons for our false-negative FISH results. One would be the failure to obtain proper cell material from the lesion, resulting in the absence of cancer cells on the slide. The other would be that the cancer cells were near-diploid, such that we could not detect aneusomy in two target chromosomes. We could probably detect more aneusomic cells using additional suitable probes for other chromosomes or chromosomal regions as reported by Romeo et al,[22] who successfully diagnosed 100% of lung cancer cases by FISH using a set of four probes. In our previous study,[23] chromosomal instability detected by FISH was associated with poor survival in patients with lung cancer. The finding of multiple chromosomal changes by FISH may be used as a prognostic factor and in the selection of patients for different therapeutic programs in the future.

In conclusion, FISH can detect lung cancer cells with aneusomy in various clinical specimens. The sensitivity was superior to that of conventional cytology. FISH should be used in conjunction with conventional cytology.

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Microwave Coagulation Therapy in Canine Peripheral Lung Tissue[1]

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Background. **New modalities for local treatments that destroy tumor effectively but which are less invasive and less damaging to normal lung tissue must be developed for patients who are unable to undergo even video-assisted thoracic surgery (VATS) due to poor cardiopulmonary function, severe adhesion, or advanced age, etc. We evaluated the use of microwave coagulation therapy (MCT), which has been used successfully for coagulation of hepatic tumors, in normal canine lung tissue to evaluate its efficacy and safety.**

Materials and methods. **Measurements of thermal response and coagulation area and histological examinations after microwave coagulation were performed in normal canine lung tissue.**

Results. **The temperature in normal canine lung tissue increased to 90–100°C at 5 mm from the electrode after 60 s and 70–80°C at 10 mm after 90 s at 40 or 60 W. The coagulation area was approximately 20 mm in diameter at 40 W and 60 W. Histological analysis demonstrated thickening of collagen fiber shortly after coagulation, stromal edema and granulation tissue after 3 months, and, finally, scar tissue was seen after 6 months.**

Conclusions. **Microwave coagulation therapy (MCT) is a useful modality for minimally invasive therapy in peripheral lung tumors.**

Key Words: **microwave coagulation; MCT, PMCT, ablation; lung tumor; peripheral lung cancer.**

INTRODUCTION

Recently, the problem of population aging on a global scale is calling for minimally invasive therapies providing good quality of life (QOL) and activity of daily living (ADL). Many investigators are looking into the problems of poor cardiopulmonary function as a result of advanced age, previous surgery, and/or synchronous or metachronous carcinoma. Meanwhile, the detection rate of early-stage carcinoma or precancerous lesions has increased due to recent advances in medical technology. In the field of chest diseases, the detection rate of tiny tumors in the peripheral lung, such as early-stage lung cancer, small metastases, or atypical adenomatous hyperplasia (AAH) has increased with the increasing use of high-resolution CT scans. Video-assisted thoracic surgery (VATS) usually is used for many of these cases. However, we believe that less-invasive therapy is necessary for patients who are inoperable due to poor cardiopulmonary function, severe adhesion, or advanced age.

There is, therefore, a need for local treatment that effectively destroys tumor but is minimally invasive and less damaging to normal tissue than surgery. In the present study, we focused on microwave coagulation therapy (MCT), which has successfully been used to coagulate hepatic tumors [1–4]. The mechanism of coagulation is dielectric heating, *i.e.*, frictional heat of water molecules. Since the dielectric heat energy cannot be generated in the presence of air, selective tumor damage may be achieved and damage to the surrounding normal air-filled lung tissue may be limited. To assess the application of PMCT for lung tumors, we evaluated its efficacy and safety in experimental studies.

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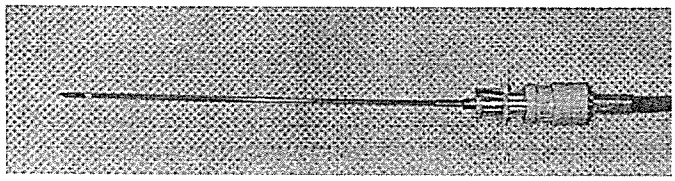


FIG. 1. A specially designed single-needle electrode, 150 mm in length and 1.6 mm in diameter, was inserted 20 mm into the normal lung.

MATERIALS AND METHODS

Measurements of Thermal Response

Animal studies were performed with the approval of the Institutional Committee for Ethical Research Animal Care. Adult beagles (10-15 kg) were given artificial respiration under general anesthesia with 30 mg/kg of phentobarbital sodium intravenously and placed in the left lateral recumbent position. Using an aseptic technique, thoracotomy was performed through the 5th intercostal space. A specially designed single-needle electrode (MD-16CBT-10/150, Azwell, Osaka, Japan, Fig. 1) that is 150 mm in length and 1.6 mm in diameter was inserted 20 mm into the normal lung. Then, tissue coagulation was performed using a microwave generator (Microtaze HSD-20W, Azwell, Fig. 2) that emitted 2450 MHz microwaves of 12 cm wavelength at a power output of 20, 40, and 60 W for 4 min. Temperature change was continuously monitored for 4 min using a K-type electric thermometer at 5 mm and 10 mm from the electrode with a sensor inserted 10 mm into the normal lung. The data of temperature were plotted for every 15 s. Measurements of temperature change were performed three times in each condition. Three beagles were used for this study.

Measurements of Coagulation Area

Microwave electrodes were inserted into normal lung tissue of beagles using the same procedure as mentioned above. Microwave coagulation was performed three times under each condition at power outputs of 20, 40, and 60 W for 1, 2, 3, and 4 min. Three beagles were used for this study. Shortly after microwave coagulation, the beagles were euthanized with an intravenous phentobarbital sodium overdose and pneumonectomy was performed. The resected canine lungs were inflated with bubbling air and 10% buffered formalin from the bronchial stump using an enema syringe pump and preserved in 10% buffered formalin for tissue fixation.

Under the same conditions, microwave coagulation was performed for normal human fresh lung tissue after resection of central type lung carcinoma, inflated with bubbling air using an enema syringe pump from the bronchial stump. Coagulation was performed once under each condition using two fresh lung lobes after resections. Informed consent was obtained in all cases. Tissue fixation was performed in the same manner as in the animal experiment.

The fixed lung tissue was transected perpendicular to the direction of the inserted electrode. The longest dimension of the maximum coagulation area of fixed lung tissue was measured.

Histological Examinations after Microwave Coagulation

Microwave coagulations were performed in three beagles at a power output of 40 W for 3 min. One beagle was euthanized with an intravenous phentobarbital sodium overdose immediately, and the other two beagles were followed up to assess histological change of the coagulated tissue. The normal activity and condition of each beagle was monitored daily. These beagles were euthanized at 3 and 6 months after the procedure. Histological changes of normal lung tissue immediately, 3 and 6 months after microwave coagulation were investigated by H-E staining and Elastica von Gieson staining.

Statistical Analysis

Data were expressed as means ± standard deviations (SD), and statistical analyses were done using Student's t test with computer software (Microsoft Excel, version 2002). A P value of less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Measurements of Thermal Response

The data of thermal response of normal canine lung tissue to microwave coagulation is shown in Fig. 3. At 5 mm from the electrode (Fig. 3A), the temperature rose rapidly over 80°C (15 s for 60 W: 83.1 ± 13.0°C; 30 s for 40 W: 83.9 ± 3.4°C), and thereafter the temperature reached a plateau around 90-100°C at both 40 and 60 W. There was no significant difference between the two groups for each time point. At 20 W, the temperature rose gradually to only 65°C and reached a plateau. At 10 mm from the electrode (Fig. 3B), the temperature rose gradually to 70°C (45 s for 60 W: 70.6 ± 11.6°C; 90 s for 40 W: 70.9 ± 13.0°C), and thereafter the temperature reached a plateau around 80°C at 40 and 60 W. There was no significant difference between the two groups for each time point. At 20 W, the temperature rose to only 50°C after 90 s and reached a plateau. It appeared that 20 W was not enough for coagulation. The same thermal re-

FIG. 2. Microwave coagulation was performed using a microwave generator that emitted 2450 MHz microwaves of 12 cm wavelength at a power output of 20, 40, and 60 W.

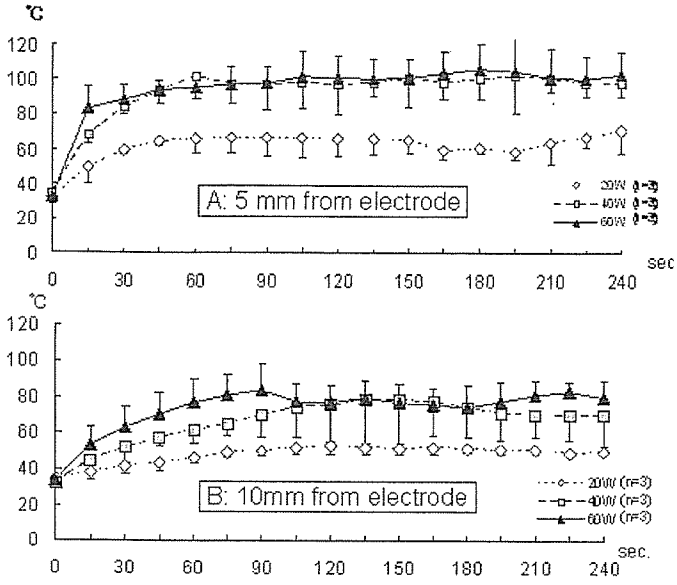


FIG. 3. (A) At 5 mm from the electrode, the temperature rose rapidly to 85°C (for 15 s at 60 W and 30 s at 40 W). The temperature reached a plateau around 90-100°C at 40 and 60 W. There was no significant difference between 40 and 60 W for each time point. (B) At 10 mm from the electrode, the temperature rose gradually to 70°C (45 s at 60 W and 90 s at 40 W). The temperature reached a plateau around 80°C at 40 and 60 W. There was no significant difference between 40 and 60 W for each time point.

sponse was obtained in the two groups of 40 W and 60 W at same distances from the electrode.

Measurements of Coagulation Area

The data of the diameter of the maximum coagulation area in the animal model is shown in Fig. 4. The maximum coagulation area increased with increased power and coagulation time. The diameter of the maximum coagulation area was 18.3 ± 10.4 mm at 40 W for 4 min and 21.7 ± 2.9 mm at 60 W for 4 min. There was no significant difference in the coagulation area at each time period when using 40 W and 60 W.

The diameter of the maximum coagulation area in normal human fresh lung tissue after resection of central-type lung cancer is shown in Fig. 5. The maximum coagulation area increased with increased power and coagulation time until 3 min. After 3 min, the diameter of the maximum coagulation area was 25 mm at 40 W and 26 mm at 60 W. At 40 and 60 W for 4 min, the diameter of the maximum coagulation area shrank to 15 mm. There was no difference between 40 and 60 W for each period of coagulation.

Histological Examinations after Microwave Coagulation

All beagles tolerated the procedure well. During 6 months of follow up to assess histological change of the coagulated tissue, the normal activity and condition of each beagle was monitored daily. There was no death due to serious complications such as hemoptysis or pneumothorax during the period.

The histological changes after microwave coagulation are shown in Fig. 6. Histological findings shortly after microwave coagulation showed degeneration and thickening of collagen fiber and exfoliation and ulceration of bronchial epithelium surrounding the electrode. No surrounding bronchioli or veins were destroyed. No blood clots or debris were observed in surrounding veins (Fig. 6A). After 3 months, histological findings showed stromal edema and loose collagen fiber, immature neoangiogenesis, progression of bronchial epithelial hyperplasia, infiltration of inflammatory cells at the boundary zone (lymphocyte > plasma cell > neutrophil) between the central coagulation area and normal tissue (Fig. 6B). After 6 months, coagulated tissue became scar tissue that showed disappearance of stromal edema, tight collagen fiber, mature capillaries, disappearance of inflammatory cells, and completion of epithelial hyperplasia (Fig. 6C).

DISCUSSION

With the increasing use of high-resolution CT scans, the rate detection of small nodules in the peripheral lung, such as early-stage lung cancer, small metastases, or AAH has increased. Kaneko *et al.* demonstrated that the detection rate of peripheral lung carcinoma by mass screening using CT scan was 0.45% (15 of 3457 examinations), 73% of which were detected by low-dose spiral CT but were not visible on standard chest radiography [5]. Noguchi *et al.* investigated 236 surgically resected small-size peripheral adenocarcinomas measuring 2.0 cm or less in greatest dimension and demonstrated that type A (localized bronchioloalveolar carcinoma: LBAC) and type B (LBAC with foci of structural collapse of alveoli) that showed ground glass opacity (GGO) on CT scanning images demonstrated

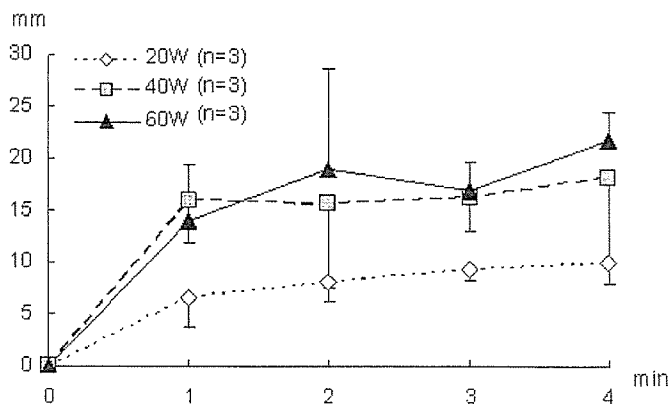
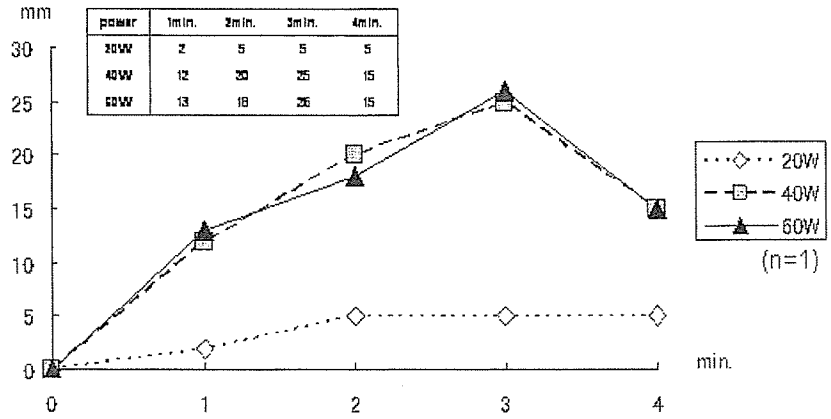


FIG. 4. The diameter of the maximum coagulation area was 18 mm at 40 W for 4 min and 22 mm at 60 W for 4 min. There was no significant difference between 40 W and 60 W for each coagulation time.



power	1min.	2min.	3min.	4min.
20W	2	5	5	5
40W	12	20	25	15
60W	13	18	26	15

FIG. 5. The diameter of the maximum coagulation area was 25 mm at 40 W and 60 W for 3 min. There was no difference between 40 and 60 W for each time. The diameter of the maximum coagulation area shrank to 15 mm at 40 and 60 W for 4 min.

no lymph node metastasis and had the best 5-year survival rate (100%) [6]. Meanwhile, according to a pathological study on lymph node metastasis of primary lung carcinoma after surgery, the rate of metastasis (N1 and N2) was 0% with a tumor 1.0 cm or less, 17% with a tumor 1.1 to 2.0 cm, and 37% with a tumor 2.1 to 3.0 cm in diameter [7]. As a result, it seems to be possible to control 83% of lung carcinomas smaller than 2.0 cm in size by local treatment. Also, the rate of local lymph node metastasis of metastatic lung tumor is known to be low.

We usually perform surgery for such patients as a possible cure, but this may lead to considerable lung damage with significant loss of function. Although VATS has widened the indication of surgery recently, some patients are unable to undergo even VATS due to poor cardiopulmonary function, severe adhesion, or advanced age, etc. Radical radiotherapy or chemotherapy or both may be offered with curative intent to such patients, but the prospect of cure is substantially worse than with surgical options, while it may also lead to considerable lung damage with significant loss of function caused by radiation fibrosis, systemic toxicity due to the anticancer agent. Therefore, new modalities for local treatment that effectively destroy tumor but are less invasive and less damaging to normal lung tissue are required. Recently, several investigators tried to use radiofrequency ablation (RFA) [8–10] or photodynamic therapy (PDT) for peripheral lung tumors [11, 12].

We are interested in microwave coagulation therapy (MCT), which has been successfully used to perform coagulation of hepatic tumors. In 1978, hepatic surgery with MCT was introduced by Tabuse [1], and recently the effectiveness of percutaneous microwave coagulation therapy (PMCT) under ultrasonography or CT scan guidance for small hepatocellular carcinoma was demonstrated [3, 4]. We considered this modality to be applicable for patients with lung tumors who are poor surgical candidates, as well as patients with hepatic tumors, and evaluated the efficacy and safety of MCT for lung tissue experimentally.

The microwave generator emits a higher frequency wavelength than electrocautery and generates dielectric heat energy due to friction of water molecules when irradiating living tissue. MCT applies this mechanism to achieve tumor necrosis. Because the dielectric heat energy cannot be generated in the presence of air, selective tumor damage may be achieved, with limited damage to the surrounding normal air-filled lung tissue. We considered it essential to know how MCT affects normal lung tissue before performing PMCT clinically for peripheral lung malignancies. An experimental study was deemed necessary to evaluate the thermal response, coagulation extent, and histological changes in the air-filled normal lung.

With regard to thermal response, the temperatures of normal canine lung tissue rose with increased microwave power and coagulation time. The temperatures in normal lung tissue rose to 90-100°C at 5 mm from the electrode after 60 s and 70-80°C at 10 mm after 90 s, thereafter reaching a plateau. A power of 20 W was not sufficient to coagulate lung tissue. These data suggested that the same thermal response could be obtained at 40 and 60 W. The coagulation area in normal canine lung tissue increased to 18 mm and 22 mm at 40 W and 60 W for 4 min, respectively. Therefore, it may be possible to coagulate a diameter of approximately 20 mm. In solid tumors, there is a possibility to achieve more extensive coagulation. In human resected normal lung with central-type lung carcinoma, the coagulation area increased to 25 mm at 40 and 60 W for 3 min and shrank to 15 mm for 4 min. This phenomenon may be explained by shrinking of lung tissue due to rapid elevation of the temperature in the tissue, because there is no radiator effect in the resected lung due to the lack of blood supply. From the current study, we concluded the optimal condition in clinical PMCT to be 40-60 W for 3–4 min of coagulation.

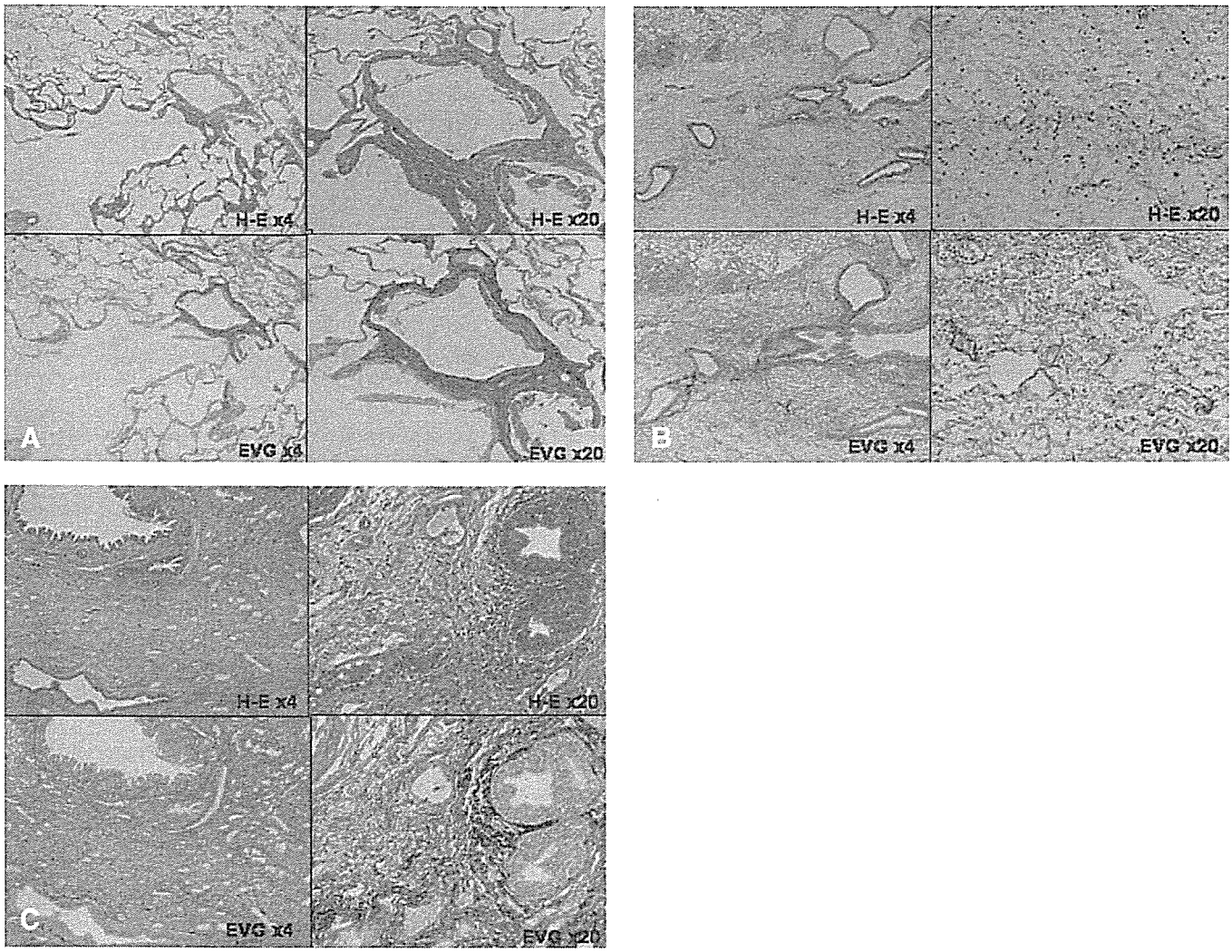


FIG. 6. (A) Histological findings shortly after microwave coagulation showed degeneration and thickening of collagen fiber and exfoliation and ulceration of bronchial epithelium surrounding the electrode. Surrounding bronchial and veins were not destroyed. (B) After 3 months, histological findings showed stromal edema and loose collagen fiber, immature neoangiogenesis, progression of bronchiolus epithelial hyperplasia, infiltration of inflammatory cells at the boundary zone between the central coagulation area and normal tissue. (C) After 6 months, coagulated tissue became scar tissue that showed disappearance of stromal edema, tight collagen fiber, mature capillaries, disappearance of inflammatory cells and completion of epithelial hyperplasia. (Color version of figure is available online.)

Histological analysis following MCT for normal canine lung tissue demonstrated exfoliation and ulcer formation of the epithelium in the bronchioli and degeneration and thickening of collagen fiber in the parenchyma by heat coagulation shortly after MCT. The coagulated lesions were gradually repaired by progression of epithelial hyperplasia and infiltration of inflammatory cells, showing stromal edema and granulation tissue after 3 months and finally becoming scar tissue after 6 months. We concluded MCT to be a safe modality for lung tissue because no destruction of bronchioles or veins was seen in the specimens during 6 months.

The present studies of MCT for peripheral lung tissue demonstrated that this new modality had no serious adverse effects and could be performed safely. However, the incidence of pneumothorax by CT-guided RFA was demonstrated to be 38.5% (3/8) in a rabbit model [8] and 33.3% (1/3) and 53.8% (7/13) in clinical cases [9, 10], which seems to be relatively high. Therefore, the development of a fine electrode with a cooling system will be necessary to prevent complications such as pneumothorax and heat sensation for clinical use.

From our experimental studies, the advantages of PMCT are the fact that this modality is minimally invasive, may be performed by local anesthesia, and is applicable for patients with poor cardiopulmonary function. In addition, the microwave generator is a very simple device, maintenance free, easy to handle, and portable, and the procedure is easy compared with RFA and PDT. The possibility of pneumothorax, heat

sensation or pain, or both during treatment and the limited coagulation area are considered the disadvantages for clinical use at present. Nevertheless, our results demonstrated the possibility of MCT for patients with small peripheral lung tumors with the intent of curative treatment. Although MCT is considered to be a useful modality as minimally invasive therapy for small peripheral lung tumors, further comparative research is necessary with other modalities such as RFA and PDT for peripheral lung tumors.

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Frequent loss of E-cadherin and/or catenins in intrabronchial lesions during carcinogenesis of the bronchial epithelium

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Plakoglobin;
Intrabronchial lesions;
Early-stage bronchial squamous cell carcinoma;
Immunohistochemistry

Summary Inactivation of the cadherin-mediated cell–cell adhesion system is believed to play a role in the initial steps of cancer invasion and metastasis. Expression of E-cadherin and its intracytoplasmic binding molecules (α-catenin, β-catenin, and plakoglobin) was examined immunohistochemically in 84 cases of intrabronchial precancerous lesions (bronchial squamous metaplasia (BSM) without atypia, BSM with atypia, dysplasia), and 21 cases of carcinoma in situ, and 4 cases of microinvasion to the bronchial wall, and 32 cases of stage I well differentiated squamous cell carcinoma (squamous cell carcinoma) to investigate the association between expression of E-cadherin and/or catenins and cancer progression. Reduced expression of E-cadherin and/or catenins was closely correlated with an atypical grade of dysplasia in the basal layer ($p < 0.05$). In particular, downregulation of E-cadherin and/or catenins was associated with an atypical grade of BSM with atypia in intrabronchial lesions ($p < 0.01$). We conclude that downregulation of α-catenin and/or β-catenin, which may reflect dysfunction of the cadherin-mediated cell–cell adhesion system, is an important marker for atypical grade during carcinogenesis of the bronchial epithelium.

1. Introduction

Cadherins are a family of cell–cell adhesion molecules that are essential for tight junctions

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