厚生労働科学研究研究費補助金

がん臨床研究事業

局所限局非小細胞肺がんの集学的治療に関する研究（臨床研究実施チームの整備）

平成17年度　総括研究報告書

主任研究者　加藤　治文

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別紙３

厚生労働科学研究費補助金（がん臨床研究事業）
総括研究報告書

局所限局非小細胞肺がんの集学的治療に関する研究

主任研究者　加藤治文　東京医科大学病院外科学第一講座教授

研究要旨：本年度は、本邦における術後補助化学療法の当該病期における妥当なレジメンを決定する大規模臨床試験（本研究Ａ）の実施計画書を作成した。本臨床第Ⅲ相試験の対象は、まず標準的外科切除行った後に、病理病期ⅠB-ⅢＡ期非小細胞肺癌と診断された症例とする。本試験は、術後４週から８週までの間に症例登録を行って、UFT 250mg/m2 の内服治療を開始し、２年間継続する治療法（標準的治療群）、もしくは欧米で評価されたプラチナ化合物を含む２剤併用化学療法の一つであるカルボプラチンとパクリタキセル（CP 療法）を４ コース行う治療法（試験治療群）のどちらかに無作為に割り付けて、それらの有効性を比較、検証する。エンドポイントは３年生存率もしくは無再発生存割合とし、予定登録は１群400 例、合計800 例、２年間で症例集積を行い、集積終了時点で中間解析を行うデザインとした。また、これと並行して、CP 療法の認容性、安全性に関わる臨床第Ⅱ相試験を個別研究として行った。試験は、進行肺癌で用いられるfull dose のCP 療法が術後補助療法として可能か否かというデザインと卵巣癌等で用いられている投与量（full dose から約 10%、投与量を削減したもの）における安全性の検証を行うデザインの２種に分かれて実施した。現時点では最終結果は得られていないが、これらの情報から第Ⅲ相試験ではより完遂率が高く安全と思われる投与量を決定する。

Ａ．採択された研究事業での研究概要

Ａ．研究目的：

１）奏効率・毒性の異なる二種類の化学療法レジメンから術前後化学療法への適性を検討し、臨床第Ⅲ試験の試験治療を決定する。

２）臨床病期（c-Stage）ⅠB-Ⅱ非小細胞肺癌（NSCLC）に対する術後化学療法の安全性および有用性を検証し、本邦における術後化学療法レジメンの妥当性を検討する。

３）臨床病期（c-Stage）ⅠB-Ⅱ非小細胞肺癌（NSCLC）に対する術前化学療法の有用性を検討する。

1.　本研究の必要性：

当該疾患の標準的治療は外科的切除もしくは外科切除＋術後補助療法であるが、治療成績は不満足であり、より安全な全身治療の強化による治療成績の向上が期待される。最近、プラチナを用いた術後化学療法の有用性が当該病期において明らかになり、世界的に術後補助化学療法が「標準的治療」の一角を担いつつある。本邦では当該病期の一部（IB）の術後補助療法としてテガフール・ウラシル（UFT）の有効性が明らかになったが、欧米では当該病期に対してプラチナ製剤を含む 2 剤併用療法を標準的レジメンとしている。後者は本邦における安全性は確立しておらず、まずは標準的治療群に組み込まれるべき治療レジメンを決定する大規模比較試験が必要である。一方、術後化学療法の治療完遂率は 50～85%であり、術前では 90%以上のそれが期待できる。プラチナを用いた化学療法の有効性、安全性を考えれば、依然術前化学療法は有望であり、その治療意義を検証する必要がある。

2.　本研究の特色：

1）　欧米では進行病期に汎用される化学療法を用いて同様の症例を対象に術前化学療法と切除単独の比較試験を開始しているが、その化学療法の妥当性については検討されていない。

2）　UFT に関する臨床試験は海外になく、欧米では同様の試験デザインで臨床試験が進む予定はない。

B. 研究方法

本研究では、まず術後の標準的化学療法レジメンを決定する比較試験（研究Ａ）を行った後、先に決定された術前化学療法＋手術群を手術＋術後補助療法群を対照とした比較試験（研究Ｂ）で検証する。エンドポイントは生存率もしくは無再発生存割合。研究Ａの予定登録は 1 群 300 例、合計 600 例； 2 年間で症例集積を行い、集積終了時点で中間解析を行う。引き続き、研究 B を行う。ここでもエンドポイントは生存率。予定登録は 1 群 150 例、合計 300 例； 2 年間で症例集積を行い、集積終了時点で中間解析を行う。5 年生存率を算定できるまで症例集積治療及び追跡を行って最終解析を行う。

（年度別研究計画）：

第１年度：前研究の結果解析。次期臨床研究 A のプロトコール作成

第２年度(当該年度)：研究 A の試験実施計画書完成

第３年度：研究 A の症例集積、治療、追跡；研究 B 試験デザインの設定

3 年計画終了時に研究継続が認められた場合、5 年生存率を算定できるまで症例集積治療及び追跡を行って最終解析を行う。

B. 採択された研究事業での研究実績

当該病期の標準的治療が昨今の報

告から外科手術単独から外科切除＋術後補助化学療法に移行するに至り、術後治療としての本邦における標準的レジメンは明らかでない。本年度の研究では、標準的治療群に組み込まれるべき術後補助化学療法のレジメンを決定する臨床第Ⅲ相試験のデザインと実施計画書の作成を行った。本臨床第Ⅲ相試験の対象は、標準的外科切除を行った後に、病理病期ⅠB-ⅢA期非小細胞肺癌と診断された症例とする。本試験は、術後4週から8週までの間に症例登録を行って、UFT 250mg/m2の内服治療を開始し、2年間継続する治療法（標準的治療群）、もしくは欧米で評価されたプラチナ化合物を含む2剤併用化学療法の一つであるカルボプラチンとパクリタキセル（CP療法）を4コース行う治療法（試験治療群）のどちらかに無作為に割り付けて、それらの有効性を比較、検証する。エンドポイントは3年生存率もしくは無再発生存割合とし、予定登録は1群400例、合計800例、2年間で症例集積を行い、集積終了時点で中間解析を行うデザインとした。本試験は先に実施したrandomized phase ⅡのO204-MFに比し群間で治療内容が大きくなるので同意が取りにくくなることが予想され、単独グループでの2年集積は困難と予想され、NPO法人である西日本胸部臨床試験機構（WJTOG）とのintergroup trialとして行う。一方、CP療法について、術後補助療法としての投与量も含め安全性情報が不足していることから、グループ関連の施設で個別に臨床第Ⅱ相試験を実施した。試験は、進行肺癌で用いられるfull doseのCP療法が術後補助療法として可能か否かというデザインと卵巣癌等で用いられている投与量（full doseから約10%、投与量を削減したもの）における安全性の検証を行うデザインの2種に分かれて実施した。現時点では最終結果は得られていないが、これらの情報から第Ⅲ相試験ではより完遂率が高く安全と思われる投与量を決定する。

（倫理面への配慮）

参加患者の安全性確保については、毒性中止・無効中止基準等の配慮がなされており、試験参加による不利益は最小化される。

また、ヘルシンキ宣言や米国ベルモントレポート等の国際的倫理原則に従い、これを遵守する。研究の監視：本研究班により、もしくは賛同の得られた他の主任研究者と協力して、臨床試験審査委員会、効果・安全性評価委員会、監査委員会を組織し、研究開始前および研究実施中の第三者的監視を行う。

臨床試験登録の際しては、この治療法が臨床試験であること、標準治療は手術単独であること、また術前治療を行うことに伴うメリット・リスク・不利益などを十分に説明がなされ、患者本人からの文書による同意を必須とする。また、試験の開始にあたり、グループ臨床試験審査委員会、参加各施設倫理委員会（IRB）の承認を得る。

C．考察

本邦からⅠ期非小細胞肺癌（腺癌）に対するUFTの術後化学療法の大規模臨床試験（N Eng J Med 2004; 350: 1713）と meta-analysis（J Clin Oncol 2005; 23: 4999）の結果が公表され、本邦においては UFT を用いた術後補助療法が少なくともⅠB 期の標準的治療戦略となりうる可能性が高いことが示された。また本剤が大腸癌や胃癌などの他癌種でも同様に補助療法として有効性が示された。従来進行肺癌での単剤としての有効性は 6～8%と言われていた薬剤が術後補助療法として有効性が示されたことは画期的である。本研究は、これを標準治療として、進行肺癌で汎用され有効性が示されている CP 療法の有効性を検討するデザインとした。これは術後補助療法として比較的毒性の少ない抗がん剤を長期投与することが良いのか、あるいは相応の毒性のある抗がん剤を進行癌と同様に短期的に投与するのが良いのかという術後補助治療コンセプトあるいは効果のメカニズムに関わる重要な情報を提供する可能性があり、研究の意義は大きい。また、この試験の結果は手術対象病期の非小細胞肺癌の標準的治療を確立するものであり、一般診療に情報還元するとともに、今後の臨床試験のデザインの礎となると予想される。

D．健康危険情報

健康危険情報として該当する事項はない。

E．その他実施した臨床研究・治験の概　要及び実績

1）2005（H17）年度に当該施設で行われた主な臨床試験

1］厚生労働省がん助成金・呼吸器悪性腫瘍に対する標準的治療確立のための多施設共同研究班：

i) JCOG0201；高分解能胸部 CT 所見に基づく肺野型早期肺癌の診断とその妥当性に関する研究：多施設共同・観察研究。研究目的ー術前の胸部薄切（thin section）CT 画像に基づく肺腺癌の質的診断を行い、縮小切除の適応となる病理学的非浸潤性肺腺癌を術前に同定できるかどうかを検討する。対象は臨床病期 IA 期の肺腺癌、またはその疑いの症例。登録症例数ー881 例。2004 年 5 月末に登録終了し、今年度はその解析を行った。当該施設から 52 例を登録。当該指導者・坪井正博は本グループの事務局として本試験の整備に関わる一方、当該施設のコーディネーターとしてその責務を果たしている。また、当該指導者・池田徳彦は研究分担医師として、その責務を果たしている。

2］臨床治験：

i) Iressa Case Control Study；非小

細胞肺癌治療におけるイレッサの投与及び非投与での急性肺傷害・間質性肺炎の発症頻度及び危険因子を検討するための国内、多施設共同、症例対照、市販後臨床試験。本件旧の目的は急性肺傷害の危険因子を同定、定量化し、イレッサの安全な使用を確保することにある。予定集積症例数；約3000 例以上、うち急性肺傷害例140 例。2003 年６月より試験開始し、2005 年6月で登録終了した。当該施設からの登録は 118 例である。当該指導者・坪井正博は、当該施設の施設責任医師として、池田徳彦は研究分担医師としてその責務を果たしている。

ii) Iressa 市販後大規模臨床第Ⅲ相試験；切除不能および再発非小細胞肺癌の second line chemotherapy としての有用性について Docetaxel を対照として比較検討する。予定集積症例数；600 例。2003 年８月より試験開始し、2005 年 9 月で登録終了した。当該施設からの登録は 21 例であった。当該指導者・坪井正博は、当該施設の施設責任医師として、池田徳彦は研究分担医師としてその責務を果たしている。

iii) EPOCH 注がん治療に伴う貧血に対する第Ⅲ相二重盲検比較試験；化学療法に伴う貧血（ヘモグロビン：11 以下）を呈する肺癌症例に対するエリスロポエチン製剤；EPOCH 注の有用性についてプラセボを対照として比較検討する。集積症例数；180 例。当該施設からの登録は 2005 年３月現在 12 例であった。当該指導者・坪井正博は、当該施設の施設責任医師として、池田徳彦は研究分担医師としてその責務を果たしている。

3] その他、いくつかの臨床治験と個別研究（医師主導）を実施、検討中である。

3） 臨床研究実施体制の活動

効果的医療技術の確立推進臨床研究事業；JCOG0204-MF などの多施設共同試験を通して集学的治療の実際を実践するとともに、指導者は試験登録、患者様への説明および同意取得に関しての指導を行った。若手医師、臨床研究協力者はこれらの指導のもとに、試験登録の補助、原資料の作成、整理および症例登録用紙の作成補助を行った。

別紙5

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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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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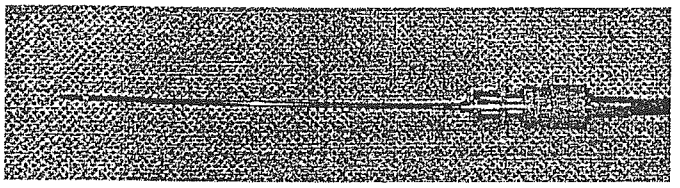


FIG. 1. A specially designed single-needle electrode, 150 mm in length and 1.6 mm in diameter, was inserted 20 mm into the normal lung.

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.

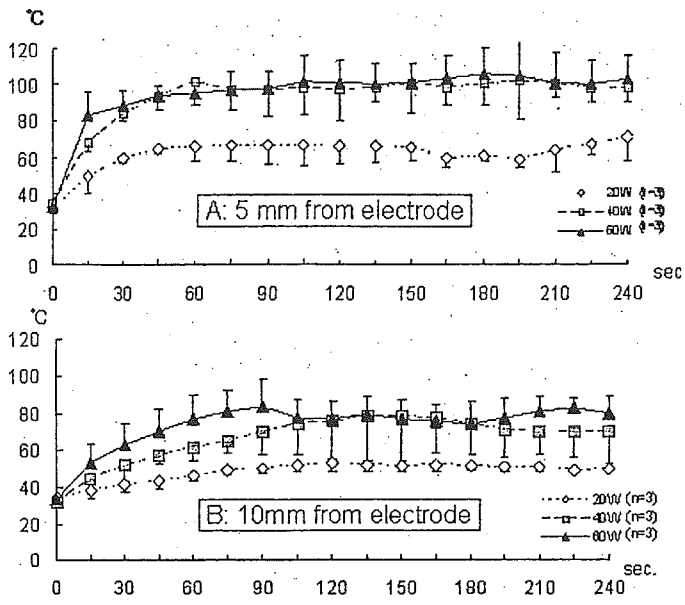


FIG. 3. (A) At 5 mm from the electrode, the temperature rose rapidly to 85°C (for 15 s at 60 W and 30 s at 40 W). The temperature reached a plateau around 90-100°C at 40 and 60 W. There was no significant difference between 40 and 60 W for each time point. (B) At 10 mm from the electrode, the temperature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

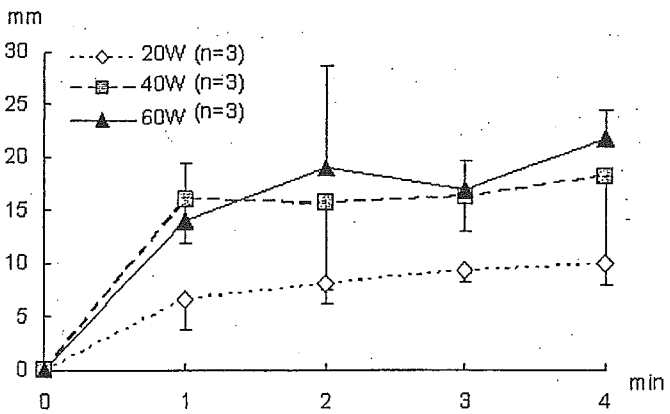
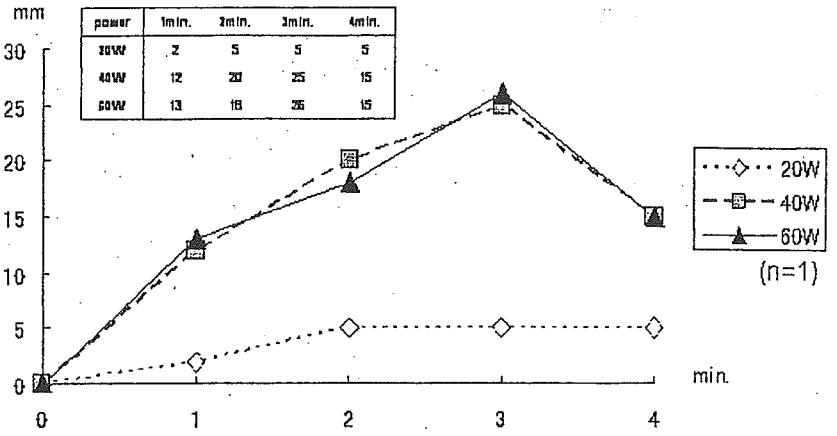


FIG. 4. The diameter of the maximum coagulation area was 18 mm at 40 W for 4 min and 22 mm at 60 W for 4 min. There was no significant difference between 40 W and 60 W for each coagulation time.



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.

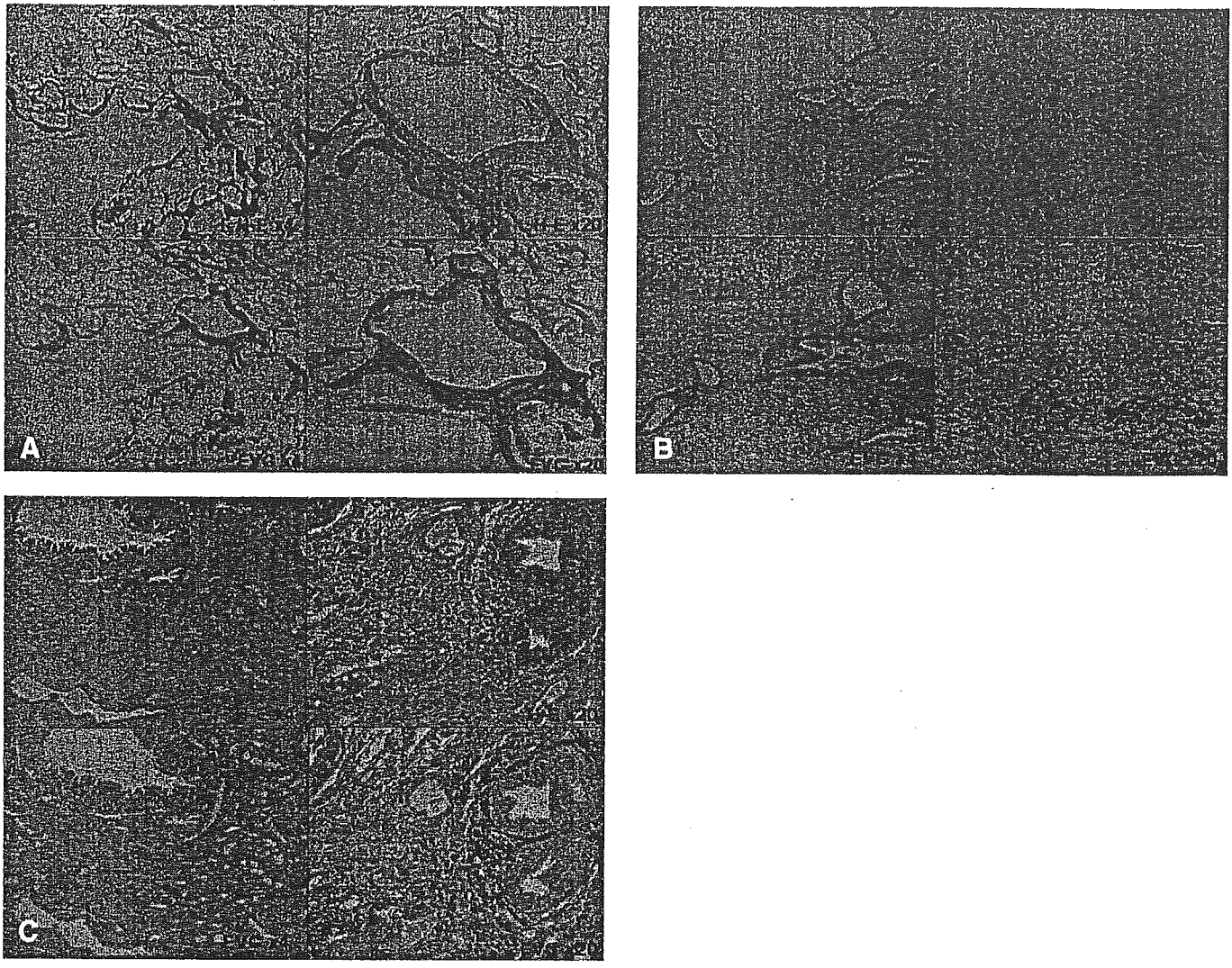


FIG. 6. (A) Histological findings shortly after microwave coagulation showed degeneration and thickening of collagen fiber and exfoliation and ulceration of bronchial epithelium surrounding the electrode. Surrounding bronchial and veins were not destroyed. (B) After 3 months, histological findings showed 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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KEYWORDS
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Plakoglobin;
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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-

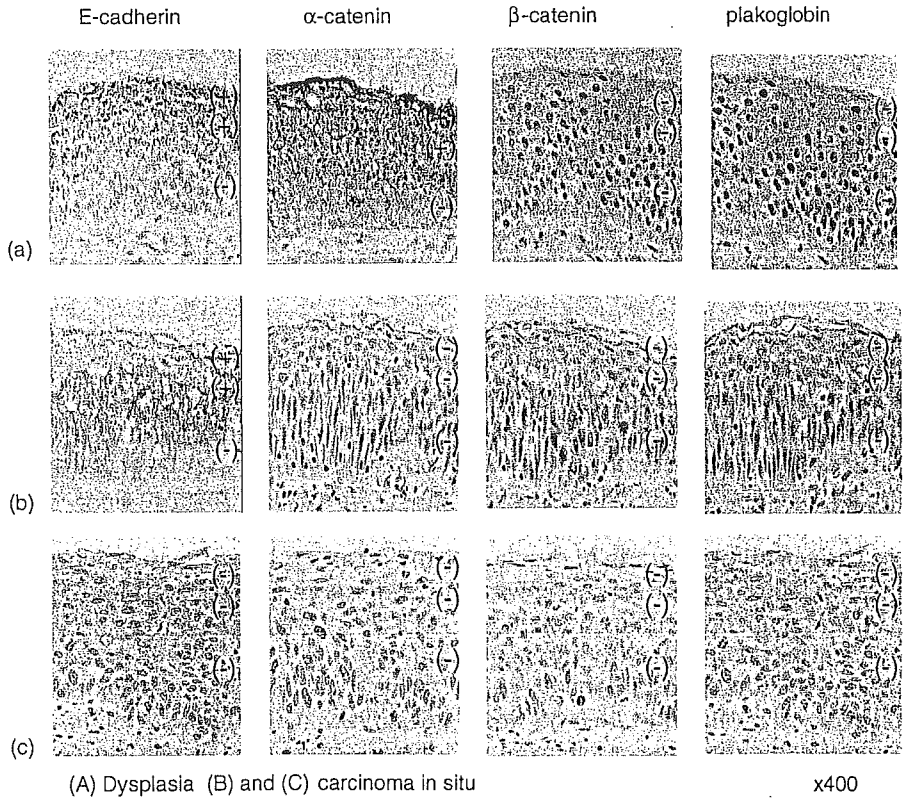

Fig. 1 (A) Representative immunohistochemical staining for E-cadherin, α-catenin, β-catenin, and plakoglobin in biopsy specimens of dysplasia (a) and carcinoma in situ (b and c). Evaluation for each layer of the intrabronchial lesions is shown at the right side of each picture ×400. (B) A borderline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

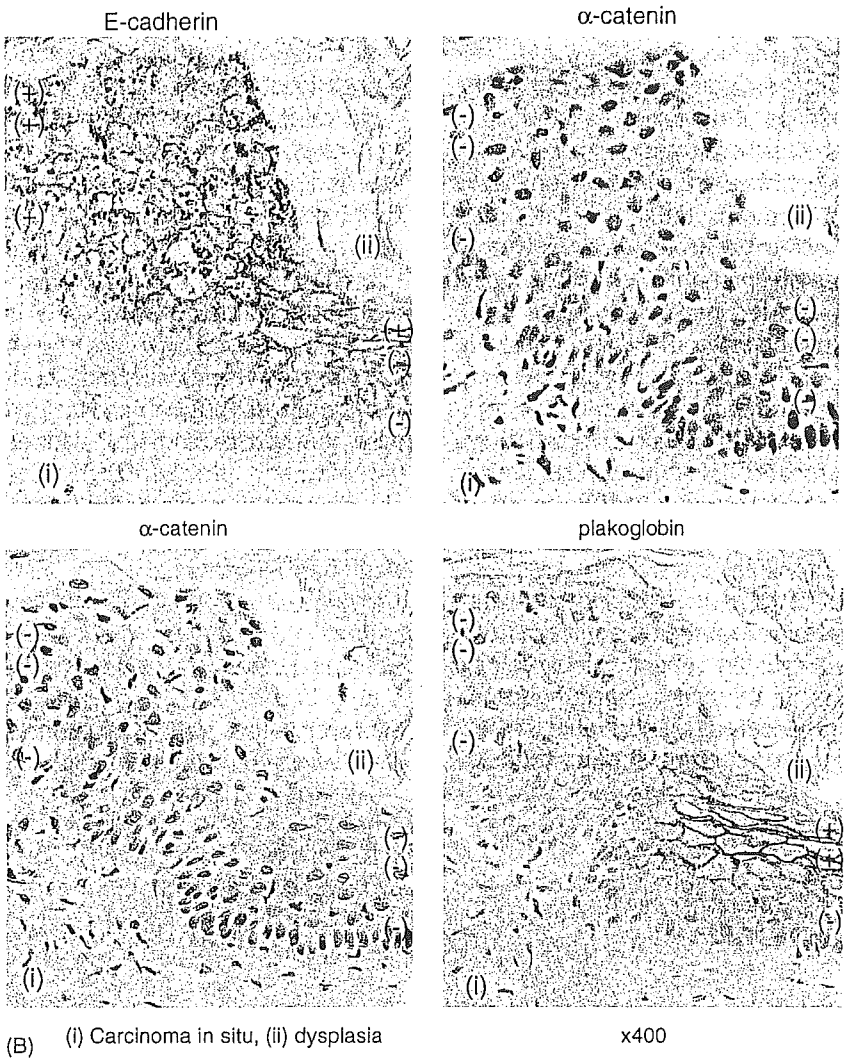

Fig. 1 (*Continued*).

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

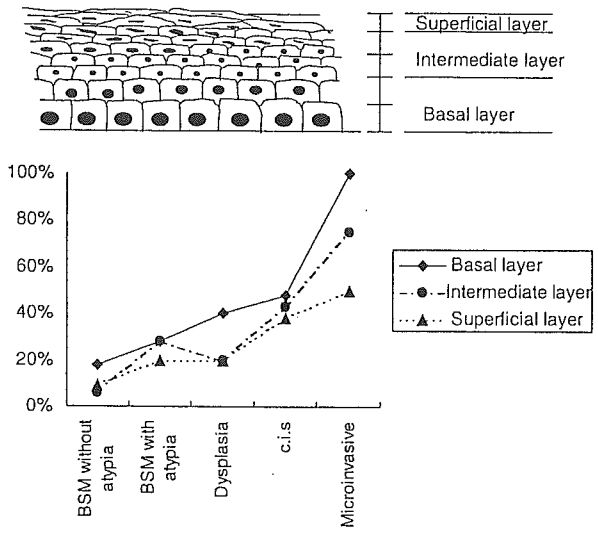

Fig. 2 Proportion of cases with reduced expression of either E-cadherin or catenins within the basal layer (♦), the intermediate layer (●) or the superficial layer (▲) of the intrabronchial lesions. The relative distribution of the different layers is shown in the upper part of the figure.

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