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Prognostic Significance of Polysialic Acid Expression in Resected Non-Small Cell Lung Cancer[1]

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ABSTRACT

Polysialic acid (PSA) is a carbohydrate attached mainly to the neural cell adhesion molecule. Because PSA is composed of a linear homopolymer of α-2–8-linked sialic acid residues and has a large negative charge, the presence of PSA attenuates the adhesive property of neural cell adhesion molecule and increases cellular motility. In an earlier study, we demonstrated that PSA and *STX*, a polysialyltransferase, were associated with tumor progression in non-small cell lung cancer (NSCLC) (F. Tanaka *et al.*, Cancer Res., *60:* 3072–3080, 2000). Therefore, in the present study, to assess the prognostic significance of PSA in resected NSCLC, a total of 236 patients who underwent complete resection for pathological (p)-stage I-IIIa disease were reviewed retrospectively. PSA was expressed in 44 of 236 (18.6%) patients, and the expression was correlated with p-stage disease. For all p-stage patients, 5-year survival rates for those with PSA-positive and PSA-negative tumors were 52.1% and 71.3%, respectively, demonstrating a significantly worse prognosis for the PSA-positive patients ($P = 0.012$). Analysis for only p-stage I patients also demonstrated a significantly worse prognosis for the PSA-positive patients; 5-year survival rates of the PSA-positive and the PSA-negative patients were 45.1% and 83.5%, respectively, ($P < 0.001$). In addition, there proved to be no difference in the postoperative survival among p-stage I, II, and IIIa patients when PSA expression was positive. Multivariate analysis confirmed that PSA expression was an independent factor to predict poor prognosis in resected NSCLC. These results suggested that PSA could be an important clinical marker and that preoperative induction and/or postoperative adjuvant therapies should be performed for PSA-positive NSCLC, even if the disease is classified as p-stage I.

INTRODUCTION

Primary lung cancer is the leading cause of cancer death in industrialized countries, and NSCLC[3] accounts for approximately 80% of primary lung cancer. However, postoperative prognosis of NSCLC patients remains poor, despite recent advances in cancer therapy. To improve the prognosis, it is important to establish new biological markers to predict prognosis other than tumor-node-metastasis (TNM) classification. Although many possible biological markers including abnormality of p53 have been reported, none of these has been recommended as a practical clinical marker in the diagnosis or therapy of NSCLC (1).

PSA is a carbohydrate composed of a linear homopolymer of α-2–8-linked sialic acid residues and is attached mainly to the NCAM (2, 3). Expression of PSA on NCAM is developmentally regulated, and the majority of NCAM in adult tissues lacks PSA, which is abundant in a variety of embryonic tissues (2–4). In some malignant tumors, including Wilms' tumor (5–7), neuroblastoma (8), natural killer cell derived-lymphoma (9), pancreatic carcinoma with neural invasion (10), and SCLC (11, 12), reexpression of the embryonic form of NCAM has been reported. Because PSA has a large negative charge, the presence of PSA attenuates the adhesive property of NCAM (13), which may allow PSA-positive cancer cells to detach from the primary tumor and form metastatic foci. In fact, Scheidegger *et al.* (12) reported that a PSA-positive subclone established from a SCLC-derived cell line demonstrated higher metastatic potential as compared with a PSA-negative subclone. However, despite the possible importance of PSA as an oncodevelopmental antigen, little has been reported concerning the clinical significance in most malignant tumors including NSCLC. In an earlier study (14), we have revealed that PSA, not NCAM, plays important roles in progression, especially nodal and distant metastases, of NSCLC, and we have suggested that PSA may be attached to molecules other than NCAM. In the present study, to clarify the prognostic significance of PSA in resected NSCLC, the expression of PSA and NCAM in completely resected pathological stage I-IIIa NSCLC was examined retrospectively.

MATERIALS AND METHODS

Clinical Characteristics of Patients. A total of 237 consecutive patients with pathological (p)-stage I-IIIa NSCLC who underwent complete tumor resection and mediastinal lymph node dissection without any preoperative therapy at Kyoto University Hospital between January 1985 and December 1990 were reviewed retrospectively (Table 1). p stage was reevaluated and determined according to the current TNM classification, as revised in 1997 (15). Histological type and tumor cell differentiation were determined according to the WHO criteria, as revised in 1999 (16). In analysis of PSA expression stratified by grade of tumor cell differentiation, well-differentiated Sq and Ad were classified as well-differentiated tumors; moderately differentiated Sq and Ad were classified as moderately differentiated tumors; La and poorly differentiated Sq and Ad were classified as poorly differentiated tumor. The other histological types were excluded from analysis stratified by cell differentiation. One patient was excluded from the study because of operation-related death, and a final total of 236 patients were evaluated. The inpatient and outpatient medical records, chest X-ray films, whole-body computed tomography films, bone and gallium scanning data, and operation records were reviewed without knowledge of the results of IHS for all patients. As described previously (17), cisplatin-based chemotherapy, radiation, and oral administration of 5-fluorouracil-derivative chemotherapeutic agents were prescribed postoperatively for 55, 35, and 58 patients, respectively. Follow-up of the postoperative clinical course was conducted by review of outpatient medical records and by inquiries by telephone or letter. The day of thoracotomy was considered the starting day for counting postoperative survival days.

Tissue Preparation and IHS. All tumor specimens cut from the primary tumor were immediately fixed in 10% (v/v) formalin and then embedded in paraffin. Serial 4-μm sections were prepared from each sample and used for routine H&E staining and IHS. All of the procedures of IHS using TSA-

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[3] The abbreviations used are: NSCLC, non-small cell lung cancer; PSA, polysialic acid; NCAM, neural cell adhesion molecule; SCLC, small cell lung cancer; p, pathological; endo-N, endoneuraminidase; IHS, immunohistochemical staining; mAb, monoclonal antibody; Sq, squamous cell carcinoma; Ad, adenocarcinoma; La, large cell carcinoma; PS, performance status.

Table 1 *Characteristics of patients and expression of PSA in NSCLC*

	No. of patients (%)	PSA expression		
		Positive	Negative	*P*
Total	236 (100)	44 (18.6%)	192 (81.4%)	
Age (mean ± SD, yrs)	62.4 ± 9.7	62.4 ± 9.9	62.4 ± 9.8	0.966
Lower age (<64 yrs)	118 (50.0)	21 (17.8%)	97 (82.2%)	0.867
Higher age (≥64 yrs)	118 (50.0)	23 (19.5%)	95 (80.5%)	
Sex				
Male	170 (72.0)	32 (18.6%)	138 (81.2%)	1.000
Female	66 (28.0)	12 (18.2%)	54 (81.9%)	
PS				
0	206 (87.3)	36 (17.5%)	170 (82.5%)	
1	28 (11.9)	7 (25.0%)	21 (75.0%)	0.328
2	2 (0.8)	1 (50.0%)	1 (50.0%)	
Histological type				
Sq	85 (36.0)	21 (24.7%)	64 (75.3%)	
Ad	130 (55.1)	21 (16.2%)	109 (83.8%)	0.282
La	13 (5.5)	1 (7.7%)	12 (92.3%)	
Others	8 (3.4)	1 (12.5%)	7 (87.5%)	
Tumor cell differentiation[a]				
Well differentiated	80 (35.1)	12 (15.0%)	68 (85.0%)	
Moderately differentiated	90 (39.5)	18 (20.0%)	72 (80.0%)	0.513
Poorly differentiated	58 (25.4)	13 (22.4%)	45 (77.6%)	
p stage				
I	138 (58.5)	19 (13.8%)	119 (86.2%)	
II	44 (18.6)	9 (20.5%)	35 (79.5%)	0.038
IIIa	54 (22.9)	16 (29.6%)	38 (70.4%)	

[a] Other histological types were excluded from the analysis.

Indirect Kit (New England Nuclear Life Science Products, Boston, MA), a sensitive IHS system (18), were performed following the manufacturer's protocol and described in the previous study (14). In brief, dewaxed sections were incubated overnight with anti-PSA mAb 12F8 (rat IgM, 500 μg/ml; PharMingen, San Diego, CA), which specifically recognizes PSA (19), diluted 1:50. To confirm "true" PSA expression, sections with and without pretreatment with endo-N, which specifically recognizes and digests PSA, were used for every IHS (20). For pretreatment with endo-N, sections were incubated with endo-N (50 μg/ml), a kind gift from Dr. Kawase (Nihon Gaishi, Handa, Aichi, Japan), for 60 min at 37°C (21, 22). To detect a nonpolysialylated form of NCAM, anti-NCAM (CD56) mAb 123C3 (mouse IgG1, 1000 μg/ml; Zymed, South San Frncisco, CA) and ERIC-1 (mouse IgG1, 200 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA) were used. Evaluation of NCAM expression was based on the results of IHS using 123C3 because the reactivity of ERIC-1 was rather weak compared with that of 123C3 (14). For every IHS, a section of a SCLC case known to show positive staining for PSA and NCAM was used as a positive control slide. In addition, a section incubated with nonimmunized rat immunoglobulin for PSA or incubated with nonimmunized mouse immunoglobulin for NCAM was used as a negative control slide.

Statistical Methods. Counts were compared by the χ^2 test. Continuous data were compared using the Student's *t* test if the distribution of samples was normal or using the Mann-Whitney *U* test if the sample distribution was asymmetrical. The postoperative survival rate was analyzed by the Kaplan-Meier method, and the differences in survival rates were assessed by the log-rank test. Multivariate analysis of prognostic factors was performed using Cox's regression model. Differences were considered significant when *P* was less than 0.05. All statistical manipulations were performed using the SPSS for Windows software system (SPSS Inc., Chicago, IL).

RESULTS

PSA Expression in NSCLC. PSA expression proved to be positive for 44 of 236 patients (18.6%; Table 1). Subset analysis revealed a significant correlation between PSA expression and p stage ($P = 0.038$); PSA expression was positive in only 19 of 138 (13.5%) p-stage I patients, whereas it was positive in 16 of 54 (29.6%) p-stage IIIa patients. The correlation between PSA expression and p stage was further analyzed in each histological type. PSA was expressed in 11 of 50 (22.0%) stage I, 4 of 17 (23.5%) stage II, and 6 of 18 (33.3%) stage IIIa patients with Sq, respectively, showing no significant difference ($P = 0.628$). In contrast, PSA expression was significantly correlated with p stage in Ad patients ($P = 0.010$); PSA was expressed in 6 of 75 (8.0%) stage I, 5 of 22 (22.7%) stage II, and 10 of 33 (30.3%) stage IIIa patients. No correlation between PSA expression and age, sex, PS, histological type, or grade of tumor differentiation was demonstrated.

PSA Expression and Postoperative Survival. Five-year survival rates for the PSA-positive and PSA-negative patients were 52.1% and 71.3%, respectively, demonstrating a significantly worse prognosis for the PSA-positive patients ($P = 0.012$; Table 2). The postoperative survival was analyzed and stratified by various patient characteristics including histological type and p stage. Five-year survival rates for the PSA-positive and PSA-negative patients with Ad were 52.2% and 69.4%, respectively, demonstrating a significantly worse prognosis for the PSA-positive patients with Ad ($P = 0.021$; Table 2). On the other hand, no significant difference between the PSA-positive and PSA-negative patients with Sq was demonstrated (Table 2). Five-year survival rates of the PSA-positive and PSA-negative patients with p-stage I disease were 45.1% and 83.5%, respectively, demonstrating a significantly worse prognosis for the PSA-positive patients with p-stage I disease ($P < 0.001$; Table 2; Fig. 1). No significant difference between the PSA-positive and PSA-negative patients with p-stage II or IIIa disease was demonstrated (Table 2). In addition, it should be noted that no difference in the postoperative survival was demonstrated among p-stage I, II, and IIIa patients when PSA expression was positive.

PSA and NCAM Expression in NSCLC. NCAM expression was positive in 17 of 236 (7.2%) patients, and PSA expression was also positive in all of the NCAM-positive patients. In the other 27 PSA-positive patients, NCAM expression was not demonstrated. Analysis of the postoperative survival according to NCAM status revealed no significant difference between the NCAM-negative and NCAM-positive patients ($P = 0.295$). To clarify the prognostic significance of PSA and NCAM expression, postoperative survival was compared after all of the patients were divided into the following three groups based on PSA and NCAM status: (*a*) PSA-positive and NCAM-positive [PSA(+)/NCAM(+)] patients; (*b*) PSA-positive and NCAM-negative [PSA(+)/NCAM(−)] patients; and (*c*) PSA-negative and NCAM-negative [PSA(−)/NCAM(−)] patients (Fig. 2). The 5-year

Table 2 *Expression of PSA and postoperative survival in NSCLC*

	Five-year survival rate (%)		*P* for survival
	PSA-positive patients	PSA-negative patients	(PSA-positive *vs.* PSA-negative patients)
All patients	52.1%	71.3%	0.012
Age			
Lower age (<64 yrs)	55.1%	77.9%	0.049
Higher age (≥64 yrs)	49.8%	64.5%	0.141
Sex			
Male	51.8%	69.9%	0.039
Female	53.0%	75.1%	0.157
PS			
0	51.2%	73.1%	0.014
1–2	57.1%	56.7%	0.677
Histological type			
Sq	57.3%	72.3%	0.547
Ad	52.2%	69.4%	0.021
La	0.0%	81.8%	0.176
Tumor cell differentiation[a]			
Well differentiated	40.0%	70.7%	0.010
Moderately differentiated	62.5%	71.1%	0.462
Poorly differentiated	53.9%	71.9%	0.497
p stage			
I	45.1%	83.5%	<0.001
II	62.9%	57.8%	0.695
IIIa	39.7%	35.6%	0.740

[a] Other histological types were excluded from the analysis.

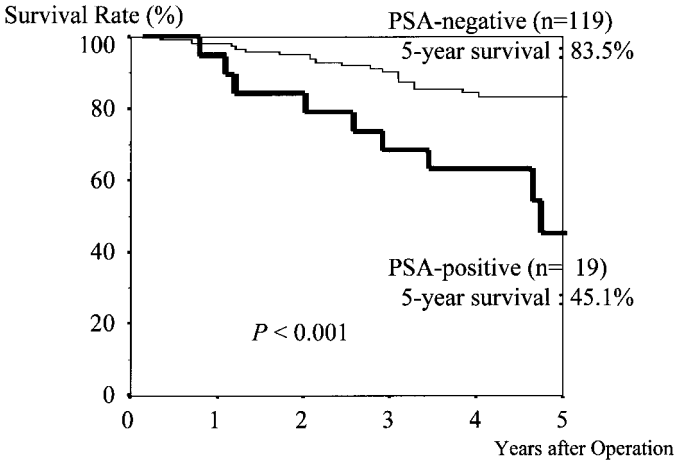

Fig. 1. Postoperative survival of patients with completely resected p-stage I-IIIa NSCLC. Comparison of postoperative survival for patients with PSA-positive tumor and patients with PSA-negative tumor.

survival rate of the PSA(+)/NCAM(−) patients was 50.4%, and these patients had the poorest prognosis among the three patient groups [$P = 0.039$ among the three groups; $P = 0.019$, PSA(+)/NCAM(−) *versus* PSA(−)/NCAM(−)]. No difference was demonstrated between the PSA(+)/NCAM(+) and the PSA(+)/NCAM(−) patients ($P = 0.686$). These results strongly suggested that PSA, not NCAM, was an important factor with which to predict poor postoperative survival.

Multivariate Analysis of Prognostic Factors. Multivariate analysis confirmed that PSA expression was an independent and significant factor to predict poor prognosis. p stage was another significant prognostic factor (Table 3). NCAM expression was not an independent prognostic factor.

DISCUSSION

Although the clinical significance of PSA in most malignant tumors had not been demonstrated, we demonstrated in the previous study that PSA played important roles in the progression of NSCLC (14). In the present article, we have revealed for the first time that PSA expression is significantly correlated with poor postoperative survival and tumor progression in NSCLC. Only a few studies on PSA expression in NSCLC have been reported. Kibbelaar *et al.* (23) reported that PSA was expressed in 3 of 33 NSCLC tumors with IHC using mAb 735, which specifically recognizes PSA. Kwa *et al.* (24) have also documented PSA expression in NSCLC (14 of 96 cases). In contrast to these studies, Lantuejoul *et al.* (25) have reported that PSA expression was specifically demonstrated in neuroendocrine lung tu-

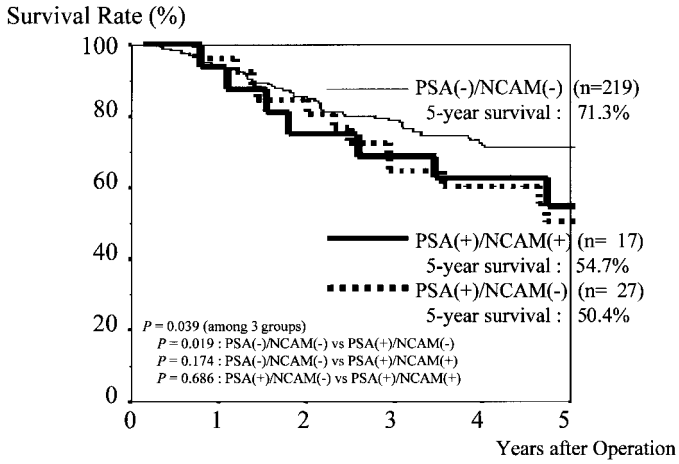

Fig. 2. Postoperative survival of patients with completely resected p-stage I-IIIa NSCLC. Comparison of postoperative survival between patients with PSA(+)/NCAM(+), PSA(+)/NCAM(−), and PSA(−)/NCAM(−) tumors.

Table 3 *Multivariate analysis of prognostic factors in NSCLC*

Prognostic factors	β	*P*	Relative hazard (95% confidence interval)
Age	0.023	0.096	1.023 (0.996–1.051)
Sex (male/female)	−0.534	0.088	0.586 (0.318–1.082)
PS (0/1/2)	0.385	0.187	1.470 (0.830–2.603)
Histological type (Non-Ad/Ad)	−0.054	0.111	0.948 (0.887–1.012)
Tumor cell differentiation (poorly/moderately/well)	0.998	0.834	0.998 (0.978–1.018)
p T factor (1/2/3)	0.400	0.044	1.491 (1.011–2.200)
p N factor (0/1/2)	0.646	< 0.001	1.908 (1.476–2.467)
PSA expression (negative/positive)	0.521	0.035	1.683 (1.036–2.733)

mors such as SCLC and that PSA expression was negative in NSCLC cases. In the present study as well as in a previous study (14), a highly sensitive IHC system was used to detect PSA expression, and the specificity was confirmed by endo-N digestion. As demonstrated in the previous study (14), PSA expressed on tumor cells may not be detected with a usual IHS system when the amount of PSA is not abundant. Because the amount of PSA expression in NSCLC is, even if present, relatively low compared with that in SCLC, PSA expressed in NSCLC may be detected only with a highly sensitive IHC system, as used in our studies. With respect to the correlation between PSA expression and postoperative survival in NSCLC, only one study conducted by Kwa *et al.* (24) has been reported; no significant correlation has been revealed, probably because of the small number of patients studied. It has already been revealed that PSA increases the motility of neural cells by attenuating the cell-cell and/or cell-matrix adhesion because of the large negative charge (2, 25–28). Considering the biological roles of PSA, it can be reasonably expected to be associated with tumor progression and a poor prognosis. In fact, it has been demonstrated experimentally that PSA increases the motility of cancer cells derived from SCLC and allows the cancer cells to detach from the primary tumor, which causes formation of metastatic foci (12). In clinical studies as well, it has been demonstrated that PSA is correlated with metastatic migration and vascular invasion in Wilms' tumor (7) and with nodal and distant metastases in neuroendocrine lung tumors (25).

The present study clearly revealed that PSA, not NCAM, is an important factor to predict poor prognosis. NCAM is a well-known carrier molecule of PSA, and some studies on NCAM expression in NSCLC have been reported. Although Lantuejoul *et al.* (25) revealed no expression of NCAM in NSCLC, others have demonstrated NCAM expression in some NSCLC cases (29–32). Whereas these studies revealed that NCAM expression was correlated with poor survival in NSCLC patients (30–32), PSA expression was not examined in these studies. The poor postoperative survival for NCAM-positive NSCLC patients might be caused by PSA expressed on NCAM, not by NCAM itself, considering the results documented in the present study. According to previous reports, the α-subunit of sodium channels is the only carrier molecule of PSA other than NCAM in mammals (28); the roles of PSA expressed on the α-subunit of sodium channels remain unknown. Although carrier molecules of PSA in NCAM-negative and PSA-positive NSCLC have not been identified, the present study clearly demonstrates that PSA itself was associated with a poor postoperative prognosis as well as tumor progression. We speculate that PSA may attenuate the adhesive property of "unknown" molecules as well as that of NCAM, which may cause the poor prognosis of NSCLC patients, regardless of the presence of NCAM.

In the present study, it was clearly demonstrated that PSA expression was a significant factor to predict poor prognosis in p-stage I NSCLC. However, the 5-year survival rate for PSA-positive patients with p-stage II or IIIa disease seemed to be slightly higher than that for PSA-negative patients, although there was no statistical significance. The main reason why PSA expression was not a significant prognostic factor in p-stage II or IIIa disease was the small number of patients. In addition, heterogeneity of patient characteristics may be another reason. Therefore, prognostic significance of PSA in NSCLC should be examined in a larger number of patients.

It should be noted that PSA-positive NSCLC patients showed a poor prognosis regardless of p stage, suggesting that preoperative induction and/or postoperative adjuvant therapies were needed for PSA-positive NSCLC, even if it was classified as p-stage I disease. Establishment of an effective therapy regimen for PSA-positive NSCLC will be needed in future. In addition, correlation between postoperative prognosis and gene expression of STX, a polysialyl transferase that plays critical roles in progression of NSCLC (14), should be examined. In conclusion, PSA, which is specifically expressed in advanced-stage NSCLC and is correlated with a poor postoperative prognosis, can be an important clinical marker in therapy for NSCLC.

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