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Postoperative adjuvant therapy for completely resected early-stage non-small cell lung cancer

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Abstract Consensus on adjuvant therapy for completely resected non-small cell lung cancer until 2002 was as follows. (1) There was no significant impact of postoperative adjuvant chemotherapy based on meta-analysis and previous clinical trials. (2) Confirmatory studies are necessary in large-scale prospective clinical trials. However, recent mega trials have introduced epoch-making changes for postoperative adjuvant chemotherapy in clinical practice since ASCO 2003. The effectiveness of UFT in N0 patients was confirmed. Patients with completely resected stage I non-small cell lung cancer, especially T2N0 adenocarcinoma, will benefit from adjuvant chemotherapy with UFT. The results of the International Adjuvant Lung Trial (IALT) have confirmed the meta-analysis in 1995. Also, both the JBR10 and Cancer and Leukemia Group B (CALGB) 9633 studies have also confirmed positive IALT results of the benefit for postoperative platinum-based chemotherapy in completely resected non-small cell lung cancer. Adjuvant chemotherapy for pathological stage IB to II, completely resected non-small cell lung cancer is standard care based on clinical trials. UFT showed the strongest evidence for IB in Japan. Platinum doublet chemotherapy with third-generation anticancer agents is also recommended. Adjuvant chemotherapy should be offered as standard care to patients after completely resected early stage non-small cell lung cancer. However, there is no evidence of the feasibility and efficacy for adjuvant chemotherapy with the platinum-based regimen in Japan. Careful management should be necessary in such treatment.

Key words Adjuvant therapy · Chemotherapy · Surgery · Non-small cell lung cancer · Early-stage lung cancer

Introduction

The 5-year survival rate after surgical treatment in the United States and Japan in each stage of non-small cell lung cancer is shown in Table 1.[1,2] Although these surgical outcomes reveal the slight difference among two groups, we are not satisfied with the results, particularly in stage IB and II, which are so-called early stage. Surgery is still the best therapeutic modality for the potential cure of the patient with non-small cell lung cancer, especially in stage I. However, in the patient with pathological stage IB, the 5-year survival rate is only about 60%. The recurrence pattern is frequently due to distant metastasis.[3] Therefore, perioperative adjuvant therapy is required for improvement of survival after surgical resection.

Meta-analysis of the randomized trials of adjuvant therapy of non-small cell lung cancer in 1995 suggested the survival benefit of cisplatin-based chemotherapy after surgery.[4] However, there are no statistical differences between postoperative adjuvant group and surgery alone,[4] and this includes a number of small trials and trials with the following disability criteria and chemotherapy regimens.

Consensus on adjuvant therapy for completely resected non-small cell lung cancer up to 2002 was as follows: (1) there was no significant impact of postoperative adjuvant chemotherapy based on meta-analysis and previous clinical trials. (2) Confirmatory studies are necessary in large-scale prospective clinical trials.[5–10] However, recent mega trials have introduced epoch-making changes for postoperative adjuvant chemotherapy in clinical practice since ASCO 2003.[11–19]

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Table 1. The surgical outcome for resected non-small cell lung cancer: 5-year survival rate (%)

Stage	Clinical staging		Pathological staging	
	Mountain	Japan	Mountain	Japan
IA	61	72.1	67	79.5
IB	38	49.9	57	60.1
IIA	34	48.7	55	59.9
IIB	24	40.6	39	42.2
IIIA	22	35.8	38	29.8
IIIB	9	28.0	3–7	19.3
IV	13	20.9	1	20.0

The new paradigm shift for the adjuvant treatment after surgery is demonstrated here by the Japanese and international trials that have been reported since 2003.

Japanese trials

A large-scale randomized phase III study of postoperative adjuvant chemotherapy with UFT for p-stage I adenocarcinoma: the JLCRG (Japan Lung Cancer Research Group) trial (presented in ASCO 2003[13])

The oral antimetabolite UFT is composed of tegafur and uracil mixed at the ratio of 1:4. This drug has been developed by Taiho Pharmaceutical Corporation, Tokyo, Japan. UFT produced higher levels of 5-FU without the toxic level of 5-FU.

Concerning adjuvant treatment using UFT, the West Japan Study Group for Lung Cancer Surgery reported that postoperative adjuvant treatment with UFT in patients with completely resected stage I–III disease prolonged survival significantly longer than observation alone. The 5-year and 10-year survival rates were 64% and 48% in the UFT group, and 49% and 32% in the control group, respectively (P = 0.02).[11] In a subgroup analysis, no statistically significant difference in the overall survival of patients with squamous cell carcinoma between the two groups was observed (P = 0.24). In contrast, the patients with adenocarcinoma in the UFT group had a significantly better survival than those in the control group (P = 0.009).[12] In addition, most patients with adenocarcinoma had stage I disease. This trial demonstrated that UFT was useful in postoperative adjuvant chemotherapy against the earlier stage of non-small cell lung cancer. However, this study involved issues with respect to study design, because enrolled subjects varied from stage I to III, with a broad range of outcome. Those results prompted us to conduct a prospective randomized trial of UFT as a postoperative adjuvant treatment for patients whose stage I adenocarcinoma was completely resected. In the confirmatory study conducted by the Japan Lung Cancer Research Group (JLCRG), patients with completely resected pathological stage I adenocarcinoma of the lung were randomized with stratification according to their pathological T status (T1 versus T2), gender, and age, which were separated between less than 65 years old and 65 years old or over, to either receive the oral administration of UFT (tegafur 250 mg/m^2/day) for 2 years or no treatment. The patients with limited resection, such as wedge resection, were excluded. A follow-up examination was performed every 3 months for the first 2 years after the patient's operation and every 6 months thereafter. The primary endpoint was overall survival.

From January 1994, through March 1997, 999 patients were entered into the study. Twenty patients withdrew their informed consent or were found to be ineligible before the start of treatment. The number of eligible randomized patients was 491 in the UFT group and 488 in the nontreatment control group. Main patient characteristics were as follows: men, 48.7%; more than 65 years old, 43.9%; pathological T1, 73.1%. There were no significant differences in the baseline characteristics of the patients. The median duration of follow-up for all 979 patients was 73 months, with range 61–94 months.

Few severe adverse reactions were associated with UFT administration. There was no grade 4 adverse reaction. In total, 10 (2%) of 482 patients developed a grade 3 adverse reaction. The percentage of compliance for UFT administration was calculated based on the number of patients who actually took UFT and the number of patients without recurrence, second cancer, or death who were expected to take UFT. The percentage of compliance was 80% [95% confidence interval (CI), 77%–84%) at 6 months, 74% (95% CI, 70%–78%) at 12 months, 69% (95% CI, 65%–73%) at 18 months, and 61% (95% CI, 77%–84%) at 24 months. The main reasons for discontinuation of UFT administration were as follows: an adverse reaction in 123 patients, patient refusal in 52, and the doctor's judgment in 34.

Overall survival between the two groups showed a statistically significant difference in favor of the UFT group based on a Kaplan–Meier analysis (P = 0.04). The 5-year survival rate (5YS) was 87.9% in the UFT group and 85.4% in the control group, respectively. Treatment failure was documented in 22.6% of the patients in the UFT group and 26.4% in the control group, respectively. The most frequent failure pattern was distant metastasis in both groups. The 5-year cancer-free survival rate was 82.8% in the UFT group and 80.4% in the control group. There is no significant difference between the two groups at P = 0.25.

Concerning subset analysis of pathological T factors, although there was no statistical difference in the T1 population, in the T2 subset, the 5-year survival rate was 84.9% in the UFT group and 73.5% in the control group. The hazard ratio was 0.0842 in the UFT group with a clear statistical difference (P = 0.0051). Concerning interaction in relation to treatment effect, treatment with UFT tended to improve the survival rate among the patients with tumors that were 2–3 cm in diameter and provided 30% survival benefit for patients with tumor that was more than 3 cm in diameter. These findings indicated that the effect of UFT might be related to certain biological factors.

In conclusion, oral demonstration with UFT in the postoperative adjuvant setting yielded a significant improvement in survival in patients with pathological stage I adenocarcinoma of the lung, particularly in stage 1B, T2 N0 M0. These results of this study may be able to confirm the previous UFT adjuvant trial.

Meta-analysis of six randomized adjuvant trials with UFT (presented at ASCO 2004[14])

Clinical trials assessing the response of non-small cell lung cancer to postoperative adjuvant chemotherapy should use survival as the primary endpoint. Response should be evaluated by means of randomized controlled studies using surgical therapy alone as control. Single studies usually do not provide clear-cut conclusions because of limited sample size. A meta-analysis of all properly randomized clinical trials comparing long-term adjuvant chemotherapy with UFT, an oral fluorinated pyrimidine derivative, with surgery alone in patients with completely resected non-small cell lung cancer was demonstrated.

Six randomized trials have been conducted that compare surgery alone with adjuvant chemotherapy with UFT. The analysis was based on individual patient data provided by the principal investigator of each trial. In data from 2003, eligible patients were analyzed on an intention-to-treat bias. The endpoint of interest was overall survival at 5 years after surgery. Major prognostic factors were well balanced between the UFT group and surgery-alone group. Most patients had early-stage non-small cell lung cancer. The distribution of pathological T1 and T2 stages among this population was 65% and 34%, respectively.

The 5-year overall survival rate and 7-year overall survival rate were 81.8% and 76.5% and 77.2% in the control group; 7-year overall survival rates were 81.8% and 76.5% and 77.2% in the control group, and 7-year overall survival was 69.5% for the surgery-alone group. The result of meta-analysis demonstrated that adjuvant chemotherapy with UFT significantly improved the overall 5-year survival rate, with hazard ratio (HR) 0.77 (95% CI, 0.63–0.94; $P = 0.011$). Heterogeneity of effect among the six studies was not significant ($P = 0.76$).

The subset analysis of this meta-analysis indicated that UFT treatment provided a definitive survival benefit in most of the subset. This meta-analysis of the T1 subset population demonstrated that treatment with UFT provided a definitive survival benefit for patients with tumor that was 2–3 cm in diameter. Therefore, on the basis of our meta-analysis, postoperative adjuvant chemotherapy with UFT has a beneficial effect on outcome in patient with curatively resected non-small cell lung cancer more than 2 cm in size. Recently, Dr. Hotta from Okayama University has also demonstrated the benefit of UFT in the postoperative adjuvant setting based on the meta-analysis of five abstracts regarding UFT adjuvant trials (HR, 0.799; 95% CI, 0.668–0.957, $P = 0.015$).[15] These results seem to confirm the previous Hamada data.

A randomized phase III study for Bestatin (Ubenimex) as postoperative adjuvant treatment in patients with stage I squamous cell lung cancer (presented at ASCO 2001[16])

In a placebo-controlled phase III trial sponsored by the Japanese NK421 Lung Cancer Study Group, the more derived immunomodulator Bestatin (Ubenimex) was used as adjuvant therapy for patients with stage I squamous cell carcinoma following completed resection.

Confirmation of the patient eligibility and the randomization were performed within 4 weeks after each operation. The oral administration started within 1 week after their randomization. One capsule of either Bestatin or placebo was administered orally after breakfast every day for 2 years postoperatively. No additional treatment was allowed until definitive recurrence or appearance of second cancer was diagnosed.

A follow-up examination was performed every 3 months for 2 years after operation and every 6 months thereafter. The primary endpoint of the study was overall survival, and the second endpoint was disease-free survival and safe assessment. The number of patients was 202 in the Bestatin group and 198 in the placebo group. There is no significant difference in baseline characteristic of patients; 97.6% and 96.3% of the projected dose of Bestatin and placebo were administered, respectively.

The median duration follow-up for 400 patients was 77 months. Overall 5-year survival rate was significantly increased for patients receiving Bestatin compared with those receiving placebo. Disease-free survival was also significantly higher in the Bestatin group compared with placebo group, 71% versus 62%. According to multivariate analysis for survival, significant prognostic factors were performance status (PS), blood transfusion, and treatment arm.

Short summary and consideration of Japanese adjuvant trials

A couple of randomized clinical trials have demonstrated survival advantage in patients predominantly with no lymph metastasis. The effectiveness of UFT in N0 patients was confirmed. The patients with completely resected stage I non-small cell lung cancer, especially T2N0 adenocarcinoma, will benefit from adjuvant chemotherapy with UFT. UFT provides 2.5% (T2, 11.4%) benefit for absolute 5-year survival rate. HR for death in patients with stage I and T2 was 0.706 and 0.48, respectively.

Future issues for UFT adjuvant chemotherapy are to be considered as follows: (1) Do patients with stage II and/or stage III disease benefit from UFT adjuvant therapy? (2) Which regimen is better, UFT or platinum-based doublet chemotherapy in the patient with stage IB and II or III? (3) Is treatment for 1 year equivalent to treatment for 2 years? (4) What is the mechanism of UFT effectiveness in the adjuvant setting? and (5) There is need for confirmatory studies in other countries. As for Bestatin, it is necessary to do another confirmatory clinical trial.

Table 2. Potential functional mechanism of UFT and bestatin

	UFT	Bestatin
Production	5-FU derivative; tegafur and uracil	Culture filtrate of *Streptomyces olivoleticuli*
Basic concept	Antimetabolic drug	Immunomodulator
Anticancer effect (possibility)	Biochemical moduration, incidence of apotosis, inhibition of angiogenesis	Inhibition of angiogenesis Introduction of apotopsis

Table 3. The efficacy of the postoperative adjuvant chemotherapy for non-small cell lung cancer based on the pathological stage

	IALT	JBR 10	CALGB 9633	JLCRG/UFT
p-stage I	Negative	Positive (IB)	Positive (IB)	Positive
p-stage II	Negative	Positive		
p-stage IIIA	Positive			
Survival benefit[a]	4.1%	15%	12% (4-year survival)	2.5% IB 11%
HR	0.86	0.70	0.62	0.71 IB 0.48
95% CI	0.76–0.98	0.52–0.92	0.41–0.95	0.51–0.98 IB 0.29–0.81

Positive, 10% improvement for the hazard ratio
HR, hazard ratio; 95% CI, 95% confidential interval
[a] Absolute difference of the 5-year survival rate between the adjuvant group and the surgery-alone group

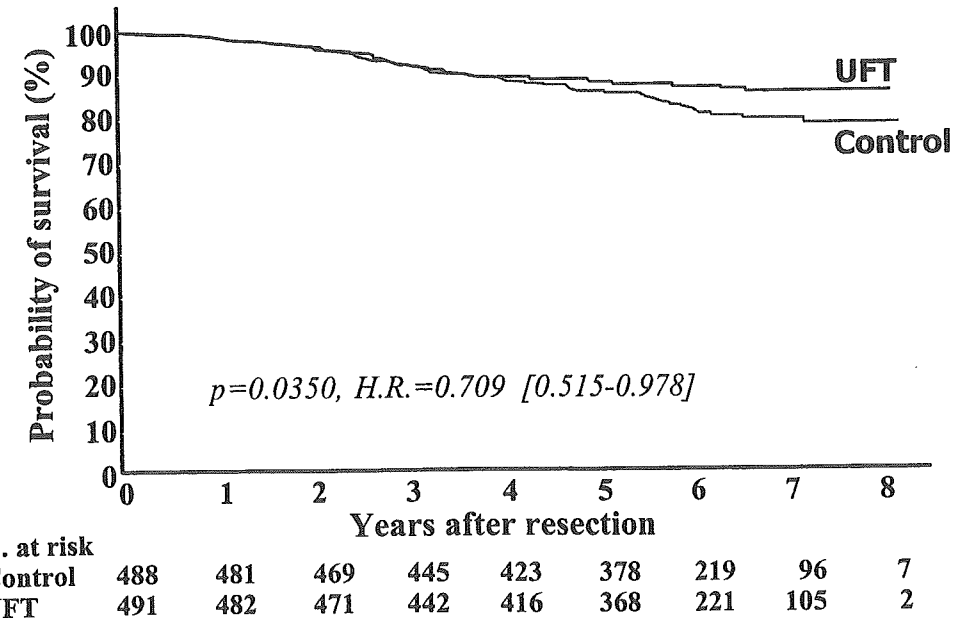

Fig. 1. Overall survival among all 979 eligible patients in the Japan Lung Cancer Research Group (JLCRG) trial. The hazard ratios indicate the risk of death in the UFT group as compared with the control group; 95% confidential intervals are shown in *brackets*. UFT, uracil-tegafur (From ref. 13 with permission)

On the basis of the comparison of mechanism between UFT and Bestatin, both drugs have been shown to inhibit angiogenesis and induce apoptosis in vivo and in vitro (Table 2). Although these data should be confirmed in future, the administration of a less cytotoxic agent and/or cytostatic drug in the adjuvant setting may improve survival for patients with early-stage non-small cell lung cancer.

Brief results of international trials

International adjuvant lung trial (IALT) (presented at ASCO 2003[17])

On the basis of a previous meta-analysis, an international adjuvant lung cancer trial was designed to evaluate the effect of cisplatin-based adjuvant chemotherapy on survival after completely resection of non-small cell lung cancer. Patients were randomly assigned either to three or four

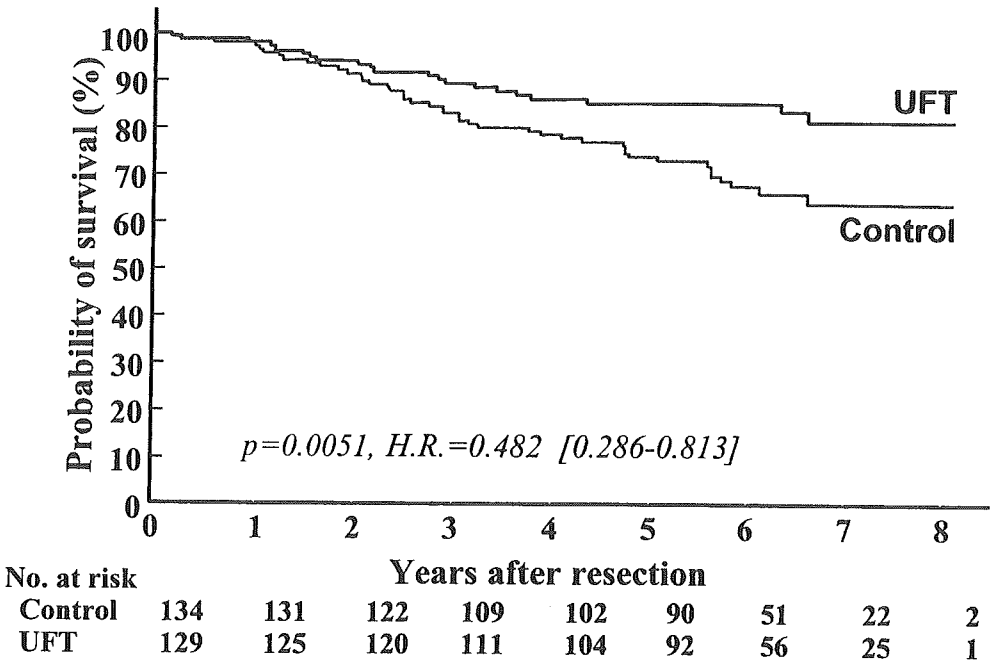

Fig. 2. Surgical outcome of T2 subset population in the JLCRG trial. UFT, uracil-tegafur (From ref. 13 with permission)

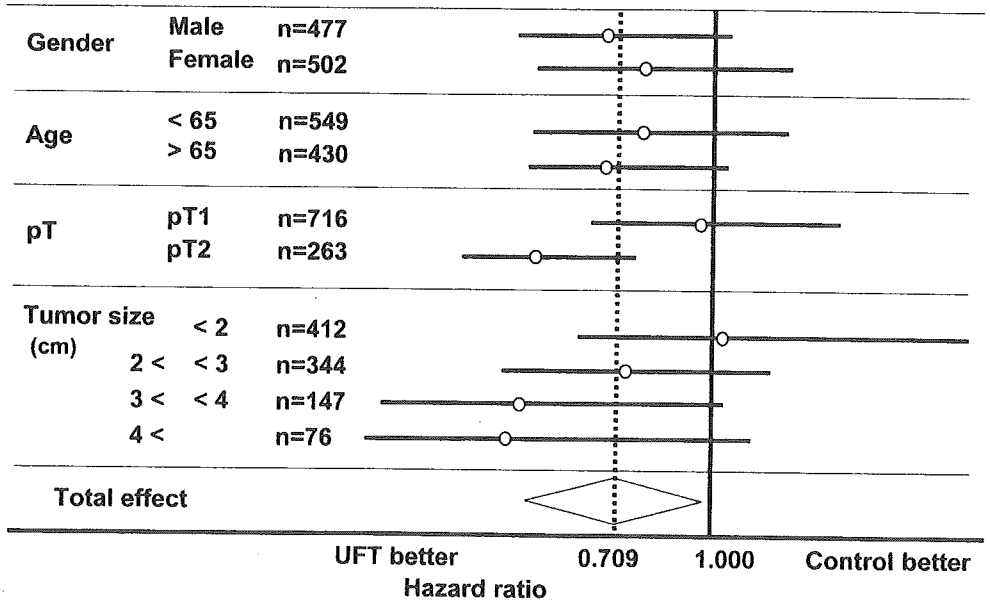

Fig. 3. Interaction in relation to treatment effect. Each *square* represents the estimated treatment effect, *horizontal lines* represent the 95% confidential intervals (CI), and the *diamond* corresponds to the 95% CI for the entire group of patients (From ref. 13 with permission)

cycles of cisplatin-based chemotherapy or to observation (without chemotherapy). Before randomization, in each center, time in the pathological stage to be included in its policy for chemotherapy and postoperative radiotherapy policy were determined. The main endpoint was overall survival.

A total of 1867 patients underwent randomization; 36.5% had pathological stage I disease, 24.2% stage II, and 39.3% stage III. The drug allocated with cisplatin was etoposide in 56.5% of patients, vindesine in 26.8%, vinblastine in 11%, and vindesine in 5.58%. Of the 932 patients assigned to chemotherapy, 73.8% received at least 240mg cisplatin per square meter of body surface area. In total, 23% of 932 patients developed a grade 4 adverse reaction. Seven patients (0.8%) died of chemotherapy-induced toxic effects. The median duration of follow-up was 56 months. Patients assigned to chemotherapy had a significant higher survival rate than those without chemotherapy (44.5% vs. 40.4% at 5 years; HR for death, 0.86; 95% CI, 0.76–0.93; $P < 0.003$). Disease-free survival rate was also significantly different between the two group (39.4% vs. 34.3% at 5 years; HR, 0.83; 95% CI, 0.74–0.94; $P < 0.003$). Seven patients (0.8%) died of chemotherapy-related toxic events. A total of 22.6% of the patients had at least one episode of

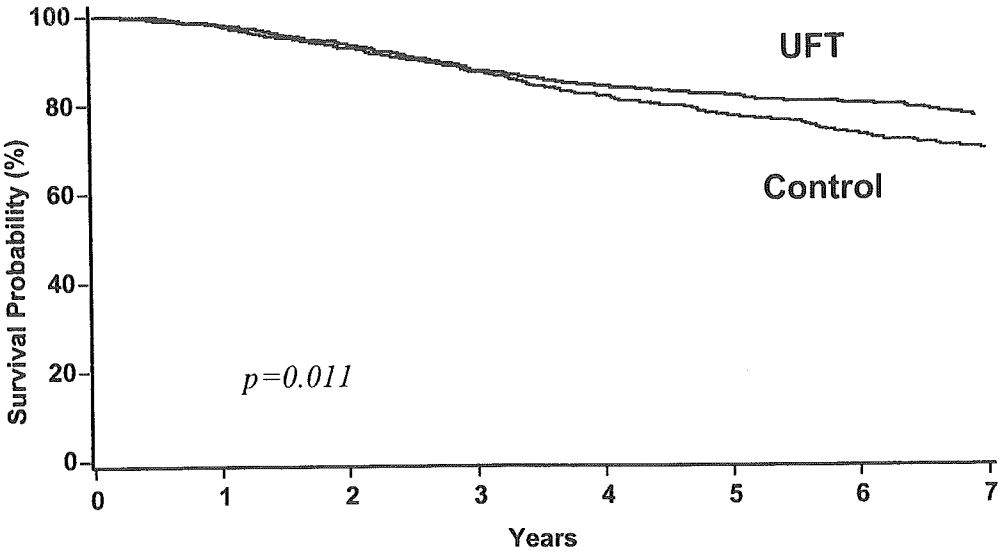

Fig. 4. Overall survival among all 2003 eligible patients in meta-analysis of six UFT trials. *P* values were calculated with stratified log-rank test (From ref. 14 with permission)

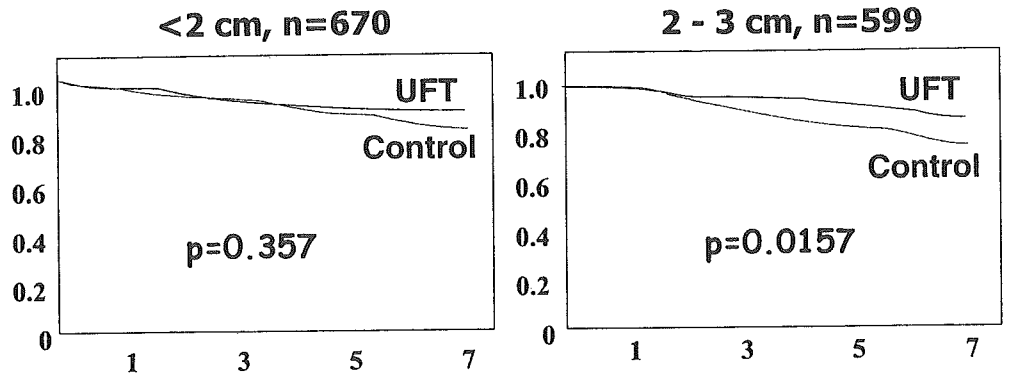

Fig. 5. Overall survival for exploratory analysis of T1 population (n = 1269) in UFT meta-analysis. *P* values were calculated with stratified log rank test (From ref. 14 with permission)

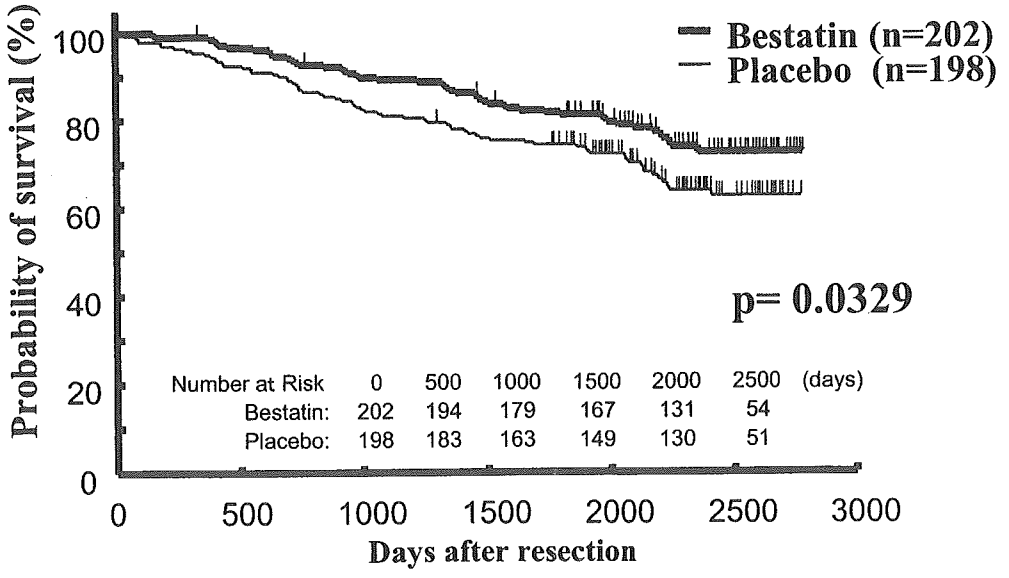

Fig. 6. Overall survival among all 400 eligible patients in Bestatin trial. *P* values were calculated with stratified log-rank test. (From ref. 16 with permission)

grade 4 toxicity, mainly neutropenia (17.5%), thrombocytopenia (2.6%), and vomiting (3.3%). These results have confirmed the meta-analysis in 1995.

NCI-Canada trial, JBR10 (presented at ASCO 2004[18])

Patients with p-stage IB and II except T3N0 were randomly assigned either to three or four cycles of cisplatin-based chemotherapy with cisplatin (50 mg/m^2, days 1, 8, every 4 weeks) or vinorelbine (25 mg/m^2, weekly to 16 weeks), or observation. A total of 344 patient underwent randomization. Stratified factors were the status of lymph node and *ras* gene. In overall survival in this study, patients with chemotherapy had a significantly higher survival rate than those with observation (69% vs. 54%, $P = 0.012$), at HR of 0.696 (95% CI, 0.524–0.923).

U.S. trial, Cancer and Leukemia Group B (CALGB) 9633 (presented in ASCO 2004[19])

Patients with p-stage IB were randomly assigned to either three or four cycles of the chemotherapy with carboplatin (AUC = 6, day 1, every 3 weeks) and paclitaxel (200 mg/m^2, day 1, every 3 weeks), or observation. A total of 482 patients underwent randomization. Stratified factors were histology, differentiation, and the status of mediastinoscopy. The median duration to follow-up was 34 months; patients assigned to chemotherapy had a significantly higher survival rate than those assigned to observation (71% vs. 59% at 4-year survival rate, $P = 0.028$). HR for this trial was 0.62 (95% CI, 0.41–0.95).

Short summary of international trials

The NCI-C and CALGB studies confirmed positive IALT results of the benefit for postoperative platinum-based chemotherapy in completely resected non-small cell lung cancer. The good results of NCI-C and CALGB trials might be due to patient selection, such as earlier-stage disease (IB and II), uniform patient population, more frequent incidence of women than ILT, and the therapeutic strategy of chemotherapy, such as a two-drug regimen with third-generation agent, better compliance, and no radiotherapy in patients without lymph node metastasis.

The summary was based on the international trial; consistent reductions in the risk of death have been observed in recent adjuvant platin-based trials and the 1995 meta-analysis. Adjuvant platinum-based chemotherapy should be recommended to completely resected non-small cell lung cancer patients with good performance status.

Consideration: future perspective

Even if completely resected stage I non-small cell lung cancer is due to recurrent disease in the majority of patients, adjuvant therapy had aimed at eradication of micrometastasis. Recent development of molecular biological techniques permits us to predict the chemotherapeutic response. In the adjuvant setting, the selection of anticancer drugs should depend on the analysis of molecular biological makers for resected materials in addition to pathological stage. In addition to cooperation with new chemotherapeutic agents, such as taxane, camptothecin, and gemcitabine, there are even newer classes of antineoplastic therapy, such as antiangiogenic inhibitor and tyrosine kinase inhibitor, that should be defined. The role of newer classes of some biological therapies with anticancer effect will be defined in coming years. The clinical benefit of platinum-based adjuvant therapy was confirmed. This paradigm is strongly recommended at stage IB and II non-small cell lung cancer. In stage IIIA, further subset analysis is necessary in the new meta-analysis, including IALT (Table 3). On the other hand, platinum-based chemotherapy has some potential of severe adverse events. Although there was no treatment-related death by carboplatin with paclitaxel in the CALGB trials, the feasibility of the platinum-based regimen in the adjuvant setting has not been confirmed yet in Japan. Careful observation after platinum-based chemotherapy is necessary.

Conclusion

Adjuvant chemotherapy for pathological stage IB to II, completely resected non-small cell lung cancer is standard care based on clinical trials. UFT showed the strongest evidence for IB in Japan. Platinum doublet chemotherapy with a third-generation anticancer agent is also recommended. Although there is no evidence of the feasibility of a platinum-based regimen in the adjuvant setting in Japan, adjuvant chemotherapy should be offered as standard care to patients after completely resected early-stage non-small cell lung cancer.

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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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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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between cells [1,2], and E-cadherin is the form most strongly expressed in epithelial cells. Cadherins form a complex with cytoplasmic proteins, known collectively as catenins. This molecular complex, together with other cytoskeletal components such as actin, constitutes the intercellular adherence junction [2–4]. The catenins are classified into two groups, α-catenins and β-catenins, and the latter group includes plakoglobin and *Drosophila* Armadillo protein as well as β-catenin itself [5,6]. Plakoglobin is isolated from the desmosomal fraction [7] and is present in both desmosomes and adherence junctions [8], and may therefore be a common regulatory molecule in cell junctions.

Cadherin-mediated cell adhesion acts as a suppressor of the invasion of cancer cells in vitro [9–11], and dysfunction of the E-cadherin system correlates with cancer cell invasion in human cancers [12,13]. The role of α-catenin in the cadherin adhesion system has been revealed by studies with cancer cells. The human lung cancer cell line PC9 expresses an aberrant α-catenin mRNA and shows very loose cell–cell association [14,15]. PC9 cells become much more closely associated and acquire an epithelioid arrangement after transfection with cDNA for a subtype of α-catenin and αN-catenin [16]. These results suggest that α-catenin is indispensable for cadherin-mediated cell–cell adhesion.

Previous immunohistochemical studies have revealed many examples of reduced and/or heterogeneous expression of E-cadherin [17–19] and α-catenin [20] in undifferentiated invasive cancers, and impaired expression of E-cadherin or α-catenin has been reported to be associated with high incidences of lymph node metastasis of human breast [21], esophageal [22], and head and neck [23] cancers. However, there have been few studies on the relationship between reduced E-cadherin expression and the prognosis of cancer patients [24–28].

The role of β-catenin and plakoglobin in determining the fate of cells has been suggested by work on a *Drosophila* homologue of this protein, Armadillo [29,30]. Moreover, it has been revealed that the association between E-cadherin and α-catenin is mediated by β-catenin [31], and that β-catenin in turn mediates the interactions of the cadherin–catenin complex with the c-erbB-2 gene product and epidermal growth factor receptor (EGF-R) [32–34]. A tumor suppressor gene product, APC protein, has been shown to interact with β-catenin and plakoglobin and to play important roles in the E-cadherin-mediated cell adhesion system and to participate in tumor invasion and metastasis.

In a previous study, we divided primary lung cancers into two groups on the basis of their expression of E-cadherin and catenins, as detected by immunohistochemistry [35]. In addition, we demonstrated a close relationship between E-cadherin-associated cell–cell adhesion, catenins, and cytologic features, in particular the formation of cellular clusters and the frequency of solitary cells. Preoperative evaluation of both cytologic features and E-cadherin-associated cell–cell adhesion may be useful for predicting the malignant characteristics of lung cancer [36].

E-cadherin and α-catenin, and also β-catenin and plakoglobin, play important roles in the cadherin-mediated cell adhesion system in various cancers. However, in the context of carcinogenesis of the bronchial epithelium, expression of E-cadherin, α-catenin, β-catenin, and plakoglobin in intrabronchial precancerous lesions has not yet been reported. In order to investigate a possible dysfunction of the E-cadherin-mediated cell adhesion system in intrabronchial lesions, we used immunohistochemistry to examine the expression of E-cadherin, α-catenin, β-catenin, and plakoglobin in biopsy specimens.

2. Materials and methods

2.1. Biopsy specimens

The biopsy samples were obtained from 109 patients with intrabronchial lesions resected between 1991 and 2000 at the Department of Surgery of Tokyo Medical University Hospital. These lesions were diagnosed pathologically as BSM without atypia in 32 cases, BSM with atypia in 25 cases, dysplasia in 5 cases, carcinoma in situ in 21 cases, microinvasion to the bronchial wall in 4 cases, and stage I well differentiated squamous cell carcinoma in 32 cases. The specimens were fixed with 10% formalin and embedded in paraffin.

2.2. Immunohistochemistry

Mouse monoclonal antibodies against human E-cadherin (HECD-1; Takara, Kyoto, Japan), α-catenin and β-catenin (anti-α-catenin and anti-β-catenin; Transduction Laboratories, Lexington, KY), and plakoglobin (CBL175; Cymbus Bioscience, Southampton, UK) were used for immunohistochemical staining. Four-micrometer-thick tissue sections were prepared from all paraffin-embedded specimens and collected on silane-coated glass slides. After deparaffinization, the formalin-fixed paraffin-embedded sections were treated with

0.01% trypsin and subjected to microwave antigen retrieval [37].

The immunohistochemical method using the avidin-biotin-peroxidase complex was described previously [35]. The reaction products were visualized with diaminobenzidine and the sections were counterstained with hematoxylin.

Negative control staining, which was performed with the same class of immunoglobulin instead of the first antibody, yielded negative results in all cases. The intensity and pattern of immunostaining with HECD-1, anti-α-catenin, anti-β-catenin, and CBL175 in intrabronchial lesions were compared with those of normal bronchial epithelium, and the immunohistochemical staining results were evaluated as described previously [35]. Levels of immunostaining were evaluated in separate compartments of the bronchial epithelium: the basal layer (the first two-fifths of the distance between the basement membrane and the free surface), the intermediate layer, and the superficial layer (the upper one-fifth of this distance). Expression of E-cadherin, α-catenin, β-catenin, and plakoglobin in each layer was judged to be normal if more than 90% of the intrabronchial lesion cells were positively stained by the appropriate antibodies. If staining was distinctly weaker than that of normal epithelium, or if less than 90% of the intrabronchial lesion cells were positively stained, the expression was judged to be reduced. Immunohistochemical staining was scored independently by two observers (Y.K., Y.E.).

2.3. Statistical analysis

The data were analyzed using the Cochran–Armitage test [38], which was conducted by a stepwise method excluding E-cadherin, α-catenin, β-catenin, and plakoglobin, since these four variables are the variables of interest. Differences at $p < 0.05$ were considered to be statistically significant.

3. Results

In bronchial epithelium, E-cadherin and all catenins were expressed at a high level. Immunohistochem-

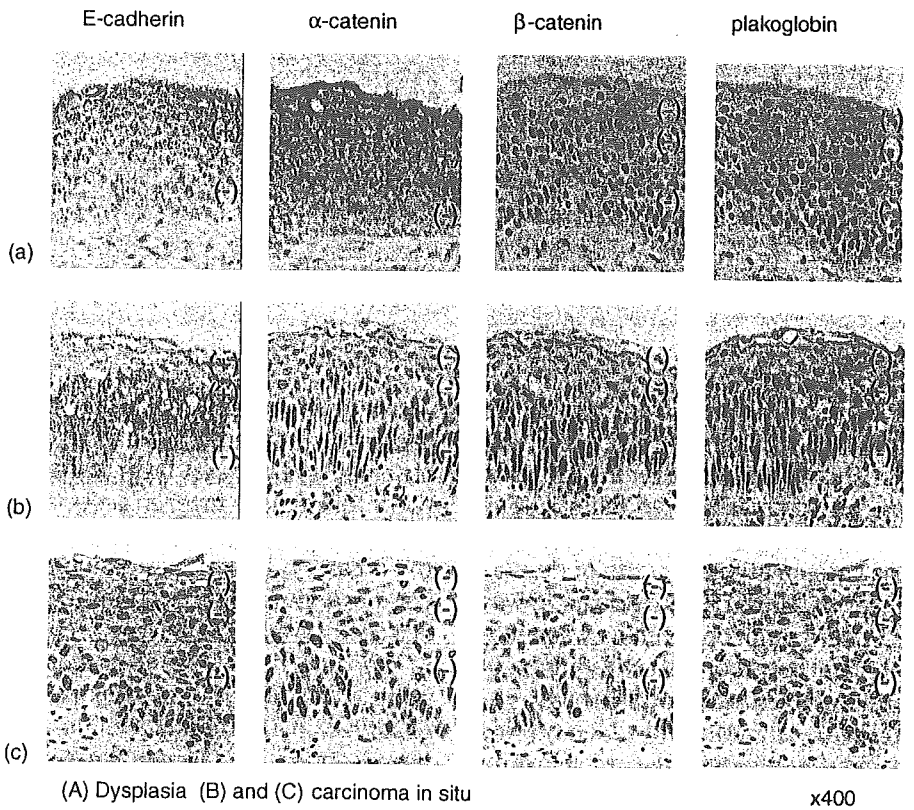

Fig. 1 (A) Representative immunohistochemical staining for E-cadherin, α-catenin, β-catenin, and plakoglobin in biopsy specimens of dysplasia (a) and carcinoma in situ (b and c). Evaluation for each layer of the intrabronchial lesions is shown at the right side of each picture ×400. (B) A borderline area between carcinoma in situ and dysplasia. Evaluation for each layer of the carcinoma in situ area is shown at the left side of each picture, and that for each layer of the dysplasia area at the right side of each picture.

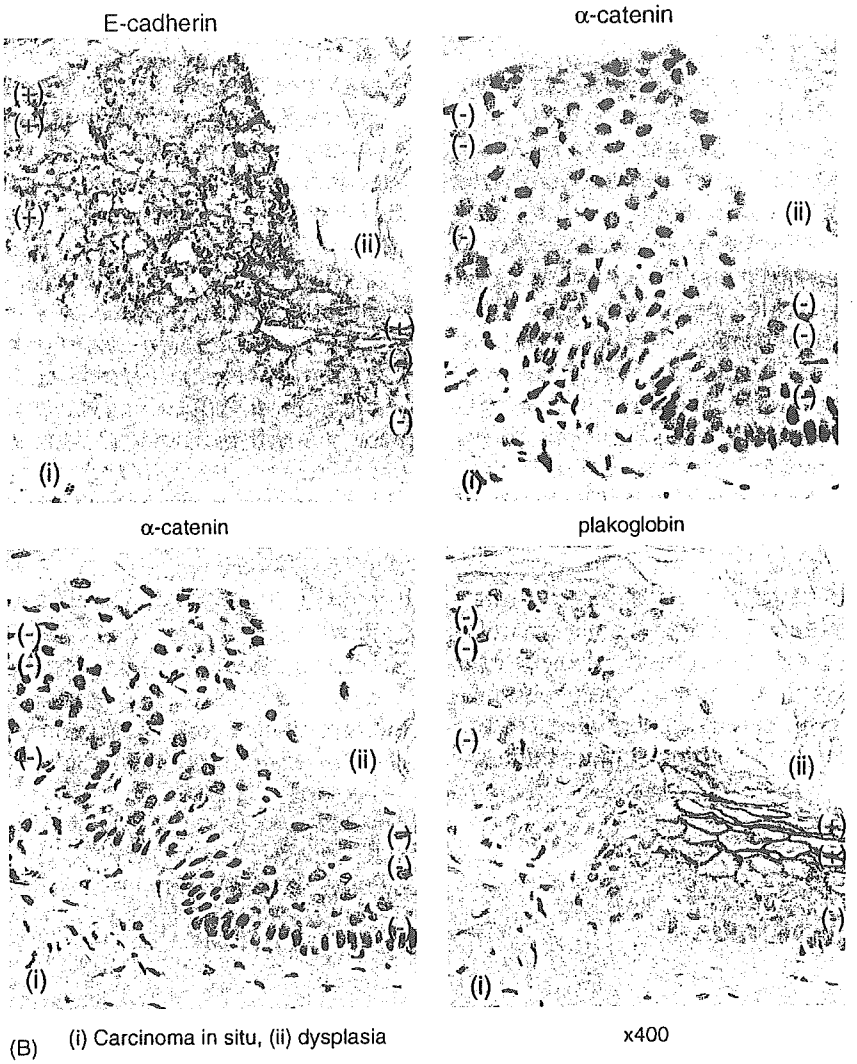

Fig. 1 (*Continued*).

ical findings for representative intrabronchial lesions are shown in Fig. 1. Cases with reduced expression of either E-cadherin or catenins in intrabronchial lesions are summarized in Table 1. 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, down-regulation of E-cadherin and/or catenins was associated with an atypical grade of BSM with atypia in intrabronchial lesions ($p < 0.01$). Additionally, reduced expression of E-cadherin and catenins was observed in squamous cell carcinoma, as shown in Table 2.

In BMS without atypia ($n = 32$ cases), loss of expression of α-catenin, β-catenin or plakoglobin was observed in the basal layer in six cases (18%), in the intermediate layer in two cases (6%), and in the superficial layer in three cases (9%). In BSM with atypia ($n = 25$ cases), loss of expression of E-cadherin, α-catenin, β-catenin or plakoglobin was observed in the basal layer in seven cases (28%), in the intermediate layer in seven cases (28%), and in the superficial layer in five cases (20%). In dysplasia ($n = 5$ cases), loss of expression of these molecules was observed in the basal layer in two cases (40%), in the intermediate layer in one case (20%), and in the superficial layer in one case (20%). In carcinoma in situ ($n = 21$ cases), loss of expression was observed in the basal layer in 10 cases (48%), in the intermediate layer in 9 cases (43%), and in the superficial layer in 8 cases (38%). In microinvasion to bronchial wall ($n = 4$), loss of expression was observed in the basal layer in four cases (100%), in the intermediate layer in three cases (75%), and in the superficial layer in two cases (50%). These results are presented in Fig. 2 and Table 3.

Table 1 Aberrant expression of E-cadherin and catenins in intrabrounchial lesions

	E-cadherin			α-Catenin			β-Catenin			Plakoglobin			Rate[a] (%)
	B	I	S	B	I	S	B	I	S	B	I	S	
BSM without atypia $n=32$	+	+	+	−	−	+	+	+	+	−	−	−	21
	+	+	+	+	+	+	−	+	+	+	+	+	
	+	+	+	+	+	+	−	+	+	+	+	+	
	+	+	+	+	+	+	+	+	−	+	+	+	
	+	+	+	+	+	+	+	+	+	−	+	+	
	+	+	+	+	+	+	+	+	+	−	+	+	
	+	+	+	+	+	+	+	+	+	−	−	−	
BSM with atypia $n=25$	−	−	+	+	+	+	+	+	+	+	+	+	28
	+	+	+	−	+	+	−	−	−	+	+	+	
	+	+	+	−	−	−	−	−	−	+	+	+	
	+	+	+	+	+	+	−	−	−	+	+	+	
	+	+	+	+	+	+	−	−	−	+	+	+	
	+	+	+	+	+	+	−	−	+	−	−	+	
	−	−	−	−	−	−	−	−	−	−	−	−	
Dysplasia $n=540\%$	−	+	+	−	+	+	−	−	−	−	−	−	40
	−	+	+	−	+	+	+	+	+	+	+	+	
	+	+	+	+	+	+	−	−	+	+	+	+	
	+	+	+	+	+	+	−	+	+	−	+	+	
	+	+	+	−	−	−	−	−	−	−	−	−	
	+	−	+	−	−	+	−	−	−	+	+	+	
Carcinoma in situ $n=21$	−	+	+	−	−	−	−	−	−	−	−	−	48
	−	−	+	−	−	−	+	+	+	+	+	+	
	−	−	−	−	−	+	−	−	−	−	−	+	
	−	−	−	−	−	−	−	−	−	+	+	+	
	−	−	−	−	−	−	−	−	−	−	−	−	
	−	−	−	−	−	−	−	−	−	−	−	−	
Microinvasion to bronchial wall $n=4$	+	+	+	−	−	+	−	−	−	+	+	+	100
	+	+	+	−	−	+	−	−	+	+	+	+	
	+	+	+	+	+	+	−	−	−	−	−	−	
	+	+	+	+	+	+	+	+	+	−	+	+	

Variable	Contrast	*p*-Value
BSM without atypia	BSM with atypia	0.097
BSM without atypia	Dysplasia	0.043
BSM without atypia	Carcinoma in situ	0.003
BSM without atypia	Microinvasive to bronchial wall	0.001

B: basal layer; I: intermedtate layer; S: superficial layer.
[a] Reduced expession rate of either E-cadherin or catenins in intrabronchial lesions.

Table 2 Reduced expression rate of E-cadherin and catenins in advanced stage of squamous cell carcinoma

	Squamous cell carcionoma, $n=32$
E-cadherin	21 (67%)
α-Catenin	26 (81%)
β-Catenin	27 (84%)
Plakoglobin	14 (44%)
Rate[a]	100%

[a] Reduced expression rate of either E-cadherin or catenins in squamous cell carcinoma.

4. Discussion

It has been established that malignant transformation can arise from an accumulation of genetic alterations. This stepwise transformation is known as multistep carcinogenesis. In general, it is known that primary lung carcinoma is one of the most malignant solid tumors, and that it has a wide range of invasive and metastatic behavior. There is a high possibility that alterations in genotype are reflected in the morphological phenotype of the bronchial epithelium. In this context, bronchial

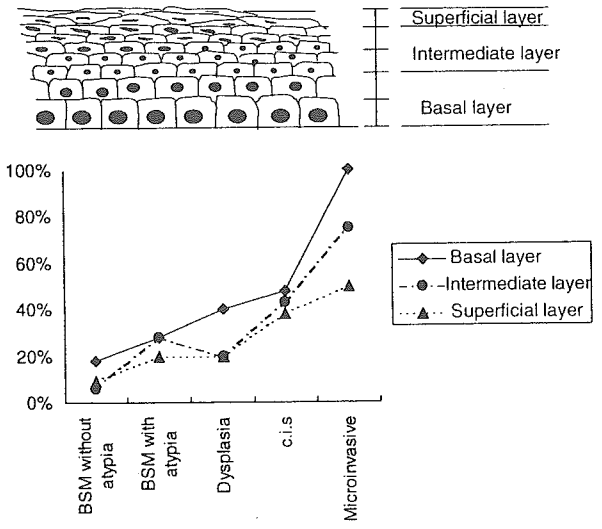

Fig. 2 Proportion of cases with reduced expression of either E-cadherin or catenins within the basal layer (◆), the intermediate layer (●) or the superficial layer (▲) of the intrabronchial lesions. The relative distribution of the different layers is shown in the upper part of the figure.

squamous metaplasia and dysplasia can be considered as precancerous lesions, mutation of the p53 tumor suppressor gene, and deletion of chromosome 17p have been reported in such lesions [39–42]. We have reported sequential changes in cell proliferation, DNA aneuploidy and accumulation of mutant p53 protein during carcinogenesis in the bronchial epithelium, and that these histochemical changes initially occurred in the basal layer [43]. We believe that the ability of cancerous cells to invade the bronchial wall will be acquired in a sequential manner during carcinogenesis. Therefore, we investigated the reduction of expression of E-cadherin and/or catenins in intrabronchial precancerous lesions and the early stages of bronchial squamous cell carcinoma. In intrabronchial lesions and squamous cell carcinoma, expression of either E-cadherin or catenins was reduced in 21% of BSM without atypia, 28% of BSM with atypia, 40% of dysplasia, 48% of carcinoma in situ, 100% of carcinoma microinvasive to the bronchial wall, and 100% of squamous cell carcinoma. We also demonstrated a positive correlation between the expression of these molecules and the grade of atypia of intrabronchial lesions. Our previous studies showed that reduced expression of E-cadherin and catenins occurs frequently in non-small cell lung carcinoma [35]. Hence, our present findings indicate that downregulation of E-cadherin and catenins may play an important role in the progression of human intrabronchial lesions and squamous cell carcinoma.

Studies on cell–cell adhesion molecules may help to clarify the mechanisms of local invasion and metastasis. Investigations of the cadherin–catenin complex have been carried out at the cellular and molecular levels [14,22,44]. It has already been reported that reduction of E-cadherin expression is caused by mutation and by inactivation of the E-cadherin gene by hypermethylation in the promoter region [45]. Dysfunction of the cadherin–catenin complex caused by reduction of the expression of these molecules implies an increased ability of cancer cells to disperse, which is the probable early step of local invasion and metastasis. Reduction of expression of E-cadherin and α-catenin is associated with local invasion and metastasis of scirrhous carcinoma in gastric cancer, breast cancer, and esophageal cancer [20,22].

In BSM with atypia and dysplasia, cells showing reduction of E-cadherin and/or catenin expression were localized mainly in the basal layer. As histological atypia increased, reduced expression of each molecule also became evident in the intermediate and superficial layers. This observation parallels the finding that proliferating cells and cells with accumulation of mutant p53 protein appeared from the basal layer to the superficial layer during carcinogenesis in the bronchus [43]. Therefore, we hypothesize that these cellular changes indicate an increased risk of eventual malignant transformation, and also that cells in the basal layer are the first to acquire the capacity for local invasion.

Our present study suggests that reduction of expression of E-cadherin and/or catenins is a rela-

Table 3 Aberrant expression rate of E-cadherin and/or catenins in intrabronchial lesions

	BSM without atypia	BSM with atypia	Dysplasia	c.i.s	Microinvasion to bronchial wall	Sq.c.ca.
Basal layer	6 (18%)	7 (28%)	2 (40%)	10 (48%)	4 (100%)	100%
Intermediate layer	2 (6%)	7 (28%)	1 (20%)	9 (43%)	3 (75%)	
Superficial layer	3 (9%)	5 (20%)	1 (20%)	8 (38%)	2 (50%)	
Whole layer	7 (21%)	7 (28%)	2 (40%)	10 (48%)	4 (100%)	
Total (cases)	32	25	5	21	4	32

tively early event in the genesis of bronchial squamous cell carcinoma, and that increasing histological atypia is accompanied by further diminution in the expression of these molecules. Finally, reduced levels of E-cadherin and/or catenins might play a critical role in local invasion beyond the basement membrane and the development of the advanced stage of squamous cell lung carcinoma.

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Smoking history before surgery and prognosis in patients with stage IA non-small-cell lung cancer—a multicenter study

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Summary The prognosis of lung cancer patients with surgically resected non-small-cell lung cancer (NSCLC) can be predicted generally from age, sex, histologic type, stage at diagnosis, and additional treatment. Nine studies have reported that a history of smoking before diagnosis influences the prognosis of the disease in lung cancer patients. In this study, a total of 3082 patients who underwent surgery and were diagnosed with primary pathological stage IA NSCLC at 36 national hospitals from 1982 to 1997 were analyzed for the effect of smoking on survival. Smoking history and other factors influencing either the overall survival or the disease-specific survival rates of patients were estimated with the Cox proportional hazards model. Multivariate analysis demonstrated significant associations between overall

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survival and age ($P<0.0001$), sex ($P=0.0002$), and performance status (PS) ($P<0.0001$). Disease-specific survival was associated with age ($P=0.0063$), sex (0.00161), and PS ($P=0.0029$). In males, disease-specific survival was associated with age ($P=0.0120$), PS ($P=0.0022$), and pack-years (number of cigarette packs per day, and years of smoking) ($P=0.0463$). These results indicate that smoking history (pack-years) is important clinical prognostic factor in estimating disease-specific survival, in male patients with stage IA primary NSCLC that has been surgically resected.

1. Introduction

The worldwide incidence and mortality from lung cancer have increased rapidly in recent decades [1]. Non-small-cell lung cancer (NSCLC) constitutes approximately 85% of all lung cancers [2]. Even after 30 years of improvements in therapeutic approaches, the 5-year mortality rate of all lung cancer remains an alarmingly high 85% [3]. The 5-year survival rate, even in the optimum surgical stage IA (T1N0M0), is 67% [4]. These poor survival rates are due primarily to recurrences [5] and second lung cancers [6].

The prognosis of lung cancer patients with surgically resected NSCLC can be predicted generally from age, sex, histologic type, stage at diagnosis, and additional treatment [4,7].

The impact of smoking history on survival is controversial. Nine studies have reported that smoking history is a negative prognostic factor in lung cancer [8–16]; whereas, others studies did not find an association [7,17–20].

Recently, Fujisawa et al. have reported that preoperative smoking history is an important clinical postoperative prognostic factor in estimating overall long-term survival in patients with primary resected stage I NSCLC [14].

The aim of this study was to evaluate the effect of smoking history on survival in patients with primary resected stage IA NSCLC.

2. Patients and methods

2.1. Patients

A Central registry for all lung cancer patients has been established in which 33,161 cases have been registered at 36 national hospitals that belong to the Japan National Chest Hospital Study Group for Lung Cancer from 1982 to 1997. We used the central registry data of surgical patients with NSCLC who had been newly diagnosed and undergone surgery. The study group comprised 3217 patients who underwent complete resection and were pathologically confirmed stage IA NSCLC. Ninety-one patients who were lack of smoking history or follow-up interval were excluded from survival analysis. In order to focus on long-term survival, 44 patients (11 with squamous cell carcinoma, 32 with adenocarcinoma, and 1 with large cell carcinoma; a total of 25 men and 19 women) who died within 1 month after surgery were excluded from the survival analyses [21]. Finally, 3082 patients were analyzed for survival analysis. The cancer histologic types included 840 squamous cell carcinomas, 2161 adenocarcinomas, and 81 large cell carcinomas. The patient group consisted of 1221 women and 1861 men who ranged in age from 22 to 89 years (mean age, 64.4 years). Histologic type and TNM classification were classified according to the criteria of World Health Organization. Performance status (PS) was classified according to the criteria of Eastern Cooperative Oncology group (ECOG). The data on smoking history (pack-years, number of packs per day, and years of smoking) were obtained from hospital records. Cause of death was reported by the doctor who followed the patient. At the last follow-up, for overall survival curves, an observation was censored if the patient was alive; for disease-specific curves, data were censored if the patient was alive or had died from a cause other than NSCLC.

2.2. Survival rate and statistical analysis

Overall survival was defined as the time between surgery and death or last follow-up evaluation. Disease-specific survival was defined as the time between surgery and cancer death or last follow-up evaluation.

Bivariate analysis was performed with Fisher's exact test. The difference in age between the two groups was analyzed with the Student's t-test. Overall survival and disease-specific survival were calculated with the Kaplan–Meier method, and the difference between survival curves was analyzed with the log-rank test. Variables in this study consist of age, sex, histologic type, tumor classification, and cigarette smoking before surgery. Multivariate analysis was performed with the Cox proportional

hazards model. All statistical analysis in this study was performed with StatView statistical software (StatView version 5.0 for Macintosh; SAS institute Inc., Cary, NC, USA). Statistical significant *P*-values were considered to be less than 0.05.

3. Results

3.1. Association between clinical features and smoking pack-years

Clinical features, including age, sex, PS, and histology, were evaluated according to smoking pack-years (Table 1). The heavy smokers group also had significantly higher population of older age, male patients, poor PS, and squamous cell carcinomas than smokers with less than 40 pack-years or non-smokers.

3.2. Cause of death

Forty-four patients died within 1 month after surgery (1.4% of 3126 patients). After a median follow-up of 3.9 years, of 3082 patients used for survival analysis, 491 patients died from recurrent or second lung cancer, and 159 patients died from non-recurrent diseases. Non-recurrent causes consisted of 27 second primary malignancies.

3.3. Overall survival and disease-specific survival

The overall and disease-specific 5- and 10-year survival curves are shown in Fig. 1. Fig. 2 demonstrates the overall survival and disease-specific survival curves according to cigarette smoking,

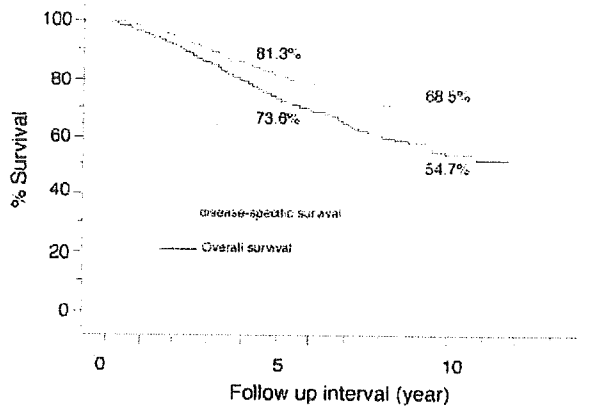

Fig. 1 Overall survival and disease-specific survival curves in patients with primary, surgically resected stage IA NSCLC.

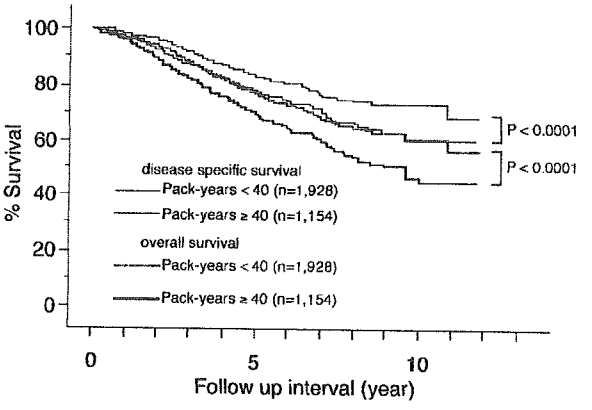

Fig. 2 Overall survival and disease-specific survival curves in patients with primary, surgically resected stage IA NSCLC, evaluated by pack-years.

Table 1 Distribution of clinical features, according to smoking pack-years

Clinical feature	Pack-years		*P*[a]
	<40	≥40	
Age (mean ± S.D.)	62.8 ± 10.3	66.9 ± 8.2	<0.0001
Sex			
Male	770	1091	<0.0001
Female	1158	63	
PS			
0	1612	855	<0.0001
≥1	306	289	
Histology[b]			
Nonsquamous cell carcinoma	1660	582	<0.0001
Squamous cell carcinoma	268	572	

[a] *P*-value for age are by Student's *t*-test and for the remainder are for Fisher exact test.
[b] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

Table 2 Overall survival and disease-specific survival, according to clinical prognostic factors

Clinical feature	No. of patients	Overall survival (%)			Disease-specific survival (%)		
		5 years	10 years	P[a]	5 years	10 years	P[a]
Age (years)							
<70	2115	77.3	60.6	<0.0001	83.1	70.9	0.0003
≥70	961	64.4	36.3		76.1	60.7	
Sex							
Male	1861	70.7	47.3	<0.0001	79.2	63.0	<0.0001
Female	1221	78.1	66.7		84.5	76.7	
Histology[b]							
Squamous cell carcinoma	840	69.8	49.8	0.0041	79.8	61.5	0.0831
Nonsquamous cell carcinoma	2242	75.1	56.6		81.8	71.2	
Performance status							
0	2467	76.8	58.1	<0.0001	82.3	69.9	<0.0001
≥1	595	61.0	41.8		76.5	62.0	
Pack-years							
<40	1928	76.5	60.5	<0.0001	83.4	73.6	<0.0001
≥40	1154	69.0	45.7		77.8	60.3	

[a] *P*-value for the log-rank test.
[b] Nonsquamous cell carcinoma is comprised of adenocarcinoma and large cell carcinoma.

and the 5- and 10-year survival rates between heavy smokers (pack-years ≥ 40) and light smokers (pack-years < 40) are both significantly different ($P < 0.0001$).

Table 2 shows the overall and disease-specific 5- and 10-year survival rates according to several variables. Significant differences in overall survival were demonstrated with age ($P < 0.0001$), sex ($P < 0.0001$), histologic type ($P = 0.0041$), PS ($P < 0.0001$), and pack-years ($P < 0.0001$). But no significant difference in disease-specific survival was found with histologic type ($P = 0.0831$). With regard to cigarette smoking, the difference between heavy smokers and light smokers was statistically significant ($P < 0.0001$) in the both overall survival and disease-specific survival.

3.4. Multivariate analysis

Multivariate analysis was conducted with the Cox proportional hazards model with the five variables. Multivariate analysis demonstrated a significant association between overall survival and age ($P < 0.0001$), sex ($P = 0.0002$), and PS ($P < 0.0001$), but no association was observed with histologic type ($P = 0.3807$) or pack-years ($P = 0.1742$) (Table 3).

Next, multivariate analysis for disease-specific survival was performed with the five variables. Multivariate analysis demonstrated a significant association of disease-specific survival with age ($P = 0.0063$), sex ($P = 0.0161$), and PS ($P = 0.0029$), and no significant association with histologic type ($P = 0.3935$) or pack-years ($P = 0.0741$) (Table 4).

We conducted a subgroup analysis for overall survival and disease-specific survival according to sex. In a subgroup analysis (Tables 3 and 4), disease-specific survival demonstrated a significant association with age ($P = 0.0120$), PS ($P = 0.0022$), and pack-years ($P = 0.0463$), and no significant correlation with histologic type ($P = 0.1971$). Similar trends were observed for overall survival among males, but pack-years was not a significant prognostic factor ($P = 0.1410$). On the other hand, the analyses for females, a considerably small proportion of heavy smokers (5.1%) gave an unstable odds ratio estimation (Tables 1, 3 and 4).

4. Discussion

In this study, more than 3000 patients with stage IA primary NSCLC that has been surgically resected were analyzed, and we found that older age, poor PS, male, and smoking history, in male, were significant unfavorable prognostic factors. We demonstrated the significant inverse correlation between cigarette smoking and long-term disease-specific survival in stage IA NSCLC patients using multivariate analysis. Even if a curative surgery has been underwent in a very early stage NSCLC, previous smoking history still was disadvantage.

The impact of smoking history on survival is still confusing. Earlier studies found no associ-