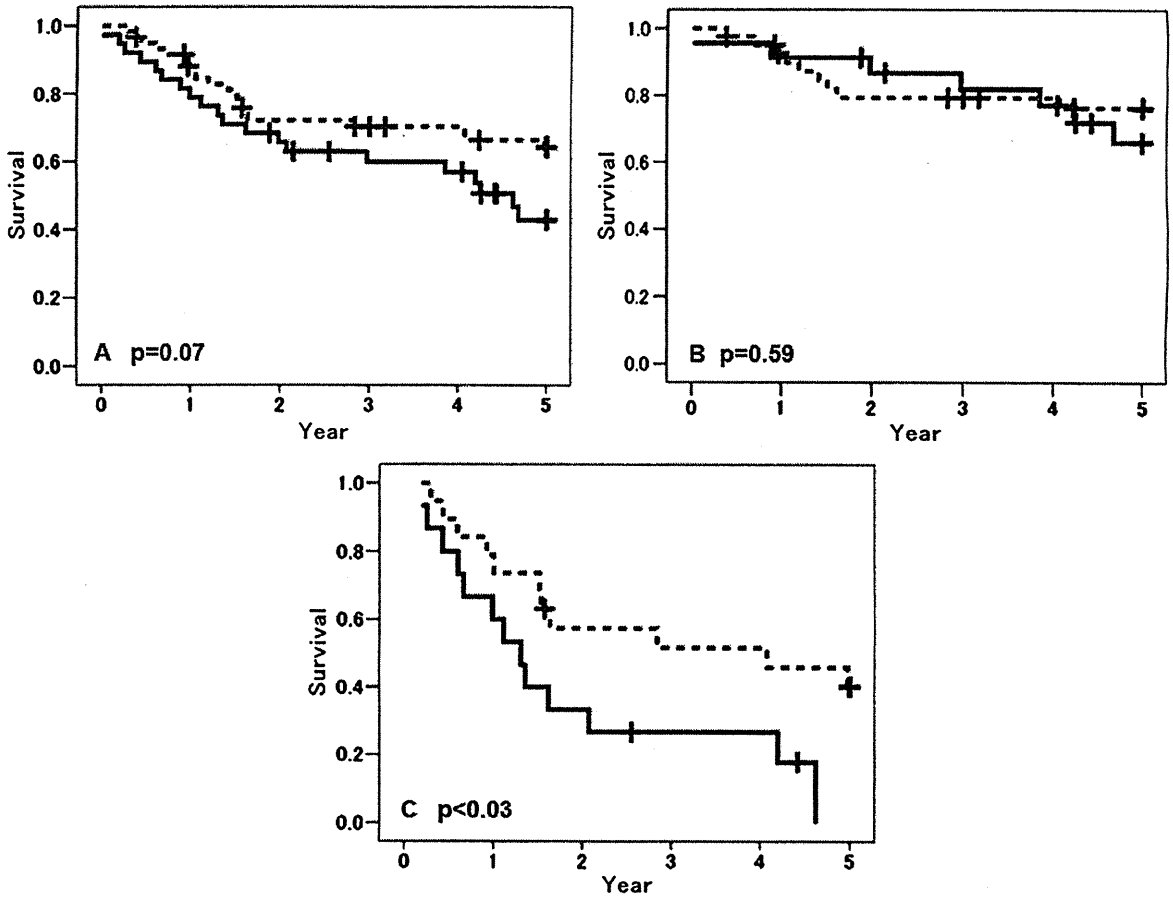


Figure 3. Disease-free survival (DFS) rates of the high (broken line) and low (solid line) WT1 expression groups. (A) DFS rates of the high (broken line) and low (solid line) WT1 expression groups for all stages. No significant difference was observed between the two groups. (B) DFS rates for patients at stages I and II of the high (broken line) and low (solid line) WT1 expression groups. No significant difference was observed between the two groups. (C) DFS rates for patients at stages III and IV of the high (broken line) and low (solid line) WT1 expression groups. In this subset, DFS rate of the low WT1 expression group was significantly lower than that of the high WT1 expression group ($p < .03$).

We evaluated association between various clinicopathological parameters and the WT1 mRNA expression. No significant association was observed between WT1 mRNA expression and age or tumor size (data not shown). In addition, no significant differences between the expression and parameters of sex, clinical stage or histological type of lung cancer were observed (data not shown).

In a multivariate analysis, significant and independent variables which influence OS were WT1 expression in the tumor tissue, pathological stages, and the absence or presence of subjective symptoms at the time of diagnosis (Table 3). As for DFS, significant and independent variables were WT1 expression, tumor size, and pathological stages (Table 4).

DISCUSSION

This is the first report that showed the relationship between WT1 gene expression and prognosis of NSCLC patients who underwent lung surgery. As for the OS and DFS of stages III and IV of NSCLC patients, WT1 expression level was a significant prognostic marker, independent of other established prognostic factors.

There have been a number of reports which show that low expression of WT1 mRNA is associated with malignant

Table 3. Multivariate Analysis of Prognostic Factors of Overall Survival

	Partial regression coefficient	p	Hazard ratio	95% CI
WT1 group	1.593	.003	4.921	1.75–13.85
Complaint at diagnosis	−1.312	.009	0.269	0.10–0.72
pathological stage	−1.203	.013	0.300	0.12–0.78

Table 4. Multivariate Analysis of Prognostic Factors of Disease-Free Survival

	Partial regression coefficient	p	Hazard ratio	95% CI
WT1 group	0.767	.025	2.152	1.10–4.22
Tumor size	0.041	.001	1.042	1.02–1.07
pathological stage	−1.261	.000	0.283	0.14–0.57

alteration. One of the growth factors whose gene expression is regulated by WT1 is vascular endothelial growth factor (VEGF). It has been reported that vegf promoter has several potential WT1 binding sites (23), and VEGF is associated with neovascularization and promotion of metastasis in lung cancer (24–26) and other solid tumors (27–29). Therefore, highly expressed WT1 might suppress expression of VEGF in lung cancers and inhibit their neovascularizaion and metastasis, resulting in favorable prognosis in patients with high expression of WT1. However, WT1 can also activate VEGF expression in a cellular context-dependent manner (23), and co-expression of WT1 and VEGF in the same area was observed in endometrial cancer tissue (30). Further study is needed to elucidate the role of WT1–VEGF pathway in lung cancers.

Moriya *et al.* reported that high level of WT1 expression was associated with suppression of lymph node metastasis in patients with SQLC, and that the invasive ability of an SQLC cell line was enhanced by suppression of WT1 gene expression (31). In all of the 27 SQLC cases in our investigation, lymph node metastasis and WT1 mRNA expression level showed significant negative correlation, which was consistent with the report by Moriya *et al.* This trend was not observed for the ADLC (antibody-dependent lymphocyte cytotoxicity) cases in our present study.

On the other hand, by *in-vitro* analysis of various types of cancers cells, there is accumulating evidence showing that the wild-type *WT1* gene is overexpressed and plays oncogenic functions, such as anti-apoptosis (32, 33) and promotion of cell migration (34). There are also a number of reports that show association between high expression of WT1 mRNA and poor prognosis. Sotobori *et al.* quantified the WT1 mRNA expression for soft tissue sarcoma in 52 patients using real-time PCR method (19). They reported that disease-specific survival rate and DFS for patients with high WT1 mRNA expression levels was significantly lower compared with that for patients with low WT1 mRNA expression levels. Srivastava *et al.* reported that high WT1 mRNA expression was associated with poor survival of patients with osteogenic sarcoma metastasis (20). As for an epithelial tumor, Miyoshi *et al.* quantified expression of WT1mRNA in breast carcinoma tissue using real-time PCR (21) and reported that poor prognosis was significantly associated with higher WT1 mRNA expression. Our data for NSCLC is apparently contradictory to the result for breast carcinoma, and the reason is unclear at present. Because cellular origin is different in NSCLC and breast carcinoma, their 5-year relative survival rates differ from one another (35). Hence, it may not necessarily be surprising that a discrepancy exists in the relationship between prognosis and WT1 gene expression. Another possibility is the difference in the induction of immune response depending on the types of tumors. Regulatory T cells as well as WT1-specific killer T cells are detected in patients with WT1-expressing tumors (36, 37). If regulatory T-cell activity differs between lung cancer and other tumors, the apparent contradictory result may be explained.

The present study showed a favorable association between WT1 expression and prognosis of NSCLC patients. This may be explained in the context of antigen-specific immune responses elicited in cancer patients. WT1 gene product is a potent pan-tumor-associated antigen, and WT1-targeting cancer immunotherapy is being demonstrated for its therapeutic potential (38). Recently, Chiba *et al.* analyzed the impact of WT1 protein expression on the prognosis of patients with recurrent or progressive glioblastoma multiforme in a phase II clinical trial of WT1 immunotherapy. The study showed that the high WT1 expression group had significantly longer OS and progression-free survival compared with the low WT1 expression group (36). These results may suggest that WT1 expression in glioblastoma cells have positive effects on their sensitivity to cytotoxic cellular immune responses targeting WT1 and correlates with favorable clinical outcome. In NSCLC, we have previously demonstrated that humoral immune responses against WT1 were elicited, as demonstrated by the enhanced production of WT1 IgG antibody (39). Interestingly, elevation in WT1 IgG antibody titers was significantly associated with longer DFS in patients with stages I–III NSCLC, suggesting that WT1-specific immune responses played an important role in anti-cancer immunity in NSCLC. In view of the above, high expression of WT1 in lung cancer cells, such as in glioblastoma cells, might have positive effects on their sensitivity to WT1-specific T cells, which correlates with favorable prognosis in advanced NSCLC.

Diversity in WT1 gene product functions may be attributable to the presence of five types of splice variants (3). One alternative splice alters the zinc finger region of WT1, resulting in modification of binding of WT1 to DNA (40). This observation suggests that each splice variant may have variable biological functions. Burwell *et al.* studied expression of different WT1 isoforms in mammary epithelial cell lines and observed that transformed phenotypes induced by transfection of the gene depended on the WT1 isoforms (41). Moriya *et al.* reported that only one isoform with a 3-amino acid deletion (–KTS) of the *WT1* gene enhanced a WT1 target gene *p21*(*Waf1*/Cip1), a gene associated with the regulation of lymph node metastasis of cancer (31). Detailed analysis of the relevancy of expression of each splice variant and prognosis of NSCLC is one of the important future issues.

In conclusion, we showed that low WT1 mRNA expression is associated with poor prognosis, and WT1 expression level will serve as a novel marker predicting prognosis of NSCLC. Moreover, our results add new information on the biological function of WT1 gene product, which may act on NSCLC as a tumor suppressor.

ACKNOWLEDGMENTS

The pathologic diagnosis of the patients who underwent lung surgery between May 2002 and March 2004 was primarily based upon the description by the late Satoru Yamamoto, MD. The authors are grateful to Ms. Reiko Hayashi for her linguistic help. This work was supported in part by a Grant-in Aid from the Ministry of Education, Science, Sports, Culture and Technology and the Ministry of Health, Labour, and Welfare, Japan.

DECLARATION OF INTEREST

The authors have no conflict of interest in connection with this paper. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeger H, Lewis WH, Jones C, Housman DE. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. Cell 1990;60:509–520.
2. Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GA. Homozygous deletion in Wilms tumours of a zinc-finger gene identified by chromosome jumping. Nature 1990;343:774–778.
3. Haber DA, Sohn RL, Buckler AJ, Pelletier J, Call KM, Housman DE. Alternative splicing and genomic structure of the Wilms tumor gene WT1. Proc Natl Acad Sci U S A 1991;88:9618–9622.
4. Dey BR, Sukhatme VP, Roberts AB, Sporn MB, Rauscher FJ, 3rd, Kim SJ. Repression of the transforming growth factor-beta 1 gene by the Wilms' tumor suppressor WT1 gene product. Mol Endocrinol 1994;8:595–602.
5. Drummond IA, Madden SL, Rohwer-Nutter P, Bell GI, Sukhatme VP, Rauscher FJ, 3rd. Repression of the insulin-like growth factor II gene by the Wilms tumor suppressor WT1. Science 1992;257:674–678.
6. Gashler AL, Bonthron DT, Madden SL, Rauscher FJ, 3rd, Collins T, Sukhatme VP. Human platelet-derived growth factor A chain is transcriptionally repressed by the Wilms tumor suppressor WT1. Proc Natl Acad Sci U S A 1992;89:10984–10988.
7. Wang ZY, Madden SL, Deuel TF, Rauscher FJ, 3rd. The Wilms' tumor gene product, WT1, represses transcription of the platelet-derived growth factor A-chain gene. J Biol Chem 1992;267:21999–22002.
8. Werner H, Re GG, Drummond IA, Sukhatme VP, Rauscher FJ, 3rd, Sens DA, Garvin AJ, LeRoith D, Roberts CT, Jr. Increased expression of the insulin-like growth factor I receptor gene, IGF1R, in Wilms tumor is correlated with modulation of IGF1R promoter activity by the WT1 Wilms tumor gene product. Proc Natl Acad Sci U S A 1993;90:5828–5832.
9. Hewitt SM, Hamada S, McDonnell TJ, Rauscher FJ, 3rd, Saunders GF. Regulation of the proto-oncogenes bcl-2 and c-myc by the Wilms' tumor suppressor gene WT1. Cancer Res 1995;55:5386–5389.
10. Luo XN, Reddy JC, Yeyati PL, Idris AH, Hosono S, Haber DA, Licht JD, Atweh GF. The tumor suppressor gene WT1 inhibits ras-mediated transformation. Oncogene 1995;11:743–750.
11. Inoue K, Ogawa H, Sonoda Y, Kimura T, Sakabe H, Oka Y, Miyake S, Tamaki H, Oji Y, Yamagami T, Tatekawa T, Soma T, Kishimoto T, Sugiyama H. Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. Blood 1997;89:1405–1412.
12. Sako M, Ogawa H, Okamura J, Tamaki H, Nakahata T, Kishimoto T, Sugiyama H. Abnormal expression of the Wilms' tumor gene WT1 in juvenile chronic myeloid leukemia and infantile monosomy 7 syndrome. Leuk Res 1998;22:965–967.
13. Tamaki H, Ogawa H, Inoue K, Soma T, Yamagami T, Miyake S, Oka Y, Oji Y, Tatekawa T, Tsuboi A, Tagawa S, Kitani T, Aozasa K, Kishimoto T, Sugiyama H, Miwa H, Kita K. Increased expression of the Wilms tumor gene (WT1) at relapse in acute leukemia. Blood 1996;88:4396–4398.
14. Osaka M, Koami K, Sugiyama T. WT1 contributes to leukemogenesis: expression patterns in 7,12-dimethylbenz[a]anthracene (DMBA)-induced leukemia. Int J Cancer 1997;72:696–699.
15. Oji Y, Miyoshi S, Maeda H, Hayashi S, Tamaki H, Nakatsuka S, Yao M, Takahashi E, Nakano Y, Hirabayashi H, Shintani Y, Oka Y, Tsuboi A, Hosen N, Asada M, Fujioka T, Murakami M, Kanato K, Motomura M, Kim EH, Kawakami M, Ikegame K, Ogawa H, Aozasa K, Kawase I, Sugiyama H. Overexpression of the Wilms' tumor gene WT1 in *de novo* lung cancers. Int J Cancer 2002;100:297–303.
16. Inoue K, Ogawa H, Yamagami T, Soma T, Tani Y, Tatekawa T, Oji Y, Tamaki H, Kyo T, Dohy H, Hiraoka A, Masaoka T, Kishimoto T, Sugiyama H. Long-term follow-up of minimal residual disease in leukemia patients by monitoring WT1 (Wilms tumor gene) expression levels. Blood 1996;88:2267–2278.
17. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, Kita K, Hiraoka A, Masaoka T, Nasu K. WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood 1994;84:3071–3079.
18. Saxena R, Sait S, Mhawech-Fauceglia P. Ewing sarcoma/primitive neuroectodermal tumor of the kidney: a case report. Diagnosed by immunohistochemistry and molecular analysis. Ann Diagn Pathol 2006;10:363–366.
19. Sotobori T, Ueda T, Oji Y, Naka N, Araki N, Myoui A, Sugiyama H, Yoshikawa H. Prognostic significance of Wilms tumor gene (WT1) mRNA expression in soft tissue sarcoma. Cancer 2006;106:2233–2240.
20. Srivastava A, Fuchs B, Zhang K, Ruan M, Halder C, Mahlum E, Weber K, Bolander ME, Sarkar G. High WT1 expression is associated with very poor survival of patients with osteogenic sarcoma metastasis. Clin Cancer Res 2006;12:4237–4243.
21. Miyoshi Y, Ando A, Egawa C, Taguchi T, Tamaki Y, Tamaki H, Sugiyama H, Noguchi S. High expression of Wilms' tumor suppressor gene predicts poor prognosis in breast cancer patients. Clin Cancer Res 2002;8:1167–1171.
22. Lung and pleural tumours. In UICC: TNM Classification of Malignant Tumours, 5th edition, L Sobin and C. Witterkind (eds). New York: John Wiley & Sons, 1997, 91–100.
23. Hanson J, Gorman J, Reese J, Fraizer G. Regulation of vascular endothelial growth factor, VEGF, gene promoter by the tumor suppressor, WT1. Front Biosci 2007;12:2279–2290.
24. Masuya D, Huang C, Liu D, Kameyama K, Hayashi E, Yamauchi A, Kobayashi S, Haba R, Yokomise H. The intratumoral expression of vascular endothelial growth factor and interleukin-8 associated with angiogenesis in nonsmall cell lung carcinoma patients. Cancer 2001;92:2628–2638.
25. Tateishi U, Kusumoto M, Nishihara H, Nagashima K, Morikawa T, Moriyama N. Contrast-enhanced dynamic computed tomography for the evaluation of tumor angiogenesis in patients with lung carcinoma. Cancer 2002;95:835–842.
26. Yuan A, Yu CJ, Luh KT, Kuo SH, Lee YC, Yang PC. Aberrant p53 expression correlates with expression of vascular endothelial growth factor mRNA and interleukin-8 mRNA and neoangiogenesis in non-small-cell lung cancer. J Clin Oncol 2002;20:900–910.
27. Fujioka S, Yoshida K, Yanagisawa S, Kawakami M, Aoki T, Yamazaki Y. Angiogenesis in pancreatic carcinoma: thymidine phosphorylase expression in stromal cells and intratumoral microvessel density as independent predictors of overall and relapse-free survival. Cancer 2001;92:1788–1797.
28. Fukata S, Inoue K, Kamada M, Kawada C, Furihata M, Ohtsuki Y, Shuin T. Levels of angiogenesis and expression of angiogenesis-related genes are prognostic for organ-specific metastasis of renal cell carcinoma. Cancer 2005;103:931–942.
29. Inoue K, Ozeki Y, Suganuma T, Sugiura Y, Tanaka S. Vascular endothelial growth factor expression in primary esophageal squamous cell carcinoma. Association with angiogenesis and tumor progression. Cancer 1997;79:206–213.
30. Dohi S, Ohno S, Ohno Y, Kyo S, Soma G, Sugiyama H, Inoue M. WT1 expression correlates with angiogenesis in endometrial cancer tissue. Anticancer Res 2010;30:3187–3192.
31. Moriya S, Takiguchi M, Seki N. Expression of the WT1 gene – KTS domain isoforms suppresses the invasive ability of human lung squamous cell carcinoma cells. Int J Oncol 2008;32:349–356.
32. Ito K, Oji Y, Tatsumi N, Shimizu S, Kanai Y, Nakazawa T, Asada M, Jomgeow T, Aoyagi S, Nakano Y, Tamaki H, Sakaguchi N, Shirakata T, Nishida S, Kawakami M, Tsuboi A, Oka Y, Tsujimoto Y, Sugiyama H. Antiapoptotic function of 17AA(+)WT1 (Wilms'

tumor gene) isoforms on the intrinsic apoptosis pathway. Oncogene 2006;25:4217–4229.
33. Tatsumi N, Oji Y, Tsuji N, Tsuda A, Higashio M, Aoyagi S, Fukuda I, Ito K, Nakamura J, Takashima S, Kitamura Y, Miyai S, Jomgeow T, Li Z, Shirakata T, Nishida S, Tsuboi A, Oka Y, Sugiyama H. Wilms' tumor gene WT1-shRNA as a potent apoptosis-inducing agent for solid tumors. Int J Oncol 2008;32:701–711.
34. Jomgeow T, Oji Y, Tsuji N, Ikeda Y, Ito K, Tsuda A, Nakazawa T, Tatsumi N, Sakaguchi N, Takashima S, Shirakata T, Nishida S, Hosen N, Kawakami M, Tsuboi A, Oka Y, Itoh K, Sugiyama H. Wilms' tumor gene WT1 17AA(-)/KTS(-) isoform induces morphological changes and promotes cell migration and invasion *in vitro*. Cancer Sci 2006;97:259–270.
35. Center for Cancer Control and Information Services, National Cancer Centre. Multiple Sites Survival. Bethesda, MD: National Cancer Institute, 2011.
36. Chiba Y, Hashimoto N, Tsuboi A, Rabo C, Oka Y, Kinoshita M, Kagawa N, Oji Y, Sugiyama H, Yoshimine T. Prognostic value of WT1 protein expression level and MIB-1 staining index as predictor of response to WT1 immunotherapy in glioblastoma patients. Brain Tumor Pathol 2010;27:29–34.
37. Lehe C, Ghebeh H, Al-Sulaiman A, Al Qudaihi G, Al-Hussein K, Almohareb F, Chaudhri N, Alsharif F, Al-Zahrani H, Tbakhi A, Aljurf M, Dermime S. The Wilms' tumor antigen is a novel target for human CD4+ regulatory T cells: implications for immunotherapy. Cancer Res 2008;68:6350–6359.
38. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, Elisseeva OA, Oji Y, Kawakami M, Ikegame K, Hosen N, Yoshihara S, Wu F, Fujiki F, Murakami M, Masuda T, Nishida S, Shirakata T, Nakatsuka S, Sasaki A, Udaka K, Dohy H, Aozasa K, Noguchi S, Kawase I, Sugiyama H. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. Proc Natl Acad Sci U S A 2004;101:13885–13890.
39. Oji Y, Kitamura Y, Kamino E, Kitano A, Sawabata N, Inoue M, Mori M, Nakatsuka S, Sakaguchi N, Miyazaki K, Nakamura M, Fukuda I, Nakamura J, Tatsumi N, Takakuwa T, Nishida S, Shirakata T, Hosen N, Tsuboi A, Nezu R, Maeda H, Oka Y, Kawase I, Aozasa K, Okumura M, Miyoshi S, Sugiyama H. WT1 IgG antibody for early detection of nonsmall cell lung cancer and as its prognostic factor. Int J Cancer 2009;125:381–387.
40. Hewitt SM, Fraizer GC, Wu YJ, Rauscher FJ, 3rd, Saunders GF. Differential function of Wilms' tumor gene WT1 splice isoforms in transcriptional regulation. J Biol Chem 1996;271:8588–8592.
41. Burwell EA, McCarty GP, Simpson LA, Thompson KA, Loeb DM. Isoforms of Wilms' tumor suppressor gene (WT1) have distinct effects on mammary epithelial cells. Oncogene 2007;26:3423–3430.

Interactive CardioVascular and Thoracic Surgery Advance Access published March 14, 2012

Interactive CardioVascular and Thoracic Surgery 0 (2012) 1–4
doi:10.1093/icvts/ivs094

INSTITUTIONAL REPORT

Surgical results and staging of non-small cell lung cancer with interlobar pleural invasion

Ryu Kanzaki[a,*], Naoki Ikeda[a], Eiji Okura[a], Naoto Kitahara[a], Yasushi Shintani[b], Akira Okimura[c], Kunimitsu Kawahara[c] and Mitsunori Ohta[a]

[a] Department of General Thoracic Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Japan
[b] Department of General Thoracic Surgery, Osaka University Graduate School of Medicine, Suita, Japan
[c] Department of Pathology, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Habikino, Japan

*Corresponding author. Department of General Thoracic Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, 3-7-1 Habikino, Habikino, Osaka 583-8588, Japan. Tel: +81-72-957-21; fax: +81-72-958-3291; e-mail: rkanzaki@tj8.so-net.ne.jp (R. Kanzaki).

Received 5 December 2011; received in revised form 28 January 2012; accepted 14 February 2012

Abstract

The aim of this study was to compare the survival rates of non-small cell lung cancer (NSCLC) with interlobar pleural invasion (IPI) with that of patients with other T2 and T3 diseases according to the seventh TNM staging system. One thousand and one patients with pathologic T2 and T3 NSCLC (according to the seventh staging criteria) treated between 1980 and 2004 were retrospectively evaluated. Among these, 682 patients were pathologically staged as T2 without IPI (T2 group), 25 as T2 with IPI (IPI group) and 294 as T3 (T3 group). The 5-year survival rate for the T2, IPI and T3 groups were 52.0, 31.1 and 36.3%, respectively. In patients without nodal involvement, the 5-year survival rates of the T2N0, IPIN0 and T3N0 groups were 60.9, 40.0 and 45.9%, respectively. The survival rate was significantly different between the T3N0 and T2N0 groups ($P < 0.001$) and between the IPIN0 and T2N0 ($P = 0.020$) groups. There was no significant difference in the survival rate between the IPIN0 and T3N0 groups ($P = 0.644$). In patients without nodal involvement, the survival of NSCLC with IPI is similar to that of the T3 disease.

Keywords: Lung cancer • Diagnosis • Staging • Pathology

INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths in the Western world [1]. Effective staging systems for lung cancer are needed to stratify the patient survival and to assess treatment results of defined patient subgroups. The seventh TNM Classification of Malignant Tumors dealt with various problems related to the sixth edition, and the proposed changes better differentiate tumours associated with different prognoses [2–4].

However, there is no agreement on whether non-small cell lung cancer (NSCLC) with interlobar pleural invasion (IPI) should be classified into T2 or T3 [5–7].

In the present study, we compared the survival rates of patients with NSCLC with IPI with that of patients with other T2 and T3 diseases according to the seventh TNM staging system. We also compared the surgical results of NSCLC with IPI with those of subgroups of T3 tumours.

PATIENTS AND METHODS

From January 1980 to December 2004, 1989 patients were operated on for NSCLC in our surgical department. Available for analysis is a database that encompasses the medical, surgical and pathology records of these patients. In our hospital, the completeness of the interlobar fissure is generally recorded in the surgical records. Pathologic staging were evaluated according to the seventh International Union Against Cancer (UICC) TNM staging system [2]. Each tumour was examined histopathologically according to the World Health Organization classification [8]. In addition to the haematoxilin and eosin stains, elastic stains are generally used to confirm the presence of IPI. In our hospital, lobectomy or larger resection with hilar and mediastinal lymph node dissection through posterolateral was performed as a standard treatment for lung cancer in this period. Patients with co-morbidities such as cardiovascular diseases or impaired pulmonary function underwent sublobar resection. Of the 1989 patients, those who underwent lobectomy or larger resection with hilar and mediastinal lymph node dissection were included while those who underwent sublobar resection were excluded. Cases of operative death or death within 30 days after the operation were excluded. Patients who underwent incomplete resection or induction of chemo- or chemoradiotherapy, patients with synchronous or metachronous multiple lung cancer, T1, T4, N3 or M1 diseases were excluded. Patients whose tumours were subsequently classified pathologically as a low-grade malignant tumour were also excluded. Thus, 1001 patients with pathological T2 and T3 NSCLC were enrolled in this retrospective study. Among the 1001 patients, those with IPI were confirmed (based on our data base (IPI group)). Then, the presence of interlobar pleura invasion was examined carefully in this group.

In the present study, IPI was regarded as a T factor in order to compare the surgical results of the IPI group with that of T2 without IPI or T3 diseases. For the purpose of comparison of the survival rates of patients with NSCLC with IPI with that of patients with other T2 and T3 diseases, patients without nodal involvement were analysed to exclude the influence of nodal involvement.

The surgical results of the IPI group were also compared with the T3 subgroups (i.e. tumours measuring >7 cm, tumour with invasion of the mediastinal structures, chest wall, diaphragm or tumours with separate nodule in the same lobe).

Patients of the IPI group underwent lobectomy with adjacent lobe segmentectomy, lobectomy with partial resection, bilobectomy or pneumonectomy. The type of resection was selected based on pulmonary reserve and localization of the tumour. Lobectomy with partial resection was preferred if complete resection with adequate margin was ensured.

Data are expressed as mean ± SD. The overall survival was analysed by the Kaplan–Meier method. Differences between groups were analysed by the log-rank test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using StatView 5.0 software (SAS Institute, Berkley, CA, USA).

RESULTS

Among the 1001 patients with pathological T2 and T3 NSCLC, 738 patients were males and 263 were females. The mean age was 65.1 ± 9.4 years (median, 67 years; range, 26–87 years). The histopathological diagnosis was squamous cell carcinoma in 391, adenocarcinoma in 521, large cell carcinoma in 60 and adenosquamous carcinoma in 29 patients. The pathological stage was T2 without IPI in 682 patients (T2 group), T2 with IPI in 25 patients (IPI group) and T3 in 294 patients (T3 group). The incidence of IPI among all the 1989 patients who were operated on for NSCLC in our department during that period was 1.3%. Table 1 shows the relation between pathological T factor and N factor. The median follow-up interval was 46 months (range, 1–301 months). Of all the 1001 patients, 596 patients underwent surgery alone, 308 patients underwent surgery and adjuvant chemotherapy, 55 patients underwent surgery and adjuvant radiotherapy, and 42 patients underwent surgery, adjuvant chemotherapy and radiotherapy.

Among the IPI group, the interlobar fissure was present and the tumour invaded across the interlobar fissure in 23 patients while the tumour invaded directly the adjacent lobe through an incomplete interlobar fissure in two patients. The primary tumour was located in the right lung in 15 patients (upper lobe in 11, middle lobe in 2 and lower lobe in 2) and in the left lung in 10 patients (upper lobe in 6 and lower lobe in 4). Fourteen (56%) patients had no lymph node metastasis, 4 (16%) had hilar–interlobar nodal metastasis (N1 disease) and 7 (28%) had mediastinal nodal metastasis (N2 disease). The type of resection was pneumonectomy in 4 patients, bilobectomy in 2, lobectomy with segmentectomy in 2 and lobectomy with partial resection in 17.

Table 1: Relationship between the pathological T and N factors

T2 ($n = 682$)		IPI ($n = 25$)		T3 ($n = 294$)	
N0M0	375	N0M0	14	N0M0	139
N1M0	170	N1M0	4	N1M0	66
N2M0	137	N2M0	7	N2M0	89

In patients without nodal involvement, the 5-year survival rates of the T2N0, IPIN0 and T3N0 groups were 60.9, 40.0 and 45.9%, respectively (Fig. 1). The survival rate was significantly different between the T3N0 and T2N0 groups ($P < 0.001$) and between the IPIN0 and T2N0 ($P = 0.020$) groups. There was no significant difference in the survival rate between the IPIN0 and T3N0 groups ($P = 0.644$).

Table 2 shows the results of analysis of T3 tumour subgroups: 87 patients had tumours >7 cm (size group), 39 with tumours invading the mediastinal structures (mediastinal pleura, parietal pericardium, the main bronchus <2 cm distal to the carina; mediastinal group), 96 with tumours invading the chest wall (chest wall group), 5 with tumours invading the diaphragm (diaphragm group) and 67 with separate nodule in the same lobe (pulmonary metastasis group).

The 5-year survival rates of the size, mediastinal, chest wall, diaphragm and pulmonary metastasis T3 subgroups were 32.5, 49.9, 40.7, 0 and 28.4%, respectively. The survival rate of the IPI group was significantly different from that of the diaphragm subgroup ($P = 0.027$), while that of the IPI group was not significantly different from the rate of the size, mediastinal, chest wall and pulmonary metastasis subgroups ($P = 0.489$, 0.097, 0.774 and 0.577, respectively).

DISCUSSION

Although there is still a debate on whether NSCLC with IPI are classified as T2 or T3, the seventh TNM staging system classifies these tumours as T2. This issue is complicated by the fact that in some cases with no interlobar fissure, invasion of the adjacent lobe can occur across the lung parenchyma without involvement of the pleura [4]. In the present study, we examined the surgical results of NSCLC with IPI according to the seventh TNM staging system and also carefully examined whether the interlobar pleura was present at the point of invasion.

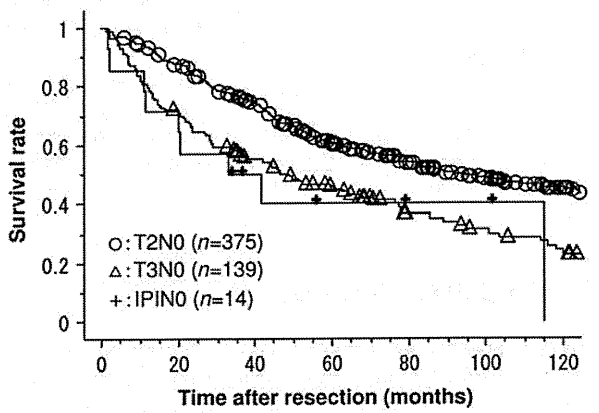


Figure 1: The 5-year survival rates of the T2N0, IPIN0 and T3N0 groups. The 5-year survival rate was significantly different between the T3N0 and T2N0 groups ($P < 0.001$) and between the IPIN0 and T2N0 ($P = 0.020$) groups, but not between the IPIN0 and T3N0 groups ($P = 0.644$).

Table 2: Subcategorization of the T3 group

	T3N0 (*n* = 139)	T3N1 (*n* = 66)	T3N2 (*n* = 89)	Total (*n* = 294)	5-year survival (*P* value)[a]
Tumours larger than 7 cm	37	17	33	87	32.5% (0.489)
Tumours invading mediastinal structures[b]	10	21	8	39	49.9% (0.097)
Tumours invading the chest wall	61	17	18	96	40.7% (0.774)
Tumours invading the diaphragm	4	1	0	5	0% (0.027)
Separate tumour nodule in the same lobe	27	10	30	67	28.4% (0.577)

[a]*P* values are log-rank comparison of survival with the IPI group.
[b]Tumours invading mediastinal structures included tumours that invaded the mediastinal pleura, parietal pericardium, tumours in the main bronchus <2 cm distal to the carina.

Several studies have examined this issue in relation to the sixth TNM staging system [9]. Miura *et al.* [5] demonstrated that the survival of patients with NSCLC-IPI was significantly better than that of patients with NSCLC with parietal pleural invasion (5-year survival rate: 34 vs. 14%, $P < 0.001$) and was not different from that of patients with NSCLC and VPI only. They concluded that NSCLC with IPI should be classified as T2. In contrast, two other investigators have reported that NSCLC with IPI should be classified as T3. Okada *et al.* [6] demonstrated that the survival rate of patients with NSCLC-IPI was similar to that of patients with NSCLC with invasion of the parietal pleura and chest wall (5-year survival rates: 37, 40 and 38%, respectively, P = NS). In the series of Demir *et al.* [7], the 5-year survival rate of patients with the NSCLC invading adjacent lobe beyond interlobar pleura disease was 36%, which was significantly poorer than the survival rate for T2 disease ($P = 0.049$), but not significantly different from the survival rate of patients with T3 disease by multivariate analysis. Joshi *et al.* [10] recently reported the surgical results of 180 patients with NSCLC that extended beyond the fissure, who underwent lobectomy based on the seventh TNM staging system. They concluded that Stage I NSCLC extending across the fissure into an adjacent lobe carries a 5-year survival rate between those of stages I and II.

In the present study, the 5-year survival rates of the T2N0 and T3N0 groups were 60.9 and 45.9%, respectively. Patients with T2N0 disease were distributed as stage IB or IIA, and these with T3N0 disease were distributed as stage IIB according to the seventh edition of the TNM classification. Our data are comparable with the surgical results of the nearly the same period (1982–2002) by another institution in Japan according to the seventh edition of the TNM classification [11] which shows 5-year survival rates of Stage IB, IIA and IIB were 64.9, 65.9 and 44.7%. In the present study, the 5-year survival rate of the IPI group was 31.1%, which is comparable with those of previous reports (range: 34–37%) [5–7]. The survival rate of patients with IPIN0 was significantly different from that of the T2N0 group. The present study indicates that the survival of the IPIN0 group is similar to that of the T3N0 group.

When compared with subgroups of T3, the survival rate of the IPI group was significantly better than that of the diaphragm subgroup but not significantly different from that of the size, mediastinal, chest wall and pulmonary metastasis subgroups. These results are in agreement with those of previous reports. In Okada's series [6], the survival rate of patients with NSCLC-IPI was similar to that of patients with NSCLC and parietal pleura or chest wall invasion. In the study of Demir *et al.* [7], the survival rate of patients with NSCLC-IPI was similar to that of patients with bronchial T3, peripheral T3 and mediastinal T3. The present study is the first to demonstrate that the survival of patients with NSCLC-IPI is similar to that of NSCLC >7 cm in size and NSCLC with a separate nodule in the same lobe.

The present study had certain limitations. First, the number of patients was relatively small. Secondly, the number of cases of NSCLC with direct invasion to an adjacent lobe through the incomplete interlobar fissure was probably underestimated. While the presence of IPI with intact interlobar fissure could be confirmed retrospectively by microscopic examination using elastic stains, which confirmed the pleural invasion of the adjacent lobe, direct invasion to an adjacent lobe through the incomplete interlobar could only be confirmed by precise description of the anatomical relationship between the tumour and the interlobar fissure. Thirdly, the study included patients who were treated over several decades and followed up with different radiological modalities and various techniques of perioperative care. Advancement of radiological modalities in this period such as multidetector computed tomography (CT) provides more accurate preoperative staging. Patients in the conventional CT era might be understaged. Adjuvant therapy might impact on the survival of patients. Unfortunately, the regimen and dose of adjuvant chemotherapy was not available in our database. Advancement of treatment for relapse, i.e. chemotherapy and radiotherapy, might influence on the survival. Patients in recent years might had better prognosis after recurrence. These factors could have influenced the results of analysis. Future data collection with precise pathological and anatomical description conducted in a multi-centre setting is needed to resolve these limitations.

CONCLUSIONS

In patients without nodal involvement, the survival of NSCLC with IPI is similar to that of the T3 disease.

Conflict of interest: none declared.

REFERENCES

[1] Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
[2] Sobin LH, Gospodarowicz MK, Wittekind C. International Union Against Cancer (UICC): TNM Classification of Malignant Tumors, 7th edn. Oxford: Wiley-Blackwell, 2009.
[3] Rami-Porta R, Crowley JJ, Goldstraw P. The revised TNM staging system for lung cancer. Ann Thorac Cardiovasc Surg 2009;15:4–9.
[4] Travis WD, Brambilla E, Rami-Porta R, Vallieres E, Tsuboi M, Rusch V *et al.* Visceral pleural invasion: pathologic criteria and use of elastic

stains: proposal for the 7th edition of the TNM classification for lung cancer. J Thorac Oncol 2008;3:1384–90.
[5] Miura H, Taira O, Uchida O, Kato H. Invasion beyond interlobar pleura in non-small cell lung cancer. Chest 1998;114:1301–4.
[6] Okada M, Tsubota N, Yoshimura M, Miyamoto Y, Matsuoka H. How should interlobar pleural invasion be classified? Prognosis of resected T3 non-small cell lung cancer. Ann Thorac Surg 1999;68:2049–52.
[7] Demir A, Gunluoglu MZ, Sansar D, Melek H, Dincer SI. Staging and resection of lung cancer with minimal invasion of the adjacent lobe. Eur J Cardiothorac Surg 2007;32:855–8.
[8] World Health Organization. Histological Typing of Lung and Pleural Tumours, 4th edn. Lyon: IARC Press, 2004.
[9] Sobin L, Wittekind C. International Union Against Cancer (UICC): TNM Classification of Malignant Tumors, 6th edn. New York: John Wiley & Sons, 2002.
[10] Joshi V, McShane J, Page R, Carr M, Mediratta N, Shackcloth M *et al.* Clinical upstaging of non-small cell lung cancer that extends across the fissure: implications for non-small cell lung cancer staging. Ann Thorac Surg 2011;91:350–3.
[11] Fukui T, Mori S, Hatooka S, Shinoda M, Mitsudomi T. Prognostic evaluation based on a new TNM staging system proposed by the International Association for the Study of Lung Cancer for resected non-small cell lung cancers. J Thorac Cardiovasc Surg 2008;136:1343–8.

ANTICANCER RESEARCH *31*: 4665-4672 (2011)

Low Dihydropyrimidine Dehydrogenase Correlates with Prolonged Survival in Patients with Lung Adenocarcinoma Treated with 5-Fluorouracil

YASUSHI SHINTANI[1], MASAYOSHI INOUE[1], YASUNOBU FUNAKOSHI[2], AKIHIDE MATSUMURA[3], MITSUNORI OHTA[4], HAJIME MAEDA[2] and MEINOSHIN OKUMURA[1]

[1]*Department of Thoracic Surgery, Osaka University Graduate School of Medicine, Osaka, Japan;*
[2]*Department of General Thoracic Surgery, National Hospital Organization Toneyama Hospital, Osaka, Japan;*
[3]*Department of General Thoracic Surgery, National Hospital Organization Kinki Chuo Chest Medical Center, Osaka, Japan;*
[4]*Department of General Thoracic Surgery, Osaka Prefectural Medical Center for Respiratory and Allergic Disease, Osaka, Japan*

Abstract. *Background: The enzyme dihydropyrimidine dehydrogenase (DPD) is involved in the metabolism of 5-fluorouracil (5-FU). The aim of this study was to clarify the correlation between the expression of DPD and the efficacy of 5-FU therapy in patients with lung adenocarcinoma (AD). Patients and Methods: We examined surgically resected specimens from 90 stage I to IIIA patients with lung ADs to determine the level of intra-tumoral DPD mRNA. Results: Administration of 5-FU improved the prognosis of patients with low DPD-expressing tumors, whereas it did not do so for patients with high DPD expressing tumors. Patients with low DPD-expressing tumors administered with 5-FU had a significantly better prognosis than those who underwent surgery alone. A Cox proportional hazards regression model revealed that administration of 5-FU was an independent variable to predict prognosis in patients with low DPD-expressing tumors. Conclusion: Quantification of DPD mRNA levels is useful for determining the subgroup of lung AD patients who would benefit most from 5-FU after surgery.*

5-Fluorouracil (5-FU) and its derivatives are widely used for treatment of various types of cancer (1). A recent study showed that postoperative oral administration of tegafur-uracil (UFT) improves survival in patients following resection of stage I lung adenocarcinoma (AD) and its administration has become standard therapy after curative resection in early non-small cell lung cancer (NSCLC) cases (2, 3). Furthermore, a novel oral form of fluorouracil S-1 was shown to have promising effects against advanced NSCLC (4). These findings indicate that 5-FU is effective for NSCLC patients and highlight the importance of detection of biomarkers for prediction of its efficacy for treating NSCLC.

Thymidylate synthase (TYMS), the target enzyme for 5-FU, catalyzes an important process for DNA biosynthesis (5, 6) and we previously reported that the prognosis of NSCLC patients is related significantly to the intratumoral *TYMS* mRNA level (7). Dihydropyrimidine dehydrogenase (DPD) is one of the key enzymes involved in the catabolism of 5-FU and its expression was found useful in predicting the efficacy of 5-FU after surgery for NSCLC based on disease-free interval during short follow-up periods (8-10). In the present study, we examined the efficacy of 5-FU in association with DPD expression in regards to prognosis, including overall survival, in patients with lung ADs over a longer follow-up period.

Patients and Methods

Ninety specimens from lung AD patients determined to be p-stage I to IIIA were obtained during surgical procedures at Osaka University Hospital, Kinki Chuo Chest Medical Center, Toneyama Hospital, and Osaka Prefectural Medical Center for Respiratory and Allergic Disease between January 1999 and March 2003. Quantification of *TYMS* and *DPD* mRNA levels in AD tissues was performed as described previously (7, 10). The obtained copy numbers of *TYMS* and *DPD* were standardized with glyceralde-hydo-3-phosphate dehydrogenase (*GAPDH*) mRNA quantity, used as an endogenous control, with the following equation: 'Result'=Log (*TYMS* 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).

Correspondence to: Yasushi Shintani, MD, Ph.D., Department of General Thoracic Surgery, Osaka University Graduate School of Medicine, 2-2-L5, Yamadaoka, Suita-city, Osaka, 565-0871, Japan. Tel: +81 668793152, Fax: +81 668793164, e-mail: yshintani@thoracic.med.osaka-u.ac.jp

Key Words: Lung adenocarcinoma, 5-fluorouracil, dihydropyrimidine dehydrogenase, thymidylate synthase, chemosensitivity.

0250-7005/2011 $2.00+.40

Table I. *Patient background data.*

Variable	Treatment		*p*-Value
	Surgery alone (control) n=60	5-FU n=30	
Age (years)	63±9.3	62±9.4	0.445
Gender			
Male	35 (58%)	20 (67%)	0.426
Female	25 (42%)	10 (33%)	
Pathologic stage			
I	38 (63%)	20 (67%)	
II	9 (15%)	6 (20%)	0.882
IIIA	13 (22%)	4 (13%)	

5-FU, 5-fluorouracil. *p*-Value, chi-square test or Mann-Whitney *U*-test.

Table II. *Thymidylate synthase (TYMS) and Dihydropyrimidine dehydrogenase (DPD) mRNA levels, and clinicopathologic factors.*

Factor	n	Log TYMS mRNA	*p*-Value	Log DPD mRNA	*p*-Value
Tumor status			0.029		0.054
pT1	49	6.90±0.29		6.89±0.48	
pT2	36	7.03±0.33		6.63±0.54	
pT3	5	7.42±0.54		6.99±0.27	
Nodal status			0.600		0.546
pN0	61	6.96±0.34		6.80±0.57	
pN1	12	7.00±0.36		6.75±0.41	
pN2	17	7.04±0.35		6.80±0.33	

p-Value, chi-square test or Mann-Whitney *U*-test.

Informed consent was obtained from all patients. Those administered UFT following surgery comprised the 5-FU group (n=30), while those who underwent surgery only, comprised the control group (n=60). UFT administration was started within 2 months after surgery and continued for more than 12 months. The dose of UFT was 300-400 mg/day and the mean duration of treatment was 21.5±7.3 months (mean±SD; range 12-26 months). The clinical backgrounds of the patients are summarized in Table I. There was no difference in clinical factors between the groups. The median follow-up period was 78±23 months (range 17-115 months) after surgery.

Chi-square, Mann-Whitney *U*, and Kruskal-Wallis tests were used to compare the results, while survival rates were estimated by the method of Kaplan and Meier and compared using log-rank test, using Statview version 5.0 for Windows (Abacus Concepts, Berkeley, CA, USA). A *p*-value of <0.05 was considered to be statistically significant.

Results

Quantification of *TYMS* and *DPD* mRNA levels in NSCLC tissues was successfully performed for all specimens. Intratumoral *TYMS* and *DPD* mRNA levels ranged from 6.28 to 8.04 (mean±SD; 6.98±0.34) and 5.36 to 8.28 (6.79±0.59), respectively. The results for *TYMS* and *DPD* mRNA levels are summarized in Table II. *TYMS* mRNA levels were associated with tumor status, while *DPD* mRNA levels were not associated with tumor or nodal status.

Thirty-five (39%) of the 90 patients developed distant metastasis after surgery. In regards to tumor stage, 12 (21%) out of 58 patients in stage I, 8 (53%) out of 15 patients in stage II, and 15 (88%) out of 17 patients in stage IIIA suffered from recurrent disease. Categorized by group, 27 (45%) out of 60 patients and 8 (27%) out of 30 in the control and 5-FU groups, respectively, had recurrence. There was no significant difference in overall survival rate between the groups (Figure 1A). Similar to a previous report (7), *TYMS* mRNA levels were significantly correlated to overall survival when dichotomized at the mean *TYMS* mRNA level (Figure 1B).

Next, we evaluated the correlation between *DPD* expression and efficacy of 5-FU. *DPD* mRNA levels were significantly correlated to overall survival in the 5-FU group, but not in the control group when dichotomized at the mean *DPD* mRNA level (Figure 2). In the 5-FU-group, the 5-year survival rate was 92% for the low DPD-expressing subgroup and 68% for the high DPD-expressing subgroup. Patients with low DPD-expressing tumors, who were administered 5-FU had a significantly better prognosis than those who underwent surgery alone (Figure 3A); the 5-year survival rates were 92% for the 5-FU group and 53% for the control group. These findings suggest that the intratumoral *DPD* mRNA level may be a possible predictor for efficacy of 5-FU administration after surgery for NSCLC. On the other hand, patients with high DPD-expressing tumors administered 5-FU had a tendency for a worse prognosis as compared to those who underwent surgery alone (Figure 3B).

We analyzed 5 variables, namely tumor status, nodal metastasis, *TYMS* mRNA expression, *DPD* mRNA expression, and 5-FU administration, using a Cox proportional hazards regression model to determine their effects on overall survival in NSCLC patients (Table IIIA). Multivariate analysis revealed that p-N2 and *TYMS* mRNA expression were independent variables for predicting overall survival (Table IIIB). Furthermore, in patients with low DPD-expressing tumors, multivariate analysis showed that *TYMS* mRNA expression and administration of 5-FU, were each independent variables predicting prognosis (Table IIIC).

Discussion

We performed quantitative assays of intratumoral *TYMS* and *DPD* mRNA levels to assess their association with clinicopathological factors, as well as the feasibility of applying them to predict the efficacy of 5-FU therapy in

patients with NSCLC over a long term. TYMS activity is necessary for cell proliferation because it catalyses an essential step in DNA synthesis, while its overexpression is reported to be associated with tumor proliferation, as well as poor prognosis, in a variety of cancer types (11, 12). As shown in Table IIIB, multivariate analysis revealed that a high level of *TYMS* mRNA was independently correlated to overall survival with a high hazard ratio, indicating that this marker can precisely perform prognosis for patients with lung AD. Determination of gene expression by RT-PCR is a useful technique for small-sized specimens, thus quantification of *TYMS* mRNA levels is clinically sensitive and useful for determining the prognosis of AD patients (7).

As DPD is a rate-limiting enzyme in the catabolism of 5-FU, its high expression in tumors is reported to result in a low sensitivity to 5-FU therapy (13). In the present study, we evaluated the efficacy of 5-FU administration as adjuvant chemotherapy, in relation to intratumoral *DPD* mRNA levels in lung AD patients. Our results revealed that *DPD* expression was significantly inversely correlated to the overall survival of patients administered 5-FU following surgery, indicating that patients with low levels of *DPD* expression in cancer tissue are sensitive to 5-FU. Furthermore, for patients with low DPD-expressing tumors, those administered 5-FU had a significantly better prognosis than those who underwent surgery alone. These findings suggest that the intratumoral *DPD* mRNA level is a possible predictor for the efficacy of 5-FU administration after surgery in lung AD patients. Interestingly, in patients with high DPD-expressing tumors, those administered 5-FU had a tendency for worse prognosis than those who underwent surgery alone (Figure 3B), suggesting that 5-FU may not have benefits for patients with high DPD-expressing tumors. Multivariate analysis showed that administration of 5-FU was an independent variable predicting prognosis of patients with low DPD-expressing lung ADs. Based on these results, determination of *DPD* mRNA levels in lung AD tumors may provide important information for clinicians to decide whether or not to proceed with 5-FU-based chemotherapy for their patients.

Based on our findings for biomarkers associated with 5-FU therapy, it is considered important to evaluate the expressions of TYMS and DPD before establishing a protocol for made-to-order chemotherapy for NSCLC patients (14). In addition, investigation of the effects of more aggressive adjuvant therapy for patients with NSCLC who have elevated *TYMS* or *DPD* mRNA levels is also necessary. Takizawa *et al.* reported that *in vitro* sensitivity to platinum-derived drugs, such as cisplatin and carboplatin, was associated with the expression of *TYMS* and *DPD* in NSCLC specimens (15). They hypothesized that these may be novel markers of DNA repair capacity and may also be linked with chemosensitivity to drugs other than 5-FU. Furthermore, it is important to clarify the roles of TYMS and DPD in regards to chemosensitivity toward various chemotherapy regimens, as their inhibition is now receiving attention for new cancer treatment drugs development. Recently, S-1, a combination of tegafur, gimeracil, and oteracil potassium (Taiho Pharmaceutical), was developed for clinical use (4). Gimeracil is a stronger inhibitor of DPD than uracil when used with UFT. However, Takeda *et al.* reported that a high level of DPD expression predicted resistance to S-1-based chemotherapy in patients with advanced NSCLC (16). Therefore, additional investigations of the effects of new

Table III.

A. Univariate analysis of overall survival in all patients.

Factors	Hazard ratio	95% CI	*p*-Value
Tumor status			
pT3 *vs.* pT1	3.71	1.05-13.2	0.042
pT2 *vs.* pT1	1.91	0.93-3.90	0.079
Nodal status			
pN2 *vs.* pN0	3.33	1.54-7.21	0.002
pN1 *vs.* pN0	1.73	0.67-4.45	0.259
TYMS mRNA			
High *vs.* low	4.17	1.81-9.03	0.001
DPD mRNA			
High *vs.* low	1.09	0.55-2.520	0.804
Administration			
5-FU *vs.* none	1.46	0.68-3.15	0.337

B. Multivariate analysis of overall survival in all patients.

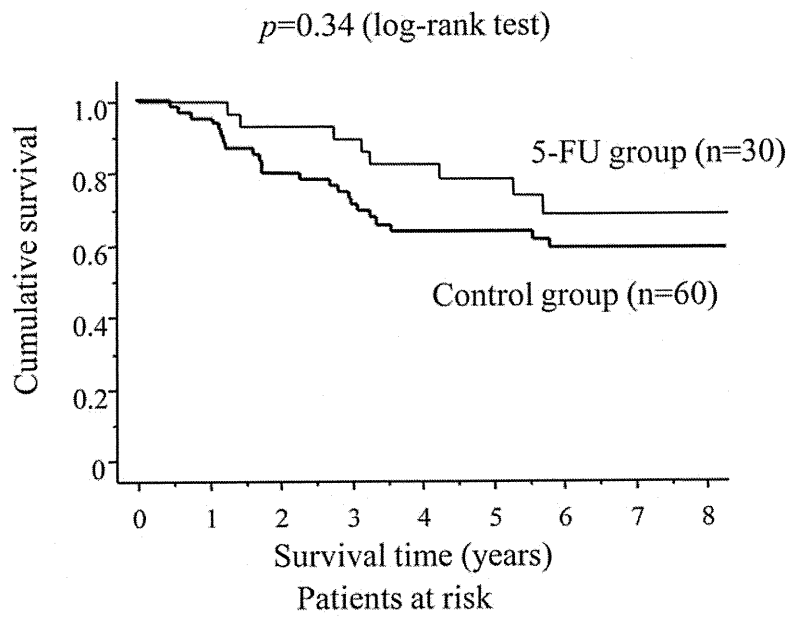
Factor	Hazard ratio	95% CI	*p*-Value
Tumor status			
pT3 *vs.* pT1	2.51	0.69-9.12	0.161
pT2 *vs.* pT1	1.27	0.59-2.75	0.546
Nodal status			
pN2 *vs.* pN0	2.56	1.16-5.66	0.020
pN1 *vs.* pN0	1.41	0.51-3.87	0.511
TYMS mRNA			
High *vs.* low	3.42	1.46-8.02	0.005

C. Multivariate analysis of overall survival in patients with low DPD-expressing tumors.

Factor	Hazard ratio	95% CI	*p*-Value
Nodal status			
pN2 *vs.* pN0	1.42	0.42-4.76	0.570
pN1 *vs.* pN0	0.85	0.22-3.27	0.816
TYMS mRNA			
High *vs.* low	5.31	1.17-24.0	0.030
Administration			
5-FU *vs.* none	7.60	1.02-56.7	0.050

CI, Confidence interval. TYMS, Thymidylate synthase. DPD, Dihydropyrimidine dehydrogenase. 5-FU, 5-fluorouracil.

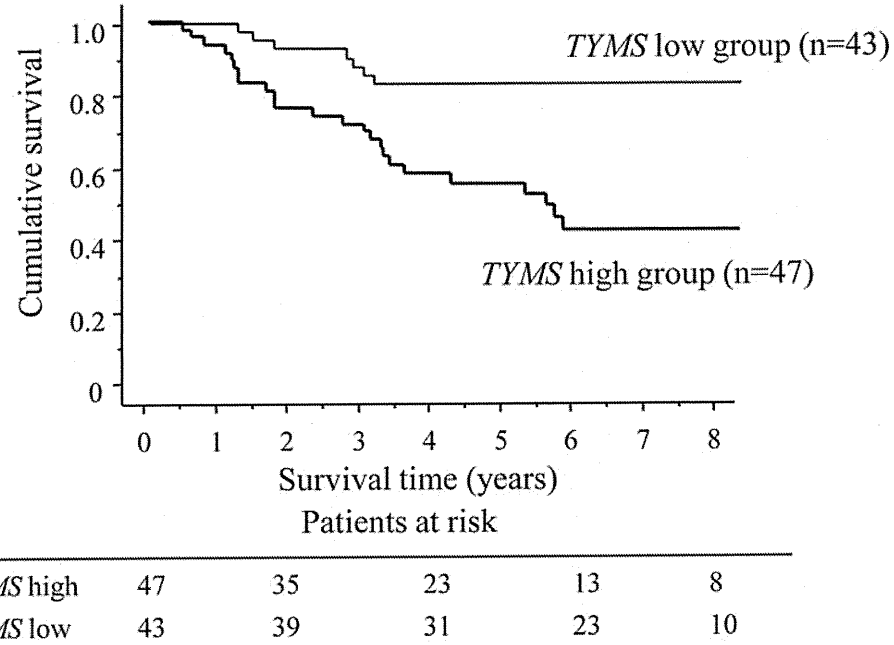
A



Patients at risk

Control	60	47	32	24	12
5-FU	30	27	22	12	6

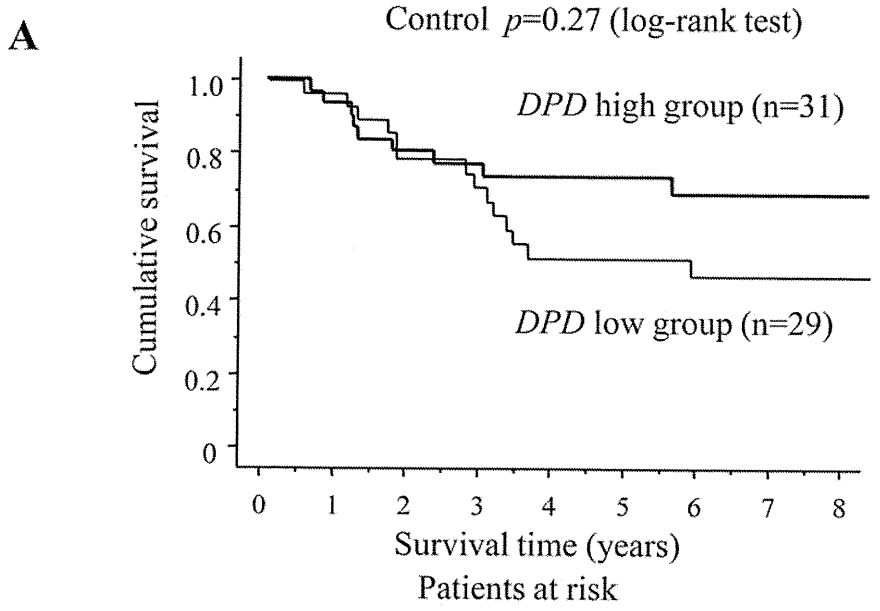
B



Patients at risk

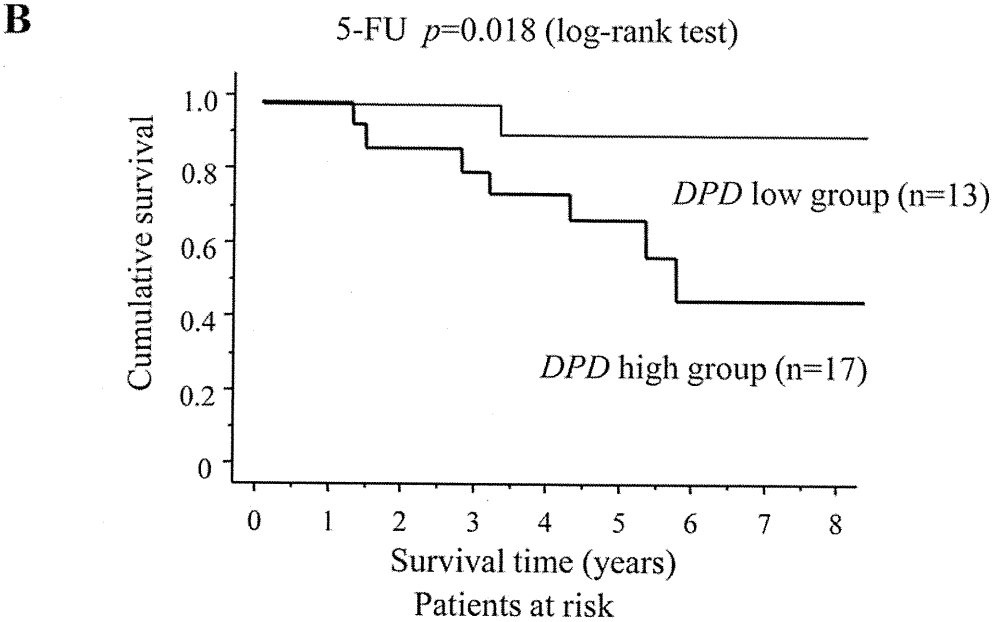
TYMS high	47	35	23	13	8
TYMS low	43	39	31	23	10

Figure 1. *A: Overall survival curves for patients administered and those not administered 5-fluorouracil (5-FU) after surgery. B: Overall survival curves for patients with high and low thymidylate synthase (TYMS) mRNA levels in resected cancer tissues when dichotomized at the mean TYMS mRNA level.*



Patients at risk

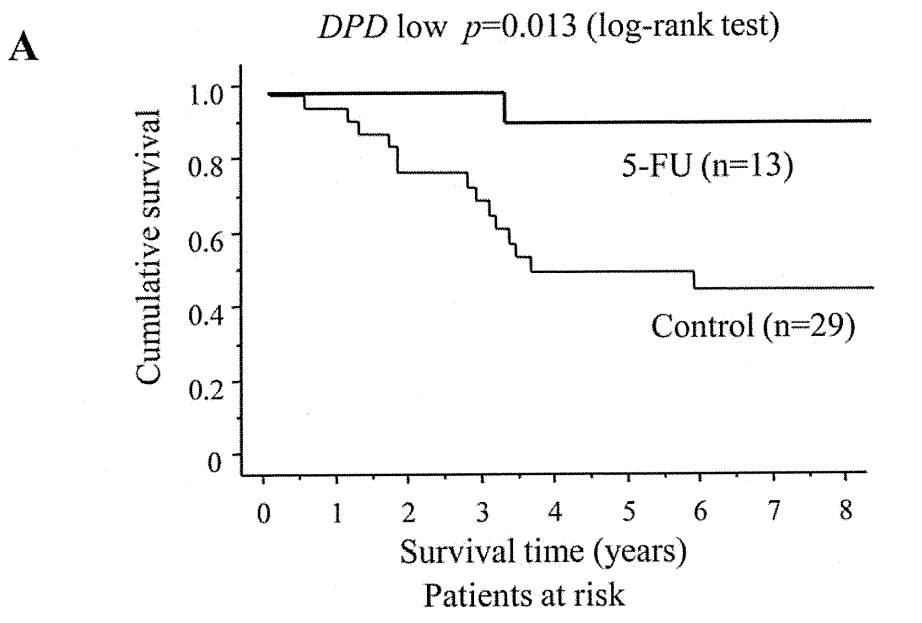
DPD high	31	25	19	14	7
DPD low	29	22	13	10	5



Patients at risk

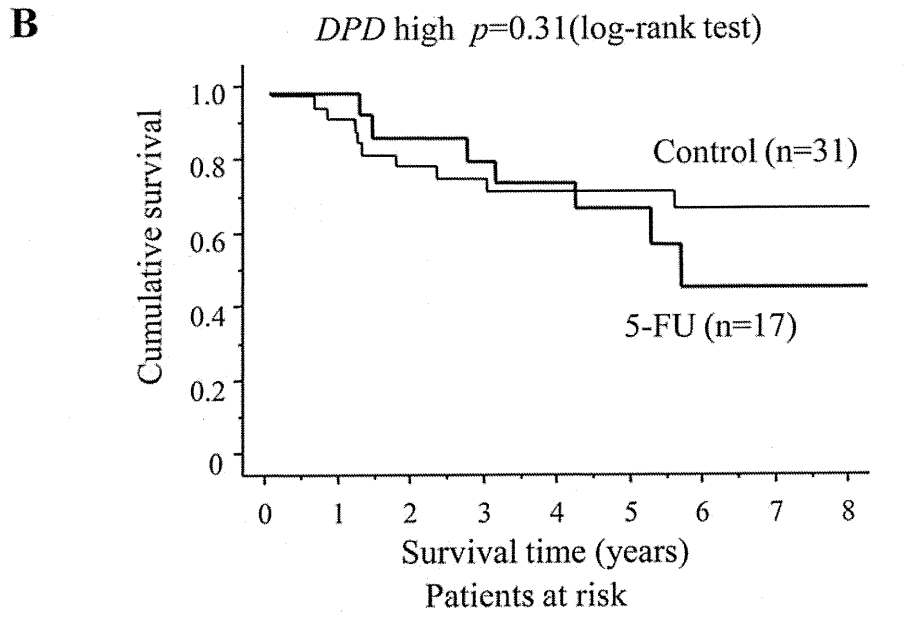
DPD high	17	14	11	3	2
DPD low	13	13	11	9	4

Figure 2. *A: Overall survival curves for patients with low and high dihydropyrimidine dehydrogenase (DPD)-expressing tumors who did not receive 5-fluorouracil (5-FU) when dichotomized at the mean DPD mRNA level. B: Overall survival curves for patients with low and high DPD-expressing tumors who received 5-FU.*



Patients at risk

Control	29	22	13	10	5
5-FU	13	13	11	9	4



Patients at risk

Control	31	25	19	14	7
5-FU	17	14	11	3	2

Figure 3. *A: Overall survival curves for patients with dihydropyrimidine dehydrogenase (DPD)-expressing tumors: comparison between those who underwent surgery alone and those who received 5-fluorouracil (5-FU) when dichotomized at the mean TYMS mRNA level. B: Overall survival curves for patients with high DPD-expressing tumors: comparison between those who underwent surgery alone and those who received 5-FU.*

regimens with other anticancer drugs and molecular targeting therapies for NSCLC patients with high DPD-expressing tumors are necessary.

In conclusion, using real-time RT-PCR, assessment of *TYMS* and *DPD* expressions in tumors from patients with NSCLC can provide precise prognostic information and predict the efficacy of 5-FU therapy after resection.

Conflicts of Interest Statement

The Authors have no conflicts of interest to declare.

References

1 Oguri T, Achiwa H, Bessho Y, Muramatsu H, Maeda H, Niimi T, Sato S and Ueda R: The role of thymidylate synthase and dihydropyrimidine dehydrogenase in resistance to 5-fluorouracil in human lung cancer cells. Lung Cancer *49*: 345-351,2005.

2 Kato H, Ichinose Y, Ohta M, Hata E, Tsubota N, Tada H, Watanabe Y, Wada H, Tsuboi M, Hamajima N and Ohta M; Japan Lung Cancer Research Group on Postsurgical Adjuvant Chemotherapy: A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. N Engl J Med *350*: 1713-1721, 2004.

3 Hamada C, Tanaka F, Ohta M, Fujimura S, Kodama K, Imaizumi M and Wada H: Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small cell lung cancer. J Clin Oncol *23*: 4999-5006, 2005.

4 Ichinose Y, Yoshimori K, Sakai H, Nakai Y, Sugiura T, Kawahara M and Niitani H: S-1 plus cisplatin combination chemotherapy in patients with advanced non-small cell lung cancer: a multi-institutional phase II trial. Clin Cancer Res *10*: 7860-7864, 2004.

5 Danenberg PV: Thymidylate synthetase: a target enzyme in cancer chemotherapy. Biochem Biophys Acta *473*: 73-92, 1977.

6 Jonston PG, Lenz HJ and Leichman CG: Thymidylate syntase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumor. Cancer Res *55*: 1407-1412, 1995.

7 Shintani Y, Ohta M, Hirabayashi H, Tanaka H, Iuchi K, Nakagawa K, Maeda H, Kido T, Miyoshi S and Matsuda H: New prognostic indicator for non-small cell lung cancer, quantitation of thymidylate synthase by real-time reverse transcription polymerase chain reaction. Int J Cancer *104*: 790-795, 2003.

8 Fischel JL, Etienne MC, Spector T, Formento P, Renee N and Milano G: Dihydropyrimidine dehydrogenase: a tumoral target for fluorouracil modulation. Clin Cancer Res *1*: 991-6, 1995.

9 Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsao-Wei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB and Danenberg PV: Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. Clin Cancer Res *6*: 1322-1327, 2000.

10 Shintani Y, Ohta M, Hirabayashi H, Tanaka H, Iuchi K, Nakagawa K, Maeda H, Kido T, Miyoshi S and Matsuda H: Thymidylate synthase and dihydropyrimidine dehydrogenase mRNA levels in tumor tissues and the efficacy of 5-fluorouracil in patients with non-small cell lung cancer. Lung Cancer *45*: 189-196, 2004.

11 Lenz HJ, Leichman CG, and Danenberg KD: Thymidylate synthase mRNA level in adenocarcinoma of the stomach: a predictor for primary tumor response and overall survival. J Clin Oncol *14*: 176-182, 1995.

12 Yamachika T, Nakanishi H, Inada K, Tsukamoto T, Kato T, Fukushima M, Inoue M and Tatematsu M: A new prognostic factor for colorectal carcinoma, thymidylate synthase, and its therapeutic significance. Cancer *82*: 70-77, 1998.

13 Harris BE, Song R, Soong SJ and Diasio RB: Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res *50*: 197-201, 1990.

14 Nakano J, Huang C, Liu D, Masuya D, Nakashima T, Yokomise H, Ueno M, Wada H and Fukushima M: Evaluations of biomarkers associated with 5-FU sensitivity for non-small cell lung cancer patients postoperatively treated with UFT. Br J Cancer *95*: 607-615, 2006.

15 Takizawa M, Kawakami K, Obata T, Matsumoto I, Ohta Y, Oda M, Sasaki T and Watanabe G: *In vitro* sensitivity to platinum-derived drugs is associated with expression of thymidylate synthase and dihydropyrimidine dehydrogenase in human lung cancer. Oncol Rep 15: 1533-1539, 2006.

16 Takeda M, Okamoto I, Hirabayashi N, Kitano M and Nakagawa K: Thymidylate synthase and dihydropyrimidine dehydrogenase expression levels are associated with response to S-1 plus carboplatin in advanced non-small cell lung cancer. Lung Cancer *73*: 103-109, 2011.

Received September 13, 2011
Revised November 17, 2011
Accepted November 18, 2011

European Journal of Cardio-Thoracic Surgery 41 (2012) 357–361
doi:10.1016/j.ejcts.2011.05.050

ORIGINAL ARTICLE

Adenosquamous carcinoma of the lung: surgical results as compared with squamous cell and adenocarcinoma cases

Hajime Maeda[a,*], Akihide Matsumura[b], Tsutomu Kawabata[c], Tetsushi Suito[d], Osamu Kawashima[e], Takehiro Watanabe[f], Kan Okabayashi[g] and Ichiro Kubota[h] for the Japan National Hospital Organization Study Group for Lung Cancer

[a] Department of General Thoracic Surgery, National Hospital Organization Toneyama Hospital, Osaka, Japan
[b] NHO Kinki-Chuo Chest Medical Center, Osaka, Japan
[c] NHO Okinawa Hospital, Okinawa, Japan
[d] NHO Ibaraki-Higashi Hospital, Ibaraki, Japan
[e] NHO Nishi-Gunma Hospital, Gunma, Japan
[f] NHO Nishi-Niigata-Chuo Hospital, Niigata, Japan
[g] NHO Fukuoka-Higashi Medical Center, Fukuoka, Japan
[h] NHO Minami-Kyushu Hospital, Kagoshima, Japan

* Corresponding author. Department of General Thoracic Surgery, National Hospital Organization Toneyama Hospital, 5-1-1 Toneyama, Toyonaka City, Osaka 560-8552, Japan. Tel: +81-6-68532001; fax: +81-6-68533127; e-mail: maedah@toneyama.hosp.go.jp (H. Maeda).

Received 16 March 2011; received in revised form 9 May 2011; accepted 13 May 2011

THORACIC

Abstract

OBJECTIVES: An adenosquamous carcinoma (ASC) of the lung is a relatively rare tumor. In this multi-institutional cohort study, we tested the hypothesis that an ASC exhibits more aggressive clinical behavior as compared to adenocarcinoma (AC) and squamous cell carcinoma (SC).

METHODS: This retrospective cohort study used a prospective database produced by the Japan National Hospital Organization Study Group for Lung Cancer over a 7-year period (operations from 1997 to 2003, follow-up data until March 2010). During that period, 4668 cases underwent an operation for various types of primary malignant lung tumors. When a sample from a tumor comprised at least 20% each of SC and AC, the case was classified as ASC. Pathologic staging was done according to the seventh edition of the International Union against Cancer (UICC) Tumor Node Matastasis (TNM) classification of malignant tumors.

RESULTS: We identified 114 patients with ASC (2.4%), 2993 with AC (64.2%), and 1369 with SC (29.3%). Kaplan-Meier survival curves for all stage cases, p-stage IA, IB, and IIIA tumors indicated that ASC cases had the least favorable survival. The 5-year survival rates for all stage cases were 23.3% for ASC, 58.0% for AC ($p < 0.0001$), and 40.8% for SC ($p < 0.0001$). The 5-year survival rates for p-stage IA were 42.0% for ASC, 81.8% for AC ($p = 0.0005$), and 63.4% for SC not significant (NS), while those for p-stage IB were 19.3%, 65.3% ($p = 0.0024$), and 46.8% (NS), respectively, and those for p-stage IIIA were 17.8%, 24.8% ($p = 0.0154$), and 18.8% (NS), respectively. There was a tendency for greater survival differences between ASC and AC in earlier tumor stages. A step-wise multivariable model demonstrated that sex, age, performance status, histology, tumor size, p-stage, operative method, and neoadjuvant/adjuvant therapy were independent prognostic factors.

CONCLUSION: ASC of the lung is more aggressive than AC and SC. The decreased survival of patients with ASC as compared with either of those single histology tumors suggests the need for a clinical trial of adjuvant chemotherapy that includes early-stage patients.

Keywords: Lung cancer • Adenosquamous carcinoma • Surgery • Prognosis

INTRODUCTION

Adenosquamous carcinoma (ASC) of the lung is a relatively rare tumor, comprising from 0.3% to 5% of non-small-cell lung cancer cases [1–8]. ASC is a mixed histologic tumor, as defined by the World Health Organization (WHO), as it has components of both adenocarcinoma (AC) and squamous cell carcinoma (SC), with each comprising at least 10% of the tumor [9]. Previously, the Japan Lung Cancer Society recommended a diagnosis of ASC when each component of the tumor comprised at least 20% of the tumor texture [10]. However, that definition was changed in 2003 to agree with that of the WHO [11]. Although several studies have suggested that an ASC of the lung is more aggressive than AC and SC [2–8], a few large series have been reported in medical literature, and the characteristics and prognosis remain poorly defined. In the present multi-institutional cohort study, we tested the hypothesis that ASCs exhibit a clinical behavior distinct from ACs and SCs of the lung,

by applying the former more rigorous histologic criteria of the Japan Lung Cancer Society.

MATERIALS AND METHODS

This retrospective cohort study used a prospective database produced by the Japan National Hospital Organization Study Group for Lung Cancer over a 7-year period (operations from 1997 to 2003, follow-up data until March 2010). Data collection and analyses were approved and the need for obtaining informed consent from each patient was waived by an institutional review board. There were 4668 patients who underwent an operation during that period for various types of primary non-small-cell lung cancer. The tumor histological type was determined according to the fifth edition of the general rules for clinical and pathologic records published by the Japan Lung Cancer Society [10]. According to these criteria, when a routine microscopic examination using hematoxylin and eosin staining shows that a sample from a tumor is comprised of at least 20% each of SC and AC, the condition is classified as adenosquamous. Pathologic staging was done according to the seventh edition of the TNM classification of malignant tumors [12].

Statistical analyses were performed using the StatView 5.0 software package (SAS Institute Inc. Cary, NC, USA). Comparisons of the characteristics of each cell type were made using the chi-square test for nominal variables and the Student's t-test for continuous variables. Survival rates were calculated using the

Table 1: Characteristics of patients with adenosquamous carcinoma, adenocarcinoma, and squamous cell carcinoma, and comparisons among adenosquamous carcinoma and single histologic carcinoma types

	ASC	AC	*p* value AC versus ASC	SC	*p* value SC versus ASC
Total no. of cases	114	2993		1369	
Age in years (range)	68.7 (34–86)	65.2 (19–93)	<0.0001	68.5 (27–91)	NS
Sex, male/female (ratio)	88/26 (3.38:1)	1591/1402 (1.13:1)	<0.0001	1254/115 (10.9:1)	<0.0001
Performance status					
0	75 (65.8)	2511 (83.8)	<0.0001	952 (69.6)	
1	34 (29.8)	449 (15.0)		377 (27.5)	
2	4 (3.5)	27 (0.9)		34 (2.5)	NS
3	1 (0.9)	5 (0.2)		4 (0.3)	
4	0 (0.0)	1 (0.1)		2 (0.1)	
Smoking history					
Yes	87 (76.3)	1393 (46.5)	<0.0001	1175 (85.8)	0.0061
No	27 (23.7)	1600 (53.5)		194 (14.2)	
Tumor size (mm)	35.0 ± 18.5	28.8 ± 16.6	<0.0001	37.9 ± 20.5	NS
T factor (%)					
T0	0 (0.0)	2 (0.1)	0.0002	0 (0.0)	
T1a	14 (12.3)	744 (25.0)		154 (11.3)	
T1b	14 (12.3)	627 (21.0)		210 (15.3)	
T2a	59 (51.7)	1166 (38.8)		573 (41.8)	NS
T2b	5 (4.4)	96 (3.2)		120 (8.8)	
T3	21 (18.4)	282 (9.4)		263 (19.2)	
T4	1 (0.9)	76 (2.5)		49 (3.6)	
N factor (%)					
N0	71 (62.2)	2253 (75.2)	0.0185	920 (67.2)	
N1	15 (13.2)	259 (8.7)		230 (16.8)	NS
N2	27 (23.7)	457 (15.3)		204 (14.9)	
N3	1 (0.9)	24 (0.8)		15 (1.1)	
Pathologic stage (%)					
0	0 (0.0)	2 (0.1)	0.0007	0 (0.0)	
IA	23 (20.2)	1165 (38.8)		299 (21.9)	
IB	30 (26.3)	809 (26.9)		375 (27.4)	
IIA	16 (14.0)	233 (7.8)		206 (15.1)	NS
IIB	12 (10.5)	177 (5.9)		173 (12.6)	
IIIA	28 (24.6)	466 (15.6)		263 (19.2)	
IIIB	1 (0.9)	38 (1.3)		21 (1.5)	
IV	4 (3.5)	103 (3.4)		32 (2.3)	
Op. method (%)					
Pneumonectomy	9 (7.9)	79 (2.6)	0.002	137 (10.0)	
Lobectomy	98 (86.0)	2613 (87.3)		1110 (81.1)	NS
Segmental/partial	7 (6.1)	301 (10.1)		122 (8.9)	
Resection (%)					
Complete	105 (92.1)	2818 (94.1)	NS	1260 (92.0)	NS
Incomplete	9 (7.9)	175 (5.9)		109 (8.0)	
Neo/adjuvant therapy					
Yes	35 (30.7)	691 (23.1)	NS	375 (37.4)	NS
No	79 (69.3)	2302 (76.9)		994 (72.6)	

ASC: adenosquamous carcinoma; AC: adenocarcinoma; SC: squamous cell carcinoma; segmental/partial: segmental or partial resection; lob: lobectomy; pneumo: pneumonectomy; Neo/adjuvant: neoadjuvant or adjuvant therapy.

Kaplan–Meier method for each carcinoma type and statistical significance was evaluated using a log-rank test for comparisons of overall differences in survival distributions. Cox-proportional hazards analysis was used to identify independent predictors of survival and prognosis. Univariate predictors were considered to be significant with a value of $p < 0.05$ and entered into a step-wise multivariable model. Statistical significance was assumed for a two-tailed p value less than 0.05.

RESULTS

We identified 114 patients with ASC (2.4%), 2993 with AC (64.2%), 1369 with SC (29.3%), and 192 (4.1%) with large cell carcinoma. We compared the patients with ASC to those with AC and SC. The clinical characteristics based on the three types are shown in Table 1. The mean age of patients with AC was younger than that of those with ASC and SC (65.2, 68.7, and 68.5 years old, respectively, $p < 0.0001$). AC patients were more likely to be women and SC patients were more likely to be men, as compared to ASC patients ($p < 0.0001$). In the distribution of performance status, significantly more patients with ASC were classified as grade 1 (29.8%) compared to AC patients (15.0%, $p < 0.0001$), while there was no difference in performance status between patients with ASC and SC. SC patients had more smoking history than ASC patients and AC patients had less ($p < 0.0001$). Mean tumor size (mm) was larger in ASC patients (35.0 ± 18.5) than AC patients (28.8 ± 16.6, $p < 0.0001$), while there was no difference in tumor size between patients with ASC and SC. Significantly, more patients with ASC were presented with T2a or T3 invasion than those with AC (51.7% vs 38.8% and 18.4% vs 9.4%, respectively; $p = 0.0002$), while there was no difference in the distribution of T factor between patients with ASC and SC. In ASC patients, the reasons for T2a consistent with tumor size in 41 (69.5%), pleural invasion in 17 (28.8%), and tumor location in one (1.7%), while in AC patients, those consistent with tumor size in 702 (60.2%), pleural invasion in 462 (39.6%), and tumor location in two (0.2%). The reason for T3 in ASC patients consistent with tumor size in nine (42.8%), pulmonary metastasis in the same lobe in one (4.8%), and chest wall invasion in 11 (52.4%), while those in AC patients consistent with tumor size in 61 (21.6%), pulmonary metastasis in the same lobe in 91 (32.3%), chest wall invasion in 126 (44.7%), and tumor location in four (1.4%). Significantly, more patients with ASC were classified as N2 (23.7%) than those with AC (15.3%, $p = 0.0185$), while there was no difference between patients with ASC and SC. Consequently, pathologic stage distribution revealed that significantly more patients with ASC were presented with stage IIIA (24.6%) than those with AC (15.6%, $p = 0.0007$), while there was no difference between patients with ASC and SC. Also, a pneumonectomy was performed significantly more frequently in patients with ASC (7.9%) than those with AC (2.6%, $p = 0.002$), while there was no difference between patients with ASC and SC. There were no differences in regard to the proportion of complete resections among the three types. Also, there were no differences in regard to the ratio of patients who received neoadjuvant therapy or adjuvant therapy among the three types.

Next, we analyzed cumulative overall survival rates according to tumor type based on pathologic stage. Including all stage cases, Kaplan–Meier survival curves indicated that ASC cases had the least favorable survival (Fig. 1), as the 5-year survival rates were 23.3% for ASC, 58.0% for AC, and 40.8% for SC, which showed statistical differences among the three types ($p < 0.0001$ for ASC to AC and SC to AC, $p = 0.0009$ for ASC to SC). We selected stage IA, IB, and IIIA for analyses, because the numbers of patients with ASC in other stages was too small for valid comparisons. Survival curves for *p*-stage IA tumors indicated that ASC cases had the least favorable survival (Fig. 2), as the 5-year survival rates were 42.0% for ASC, 81.8% for AC, and 63.4% for SC, which showed statistical differences between ASC and AC patients ($p = 0.0005$), and between AC and SC ($p < 0.0001$). Furthermore, the survival curves for p-stage IB cases also indicated that ASC had the least favorable survival (Fig. 3), as those 5-year survival rates were 19.3%, 65.3%, and 46.8%, respectively, with a significant difference observed between ASC and AC ($p = 0.0024$), and between AC and SC ($p < 0.0001$). Finally, the survival curves for *p*-stage IIIA tumors also indicated that ASC had the least favorable survival (Fig. 4), with 5-year survival rates of 17.8%, 24.8%, and 18.8%, respectively, and a significant difference between ASC and AC ($p = 0.0154$).

A step-wise multivariable model demonstrated that sex, age, performance status, histology, tumor size, *p*-stage, operative method, and neoadjuvant/adjuvant therapy were independent prognostic factors (Table 2).

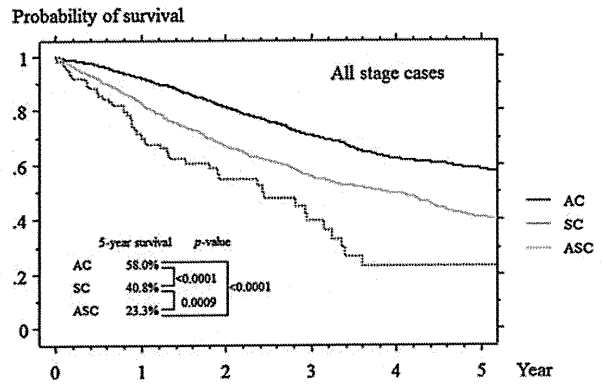


Figure 1: Survival curves following resection for patients with all stage cases of adenocarcinoma (AC), squamous cell carcinoma (SC) and adenosquamous carcinoma (ASC).

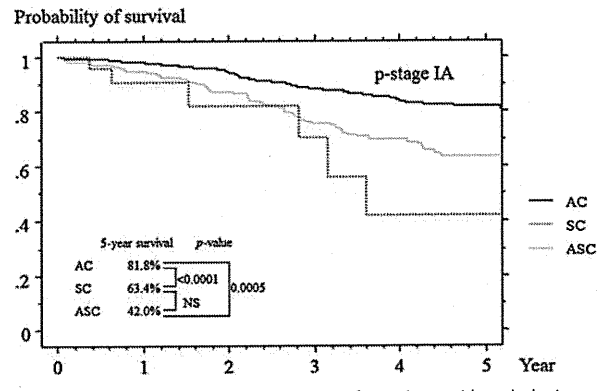


Figure 2: Survival curves following resection for patients with pathologic stage IA adenocarcinoma (AC), squamous cell carcinoma (SC) and adenosquamous carcinoma (ASC).

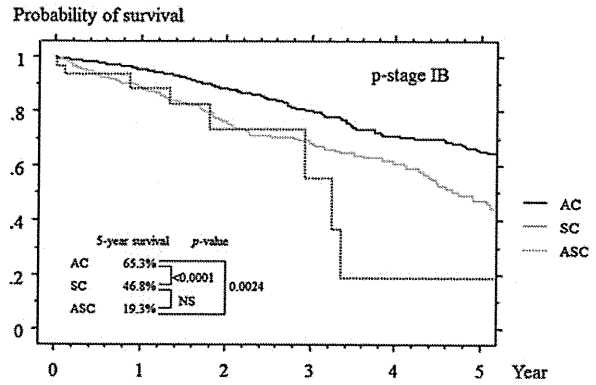


Figure 3: Survival curves following resection for patients with pathologic stage IB adenocarcinoma (AC), squamous cell carcinoma (SC) and adenosquamous carcinoma (ASC).

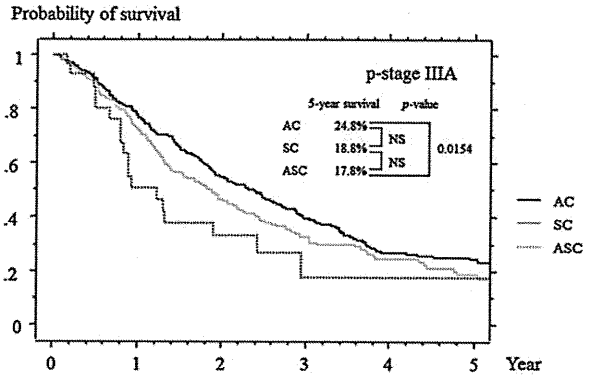


Figure 4: Survival curves following resection for patients with pathologic stage IIIA adenocarcinoma (AC), squamous cell carcinoma (SC) and adenosquamous carcinoma (ASC).

DISCUSSION

ASC of the lung is a relatively uncommon subtype of lung cancer and prior reports have suggested that it represents 0.3–5% of lung cancer cases [1–8]. We found ASC in 2.4% of 4668 patients with non-small-cell lung cancer who underwent surgery, using the rigorous histologic criteria formally presented by the Japan Lung Cancer Society. Although a few studies have reported nonsignificant differences in regard to postoperative survival between ASC and other histologic types [1,13], many more have indicated that ASC has a more aggressive behavior and worse prognosis than other histologic types of non-small-cell lung cancer [2–8]. However, a few large series have been presented in medical literature, thus the characteristics and prognosis of this entity remain poorly defined.

In regard to the more aggressive behavior and worse prognosis of ASC, Takamori and colleagues evaluated 2160 patients who underwent resection for primary lung cancer, and found 56 patients (2.6%) with ASC. The survival curves indicated that the outcome of ASC was worse than that of AC and SC, particularly in stage I and II cases [3]. Shimizu and colleagues [4] examined 1284 patients who underwent resection for primary lung cancer, including 44 cases (3.4%) of ASC. The 5-year survival rate for the ASC cases was 18.5%, which was significantly worse than that for AC (39.2%) and SC (38.7%) cases. Nakagawa and colleagues reviewed outcomes in 30 cases of resection for ASC. The cumulative survival rate for patients with ASC and pathologic stages IA–IIB in their study population was similar to that of patients with stage IIIA AC or SC [6]. Gawrychowski and colleagues [7] examined data for 96 patients with ASC, and found that the cumulative postoperative survival rate for patients with ASC at 5 years was 25.4% and after 10 years was 19.2%, as compared with 42.5% and 39.1%, respectively, for a contemporaneous cohort of patients with AC. In addition, their analysis of survival according to pathologic stage revealed that stage IB patients treated surgically for ASC showed significantly worse outcomes than patients who underwent surgery for AC, as the 5-year survival rate was 31.8% for ASC in comparison with 56.3% for AC. On the other hand, survival rates did not differ significantly between ASC and AC patients classified as stage IA, IIA, or IIB. Cooke and colleagues [8] examined a national database of patients surgically treated in the United States and identified 872 diagnosed with ASC. Their study cohort was limited to patients who underwent a lobectomy for early-stage and node-negative disease, and those with ASC represented 4.1% of the 21361 patients examined. Overall survival was significantly reduced for both ASC and SC cases, as compared with AC. The 5-year survival rate for patients with stage I tumors was 62.0% for ASC, 69.2% for SC, and 73.2% for AC ($p < 0.0001$). Although cases of ASC stage II showed a trend toward worse survival, there were no statistical differences among the three groups.

Table 2: Multivariate analysis of prognostic factors for survival

Variable	HR	CI	*p* Value
Sex (female vs male)	0.593	0.514–0.684	<0.0001
Age (<64 vs >65)	0.629	0.555–0.713	<0.0001
PS (0, 1 vs 2–4)	0.444	0.326–0.606	<0.0001
Histology (AC vs SC vs ASC)			
Adenocarcinoma	0.481	0.361–0.641	<0.0001
Squamous cell carcinoma	0.515	0.386–0.688	<0.0001
Tumor size (≤30 vs ≥31 mm)	0.603	0.531–0.683	<0.0001
p-Stage (0, I vs II vs III vs IV)			
p-Stage 0, IA, IB	0.225	0.175–0.288	<0.0001
p-Stage IIA, IIB	0.464	0.359–0.599	<0.0001
p-Stage IIIA, IIIB	0.893	0.702–1.136	0.3572
Op. method (seg/part vs lob vs peumo)			
Segmental/partial	1.075	0.829–1.393	0.5865
Lobectomy	0.750	0.613–0.917	0.0051
Neo/adjuvant (no vs yes)	0.859	0.756–0.976	0.0195

HR: hazard ratio; CI: confidence interval; PS: performance status; seg/part: segmental or partial resection; lob: lobectomy; pneumo: pneumonectomy; Neo/adjuvant: neoadjuvant or adjuvant therapy.

Similar to these studies, our analysis of survival found that ASC tumors resulted in a worse prognosis than AC and SC (Figs. 1–4). Including all stage cases, survival curves indicated that ASC cases had significantly the least favorable survival compared to other cell types. According to the tumor type based on pathologic stage, ASC cases in stage IA, IB, and IIIA revealed significantly worse survival compared to AC, while in comparison to SC, ASC showed a trend toward worse survival, though it was not significant. We found a tendency for larger differences in survival between ASC and single histology AC cases in earlier stages.

The reason why ASC tumors have more aggressive behavior than AC and SC tumors remains unresolved. As for the histogenesis of ASC, there are many possibilities, including AC with

squamous metaplasia, collision tumor, and bipotential undifferentiated cell origin [3]. Niho and colleagues [14] analyzed the clonality of ASC and reported that squamous cell and AC components showed identical monoclonal patterns, which suggested that both originated from the same cell type. Kanazawa and colleagues [15] also suggested monoclonal transition from SC to AC in ASC. These findings support the hypothesis that ASC originates from a monoclonal expansion of a single mutant progenitor cell clone, which is different from AC and SC. Several studies have reported distinctive characteristics of ASC found in clinicopathologic examinations. Ruffini and colleagues [5] found that among ASC cases, high cell grading, advanced stage, and intratumoral perineural invasion were significantly more evident than in the single histology population. Cakir and colleagues [16] also found that advanced stage, vascular invasion, and parietal pleural involvement was significantly more evident than in the single histology group. Furthermore, Bastide and colleagues performed comparative transcriptome analysis and suggested that ASC tumors are more complex than simple mixes of AC and SC components. They proposed that neuroendocrine differentiation and extracellular signalregulated kinase (ERK) proliferation pathways may be preferentially deregulated in ASC as compared to AC and SC, respectively, which could explain the high clinical aggressiveness of ASC [17].

We could not analyze details of the pathologic characteristics of ASC, such as cell grading, vascular invasion, or ratio of the component of AC and SC. Several authors mentioned that these factors are supposed to affect on survival, so further study is needed.

In conclusion, ASC of the lung is more aggressive than AC and SC. The decreased survival of patients with ASC as compared with the single histology AC and SC suggests the need for a clinical trial of adjuvant chemotherapy, including early-stage patients.

Conflict of interest: none declared.

REFERENCES

[1] Sridhar KS, Bounassi MJ, Raub W, Richman SP. Clinical features of adenosquamous lung carcinoma in 127 patients. Am Rev Respir Dis 1990; 142:19–23.

[2] Naunheim KS, Taylor JR, Skosey C, Hoffman PC, Ferguson MK, Golomb HM, Little AG. Adenosquamous lung carcinoma: clinical characteristics, treatment, and prognosis. Ann Thorac Surg 1987;44: 462–6.

[3] Takamori S, Noguchi M, Morinaga S, Goya T, Tsugane S, Kakegawa T, Shimosato Y. Clinicopathologic characteristics of adenosquamous carcinoma of the lung. Cancer 1991;67:649–54.

[4] Shimizu J, Oda M, Hayashi Y, Nonomura A, Watanabe Y. A clinicopathologic study of resected cases of adenosquamous carcinoma of the lung. Chest 1996;109:989–94.

[5] Ruffini E, Rena O, Oliaro A, Filosso PL, Bongiovanni M, Arslanian A, Papalia E, Maggi G. Lung tumors with mixed histologic pattern. Clinico-pathologic characteristics and prognostic significance. Eur J Cardiothorac Surg 2002;22:701–7.

[6] Nakagawa K, Yasumitsu T, Fukuhara K, Shiono H, Kikui M. Poor prognosis after lung resection for patients with adenosquamous carcinoma of the lung. Ann Thorac Surg 2003;75:1740–4.

[7] Gawrychowski J, Brulinski K, Malinowski E, Papla B. Prognosis and survival after radical resection of primary adenosquamous lung carcinoma. Eur J Cardiothorac Surg 2005;27:686–92.

[8] Cooke DT, Nguyen DV, Yang Y, Chen SL, Yu C, Calhoun RF. Survival comparison of adenosquamous, squamous cell, and adenocarcinoma of the lung after lobectomy. Ann Thorac Surg 2010;90: 943–8.

[9] Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E. In collaboration with L.H. Sobin and pathologists from 14 countries. Histological typing of lung and pleural tumours (International Histological Classification of Tumours), Geneva: World Health Organization; 1999:1–55.

[10] The Japan Lung Cancer Society. General rules for clinical and pathological recording of lung cancer, 5th ed., Tokyo: Kanehara; 1999.

[11] The Japan Lung Cancer Society. General rules for clinical and pathological recording of lung cancer, 6th ed., Tokyo: Kanehara; 2003.

[12] Goldstraw P, executive editor. IASLC staging handbook in thoracic oncology. Editorial Rx Press; 2009.

[13] Hsia JY, Chen CY, Hsu CP, Shai SE, Wang PY. Adenosquamous carcinoma of the lung. Surgical results compared with squamous cell and adenocarcinoma. Scand Cardiovasc J 1999;33:29–32.

[14] Niho S, Yokose T, Kodama T, Nishiwaki Y, Mukai K. Clonal analysis of adenosquamous carcinoma of the lung. Jpn J Cancer Res 1999;90: 1244–7.

[15] Kanazawa H, Ebina M, Ino-oka N, Shimizukawa M, Takahashi T, Fujimura S, Imai T, Nukiwa T. Transition from squamous cell carcinoma to adenocarcinoma in adenosquamous carcinoma of the lung. Am J Pathol 2000; 156:1289–98.

[16] Cakir E, Demirag F, Aydin M, Unsal E. Clinicopathologic features and prognostic significance of lung tumours with mixed histologic patterns. Acta Chir Belg 2009;109:489–93.

[17] Bastide K, Ugolin N, Levalois C, Bernaudin JF, Checillard S. Are adenosquanous lung carcinomas a simple mix of adenocarcinomas and squamous cell carcinomas, or more complex at the molecular level? Lung Cancer 2010;68:1–9.