Table 1 Relationship between results of mediastinoscopy and number of stations evaluated as c-N2 on CT.

Results of mediastinoscopy	numbers of station evaluated as c-N2 on CT		p-value
	1 station	More than 2 stations	
positive	68	65	P=0.002
negative	50	17	

Table 2 Final p-n status in patients with c-N2, mediastinoscopically negative lung cancer.

p-n0	31
p-n1	18
p-n2	10
total	59

Table 3 Histopathological findings of lymph nodes obtained by mediastinoscopy in p-n0, 1 patients with c-N2 by CT, but mediastinoscopically negative lung cancer.

Silicotic change	6 cases
Sarcoid reaction	3 cases
Tuberculous change	1 case
No particular change	39 cases
total	49 cases

c-N2，縦隔鏡陰性例中のp-n0，n1計49例のうち，縦隔鏡下に採取したリンパ節の病理組織学的検索において，珪肺性変化を6例，サルコイド反応を3例，結核性変化を1例認めた．その他の39例では特記すべき病理組織所見は認められなかった（Table 3）．かかる39例のうち，9例で閉塞性肺炎，4例でそれぞれ肺結核，肺非定型抗酸菌症，珪肺，間質性肺炎を合併していた．その他の26例（53.1%）では，術前に肺癌以外のリンパ節腫大を来しうる誘因を有していなかった（Table 4）．

c-N2，縦隔鏡陰性例中のp-n2例10例において，縦隔鏡非到達域の転移を5例（部位：右側大動脈弓のため右#4の検索が不十分に終わった特殊例1例，#5が2例，#8が1例，#6と#9が1例であった），縦隔鏡到達困難な#7下方の転移を2例認めた．また，縦隔鏡到達可能域（#1，2，3，4，7浅部）での偽陰性例は術中出

Table 4 Complicating diseases of p-n0, 1 cases in c-N2 by CT, but mediastinoscopically negative patients without specific histopathological findings in LN.

Obstructive pneumonia	9 cases
Pulmonary tuberculosis	1 case
Non-tuberculous mycobacteriosis	1 case
Silicosis	1 case
Interstitial pneumonia	1 case
None	26 cases
total	39 cases

Table 5 Reasons for the discrepancy between mediastinoscopic-n status and final p-n status in p-n2 lung cancer patients with c-N2 by CT, but mediastinoscopically negative lung cancer.

Metastasis beyond mediastinoscope accessible region	5
Metastasis to the # 7 lower part where mediastinoscope hardly accessible	2
Failure to confirm metastasis to mediastinoscope accessible region	2
Incomplete study because of the hemorrhage	1
	n = 10

血のため検索不十分に終わった1例を含め3例であった（Table 5）．

縦隔鏡非到達域（#5，6，8，9）いずれかのみのc-N2例6例における，縦隔鏡ならびに開胸術時の縦隔リンパ節転移状況をみた（Table 6）．6例中2例で縦隔鏡にて，CTでのc-N2部以外への転移を認めた．また，6例中3例はCT疑陽性例であった．

縦隔鏡到達可能域のみを対象とした場合，sensitivityは97.7%，specificityは100%，accuracyは98.4%，negative predictive valueは94.9%，positive predictive valueは100%であった（Table 7）．縦隔鏡到達可能域以外のp-n2例も含めた場合，sensitivityは92.8%，specificityは100%，accuracyは94.7%，negative predictive valueは83.1%，positive predictive valueは100%であった（Table 8）．

考　察

肺癌では，リンパ節転移の有無により病期が変わり，治療方針，予後が大きく変わる．縦隔鏡検査は縦隔リンパ節転移の有無に関する質的診断が可能で，特に術

Table 6 Results of mediastinoscopy and operation in lung cancer patients evaluated as c-N2 only in station #5, 6, 8, or 9 on CT.

LN stations evaluated as c-N2 on CT	Confirmed positive stations by mediastinoscopy	Confirmed positive stations at thoracotomy
5	4, 7, contra. 4	2, 3, 4, 5, 7, 8, contra. 4
5	none	none
5	none	none
5, 6	3, 4	2, 3, 4, 5, 6
5	none	none
5, 6	none	3, 4, 5

LN: lymph node; contra.: contralateral.

Table 7 Results of the mediastinoscopic evaluation in c-N2 lung cancer patients (when having intended for only the mediastinoscope accessible level (#1, #2, #3, #4, and upper part of #7)).

Sensitivity	97.7%
Specificity	100%
Accuracy	98.4%
Negative predictive value	94.9%
Positive predictive value	100%

Table 8 Results of the mediastinoscopic evaluation in c-N2 lung cancer patients (when having intended for all mediastinal lymph nodes).

Sensitivity	92.8%
Specificity	100%
Accuracy	94.7%
Negative predictive value	83.1%
Positive predictive value	100%

前治療の対象となりうるc-N2症例においては，その正確な病期決定に有用とされている．今回我々は，術前CT検査にてc-N2と診断され，縦隔鏡検査にて陰性であった症例の既往歴および採取リンパ節の病理組織所見，ならびにc-N2症例の縦隔鏡検査成績をretrospectiveに検討した．

本検討において，CTにて腫大を認めた縦隔リンパ節のstation数が多い症例ほど，p-n2例が多いことが判明した．術前CTで複数のstationの縦隔リンパ節腫大を認める症例には，積極的に縦隔鏡検査での病理組織学的検索が必要であると考えられた．

CTにてc-N2と診断された59例のうち，49例（83.1%）において，縦隔鏡検査を施行することによりN2を否定できた．これらは画像のみで縦隔リンパ節転移ありと診断された場合，もしそれらがn0，1例であれば，本来first choiceであるべき手術療法が選択されない危険性のある一群である．このような危険性を下げるべく，かかる症例の既往歴や採取リンパ節の病理組織所見を検討したところ，術前に肺炎，感染症の合併など肺癌以外のリンパ節腫大誘因を有する例もあるが，むしろ誘因を考慮しがたい症例の方が多いことが明らかとなった．術前に肺炎，感染症を合併している症例では転移との鑑別のために，また明らかなリンパ節腫大誘因を有していない症例でも，縦隔鏡による組織学的検索が必要であると考えられた．

一般的に気管分岐部リンパ節（#7）への転移に関しては，その深部まで縦隔鏡を到達させることが困難なため偽陰性例が多く[1]，我々も縦隔鏡到達可能域のリンパ節を上縦隔，左右気管傍，気管前，左右気管気管支，気管分岐部の浅部としている[2]．本検討では，c-N2，縦隔鏡陰性で，最終的にp-n2であった10例のうち，#7深部を含めた縦隔鏡非到達域への転移を7例に認めたが，残る3例は術中出血のためにincomplete studyに終わった1例と到達可能域での見逃し例2例の，いずれも技術の向上により回避しうる症例であった．一方，縦隔鏡非到達域（#5，6，8，9）のみのc-N2例に対する診断，治療方針は議論の分かれるところである．当科では，腫大station部位にかかわらず，c-N2例においては全例縦隔鏡検査を施行しており，かかる症例を6例認め；うち2例で縦隔鏡にて，CTでのc-N2部以外への転移を認めた．また，6例中3例はCT疑陽性例であった．症例数は少ないものの，かかる症例に対しては，胸腔鏡などの他のアプローチによる腫大リンパ節の病理組織学的検索も考慮すべきであると考えられた．

CTによる縦隔リンパ節転移の診断は，リンパ節の大きさのみを診断根拠にしているので，CTに比し縦

隔鏡の診断率が優れていることは明らかであるが，近年，新しい肺癌の縦隔リンパ節転移の診断手段として，Positron emission tomography (PET)[3] や，PET-CT[4] の有用性に関する報告が散見されており，Dwamena らのメタアナリシスの報告によると，CT，FDG-PET の感度，特異度はそれぞれ60%，77%と，79%，91% であった[5]．本検討においては，縦隔鏡は，sensitivity が93%で，また false positive がないので，specificity は100%となり，共に CT，FDG-PET より良好な成績であった．さらなる診断精度の向上により，非侵襲性に縦隔リンパ節転移診断が可能となりうるが，少なくとも現時点では治療方針へ寄与する質的診断には縦隔鏡検査が有用であると考えられた．

結　語

CT にて c-N2 と診断された症例の83.1%において，縦隔鏡検査を施行することにより N2 を否定できた．これらの症例には術前に肺炎，感染症の合併など肺癌以外のリンパ節腫大誘因を有する例もあるが，誘因を考慮しがたい症例も多い．以上より，c-N2 でただちに手術非適応としたり，induction therapy を行うことは妥当ではない．非到達域の存在という弱点も有するが，質的診断には縦隔鏡検査が有用である．特に，複数の station に腫大を認める症例には，積極的に縦隔鏡検査を施行すべきである．なお，縦隔鏡における偽陰性例を減少すべく，さらなる技術の向上が必要であると考えられた．

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Benefits of Surgery for Patients With Pulmonary Metastases From Colorectal Carcinoma

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Background. A pulmonary metastasectomy for colorectal carcinoma is a generally accepted procedure, although several prognostic predictors have been reported. The aim of this study is to define the patients who benefit from pulmonary metastasectomy for colorectal carcinoma.

Methods. Retrospective survival analysis was performed using 128 patients who underwent curative pulmonary resection.

Results. The overall 5-year survival rate was 45.3%. Univariate analysis showed the number of metastases, location (unilateral or bilateral), prethoracotomy carcinoembryonic antigen (CEA) level, hilar or mediastinal lymph-node metastasis, and Dukes' stage to be considerable prognostic factors. Among these, Dukes' A for the primary lesion and unilateral pulmonary metastasis were shown to be independent predictors of longer survival by multivariate analysis ($p = 0.0093$ and $p = 0.0182$, respectively). In patients treated with both pulmonary and hepatic metastastasectomies, a better prognosis was observed in those who received metachronous resection. Recurrence after a pulmonary metastasectomy frequently occurred in the thorax and the 3-year survival rate was 44.6% in patients who underwent a repeat thoracotomy.

Conclusions. Patients with unilateral metastasis and Dukes' A for the primary tumor benefit most from the resection of pulmonary metastasis from colorectal carcinoma. Further, the number of metastases, prethoracotomy CEA level, and hilar or mediastinal lymph-node involvement should be considered to determine the operative indication. Finally, periodic follow-up examinations for thoracic recurrence should be carefully performed as these patients may have a heightened risk of requiring a repeat thoracotomy.

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The lung is one of the most frequent metastatic sites of colorectal carcinoma and some patients have undergone resection for pulmonary metastasis, since Blalock first reported the successful surgical treatment of pulmonary metastasis from colorectal carcinoma in 1944 [1]. Thereafter, Thomford noted criteria indicating the resection of metastatic lung tumors: (i) the patient must be a good risk for surgical intervention, (ii) the primary malignancy is controlled, (iii) there is no evidence of metastatic disease elsewhere, and (iv) the roentgenologic evidence of pulmonary metastasis is limited to one lung [2]. Morrow [3], Mountain [4], Takita [5], and The International Registry of Lung Metastases [6] then evaluated the surgical results for metastatic lung tumors, whereas McCormack discussed the operative indication for pulmonary metastasectomy associated with colorectal carcinoma [7]—one of the most frequent metastatic lung tumors that general thoracic surgeons encounter. Operative indications must be considered for all aspects, as pulmonary metastasis is a distant lesion and pulmonary resection always causes the deterioration of respiratory function. Thus, it is very important to identify the patients who can benefit from surgery.

We previously reported that the resection of pulmonary metastasis from colorectal carcinoma is effective in patients with a normal CEA level and without lymph-node metastasis [8], although several points, such as the relationship between survival and the number of metastatic tumors and/or stage of the primary tumor, the recurrence pattern after pulmonary metastasectomy, and the effectiveness of repeat pulmonary metastasectomy, as well as others, are still unclear because of the small number of patient cases. In this report, we conducted a retrospective multicenter analysis of patients treated with pulmonary metastasectomy for colorectal carcinoma. From our results, we discuss the prognostic factors, patterns of hilar or mediastinal lymph-node in-

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volvement, the outcomes of patients with pulmonary metastasis found during treatment for the primary lesion, the surgical results of patients treated for both hepatic and pulmonary metastases, the recurrence sites after pulmonary metastasectomy, and the significance of repeat pulmonary metastasectomy in an attempt to define which patients can benefit from a metastasectomy associated with colorectal carcinoma.

Patients and Methods

The records of all patients with pulmonary metastases from colorectal carcinoma who underwent a pulmonary metastasectomy between March 1979 and May 2002 at Toneyama National Hospital, Osaka University Hospital, Osaka Prefectural Habikino Hospital, and National Kinki Central Hospital were reviewed. All resected specimens were pathologically confirmed to be pulmonary metastases from colorectal carcinoma by each pathology division. Preoperative evaluation was performed using chest roentgenogram and computed tomography (CT) images for pulmonary nodules. Only those patients whose metastatic sites were confined to the lung or with extrathoracic metastases that could be completely resected after surgery were included in this study. Three patients underwent incomplete resection and were excluded from this study. Patients were evaluated for age, gender, primary site, number and largest diameter of pulmonary metastases, existence of metastases on the unilateral or bilateral lungs at diagnosis, prethoracotomy carcinoembryonic antigen (CEA) level, hilar or mediastinal lymph-node metastases, and Dukes' stage of the primary tumor to define prognostic factors. Because the chance of detection of pulmonary recurrence depended on how frequently the attending physician checked the chest roentgenogram or CT images, we did not use disease-free interval as a variable. We also analyzed the surgical results of patients with both hepatic and pulmonary metastases and patients with pulmonary metastasis found during surgery for the primary. Furthermore, we surveyed all patients who suffered from recurrence after pulmonary metastasectomy and evaluated the results of the repeat thoracotomy. Survival after pulmonary resection was estimated according to the method of Kaplan–Meier. The prognostic influence of variables on survival was analyzed using the log-rank test and Cox proportional hazards model. All variables tested by univariate analysis were applied to multivariate analysis.

Results

Patient characteristics are shown in Table 1. Only those who underwent complete resection of pulmonary metastases were included. A thoracotomy without a macroscopic residual lesion was defined as complete resection. The number of metastatic lesions was evaluated using conventional CT scanning and operative palpation. Three patients had extrathoracic lesions at the time of thoracotomy: 2 with hepatic metastasis were treated with simultaneous hepatectomy and transcatheteric arterial embolization and 1 with adrenal metastasis underwent sequential adrenalectomy. The resection margins were evaluated by histology or cytology. The mean follow-up period was 85.9 months and 7 patients were lost to follow-up. The estimated 5-year survival rate for all patients was 45.3% (Fig 1). The 5-year survival rate of patients with solitary pulmonary metastasis was 50.6% as compared with 29.5% for those with multiple metastases ($p = 0.0163$). The prognosis of patients with a solitary metastasis was considerably better than with two or

Table 1. Patient Characteristics

Age	39–78 years (mean 61.8)	
Gender	male	85
	female	43
Primary	colon	73
	rectum	54
	colon + rectum	1
Prethoracotmy CEA	elevated	56
	normal	65
	not measured	7
Metastatic site[a]	right upper lobe	47
	right middle lobe	19
	right lower lobe	36
	left upper lobe	40
	left lower lobe	31
Operation mode	pneumonectomy	2
	lobectomy	69
	segmentectomy or partial resection (video-assisted thoracoscopic surgery 10)	57
Metastatic number	solitary	95
	multiple (bilateral metastasectomy 12: simultaneous 2, sequential 10)	33

[a] All metastatic lesions for each pulmonary lobe were counted.
CEA = carcinoembryonic antigen.

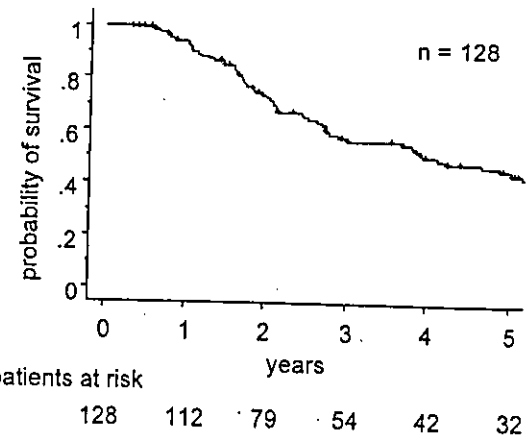

Fig 1. Overall survival of 128 patients who underwent curative resection for pulmonary metastases from colorectal carcinoma. The 5-year survival rate was 45.3%. Median survival time was 49.5 months.

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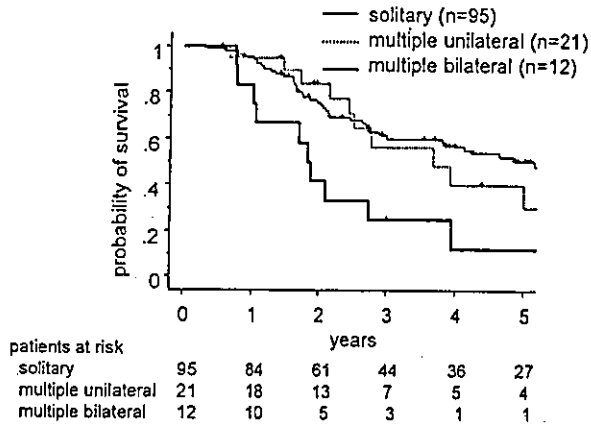

Fig 2. Probability of survival according to the number and location (unilateral or bilateral) of metastatic lesions. The 5-year survival rate for patients with solitary, multiple unilateral, and multiple bilateral metastases was 50.6%, 40.3%, and 12.5%, respectively. The survival rate for patients with multiple bilateral metastases was worse than with a solitary lesion (p = 0.0028) and with multiple unilateral lesions (p = 0.0727).

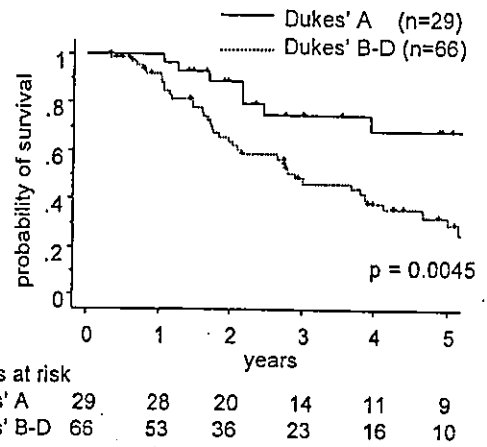

Fig 3. Probability of survival by Dukes' stage. The 5-year survival rate for patients with Dukes' A was 68.7% compared with 32.8% for patients with Dukes' B–D (significant, p = 0.0045).

more. When considering both the number and location of metastasis, the 5-year survival rate for patients with solitary, multiple unilateral, and multiple bilateral metastases was 50.6%, 40.3%, and 12.5%, respectively (Fig 2). The survival rate for patients with multiple bilateral metastases was worse than with a solitary lesion and with multiple unilateral lesions. Patients with a unilateral lesion demonstrated better outcome results.

The serum CEA level was measured before pulmonary metastasectomy in 121 patients, of which 56 (46.3%) had an elevated prethoracotomy CEA level. The cutoff value for each institute varied from 2.5–7.0 ng/ml. The 5-year survival rate for patients with normal CEA was 51.9% as compared with 38.3% for patients with elevated levels ($p = 0.0563$). When a limit of a twofold cutoff for each institute was used, the 5-year survival rate for 93 patients with a low CEA level was 51.7% which is significantly better than the 27.5% for patients showing a high CEA level ($p = 0.0184$).

In the last decade, we have performed lymph-node dissection, or at least sampling, and found mediastinal or hilar lymph-node metastases in 21 out of 89 patients (23.6%). The 5-year survival rate for patients without lymph-node involvement was 50.8% as compared with 19.3% for node-positive patients ($p = 0.0047$). Most positive nodes were located at the metastatic lobes or nearby sites in the mediastinum [9]. The level of node involvement, whether hilar or mediastinal, had no prognostic implications.

We also analyzed patient survival according to Dukes' stage as determined during surgery for a primary colorectal lesion in 95 patients (Dukes' A, 29; B, 8; C, 48; D, 10). The 5-year survival rates for patients with Dukes' A, B, C, and D were 68.7%, 38.1%, 31.9%, and 27.8%, respectively. Because the number of Dukes' B and D patient cases was small, we compared Dukes' A versus B–D and found that the 5-year survival rate for those with Dukes' A was 68.7% as compared with 32.8% for those with B–D (Fig 3).

The potential prognostic factors were tested by univariate and multivariate analyses. Univariate analysis using the variables listed in Table 2 showed that the number of metastases, location (unilateral or bilateral), CEA level, mediastinal or hilar lymph-node involvement, and Dukes' stage were considerable prognostic factors. Regarding CEA level, when positive cases were compared with negative, we found a trend, however, the limit of twice the cutoff revealed survival significance between CEA high and low groups. Solitary metastasis, unilateral location, a CEA level less than twice the cutoff, no lymph-node metastasis, and Dukes' A were better prognostic predictors in univariate analysis. Using all variables tested by univariate analysis, we also performed multivariate analysis (Table 3). As a result, Dukes' stage and pulmonary metastasis location were shown to be

Table 2. Potential Prognostic Factors by Univariate Analysis in Patients With Pulmonary Metastasis From Colorectal Carcinoma

Variables	Hazard Ratio	95% CI	p Value
Age	1.004	0.979–1.030	0.7367
Gender (female:male)	0.933	0.555–1.568	0.7930
Primary (colon:rectum)	0.783	0.481–1.275	0.3256
Number (> 2: solitary)	1.866	1.113–3.129	0.0180
Size[a] (> 3 cm: < 3 cm)	1.300	0.796–2.122	0.2939
Location (bilateral: unilateral lung)	2.700	1.368–5.329	0.0042
CEA (elevated: WNL)	1.625	0.982–2.689	0.0587
(>:<2 × cutoff)	1.920	1.106–3.335	0.0205
LN (positive: negative)	2.266	1.266–4.058	0.0059
Dukes' stage (A: B–D)	0.348	0.162–0.745	0.0066

[a] Size = maximum diameter of the pulmonary metastases.

CI = confidence interval; LN = lymph node metastasis; WNL = within normal limits.

Table 3. Potential Prognostic Factors by Multivariate Analysis in Patients With Pulmonary Metastasis From Colorectal Carcinoma

Variables	Hazard Ratio	95% CI	*p* Value
Age	1.008	0.972–1.045	0.6797
Gender (female:male)	0.589	0.271–1.280	0.1811
Primary (colon:rectum)	0.832	0.398–1.741	0.6257
Number (> 2: solitary)	0.908	0.408–2.020	0.8124
Size[a] (> 3 cm: < 3 cm)	1.589	0.817–3.092	0.1728
Location (bilateral: unilateral lung)	4.206	1.277–13.856	0.0182
CEA (elevated: WNL)	1.354	0.663–2.764	0.4050
LN (positive: negative)	2.217	0.951–5.170	0.0653
Dukes' stage (A: B–D)	0.326	0.140–0.759	0.0093

[a] Size = maximum diameter of the pulmonary metastases.

CI = confidence interval; LN: lymph node metastasis; WNL = within normal limits.

independent prognostic predictors. No lymph-node involvement showed a tendency to be a better prognostic factor. The regression coefficient of metastatic number to location was 0.470 and metastatic number was not detected as a prognostic factor in the multivariate analysis. Thus, patients with Dukes' A and a unilateral pulmonary metastatic lesion were determined to most likely benefit from a metastasectomy.

Thirteen patients underwent both pulmonary and hepatic metastasectomy, whereas metachronous and synchronous resections were performed in 10 and 3 patients, respectively. The most frequent metastasectomy pattern for both the lung and liver was metachronous resection (primary lesion—hepatic and pulmonary metastasis) in 9 patients and surgical results produced 47 months of median survival time (MST). The interval between hepatectomy and pulmonary resection ranged from 12–73 months (average 35.2 months). One patient who underwent a resection (primary lesion—pulmonary and hepatic metastasis) was alive after 87 months. In contrast, patients treated with synchronous resections had a poor outcome, although the number of patient cases was small. Two patients who underwent a resection (primary lesion—liver and lung metastases) died after 12 and 14 months and 1 patient has survived for 6 months. On the other hand, the 5-year survival rate for 115 patients with only pulmonary metastases was 45.5% and the MST was 49.5 months.

Nine patients were diagnosed with colorectal carcinoma with pulmonary metastasis before treatment for the primary lesion (ie, Dukes' D, colorectal carcinoma). The primary site was the colon in 6 patients and the rectum in 3 patients. The tumor diameter ranged from 1–10 cm (average 3 cm). A lobectomy was performed in 5 patients and 4 patients underwent partial resection. The survival for these patients ranged from 13–62 months (MST: 26 months), whereas 3 patients survived for more than 4 years. Recurrence after pulmonary resection occurred in the lung in 3 patients and the brain in 2 patients.

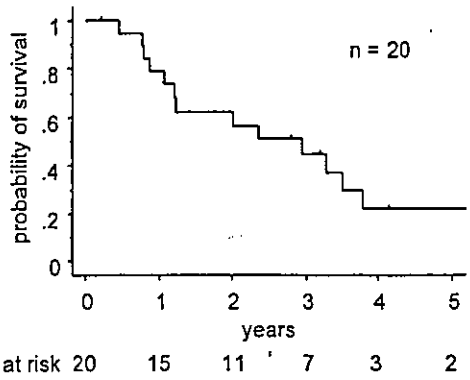

Fig 4. Probability of survival for patients who underwent a repeat pulmonary metastasectomy. The 3- and 5-year survival rates were 44.6% and 22.3%, respectively. Median survival time was 35.4 months.

We also thoroughly surveyed the outcomes after pulmonary metastasectomy. Interestingly, intrathoracic recurrence, especially in the lung, was most frequent and occurred in 46 (53%) of the patients that showed evidence of recurrence. Recurrence in the liver, brain, bone, and peritoneum was detected in 12, 9, 9, and 8 patients, respectively. Among those suffering from intrathoracic recurrence, 20 patients underwent a repeat thoracotomy and their survival curve is shown in Figure 4. The 3- and 5-year survival rates after the second operation were 44.6% and 22.3%, respectively, with an MST of 35.4 months.

Comment

Surgical results for pulmonary metastasis related to colorectal cancer have been reported from several institutes with 5-year survival rates ranging from 22.0%–48.0% [7, 8, 10–18]. From their findings, with the results of our study, and because no other effective treatment for this disorder has been established, we suppose that pulmonary metastases from colorectal carcinoma should be resected according to the following criteria: (i) pulmonary nodules consistent with metastases, (ii) no residual tumor at the primary site, (iii) all nodules potentially resectable with planned surgery, (iv) adequate postoperative pulmonary reserve anticipated, and (v) no extrathoracic metastases. In addition to these criteria, we propose the following five prognostic predictors to consider surgical indication: (i) location (unilateral or bilateral) and Dukes' stage as independent prognostic factors, (ii) the number of metastases, (iii) prethoracotomy CEA level, and (iv) intrathoracic lymph-node involvement as reference factors. From these findings, we have redefined the Dukes' stage as a prognostic predictor in patients with pulmonary metastasis from colorectal carcinoma. This study revealed that the prognostic impact of tumor location and Dukes' stage was greater than that of CEA level or lymph-node involvement which was shown to be prognostic factors in our previous study with a small number of patients [8].

Sakamoto reported that no considerable differences in survival rate were found among patient groups for solitary, ipsilateral multiple, and bilateral metastases [13]. In this study, although only 12 patients (9.4%) suffering bilateral metastases underwent a metastasectomy, the 5-year survival rate was substantially worse than that for patients with unilateral metastasis. It is expected that the survival rate will decrease in association with an increase in the number of metastases in patients with bilateral metastases even if all metastatic nodules are macroscopically extirpated. The mean number of lesions resected in these patients with bilateral metastases was 4.2, therefore, we considered that such bilateral multiple metastatic patients had a minimal depressive effect on the survival curve of the bilateral metastases group. Furthermore, multivariate analysis revealed that tumor location (unilateral or bilateral) and not metastatic number was a most powerful and independent prognostic factor suggesting that patients with hemithoracic pulmonary lesions could be good candidates for resection. However, patients with bilateral metastases will gain no surgical benefit.

We found that patients with a solitary pulmonary metastasis had a considerably better survival rate than those with two or more by univariate analysis, although multivariate analysis did not show the metastatic number to be an independent prognostic factor. The number of pulmonary metastases has often been reported as an independent prognostic factor [10–12, 16, 19, 20], whereas some studies have found no marked difference [7, 14]. CT imaging has recently been improved and is able to detect a tiny nodule allowing for preoperative knowledge of the exact number of pulmonary metastases. Thus, an accurate preoperative evaluation of nodule number, which may have an influence on prognosis, has become increasingly important. The size of the metastases was not a prognostic factor in our study and most other reports found no substantial relationship between tumor size and patient prognosis [12, 14–16, 20], although Shirouzu [10] and Okumura [11] reported that patients with pulmonary metastases 3 cm or greater in diameter had worse survival than those with tumors less than 3 cm. In the analysis by Shirouzu, the results may have been influenced by the bias that 57% of the patients with metastases 3 cm or greater in diameter suffered from lymph-node metastases.

Sakamoto [13], Saito [18], Rena [21], Pfannschmidt [22], Higashiyama [23], and Headrick [24] have reported CEA to be a prognostic factor in patients with pulmonary metastasis from colorectal carcinoma. In this study, the outcome of patients with an elevated CEA level was inclined to be poor compared with those having a normal level (Table 2). However, when analyzing with a twofold cutoff value limit, the survival of the CEA high group was considerably worse than that of the CEA low group with a less than twofold cutoff (Table 2). Interestingly, recent reports by Saito [18] and Higashiyama [23] similarly showed that a prethoracotomy CEA greater than 10 ng/ml was a predictor of poor prognosis, whereas their cutoff values were set at 5 ng/ml. Namely, poor prognosis could be predicted in patients with a distinct elevation of CEA.

As for hilar or mediastinal lymph-node involvement in patients with pulmonary metastasis from colorectal carcinoma, the previous analysis from a single institute resulted in a substantially better prognosis for patients without lymph-node metastasis [8]. Okumura, Saito, Pfannschmidt, and Headrick also reported the prognostic impact of lymph-node involvement [11, 18, 22, 24]. We confirmed those results in this study using a large number of patients and concluded that lymph-node metastasis was a prognostic factor (Table 2). However, multivariate analysis showed lymph-node involvement not to be an independent prognostic predictor when analyzed together with Dukes' stage (Table 3). We interestingly observed that most patients with lymph-node metastases were diagnosed with Dukes' B–D for the primary lesion except for 2 patients. There may be some biological relationship between the invasiveness of the primary tumor and the intrathoracic lymph-node involvement. In addition, the metastatic site of nodes corresponded to the regional segmental or lobar nodes supporting the hypothesis of node metastasis from pulmonary metastatic lesions. Thus, we suppose that sufficient evaluation of intrathoracic lymph nodes, for instance, using CT, fluorodeoxyglucose-positron emission tomography, fiberscopic transbronchial needle aspiration, or mediastinoscopy is important in aiding the decision of the operative indication, as lymph-node involvement is not rare in patients with pulmonary metastasis from colorectal carcinoma. The results of this study showed that patients with proven node metastasis had no operative indication. We recommend lymph-node dissection or sampling to predict prognosis.

The invasiveness of colorectal carcinoma is generally classified using Dukes' stage in which a tumor limited to the muscularis propria is considered to be Dukes' A, that beyond the muscularis propria but without lymph-node metastasis is considered to be Dukes' B, that with lymph-node metastasis is considered to be Dukes' C, and a tumor remaining locally or with distant metastasis is considered to be Dukes' D [25]. Dukes' classification is reported to be a prognostic factor for patients undergoing hepatic metastasectomy for colorectal carcinoma [26], however, the prognostic impact of Dukes' stage for pulmonary metastasis has not been sufficiently assessed. Sauter found a better, though statistically insignificant, disease-free survival in Dukes' B compared with Dukes' C patients who underwent pulmonary resection of metastatic colorectal carcinoma [27]. We found the Dukes' stage to be an independent prognostic factor (Table 3). These results suggest that the degree of local invasiveness can influence the prognosis of patients with distant metastasis in colorectal carcinoma. We believe that Dukes' classification is useful to predict prognosis and should be considered for operative indication in patients with pulmonary metastasis.

Regarding the pulmonary and hepatic metastasectomy procedure, Gough reported 9 patients with a median survival of 27 months [28] and Lehnert noted that se-

quential resection was warranted in carefully selected patients with a 2-year survival rate of 70% [29]. Kobayashi also reported a 5-year survival rate of 31% in 47 patients and found that patients with a solitary pulmonary metastasis or a small number of hepatic metastases were good candidates for metastasectomy [30]. Further, a study by Robinson revealed that patients treated with metachronous resection survived longer as compared with synchronous resection [31]. Similarly, patients treated with metachronous resection showed a reasonably good outcome in this study. Thus, we consider that patients suffering from metachronous metastases to the liver and lung are potential operative candidates.

The thorax was the most frequent site of recurrence after pulmonary metastasectomy. Mori also reported similar results which showed intrathoracic recurrence in 58% of 35 patients including 79% in the lung and 21% in the lymph nodes [14]. From these results and the feasible outcome after repeated metastasectomy, we concluded that patients undergoing pulmonary metastasectomy from colorectal carcinoma should be carefully followed up, especially in the chest as they may have a heightened need for repeat thoracotomy. Our recommendation for follow-up is a chest roentgenogram at least every 3 months and a CT scan every year. The serum CEA level may also be useful to predict recurrence, because 5 patients who underwent repeated metastasectomy showed CEA elevation. However, no applicable prognostic predictor to select candidates for repeat metastasectomy was found among the variables tested in this study. Therefore, we would consider repeat metastasectomy for good-risk patients whose lesions are resectable.

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Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA levels in tumor tissues and the efficacy of 5-fluorouracil in patients with non-small-cell lung cancer

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Real-time RT-PCR

Summary We examined 116 stage I–IIIA non-small-cell lung cancer (NSCLC) patients for intra-tumoral expression of thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) using TaqMan reverse transcription polymerase chain reaction (RT-PCR) assay to clarify the correlation between gene expression and the efficacy of 5-fluorouracil (5-FU) in patients with NSCLC. Patients who were administered 5-FU alone after surgery comprised the 5-FU group ($n = 30$), and those who underwent only surgery comprised the control group ($n = 86$). When dichotomized at the mean TS and DPD mRNA level, patients with low-DPD tumors who were administered 5-FU had a significantly better prognosis than those who did not receive adjuvant treatment ($p = 0.041$). In addition, in the 5-FU group, 10 patients with both low-TS and low-DPD tumors have not had any relapse, whereas 8 of the 20 patients with either high-TS or high-DPD tumors developed distant metastasis after surgery. Based on these results, the quantitation of TS and DPD mRNA levels may predict the efficacy of 5-FU after surgery for patient with NSCLC.
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1. Introduction

Thymidylate synthase (TS) is the target enzyme for 5-fluorouracil (5-FU) and catalyzes the methylation of fluorodeoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), which is an important process for DNA biosynthesis [1,2]. Recent studies have demonstrated that the prognosis of cancer patients is significantly related to TS intra-tumoral immunohistochemical expression and mRNA levels [3,4]. We had previously shown that the quantitation of TS mRNA levels is clinically sensitive and useful for determining

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the prognosis of stage I and II non-small-cell lung cancer (NSCLC) patients [5]. On the other hand, dihydropyrimidine dehydrogenase (DPD) is a key enzyme in the catabolism of 5-FU and its high expression in a tumor is thought to reduce the efficacy of 5-FU [6,7]. Therefore, the activity of these enzymes in tumor tissues is considered to be associated with the chemosensitivity to 5-FU [8,9].

5-FU has been used in gastric cancer, colorectal cancer, breast cancer, and NSCLC. Recently, several reports showed that oral UFT (a combination of tegafur and uracil in a molar ratio of 1:4, Taiho Pharmaceutical Co. Tokyo, Japan) is effective postoperative adjuvant chemotherapy for NSCLC [10,11]. In addition, it is reported that DPD expression may predict the efficacy of UFT after surgery for NSCLC with immunohistochemical clinical investigation [12,13]. It is also reported that DPD activities evaluated by radioenzyme assay correlated with in vitro sensitivity to 5-FU [14]. Since there is no study on TS and DPD gene expression in NSCLC tissues, we measured both TS and DPD mRNA levels tissues, and examined the association of these enzyme mRNA levels with clinicopathological factors and the efficacy of 5-FU.

2. Materials and methods

2.1. Specimens

One hundred and sixteen specimens from 116 lung cancer patients that were determined to be p-stage I–IIIA [15] were obtained at Osaka University Hospital, National Kinki Central Hospital, Toneyama Hospital, Osaka Prefectural Habikino Hospital, and Osaka Police Hospital between January 1999 and March 2003. The specimens were frozen in liquid nitrogen as soon as possible and stored at −70 °C until extraction of RNA. Informed consent was obtained from all patients. Patients who were administered UFT alone comprised the 5-FU group ($n = 30$), and those who underwent only surgery comprised the control group ($n = 85$). UFT administration was started within 2 months after surgery and continued more than 6 months. The dose and mean duration of UFT were 300–400 mg/body weight per day and 19.4 ± 7.3 months (mean ± S.D.; range: 6–24 months), respectively. The clinical background of patients is summarized in Table 1. There was no difference in clinical factors between the 5-FU group and the control group. Median follow-up of the patients was 42 months (range: 6–57 months) after surgery.

Table 1 Patients background

Variable	Treatment		P-value
	Surgery alone ($n = 86$)	5-FU ($n = 30$)	
Age (mean ± S.D., years)	64.7 ± 9.7	62.8 ± 9.0	0.346[a]
Gender			
Male	61	18	0.347[b]
Female	25	12	
Pathological stage			
IA	32	14	
IB	26	7	
IIA	3	2	0.795[b]
IIB	10	3	
IIIA	15	4	
Histologic type			
Adenocarcinoma	58	23	
Squamous cell carcinoma	25	5	0.347[b]
Others	3	2	
Performance status			
0	75	27	0.687[b]
1	11	3	

[a] Mann–Whitney U-test.
[b] Chi-square test.

Table 2 Sequences of PCR primers and sequence-specific probes for TS, DPD and GAPDH

	Sequence (5′–3′)
TS	
Forward primer	GAATCACATCGAGCCACTGAAA
Reverse primer	CAGCCCAACCCCTAAAGACTGA
Probe	FAM-TTCAGCTTCAGCCAGAACCCAGA-TAMRA
Standard reverse primer	TTT TTT TTT TTT TTT GGC TAC CAC ATG AGT CGA GGG AGT
DPD	
Forward primer	AATGATTCGAAGAGCTTTTGAAGC
Reverse primer	GTTCCCCGGATGATTCTGG
Probe	FAM-TGCCCTCACCAAAACTTTCTCTCTTGATAAGGA-TAMRA
Standard reverse primer	TTT TTT TTT TTT TTT GGT GGT TCC CCC GGA TGA TTC TGG
GAPDH	
Forward primer	GAAGGTGAAGGTCGGAGTC
Reverse primer	GAAGATGGTGATGGGATTTC
Probe	TET-CAAGCTTCCCGTTCTCAGCC-TAMRA
Standard reverse primer	TTT TTT TTT TTT TTT GAA GAT GGT GAT GGG ATT TC

2.2. Real-time quantitative RT-PCR for TS, DPD and GAPDH mRNA

The quantitation of TS and DPD mRNA levels in NSCLC tissues was performed, as described previously [5]. Briefly, to construct RNA standards for the real-time reverse transcription polymerase chain reaction (RT-PCR) assays for TS, DPD and GAPDH mRNA, the region of each mRNA was amplified with each forward primer and the standard reverse primer (Table 2) using cDNA derived from the RNA of a healthy volunteer as a template. Amplified fragments were cloned into pCR2.1 (Invitrogen, The Netherlands), and the plasmid was transformed into *E. coli* (Toyobo, Tokyo, Japan). The RNA fragment used for the preparation of RNA standards was synthesized in vitro from the plasmid that had been linearized with an RNA polymerase T7 RNA synthesis kit (Wako, Tokyo, Japan). The RNA standards were constructed by sequential dilution in a range of 10^4 and 10^{10} copies/μl, following calculation of the RNA copy number.

Total RNA was extracted from 100 mg of each tissue sample using an AGPC method. A 1 μg aliquot of total RNA solution including oligo-dT primer in a total volume of 7 μl was incubated at 80 °C for 5 min. After cooling on ice, RT–enzyme mixture was added, and the resulting mixture was incubated at 42 °C for 60 min, then denatured at 95 °C for 5 min.

PCR reactions were carried out using a reaction mixture containing TaqMan Universal PCR Master Mix (PE Applied Biosystems, Foster City, USA), the primer mixture, a labeled probe (Table 2), and sample cDNA. Real-time PCR assays were run on an ABI PRISM 7700 sequence detection system (PE Applied Biosystems) with the following protocol: 50 °C for 2 min, 95 °C for 10 min, and then 50 cycles at 95 °C for 15 s and 60 °C for 2 min.

The amount of mRNA levels in the sample specimen was calculated from the threshold cycle (C_t) of the sample and the RNA standard curve. The obtained copy number of TS or DPD was then standardized with GAPDH mRNA quantity as the endogenous control using the equation: result = log(TS or DPD RNA copy number in tumor)/(GAPDH RNA copy number in tumor) × (6.1×10^9; GAPDH RNA copy number in 1 μg of total RNA extracted from the peripheral blood of 30 healthy volunteers).

2.3. Immunohistochemical staining for TS and DPD

Formalin-fixed and paraffin-embedded 3 μm thick tissue sections were deparaffinized and rehydrated. For antigen retrieval steps, the sections were treated by microwave for 10 min in a 10 μM hot citrate buffer solution at pH 6.0. After quenching the endogenous peroxidase activity with 0.3% H_2O_2 for 20 min, the sections were pre-incubated with peroxidase blocking reagent (DAKO, Kyoto, Japan) for 15 min. The sections were then incubated overnight at 4 °C with anti-TS [16] and anti-DPD polyclonal antibodies [17] provided by Taiho Pharmaceutical Co. Ltd., Saitama, Japan (diluted to 1:500). Biotinylated goat anti-rabbit IgG (Dako, Kyoto, Japan) was applied as a secondary antibody for 20 min at room temperature, followed by streptavidin–biotinylated peroxidase complex

for 20 min at room temperature. Peroxidase activity was visualized with a diaminobenzidine tetrahydrochloride solution for 5 min at room temperature. The sections were then counter-stained with hematoxylin. Slides were examined separately by two independent investigators who were unaware of the clinical characteristics of the patients. Five areas selected at random were scored by "HSCORE", which reflects both the intensity and percentage of cells staining at each intensity according to the method described by McCarty et al. [18]. Intensity was classified as 0 (no staining), +1 (weak staining), +2 (distinct staining), or +3 (very strong staining). A value designated the "HSCORE" was obtained by the following algorithm: HSCORE = $\sum$ (intensity × percentage of cells that stain at each intensity). When the HSCORE was ≥30, the sample was classified as high TS, and it was <30, the sample was classified as low TS [5,12]. In addition, when the HSCORE of DPD in a given specimen was ≥50, the sample was classified as high DPD, and it was <50, the sample was classified as low DPD [12].

2.4. Statistical analysis

Chi-square test, Mann–Whitney *U*-test and Kruskal–Wallis test were used to compare the results, and free disease survival rates were estimated by the method of Kaplan and Meier [19] and compared with results of a log-rank test using Statview version 5.0 for Windows (Abacus Concepts, Berkeley, CA) and a *P*-value of <0.05 was considered to be statistically significant.

3. Results

3.1. TS and DPD mRNA levels in tumors

The quantitation of TS and DPD mRNA levels in NSCLC tissues was successfully performed in all specimens. Intra-tumoral TS and DPD mRNA levels varied from 6.279 to 8.041 (mean ± S.D.: 6.97 ± 0.33) and from 5.176 to 8.279 (6.62 ± 0.58).

3.2. Clinicopathologic factors and TS and DPD mRNA levels

The results for TS and DPD are summarized in Table 3. TS mRNA levels were associated with the advance of tumor status, and those in advanced cancer tissues were significantly higher than those in early cancer tissues. In contrast, the reverse association was observed in DPD mRNA levels. According to nodal status, there was no significant difference in TS or DPD mRNA levels. In regard to histological types, DPD mRNA levels in adenocarcinoma showed significantly higher than those in squamous cell carcinoma.

3.3. TS and DPD mRNA levels and TS and DPD immunohistochemical evaluation

We conducted an immunohistochemical investigation on intra-tumoral expression of both TS and DPD using 92 available specimens. Fifty (54%) and 44 (48%) of the specimens were TS-positive and DPD-positive, respectively, by immunoreaction (Fig. 1). The correlation between mRNA levels and protein expression evaluated by immunohistochem-

Table 3 Clinicopathologic factors and TS and DPD mRNA levels

Factor	*n*	log(TS mRNA)	*P*-value[a]	log(DPD mRNA)	*P*-value[a]
Tumor status					
pT1	56	6.88 ± 0.28	0.003	6.75 ± 0.56	0.024
pT2	52	6.99 ± 0.32		6.47 ± 0.59	
pT3	8	7.39 ± 0.42		6.61 ± 0.58	
Nodal status					
pN0	85	6.96 ± 0.33	0.749	6.60 ± 0.64	0.199
pN1	14	6.50 ± 0.38		6.50 ± 0.38	
pN2	17	6.99 ± 0.34		6.81 ± 0.32	
Histologic type					
Adenocarcinoma	81	6.98 ± 0.34	0.680	6.79 ± 0.51	<0.0001
Squamous cell carcinoma	30	6.95 ± 0.31		6.17 ± 0.58	
Others	5	6.75 ± 0.37		6.51 ± 0.37	

[a] Kruskal–Wallis test.

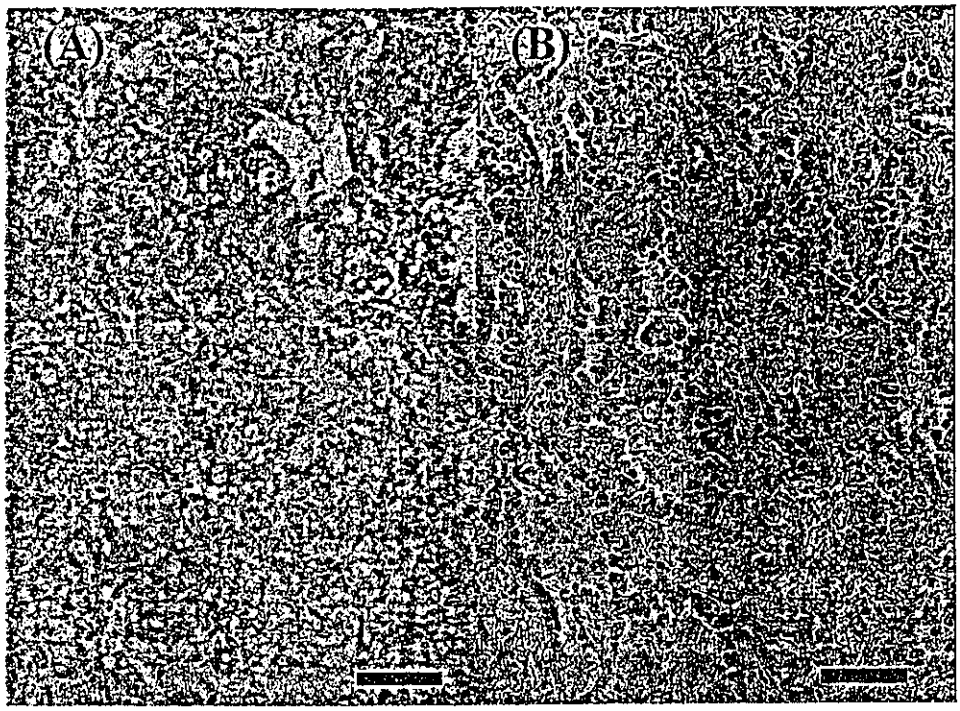

Fig. 1 Immunohistochemical staining of surgically resected specimens from lung cancer patients using anti-thymidylate synthase (A) and anti-dihydropyrimidine dehydrogenase (B) polyclonal antibodies. Note the diffuse positive staining present in the cytoplasm of the cancer cells (100× magnification). Scale bar = 100 μm.

ical staining is shown in Fig. 2. The mean TS mRNA level in the TS-positive group was 7.095, as compared with 6.796 in the TS-negative group, which was statistically different ($P < 0.0001$), whereas the mean DPD mRNA level in the DPD-positive group was 6.645, as compared with 6.467 in the DPD-negative group, which did not reach statistical significance ($P = 0.108$).

3.4. Disease-free survival and TS and DPD mRNA levels

Thirty-three (28%) of the 116 patients developed distant metastasis after surgery. In regard to tumor stage, 12 (15%) of the 79 patients in stage I, 7 (39%) of the 18 patients in stage II, and 14 (74%) of the 19 patients in stage IIIA suffered from

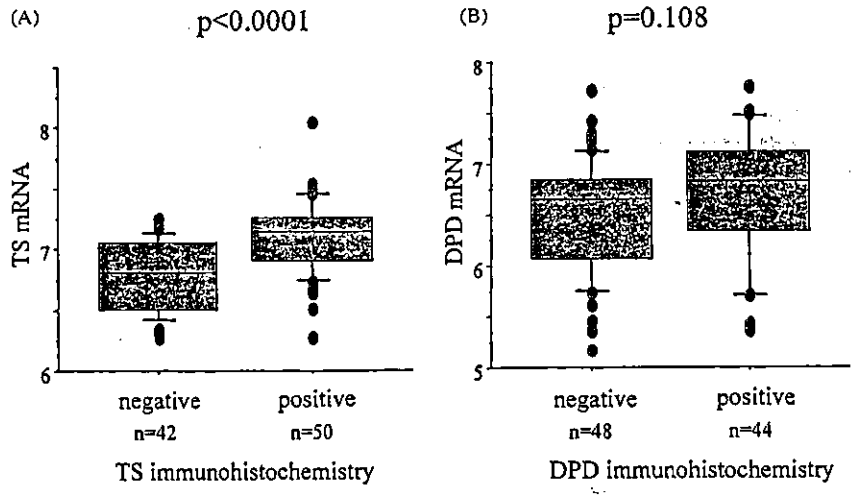

Fig. 2 Association of mRNA levels with immunohistochemistry findings. Bars for the box extend from the 25th to 75th percentile of the data, and the line in the middle represents the median. The upper and lower bars represent the distance from the 10th to 90th percentile from the median. The dots represent outlier values in the data. (A) There was a significant difference in mRNA levels in tumor tissues between the TS-positive and TS-negative patient groups, as evaluated by immunoreaction. (B) According to DPD immunohistochemical evaluations, there was no significant difference in DPD mRNA levels.

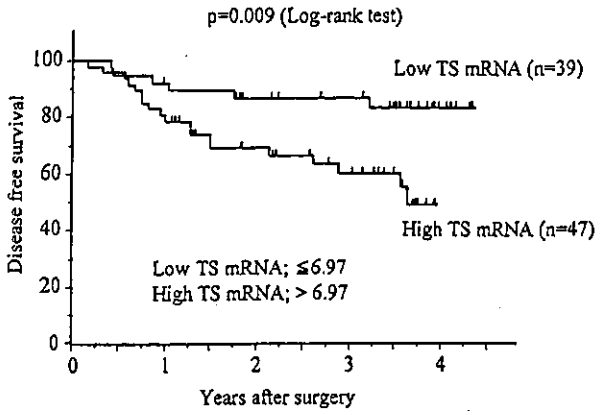

Fig. 3 Disease-free survival curves for patients who underwent only surgery, with high TS mRNA and low TS mRNA levels in lung cancer tissue when dichotomized at the mean TS mRNA level. TS mRNA levels were significantly correlated to disease-free survival.

recurrent disease. In the control group, 25 (29%) of the 86 patients had relapses of disease, and TS mRNA levels were significantly correlated to disease-free intervals for the control group ($P = 0.009$, log-rank test; Fig. 3), whereas DPD mRNA levels were not correlated ($P = 0.367$), when dichotomized at the mean TS and DPD mRNA level, respectively. In the 5-FU group, 8 (27%) of the 30 patients had recurrences, and DPD mRNA levels were significantly correlated to disease-free intervals ($P = 0.016$; Fig. 4), whereas TS mRNA levels were not correlated ($P = 0.114$).

For the 63 patients who had high-DPD tumors, there was no significant difference in disease-free survival rate between the 5-FU group and the control group ($P = 0.20$). However, for the 53 patients who had low-DPD tumors, those who were administered 5-FU had a significantly better prognosis

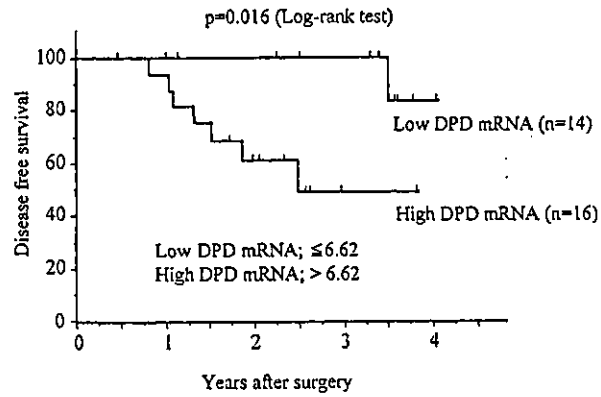

Fig. 4 Disease-free survival curves for patients who were administered 5-FU alone after surgery, with high DPD mRNA and low DPD mRNA levels in lung cancer tissue. DPD mRNA levels were significantly correlated to disease-free survival in the 5-FU group.

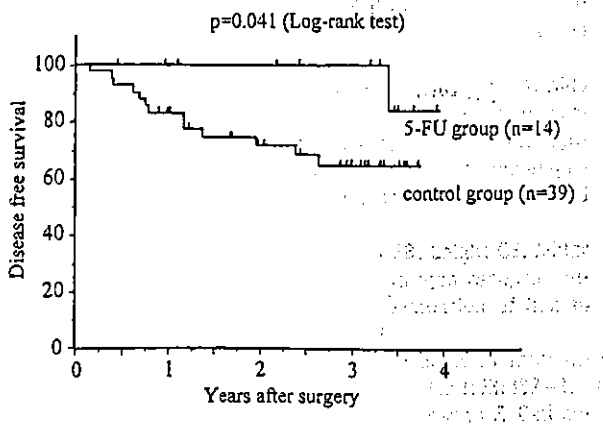

Fig. 5 Disease-free survival curves for patients with low-DPD tumors. Patients who were administered 5-FU after surgery had a significantly better prognosis than those who did not receive adjuvant treatment.

than those who did not receive adjuvant treatment ($P = 0.041$; Fig. 5).

In addition, in the 5-FU group, 10 patients with both low-TS and low-DPD tumors have not had any relapse, whereas 8 of the 20 patients with either high-TS or high-DPD tumors developed distant metastasis after surgery (Fig. 6).

4. Discussion

We performed quantitative assay of intra-tumoral TS and DPD mRNA levels to assess the association of their levels with clinicopathological factors and the feasibility of using them to predict the efficacy

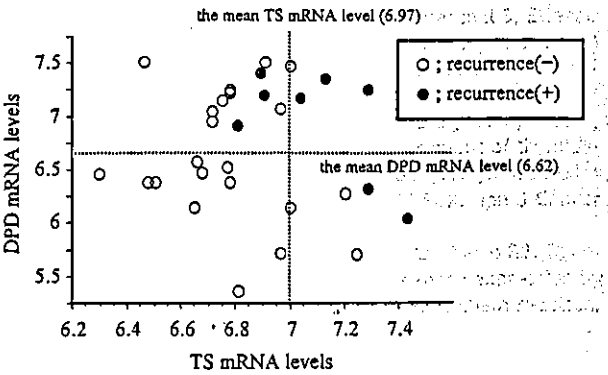

Fig. 6 Correlation between relapses of disease and TS and DPD mRNA levels in 30 NSCLC tissues in the 5-FU group. The dotted lines indicate the mean value of each mRNA level. The open circles represent the patient without a relapse of disease, and the closed circles represent the patient with a relapse of disease. Ten patients with both low-TS and low-DPD tumors have not had any relapse, whereas 8 of the 20 patients with either high-TS or high-DPD tumors developed distant metastasis after surgery.

of 5-FU in patients with NSCLC. The expression of TS controls cell proliferation in a positive manner, as an increase in TS expression in synchronous cells as they enter the S phase from the G_0 phase has been demonstrated [20]. It is therefore reasonable to expect that TS mRNA levels increases in accordance with tumor status, and that a high expression of TS was associated with a poor prognosis. As shown in Fig. 3, there was a significant difference between the groups with low and high TS mRNA levels for patients who underwent only surgery. In contrast, DPD mRNA levels in cancer tissues of advanced tumor status were significantly lower than those in early cancer tissues. It is reported that DPD mRNA levels tended to decrease during cell growth in vitro [21]. These findings suggest the possibility of down-regulation of DPD expression during tumor growth.

As DPD is a rate-limiting enzyme in the catabolism of 5-FU, its high expression in a tumor is thought to result in low sensitivity to 5-FU [6–9,22]. In this study, we evaluated the efficacy of 5-FU administration as adjuvant chemotherapy in relation to intra-tumoral DPD mRNA levels of patients with NSCLC. The results showed that DPD mRNA levels were significantly correlated to disease-free intervals for patients who were administered 5-FU. Furthermore, for patients with low-DPD tumors, those who were administered 5-FU had a significantly better prognosis than those with surgery alone. These findings suggest that intra-tumoral DPD mRNA levels serve as a possible predictor for the efficacy of 5-FU administration after surgery for NSCLC. The present study is the first known to report DPD gene expression in NSCLC patients based on comparisons of DPD mRNA levels and the efficacy of 5-FU administration. As for histological type, DPD mRNA levels in adenocarcinoma were significantly higher than those in squamous cell carcinoma. Several investigators have also reported that squamous cell carcinoma exhibits a lower level of DPD than adenocarcinoma evaluated by immunohistochemistry or radioenzyme assay [13,23]. These results indicated that patients with squamous cell lung carcinoma may be more susceptible to treatment with 5-FU than those with lung adenocarcinoma.

The role of TS in sensitivity to 5-FU is still controversial. TS expression has been reported to be associated with response to 5-FU treatment [2,3,24]. On the other hand, some investigators have reported that there was no correlation between TS activity and sensitivity to 5-FU [25,26]. However, at least, our results showed that TS expression is associated with disease-free survival rate for patients with NSCLC. Furthermore, in the 5-FU group, no patient with both low-TS and low-DPD mRNA levels had a relapse of disease, whereas 8 of the 20 patients with either high-TS or high-DPD tumors developed distant metastasis after surgery, when the cut-off level was at the mean value for each mRNA level. Our results suggest that TS mRNA levels as well as DPD mRNA levels are associated with response to 5-FU treatment and disease-free survival after complete resection of NSCLC. Fujiwara et al. also reported that in vitro drug sensitivity test showed an inverse correlation between 5-FU sensitivity and each mRNA expression in the clinical samples of gastric cancer [27]. Based on these results, TS and DPD mRNA levels in tumors would permit more rational decisions on whether or not to proceed with 5-FU-based chemotherapy.

DPD protein levels are not always in parallel with DPD mRNA levels in clinical specimens [13,14]. The present results also showed that DPD protein content, evaluated using an immunohistochemistry method, was independent of DPD mRNA level. DPD protein levels in tumors change in a circadian rhythm, therefore DPD mRNA levels are thought to be a better parameter than DPD activity or DPD protein content [21]. In addition, a quantitative real-time RT-PCR assay is able to determine mRNA levels from nanogram amounts of total RNA [28], thus it is possible to measure the mRNA level of each enzyme in biopsy specimens obtained with a bronchofiberscope or needle biopsy. If tumor progression or prognosis can be determined preoperatively, the quantitation of TS and DPD mRNA levels in biopsy specimens may be useful for forming an effective preoperative therapeutic strategy.

In conclusion, an assessment of TS and DPD expression using real-time RT-PCR in patients with NSCLC can not only provide precise prognostic information but also predict the efficacy of 5-FU after surgery for patient with NSCLC. We consider it necessary to investigate the effects of preoperative aggressive adjuvant therapy for patients with high-TS or high-DPD NSCLC.

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Tracheal Ring Regeneration

To the Editor:

I was delighted to read the interesting article about replacement of the trachea with an autologous aortic graft by Martinod and associates [1]. Their results confirmed and their study duplicated the experimental work done by my associates and me [2] more than 30 years ago. There were four similarities between their experiments and ours. The new "trachea" developed in autologous tissue; the new "trachea" developed rings, which were incomplete posteriorly; it was external to a prosthetic device, a silicon tube in our experiments and a nitinol or Endoxane stent in theirs; and follow-up was up to 3 years. The dissimilarity was that they used sheep and we used dogs in the experiments.

Another coincidence is that both articles were discussed by eminent doctors from Massachusetts General Hospital, Dr Hermes C. Grillo for ours and Dr Douglas Mathisen [3] for theirs. Dr Grillo's discussion of our 1973 article mentioned some interesting embryological facts as well [2].

Even though it took more than 30 years for our work to be confirmed and duplicated, I look forward to seeing more young investigators interested in the subject of autologous substitution for the trachea as well as other organs.

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Reply

To the Editor:

We thank Dr Demos for his interest in our article [1] and for the mention of his "historical" report [2] on the reconstruction of the trachea using a silicone prosthesis. Although our approach was different because we were looking for biological reconstruction rather than prosthetic substitution (the prosthesis was used as a temporary stent), we both sought the same goal of a reliable tracheal replacement, and we were both criticized by "nonbelievers." We will not forget to mention his interesting pioneering work in our future studies.

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Adenosquamous Carcinoma of the Lung and Visceral Pleural Invasion

To the Editor:

Nakagawa and colleagues [1] reported their findings on the poor prognosis for patients with adenosquamous carcinoma (ASC) of the lung after surgical resection. The authors concluded that patients with stage IA or IB ASC had a prognosis similar to that of patients with stage IIIA adenocarcinoma or squamous cell carcinoma. Nakagawa and co-authors also found significant differences in pathological pleural invasion characteristics between the two groups; the visceral and parietal was invaded (p2–p3) in 50% (15/30) of patients with ASC versus 26.8% (327/1,219) of those with adenocarcinoma or squamous cell carcinoma.

In 2001, one group [2] presented a study of 69 patients who underwent surgical resection for ASC of the lung. This study also confirmed the aggressive biological behavior of ASC. The other main characteristic of ASC was its propensity to invade the visceral pleura (p1–p2) (41%) and the parietal pleura (p3) (22%), a finding similar to that observed by Nakagawa and associates. Our study also demonstrated that peripheral location of ASC and visceral pleural invasion (VPI) were the only factors associated with a poor prognosis in the multivariate analysis. We postulated that such characteristics allow tumor cells to exfoliate within the pleural cavity and that the exfoliated tumor cells are absorbed by the parietal pleural lymphatics and reach the bloodstream, thus contributing to dissemination of cancer.

We [3] also observed that such a phenomenon was possible whatever the histology of the peripheral lung cancer but that VPI was twice more frequent in ASC. Furthermore, for the stage IIIA N2 subset, the survival rates of patients with one-station N2 involvement with VPI was close to that observed for patients with N2 involvement of two or more stations with or without VPI [3]. These survival results are comparable to those of Nakagawa and co-workers and suggest that reabsorption of parietal pleural lymphatic tumor cells behave as a metastatic mediastinal lymph node chain in the presence of VPI and that VPI can be likened to one-station N2 disease. This is the case whatever the lung cancer histology but appears particularly important in ASC because of the high frequency of VPI in this histological subgroup.

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Reply

To the Editor:

Thank you for reading our report [1]. I agree that the prognosis for patients with adenosquamous carcinoma is poorer than that for patients with other histological types of lung cancer. I originally had assumed that pleural invasion was an independent prognostic factor, but this was not substantiated by the multivariate analysis. However, I think your opinion about pleural invasion is very useful and informative. I have encountered several patients with histopathological evidence of lymphatic invasion directly beneath the visceral pleura. The reason for the high frequency of pleural invasion associated with adenosquamous carcinoma compared with other histological types of lung cancer is unclear. Such subpleural lymphatic invasion may be an index of the aggressive behavior of adenosquamous carcinoma of the lung.

For patients with stage IIIA disease, which was treated by complete resection in our hospital between September 1976 and August 1998, the 5-year survival rate was 7.4% for patients who had involvement of only one mediastinal lymph node station (N2) with visceral pleural invasion (n = 71) and 27.2% for those who had involvement of one mediastinal lymph node station (N2) without visceral pleural invasion (n = 47). The 5-year survival rate was 16.1% for patients with involvement of two or more mediastinal lymph node stations (N2) irrespective of the status of visceral pleural invasion (n = 128). The outcome of patients who had involvement of only one lymph node station without visceral pleural invasion was significantly better than that of patients with other types of N2 disease.

As in the study of Riquet and coauthors [2], outcome did not differ significantly between patients who had involvement of one mediastinal lymph node station with pleural invasion and those who had involvement of two or more mediastinal lymph node stations irrespective of the status of visceral pleural invasion. In the patients with visceral pleural invasion, it can be imagined that tumor cells invading the parietal pleural lymph nodes were reabsorbed and transferred to the mediastinal lymph nodes; however, no direct evidence is available to corroborate this. It is an open question whether reabsorption of tumor cells in the parietal pleural can be verified clinically or experimentally.

The reasons for the poor prognosis for patients with adenosquamous carcinoma of the lung remain unclear. Perhaps techniques such as gene analysis will help to identify the most critical factors.

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Octreotide in Bronchobiliary Fistula Management

To the Editor:

Singh and colleagues [1] described the management of bronchobiliary fistula (BBF) by endoscopic retrograde cholangiography and medical therapy. Their use of octreotide for BBF was not previously documented according to our MEDLINE search. We report 2 cases of patients demonstrating the therapeutic utility of octreotide in BBF management.

A 56-year-old woman was seen with chest pain, fever, and bilioptysis. History included percutaneous liver abscess drainage and thoracotomy for iatrogenic hemothorax 4 years earlier. There was no jaundice or pallor. Crackles were heard at the base of the right lung. The level of alkaline phosphatase was slightly elevated (154 IU/L). A biliary scan showed activity above the diaphragm, and endoscopic cholangiography revealed a filling defect in the intrahepatic ductal system with contrast material extravasating into the right lung. Multiple bile duct stones were extracted, and a stent was inserted into the common bile duct for biliary decompression, but bilioptysis persisted 4 days later. A repeat cholangiogram confirmed a persistent BBF. A 28-day trial of octreotide, 100 μg subcutaneously three times a day, was initiated, during which progressive reduction in bilioptysis was seen until resolution on day 17, and repeat biliary scanning confirmed closure of the fistula. The patient remained asymptomatic after discontinuance of octreotide and removal of the endobiliary stent.

A 71-year-old woman was seen with painless bilioptysis 4 months after undergoing a right hepatectomy for colon metastases. Decreased air entry and dullness to percussion were noted at the base of the right lung. The alkaline phosphatase level was elevated (431 IU/L). The presence of a BBF was suggested by a right subphrenic accumulation of fluid on a computed tomographic scan and by isotope concentration above the right hemidiaphragm on a biliary scan. An endoscopic cholangiogram confirmed the diagnosis of BBF, and a common bile duct stent was inserted, but bilioptysis continued for 4 days. Octreotide was then initiated at 100 μg subcutaneously three times a day with marked reduction in frequency and production of bilioptysis; however, follow-up biliary scanning showed a persistent BBF. Octreotide was continued until the patient underwent operation to repair the BBF 6 weeks later. A laceration of the inferior vena cava resulted in uncontrollable hemorrhage and cardiac arrest. Postmortem examination revealed a patent malignant BBF.

Reports of BBF secondary to choledocholithiasis or right

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