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横山, 秀樹

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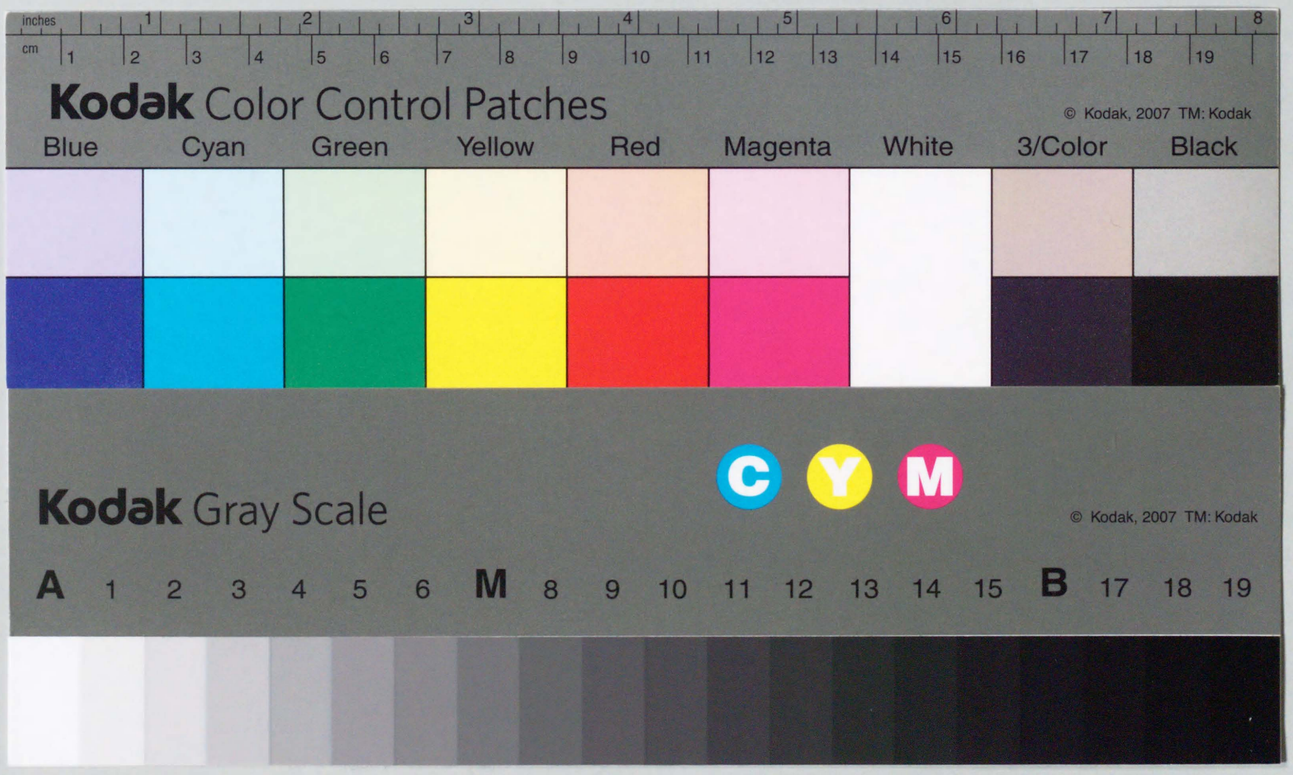
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Immunohistochemical Evidence that P-Glycoprotein in Non-Small Cell Lung Cancers is Associated with Shorter Survival

HIDEKI YOKOYAMA, TERUYOSHI ISHIDA, KENJI SUGIO, TAKASHI INOUE, and KEIZO SUGIMACHI

Department of Surgery II, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Abstract: The expression of P-glycoprotein in 159 non-small cell lung cancers was immunohistochemically examined using a monoclonal antibody (MoAb C219). A total of 93 (60%) cancers were found to be positive for P-glycoprotein. The 5-year survival rates of patients with P-glycoprotein (P-gp+) and those without P-glycoprotein (P-gp−) were 47.6% and 73.6%, respectively ($P < 0.05$). According to a univariate analysis, P-gp+ was associated with a poor prognosis for males, those with stage I cancer, those who underwent complete resection, and those with adenocarcinoma or squamous cell carcinoma. A multivariate study using the Cox regression analysis indicated that the expression of P-glycoprotein is useful for predicting the prognosis. Among 24 patients who underwent complete resection and postoperative adjuvant chemotherapy, 18 were P-gp+ and the remaining 6 were P-gp−. Of the 18 with P-gp+ cancer, 11 relapsed and 9 died from tumor-related causes, while the other 7 remain free from tumor recurrence; however, all with P-gp− cancer are alive without recurrence. These observations suggest a bias toward a shorter survival for patients with P-gp+ cancer because P-glycoprotein may be associated with chemoresistance. Thus, detection of the expression of P-glycoprotein will aid in planning appropriate adjuvant chemotherapy for patients with non-small cell lung cancer.

Key Words: P-glycoprotein, multidrug resistance, lung cancer, immunohistochemistry, postoperative chemotherapy

Introduction

Lung cancer is one of the leading causes of death from disease in Japan and its incidence is increasing rapidly.[1] The length of survival strongly depends on the stage of the disease, such as TNM status and the curability of surgery.[2] However, recurrence, regional and distant, frequently develops in patients who have undergone complete resection.[3,4] Patients with stage I disease have 5-year survival rates between 50% and 70%.[2,5] A more favorable prognosis can often be achieved if postoperative adjuvant chemotherapy is prescribed;[6] however, adjuvant chemotherapy has been found to be of little benefit for patients with non-small cell lung cancer.[7,8]

Tumor cells are often resistant to various drugs that are functionally and/or structurally different. One of the most widely studied types of drug resistance is the phenomenon of an overexpressed multidrug resistance gene (*mdr1*) product, a 170 kDa membrane-spanning protein, usually referred to as P-glycoprotein.[9] A number of efforts have been made to determine the mechanisms associated with *mdr1* or P-glycoprotein, and it has been revealed that P-glycoprotein functions as an energy-dependent efflux pump to decrease intracellular drug accumulation.[10] This property has the potential of influencing the outcome of chemotherapy, because a significant number of anticancer drugs are substrates for transport by this protein.[11,12]

To investigate its clinical significance, we immunohistochemically examined the expression of P-glycoprotein in resected non-small cell lung cancers.

Materials and Methods

Patients and Materials

Materials were obtained from 159 patients who underwent surgery for non-small cell lung cancer in the Department of Surgery II, Faculty of Medicine, Kyushu University, Fukuoka, between 1974 and 1992. There were 111 men and 48 women ranging in age from 42 to 75 years with a mean age of 60 years. Data on patients

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who died within the first postoperative month or who underwent exploratory thoracotomy were excluded from the analysis in the present study. None of the patients had received preoperative chemotherapy and/or radiotherapy, but 24 had been given postoperative adjuvant chemotherapy.

For the assessment of the TNM classification for lung cancer, we followed the guidelines of the International Union Against Cancer. There were 77 patients with stage I, 9 with stage II, 44 with stage IIIA, 18 with stage IIIB, and 10 with stage IV cancer. Histology of the disease was determined according to the WHO classification.[13] There were 92 patients with adenocarcinoma, 57 with squamous cell carcinoma, and 10 with large cell carcinoma. Complete resection of the tumor by lobectomy or pneumonectomy combined with hilar and mediastinal lymphadenectomy was performed whenever feasible, as the standard surgical treatment.

Immunohistochemistry

The primary monoclonal antibody (MoAb C219) was obtained from Centcor Diagnostics (Malvern, PA, USA). MoAb C219 is a murine monoclonal antibody, subclass IgG2a, which binds to a highly conserved cytoplasmic epitope of P-glycoprotein.[14] Indirect staining was done using the labeled avidin-biotin (LAB) method.[15] Immunohistochemical staining was conducted by employing the following process. After 4-μm-thick sections had been cut and fixed in cold acetone for 15 min, they were incubated with primary monoclonal antibody (MoAb C219) at a concentration of 15 μg/ml for 2 h. The sections were then exposed to a biotinylated secondary antibody and avidin with horseradish peroxidase (Nichirei, Tokyo, Japan) for 30 min and 10 min, respectively. After this treatment, visualization of the peroxidase was achieved by the diaminobenzidine method. Each section was stained with methyl green and examined under a transmission light microscope.

Nonimmune mouse serum was employed for the controls. Using MoAb C219, purified P-glycoprotein conjugates, and diluted antiserum (1:50), the specificity of MoAb C219 was tested by an absorption test. When the sections were incubated with purified P-glycoprotein conjugate, then with anti-P-glycoprotein serum, the staining result was negative.

Counting Procedure

For each section, we examined ten high-power fields at random, and in each field we checked the immunoreactivity of 100 tumor cells. The extent of anti-P-glycoprotein immunoreactivity was scored as negative when less than 25% of the tumor cells were stained, and as positive when 25% or more of the tumor cells were stained.

Statistical Analysis

The BMDP statistical package program (BMDP, Los Angeles, CA, USA) for the IBM (Armonk, NY, USA) 4381 mainframe computer was used for statistical analyses.[16] The chi-squared test or Fisher's exact test was used to analyze the statistical significance of differences between the immunohistoreactivity for P-glycoprotein and the factors of sex, TNM stage, curability of the operation, histological type, and the degree of differentiation. Fisher's exact test was used when there were six or fewer items in a group, and survival rates were calculated by the Kaplan-Meier method.[17] Comparison among survival rates was made using the log-rank test.[18] The BMDP P2L program was used for the multivariate adjustment of covariates, such as sex, age, Brinkman's index, TNM status, stage, size of the tumor, histology, degree of differentiation, and curability of the operation, simultaneously, by the Cox regression analysis.[19] In all analyses, a critical level of significance of 0.05 was chosen.

Results

Immunohistochemistry

Immunoreactivities for P-glycoprotein were in the cell membrane and cytoplasm of the malignant cells (Fig. 1A,B), but the normal alveolar epithelium and stroma were not stained. Of the 159 patients examined, there were 96 (60%) with a positive immunoreactivity for P-glycoprotein and 63 (40%) with a negative immunoreactivity, as shown in Table 1. Data assessed included the factors of sex, tumor status (T), node status (N), metastasis status (M), stage, histology, and curability of the operation, according to the extent of anti-P-glycoprotein immunoreactivity. There was no statistical significance in the incidence of anti-P-glycoprotein immunoreactivity in any of the factors, except for the histological type. In large cell carcinomas, the incidence of positive immunoreactivity was less than that in adenocarcinomas or squamous cell carcinomas ($P < 0.05$).

Prognostic Studies

As shown Fig. 2, the 5-year overall survival rates of patients with glycoprotein (P-gp+) and those without glycoprotein (P-gp−) were 48% and 74%, respectively ($P < 0.05$). The 5-year survival rates, according to the clinicopathological factors, of patients separated by the anti-P-glycoprotein immunoreactivity for the various factors are given in Table 2. When factors such as male sex, adenocarcinoma, squamous cell carcinoma, and complete resection were assessed, statistically significant differences were found in the survival rates of P-gp+ patients and P-gp− patients. We also evaluated the pattern of immunoreactivity for P-glycoprotein, but there were no statistically significant differences in the survival rates of patients with focally positive P-glycoprotein, those with diffusely positive P-glycoprotein, and those without P-glycoprotein (data not shown).

Table 3 summarizes the results of the Cox regression analysis of the dependence of survival upon the expression of P-glycoprotein and other covariates. The expression of P-glycoprotein was seen to have prognostic significance when all covariates were included in the Cox regression analysis.

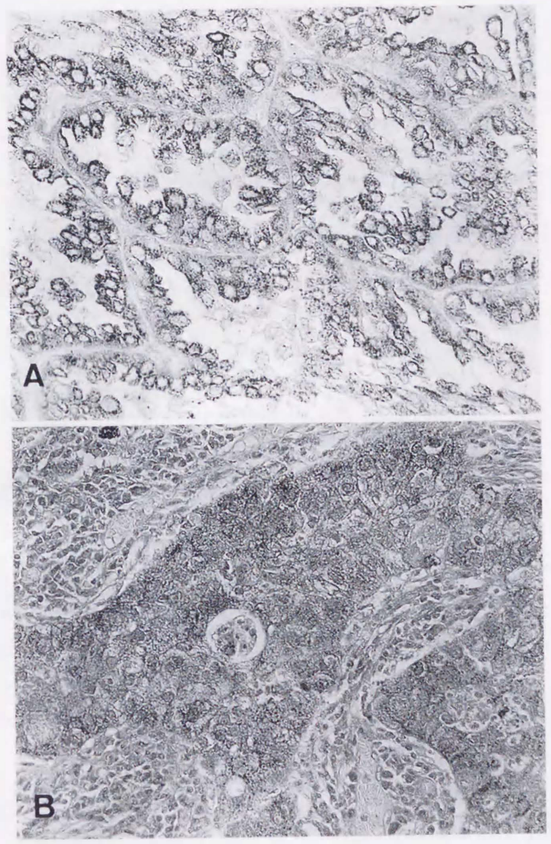

Fig. 1A,B. Immunohistochemistry of adenocarcinoma, showing diffuse cytoplasmic and membrane staining for P-glycoprotein (**A**, ×550), in squamous cell carcinoma (**B**, ×550)

Table 1. Relationships among the immunoreactivities of P-glycoprotein and various clinicopathological factors in patients with non-small cell lung cancer

Variables	No. of patients	P-glycoprotein +	P-glycoprotein −
Sex			
male	111	64	47
female	48	32	16
Tumor			
1	54	30	24
2	64	39	25
3	18	10	8
4	21	15	6
Nodes			
0	98	56	42
1	11	9	2
2	48	29	19
3	1	1	0
Metastasis			
0	148	88	60
1	10	7	3
Stage			
I	77	45	32
II	9	7	2
IIIA	44	24	20
IIIB	18	12	6
IV	10	7	3
Histology			
adenocarcinoma	92	62	30
squamous cell carcinoma	57	31	26 *
large cell carcinoma	10	3	7
Degree of differentiation			
well	67	42	25
moderately	63	37	26
poorly	28	16	12
Curability of operation			
complete	125	77	48
incomplete	34	19	15
Total	159	96	63

+, staining of 25% or more than 25% of tumor cells
−, staining of less than 25% of tumor cells
*$P < 0.05$

The postoperative courses of the 24 patients who underwent complete resection and postoperative adjuvant chemotherapy are shown in Fig. 3. These patients were treated with two or three cycles of PAP chemotherapy, comprised of pepleomycin 5 mg/m², adriamycin 30 mg/m², and cisplatin 50 mg/m², or CVP chemotherapy, comprised of cyclophosphamide 400 mg/m², vindesin 4 mg/m², and cisplatin 50 mg/m². Five of these patients were also treated with 30–45 Gy radiotherapy. Among the 24 patients treated with chemotherapy, 18 were P-gp+ and the others were P-gp−. Of the 18 who were P-gp+, 11 developed a recurrence and 9 died from tumor-related causes. Conversely, none of the patients who were P-gp− developed recurrence and all were alive at the time of writing.

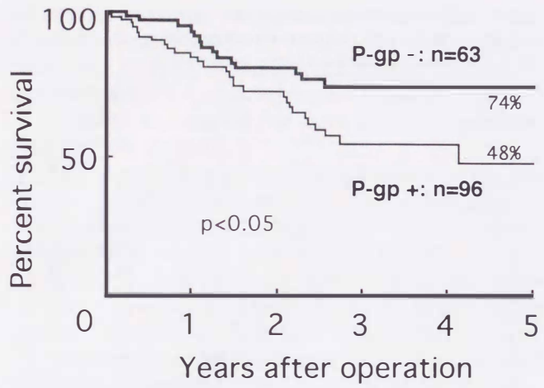

Fig. 2. Survival curves of patients with non-small cell lung cancer, according to immunoreactivity for P-glycoprotein

Discussion

P-Glycoprotein is a 170kDa transmembrane protein associated with the chemoresistance of cancer cells, which is encoded by the multidrug resistance gene (*modr1*).[9] This protein is an energy-dependent efflux pump capable of decreasing the intracellular concentration of a broad range of cytotoxic compounds.[20,23] We obtained immunohistochemical evidence that the expression of P-glycoprotein was associated with shorter survival in patients with non-small cell lung cancer who underwent complete resection. Furthermore, of a total 24 patients who received postoperative adjuvant chemotherapy, 11 out of 18 patients with P-gp+ cancer had a relapse and 9 died from tumor-related causes. On the other hand, all with P-gp− cancer are alive without recurrence. Although the number of patients in this

Table 2. Survival rates according to various prognostic factors based on the detection of P-glycoprotein

Variables	No. of patients	Percent survival (years) 1	2	3	4	5	*P* Value
Sex							
male							
P-gp+	64	81	65	48	48	—	0.0478*
P-gp−	47	90	75	69	—	—	
female							
P-gp+	32	92	87	66	52	52	0.1394
P-gp−	16	100	93	85	85	85	
Tumor							
1							
P-gp+	30	100	95	81	81	81	0.2600
P-gp−	24	100	95	95	95	95	
2							
P-gp+	39	80	70	54	54	36	0.1308
P-gp−	25	96	85	73	73	73	
3							
P-gp+	10	90	51	13	—	—	0.3720
P-gp−	8	88	50	50	50	—	
4							
P-gp+	15	71	61	51	—	—	0.7641
P-gp−	6	67	50	33	—	—	
Nodes							
0							
P-gp+	56	94	77	67	67	67	0.0737
P-gp−	42	94	89	85	85	85	
1							
P-gp+	9	100	100	67	33	33	0.3642
P-gp−	2	100	100	100	100	100	
2							
P-gp+	29	67	62	34	34	—	0.4095
P-gp−	19	89	62	48	—	—	
Metastasis							
0							
P-gp+	88	88	75	61	61	53	0.1141
P-gp−	60	93	81	74	74	74	
1							
P-gp+	6	71	71	0	0	0	0.3034
P-gp−	3	100	67	67	—	—	

Table 2. *Continued*

Variables	No. of patients	Percent survival (years) 1	2	3	4	5	*P* Value
Stage							
I							
P-gp+	45	95	88	78	78	78	0.0138*
P-gp−	32	100	100	100	100	100	
II							
P-gp+	7	100	100	100	0	0	0.3137
P-gp−	2	100	100	100	100	100	
IIIA							
P-gp+	24	81	57	28	—	—	0.3314
P-gp−	20	90	63	51	51	—	
IIIB							
P-gp+	12	72	60	60	60	60	0.5714
P-gp−	6	67	50	33	—	—	
IV							
P-gp+	7	71	71	0	0	0	0.3034
P-gp−	3	100	67	—	—	—	
Histology							
adenocarcinoma							
P-gp+	62	85	77	59	59	51	0.0469*
P-gp−	30	96	84	79	79	79	
squamous cell carcinoma							
P-gp+	31	88	64	38	38	38	0.0474*
P-gp−	26	96	82	73	73	73	
large cell carcinoma							
P-gp+	3	50	50	50	50	50	0.9287
P-gp−	7	71	57	57	57	—	
Degree of differentiation							
well							
P-gp+	42	95	85	66	66	57	0.0944
P-gp−	25	95	91	86	86	86	
moderately							
P-gp+	37	83	68	41	41	—	0.0713
P-gp−	26	96	78	71	71	71	
poorly							
P-gp+	12	63	47	47	47	47	0.9458
P-gp−	6	81	58	46	46	—	
Curability of operation							
complete resection							
P-gp+	77	89	76	60	50	50	0.0038*
P-gp−	48	100	90	87	87	87	
incomplete resection							
P-gp+	19	69	59	30	30	30	0.6832
P-gp−	15	72	51	34	34	—	
Total							
P-gp+	96	84	73	54	48	48	0.0357*
P-gp−	63	93	80	74	74	74	

P-gp+, patients with P-glycoprotein; P-gp−, patients without P-glycoprotein
* Statistically significant

Table 3. Patient characteristics related to survival according to a multivariate analysis using the Cox regression model

Prognostic factor	Regression coefficient	Chi-squared	*P* Value
Size of tumor	0.0228	27.947	0.000
Curability of operation	0.7635	16.331	0.000
P-Glycoprotein	1.3892	6.659	0.010
Sex	1.6806	11.823	0.001
Nodal status	0.4158	5.490	0.019

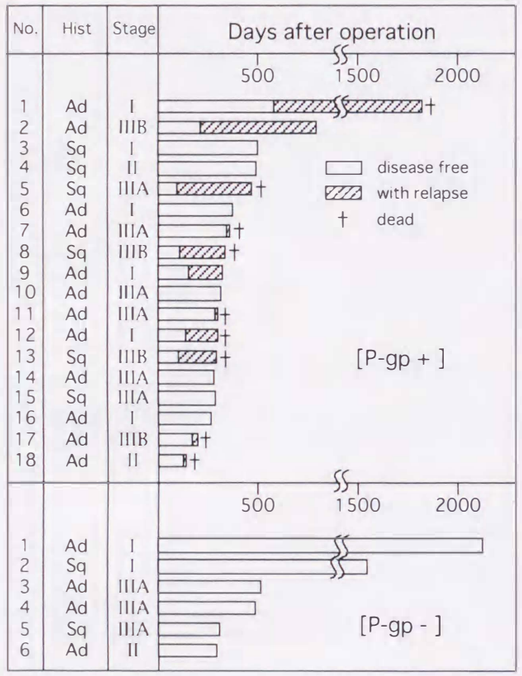

Fig. 3. Clinical courses of patients who underwent complete resection and postoperative adjuvant chemotherapy, according to immunoreactivity for P-glycoprotein

series was limited, these findings suggest that the prescription of postoperative chemotherapy reflects the prognosis of patients with P-gp+ non-small cell lung cancer. Therefore, P-glycoprotein may serve as a chemotherapeutic indicator.

Many researchers have reported that the strongest expression of P-glycoprotein is found in normal tissues of the kidney, adrenal gland, liver, and intestine,[22,23] which indicates that the primary role of P-glycoprotein in normal tissue is to secrete physiological metabolites and ingested chemicals into bile, urine, and the lumen of the intestinal tract. P-Glycoprotein is also expressed in lung, colon, breast, and ovarian cancers, and in leukemia.[24,25]

Sugawara reported that the monoclonal antibody (MRK16) for P-glycoprotein revealed immunohistochemical reactivities in all cases of functional hormone-producing adrenal tumors, 1 case of insulinoma, 2 cases of untreated colonic cancer, 1 case each of gastric cancer and breast cancer, 6 cases of renal cell carcinoma, and 17 cases of bladder cancer.[23] He also reported that the expression was found in one of 10 cases of lung cancer. This incidence of immunoreactivity is much lower than that observed in our present study; however, the different monoclonal antibodies for P-glycoprotein that were used in each study would presumably account for the discrepancy.

Mizoguchi et al. reported that well-differentiated colorectal carcinomas contained significantly higher concentrations of *mdr1* RNA than moderately differentiated colorectal carcinomas.[24] In our study, there was no significant difference in the immunohistochemical expression of P-glycoprotein among well-differentiated, moderately differentiated, and poorly differentiated tumor cells. However, in cases of large cell carcinoma, the incidence of P-gp+ was less than that of P-gp−. Large cell carcinoma has many clinicopathological properties that are quite different from those of adenocarcinoma or squamous cell carcinoma. Thus, the poor sensitivity to chemotherapeutic agents and the poor prognosis associated with large cell carcinoma may be attributable to factors other than P-glycoprotein.

A direct correlation between P-glycoprotein and drug resistance has been studied by many researchers. In studies on breast carcinomas, P-glycoprotein was rarely found in patients not given prior chemotherapy.[26,27] In other malignancies, such as ovarian carcinomas[10,28] and pediatric tumors.[29,30] P-glycoprotein was detected only after the patients had ingested anticancer drugs. On the other hand, none of our patients had been given preoperative chemotherapy, yet they showed a higher positive rate of immunoreactivity for P-glycoprotein. Wishart et al. reported that 26 of 29 patients with treated breast cancer showed a positive immunoreactivity for the monoclonal antibody C219.[31] They stated that significant levels of P-glycoprotein expression may be present in breast cancer before exposure to drugs and is associated with multidrug resistance. Our findings support this point of view.

There are few reports correlating immunohistochemical results with the clinical outcome of patients. Verrelle et al. reported that P-glycoprotein-positive staining in 17 patients with breast cancers treated with chemotherapy was significantly correlated with no initial response to chemotherapy and with a shorter progression-free survival.[25] Chen et al. analyzed the outcome of 67 patients with neuroblastomas and found that the group with negative staining for P-glycoprotein had significantly longer relapse-free and overall survival than the group with positive staining.[32] Although there are many other factors, P-glycoprotein is one of the most promising factors affecting the prognosis of patients with non-small cell lung cancer.

In conclusion, the findings of this study on the P-glycoprotein expression of non-small cell lung cancers have important implications for clinical oncology. Thus, we believe that examining tumors for P-glycoprotein will aid in planning adjuvant therapy for patients with non-small cell lung cancer.

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References

1. Ministry of Health and Welfare (1995) Vital statistics of Japan 1993, vol. 3. Ministry of Health and Welfare, Japan
2. Ishida T, Yokoyama H, Kaneko S, Sugio K, Sugimachi K (1990) Long-term results of operation for non-small cell lung cancer in the elderly. Ann Thorac Surg 50:919–922
3. Ishida T, Yano T, Maeda K, Kaneko K, Tateishi M, Sugimachi K (1990) Strategy for lymphadenectomy in lung cancer; three centimeters or less in diameter. Ann Thorac Surg 50:708–713
4. Tsubota N, Yoshimura M (1996) Skip metastasis and hidden N2 disease in lung cancer: how successful is mediastinal dissection? Surg Today 26:169–172
5. Mountain C (1986) A new international staging system for lung cancer. Chest 89(Suppl.):225s–233s
6. Homes EC, Hill LD, Gail M (1985) A randomized comparison of the effect of adjuvant therapy on resected stage II and stage III non-small cell carcinoma of the lung. The Lung Cancer Study Group. Ann Surg 202:335–341
7. Shields TW, Higgins GA, Humphrey EW, Matthews MJ, Keehn RJ (1982) Prolonged intermittent adjuvant chemotherapy with CCNU and hydroxyurea after resection of carcinoma of the lung. Cancer 50:1713–1721
8. Hara N, Ohta M, Ichinose Y, Motohiro A, Ishida T, Noge S, Miyake J (1985) Surgical adjuvant chemotherapy for lung cancer. In: Ishigami J (ed) Recent advances in chemotherapy: proceedings of the 14th International Congress on Chemotherapy University of Tokyo Press, Tokyo, pp 155–157
9. Gros P, Neriah YB, Croop JM, Housman DE (1986) Isolation and expression of a complementary DNA that confers multidrug resistance. Nature 323:728–731
10. Chen CJ, Chin JE, Veda K, Clark DP, Pastan I, Gottesman MM, Roninson IB (1986) Internal duplication and homology with bacterial transport proteins in the mdrl (P-glycoprotein) gene from multidrug-resistant human cells. Cell 47:381–389
11. Licht T, Pastan I, Gottesman M, Hermann F (1994) P-glycoprotein-mediated multidrug resistance in normal and neoplastic hematopoietic cells. Ann Hematol 69:159–171
12. Ling V (1995) P-glycoprotein; its role in drug resistance. Am J Med 99(Suppl):31S–34S
13. World Health Organization (1982) Histological typing of lung tumors (WHO), edn 2. Am J Clin Pathol 77:123–136
14. Grogan T, Dalton W, Rybski J, Spier C, Meltzer P, Richter L, Gleason M, Pindur J, Cline A, Scheper R, Tsuruo T, Salmon S (1990) Optimization of immunocytochemical P-glycoprotein assessment in multidrug-resistant plasma cell myeloma using three antibodies. Lab Invest 63:815–824
15. Giorno R (1984) A comparison of two immunoperoxidase staining methods based on the avidin-biotin interaction. Diagn Immunol 2:161–166
16. Dixon WJ (ed) (1988) BMDP statistical software. University of California Press, Berkeley, B34, pp 187–220
17. Kaplan EL, Meier P (1965) Non-parametric estimation from incomplete observation. J Am Stat Assoc 53:203–224
18. Petp R, Pike MC, Armitage P, Brestlow NE, Cox DR, Howard SV, Mantel N, McPherson K, Peto J, Smith PG (1977) Design and analysis of randomized clinical trials requiring prolonged observation of each patient: II. Analysis and examples. Br J Cancer 35:1–39
19. Cox DR (1972) Regression models and life tables. J R Stat Soc B 34:187–220
20. Gottesman MM, Pastan I, Ambudkar SV (1996) P-glycoprotein and multidrug resistance. Curr Opin Genet Dev 6:610–617
21. Bosch I, Croop MJ (1996) P-glycoprotein multidrug resistance and cancer. Biochim Biophys Acta 1288:F37–F54
22. Sugawara I, Kataoka I, Morishita Y, Hamada H, Tsuruo T, Itoyama S, Mori S (1988) Tissue distribution of P-glycoprotein encoded by a multidrug-resistant gene as revealed by a monoclonal antibody, MRK16. Cancer
23. Sugawara I (1990) Expression and functions of P-glycoprotein (mdrl gene product) in normal and malignant tissues. Acta Pathol Jpn 40:545–553
24. Mizoguchi T, Yamada K, Furukawa T, Hidaka K, Hisatsugu T, Shimazu H, Tsuruo T, Sumizawa T, Akiyama S (1990) Expression of the MDR1 gene in human gastric and colorectal carcinomas. J Natl Cancer Inst 82:1679–1683
25. Verrelle P, Meissonnier F, Fonck Y, Feillel V, Dionet C, Kwaitkowski F, Plagne R, Chassagne J (1991) Clinical relevance of immunohistochemical detection of multidrug resistance P-glycoprotein in breast carcinoma. J Natl Cancer Inst 83:111–116
26. Scotto KW, Bielder JL, Melera PW (1986) Amplification and expression of genes associated with multidrug resistance in mammalian cells. Science 232:751–755
27. Bell DR, Gerlach JH, Kartner N, Buick RN, Ling V (1985) Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. J Clin Oncol 3:311–315
28. Rutledge M, Robey-Cafferty S, Silva E, Bruner J (1989) Monoclonal antibody (C219) detection of the multidrug resistant protein (P-glycoprotein) in routinely processed tissues: a study of 36 cases of ovarian carcinoma. Lab Invest 60:80A
29. Chan HS, Haddad G, Thorner PS, Deboer G, Lin YP, Ondrusek N, Yeger H, Ling V (1991) P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. N Engl J Med 325:1608–1614
30. Dalton WS (1993) Drug resistance modulation in the laboratory and the clinic. Semin Oncol 20:64–69
31. Wishart GC, Plumb JA, Going JJ, McNicol AM, McArdle CS, Tsuruo T, Kaye SB (1990) P-glycoprotein expression in primary breast cancer detected by immunocytochemistry with two monoclonal antibodies. Br J Cancer 62:758–761
32. Chen HSL, Haddad G, Thorner PS, DeBoer G, Lin YP, Ondrusek N, Yeger H, Ling V (1991) P-glycoprotein expression as a predictor of the outcome of therapy for neuroblstoma. N Engl J Med 325:1608–1614