Loss of Heterozygosity at 3p in Non-Small Cell Lung Cancer and Its Prognostic Implication[1]

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ABSTRACT

We examined 110 patients with non-small cell lung cancer who underwent consecutive pulmonary resection for loss of heterozygosity (LOH) at the short arm of chromosome 3 (3p). We performed a PCR-based microsatellite polymorphism analysis for detection of LOH. The microsatellite markers used were *D3S966* (3p21.3), *D3S1007* (3p21.3–22), and *D3S1228* (3p14.1–14.3). Of 98 informative cases, 3p LOH was found in 45 (46%). 3p LOH was more prevalent in squamous cell carcinoma (24/35, 69%) than in adenocarcinoma (18/52, 35%; $P = 0.0019$). There was no significant association between 3p LOH and sex, disease stage, or grade of differentiation. However, patients with 3p LOH tended to survive for a shorter period of time ($P = 0.0631$, log rank test). There was no such tendency in squamous cell carcinoma ($P = 0.7513$), but in adenocarcinoma, the difference of survival was significant ($P = 0.0015$). Cox's proportional hazards model also predicted that 3p LOH was an independent poor prognostic marker in adenocarcinoma ($P = 0.0502$) but not in squamous cell carcinoma or in the entire cohort ($P = 0.7866$ and 0.1371, respectively). LOH at 3p may help to identify non-small cell lung cancer patients with a poor prognosis, who thus need an intensive postoperative follow-up protocol or who are suitable for novel investigational therapeutic approaches. It is also suggested that the putative tumor suppressor gene at 3p may have a different role in squamous cell carcinoma and adenocarcinoma of the lung.

INTRODUCTION

Loss of genetic material at a certain chromosomal locus resulting in LOH[3] is frequently seen in various types of human malignancies, and it is considered as a hallmark of a tumor suppressor gene (1, 2). In lung cancer, LOH is frequently present at many chromosomal arms including 1p, 2q, 3p, 5q, 8p, 9p, 11p, 13q, 17p, and 18q (3–5). Among these abnormalities, LOH at multiple loci on the short arm of chromosome 3 (3p) is the most frequent genetic lesion in lung cancer, which was first identified by Whang-Peng *et al.* (6) by means of cytogenetics. Of interest, it is the earliest appearing molecular abnormality described to date in the pathogenesis of lung cancer (7, 8). Subsequent numerous studies using RFLP revealed that (*a*) allelic loss of 3p occurs in a significant fraction of NSCLC and in almost all SCLC (4, 5, 9–15), and (*b*) there are at least three distinct regions located at 3p25, 3p21.3, and 3p14-cen commonly deleted in lung cancer (14). However, corresponding tumor suppressor genes have not been cloned.

Previous studies suggested that the 3p LOH is associated with less differentiated histology or with the advanced stage in adenocarcinoma of the lung (15) and with a trend toward a poor prognosis (16). However, clinical implication or a prognostic impact of the 3p LOH has not been established. In this study, as a part of systematic search for molecular prognostic markers in NSCLC (17–20), we examined 110 patients with NSCLC for 3p LOH using PCR-based microsatellite polymorphism analysis. We correlated the finding with various clinical features including patient survival.

MATERIALS AND METHODS

Patients and DNA. During a 21-month period from July 1991 to April 1993, 143 consecutive patients underwent pulmonary resection for treatment of NSCLC at the Department of Surgery II, University of Occupational and Environmental Health as a routine clinical practice. Of them, 110 patients (77%) were studied. The criterion of the patients for inclusion in this study was solely availability of paired normal lung tissue and tumor materials. There were 61 adenocarcinomas, 38 squamous cell carcinomas, 7 large cell carcinomas, and 4 other types. Thirty-eight patients had stage I disease, 8 had stage II, 38 had stage IIIa, 15 had stage IIIb, and 11 had stage IV disease. For postoperative follow-up, patients were asked to visit our clinic for examinations including chest X-ray and tumor marker determination every month for the first year, every other month for the second year, and then every 3 months for the third year and so on. Computed tomographic scanning and bone scintigram were performed at least once every year after the opera-

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[3] The abbreviations used are: LOH, loss of heterozygosity; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer.

Table 1 Association between 3p LOH and various clinical factors

Factors	Category	No. of informative cases (98)	No. of cases with LOH (45)	% (46)	*P*
Sex	Male	74	36	49	0.3409
	Female	24	9	38	
Smoking status	Smoker	68	36	53	0.0235
	Nonsmoker	26	7	27	
Histology	Squamous cell carcinoma	35	24	69	0.0019[a]
	Adenocarcinoma	52	18	35	
	Large cell carcinoma	7	0	0	
	Other	4	3	75	
Differentiation	Well or moderately	65	30	46	0.4887
	Poorly	20	11	55	
Stage	I–II	40	16	40	0.3289
	III–IV	58	29	50	

[a] *P* value for squamous cell carcinoma *vs.* adenocarcinoma.

tion. Samples obtained at surgery were kept frozen at −80°C, and genomic DNA was extracted using standard techniques.

Microsatellite Polymorphism. Microsatellite markers at the short arm of chromosome 3 were amplified by PCR for tumor and normal lung DNA. Three microsatellite markers used were *D3S966* (3p21.3), *D3S1007* (3p21.3–22), and *D3S1228* (3p14.1–14.3; Ref. 21). PCR was performed in a 10-μl reaction containing 4 pmol of each primer, 200 μM 4 deoxyribonucleotide triphosphates, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 gelatin (w/v)%, 5 μl [α-^{32}P]dCTP (specific activity, 3000 Ci/mmol; Amersham Corp., Arlington Heights, IL), 0.5 μl genomic DNA, and 0.05 units *Taq* DNA polymerase. *D3S966* and *D3S1228* were amplified in the same reaction mixture. Reactions were heated at 94°C for 10 min and then cycled for 35 times; each cycle consisted of 1 min at 94°C, 2 min at the annealing temperature, and 1 min at 72°C. The annealing temperatures were 65°C for *D3S1007* and 60°C for *D3S966* and *D3S1228*. After the PCR, 2 μl of the reaction were mixed with 4 μl loading buffer, heated at 94°C for 5 min, and electrophoresed through a 6% polyacrylamide-7 M urea gel at 60 W for 2 to 3 h at room temperature. After electrophoresis, the gel was dried, and the DNA bands were visualized using a BAS 2000 Bioimage Analyzer (Fuji Photo Film Co. Ltd., Tokyo, Japan). Intensities of each band were semiquantitated using densitometry.

Statistical Analysis. Comparison of proportions was performed using the χ^2 test. The Kaplan-Meier method was used to estimate the probability of survival as a function of time (22), and survival differences were analyzed using the log rank test (23). The median follow-up period was 701 days, and 39 patients died during this observation period. Cox's proportional hazards modeling technique was used to identify which independent factors had a jointly significant influence on overall survival (24).

RESULTS

Microsatellite Polymorphism Assay for Determination of LOH. DNA was amplified across the polymorphic site, and the amplified DNA was subjected to electrophoresis in a polyacrylamide gel. If the patient was heterozygous at the test locus, the two bands were represented (alleles 1 and 2). Because contamination of normal stromal cells was almost inevitable in tumor samples, it was occasionally difficult to diagnose LOH with only a visual inspection. We therefore calculated the ratio between the amounts of DNA in allele 1 and allele 2 quantitated using densitometry for all tumor and normal lung DNA samples according to Ganly *et al.* (25). Even in normal lung DNA samples, the intensities of allele 1 were not always the same as those of allele 2, probably due to a different efficiency of amplification of PCR product of a different size. For normal lung DNA samples, the mean, 5th percentile, and 95th percentile of the allelic ratios were 1.22 (0.97–1.54) for *D3S966*, 1.17 (0.91–1.53) for *D3S1007*, and 1.30 (0.88–2.04) for *D3S1228*. LOH was recorded when the allelic ratio of a tumor sample fell outside this range of the normal lung allelic ratio. This criterion of LOH corresponded well with that obtained by visual inspection in most of the cases.

Incidence of LOH at 3p in Lung Cancer Specimens. Fifty-seven percent, 54%, and 48% of the samples were informative at the *D3S966*, *D3S1007*, and *D3S1228* loci, respectively. These levels of heterozygosity were somewhat lower than originally reported (*i.e.*, 77%, 77%, and 70%, respectively; Ref. 21). This probably may be due to the fact that most of our patients were from a relatively small area of Japan. Incidence of 3p LOH was 47%, 41%, and 40% at *D3S966*, *D3S1007*, and *D3S1228*, respectively. When the relationship of LOH among these three loci was evaluated, there was 84% concordance between *D3S966* and *D3S1007*, 77% concordance between *D3S1007* and *D3S1228*, and 77% concordance between *D3S1228* and *D3S966*. Ninety-eight (89%) of the 110 surgical specimens were informative, and 45 of them showed 3p LOH (46%) at least at one of the three loci.

Relationship between 3p LOH and Various Clinical Features. Table 1 summarizes a relationship between 3p LOH and various clinical factors. Twenty-four (69%) of 35 squamous cell carcinomas lost heterozygosity at 3p, as did 18 (35%) of 52 adenocarcinomas, and this difference was statistically significant ($P = 0.0019$). We also noted that 3p LOH was more frequent in smokers (53%) than in lifetime nonsmokers (27%, $P = 0.0235$). However, 3p LOH was not associated with sex, grade of differentiation, or stages of the disease ($P = 0.3409$, 0.4887, 0.3289, respectively).

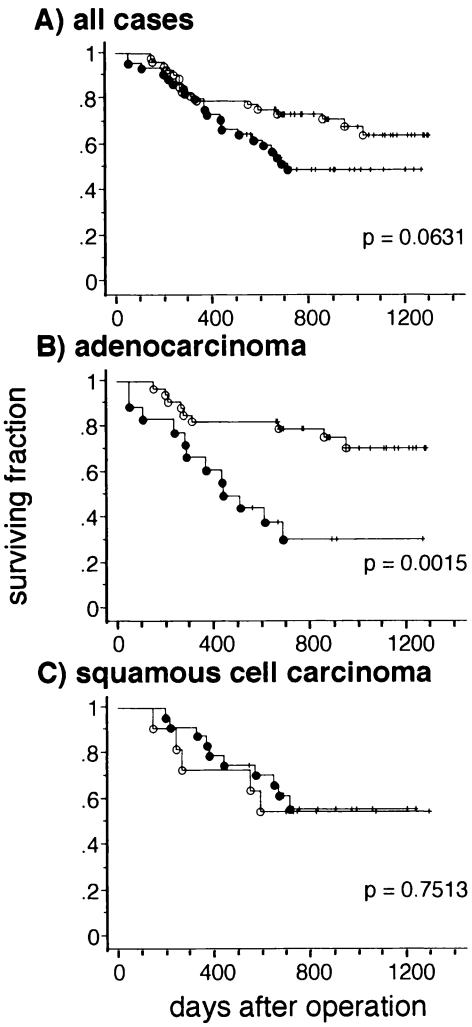

Fig. 1 Kaplan-Meier curve for overall survival with respect to 3p LOH in all patients with NSCLC (*A*), in those with adenocarcinoma (*B*), and in those with squamous cell carcinoma (*C*). ●, patients with 3p LOH; ○, patients without 3p LOH.

Effect of 3p LOH on the Prognosis of the Patients. We next asked whether 3p LOH was a prognostic factor (Fig. 1). When all patients were considered, patients with 3p LOH tended to survive for a shorter period of time ($P = 0.0631$; Fig. 1*A*). No statistically significant association between the presence of 3p LOH and a poor prognosis was observed either in stage I/II or stage III/IV disease subsets ($P = 0.1061$ and 0.4016, respectively).

Since a difference in incidence of 3p LOH between adenocarcinoma and squamous cell carcinoma may suggest a distinct effect of 3p LOH on the prognosis in patients with different histological subtypes, we analyzed the data accordingly. In the adenocarcinoma subset, patients with 3p LOH had a significantly worse prognosis ($P = 0.0015$; Fig. 1B). However, there was no survival impact of 3p LOH in squamous cell carcinoma ($P = 0.7513$; Fig. 1*C*). Multivariate analysis using Cox's proportional hazards model was performed to assess the interrelationship of potential prognostic factors including sex, age, stage, histological subtypes, and 3p LOH in the entire cohort (Table 2). We also performed multivariate analysis for adenocarinoma and squamous cell carcinoma subsets (Table 2). The model predicted that 3p LOH ($P = 0.0502$) was an independent variable that decreased survival in patients with adenocarcinoma, but this is not the case in the entire group or the squamous cell carcinoma subset ($P = 0.1371$ and 0.7866, respectively).

DISCUSSION

We detected 3p LOH in 45 (46%) of 98 informative NSCLC cases. LOH at 3p was more frequent in squamous cell carcinoma (69%) than in adenocarcinoma (35%). This is in agreement with previous studies reporting that 3p LOH is more frequent in squamous cell carcinoma than in adenocarcinoma; *i.e.*, 75% in squamous cell carcinoma *versus* 50% in adenocarcinoma (10), 50% *versus* 38% at 3p21 (26), 61% *versus* 36% (16), 81% *versus* 40% (4), 83% *versus* 61% (5), or 100% *versus* 67% (15). It is not surprising to see that there is a difference in the incidence of a certain genetic change by histological type of lung cancer. For example, *ras* mutations are found predominantly in adenocarcinomas (27), whereas *p53* gene mutations are more prevalent in squamous cell carcinoma than in adenocarcinoma (19, 20, 28). Inactivation of the *rb* and *p53* genes is almost always present in small cell lung cancer (29–32), whereas the *ras* mutation is exceptional in this histological type (33).

We were able to identify 3p LOH as a significant poor prognostic factor in patients with adenocarcinoma, but not in those with squamous cell carcinoma or NSCLC. This differential effect by histological types may be supported by the evidence that correlation between the frequency of 3p LOH and histopathological grade or pathological stage are reported to be seen only in adenocarcinoma (15), although it was not the case in the present study. Our findings are a confirmation and extension of the observation reported by Horio *et al.* (16), who for the first time suggested the possible association between 3p LOH and a shortened overall survival in patients with NSCLC with a borderline significance ($P = 0.084$), although they did not analyze their data by histological type. This situation is analogous to the case of *p53* gene mutation (20, 34) or $p185^{neu}$ overexpression (35), where these genetic abnormalities are a significant poor prognostic marker in adenocarcinoma but not in squamous cell carcinoma. This evidence strongly suggests that these various genetic lesions have different roles in different histological subtypes of NSCLC in which distinct but partly overlapping sets of genetic alterations may be necessary to occur in different timing.

LOH at 3p appears to predict a poor prognostic outcome in patients with NSCLC, especially in those with adenocarcinoma. At the same time, our data offer additional evidence of the concept that squamous cell carcinoma and adenocarcinoma may have a somewhat different pathogenesis and prognosis. In the future, we may be able to apply our expanding knowledge of the molecular genetic lesions in lung cancer to improve the survival and quality of life of patients with this disease.

Table 2 Cox's proportional hazards model for factors associated with the overall survival of patients with nonsquamous cell carcinoma

Variable	Category	*P*	Hazards ratio[a]	95% confidence interval[b]
All histological types				
Sex	Male		1	
	Female	0.5619	0.785	0.347–1.778
Age (yr)	65<		1	
	≤65	0.4147	0.750	0.376–1.496
Stage	III–IV		1	
	I–II	0.0018	0.284	0.129–0.626
Histology	Nonsquamous		1	
	Squamous	0.6307	0.839	0.411–1.715
3p	Retained		1	
	LOH	0.1371	1.69	0.846–3.378
Adenocarcinoma				
Sex	Male		1	
	Female	0.9941	1.004	0.381–2.647
Age	65<		1	
	≤65	0.3213	0.634	0.257–1.562
Stage	III–IV		1	
	I–II	0.0014	0.086	0.019–0.388
3p	Retained		1	
	LOH	0.0502	2.558	0.999–6.536
Squamous cell carcinoma				
Sex	Male		1	
	Female	0.4759	0.467	0.058–3.789
Age	65<		1	
	≤65	0.6958	0.809	0.280–2.341
Stage	III–IV		1	
	I–II	0.4138	0.634	0.212–1.893
3p	Retained		1	
	LOH	0.7866	1.162	0.291–2.545

[a] The model parameters (b_i) were converted to relative risks by computing exp(b_i), where exp(a) = 2.7182[a].

[b] The 95% confidence interval for the relative was computed as [exp(b_{iL}), exp(b_{iH})], where $b_{iL} = b_i - 1.96$ [estimated SE (b_i)] and $b_{iH} = b_i + 1.96$ [estimated SE (b_i)].

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REFERENCES

1. Marshall, C. J. Tumor suppressor genes. Cell, *64:* 313–326, 1991.

2. Karp, J. E., and Broder, S. Molecular foundations of cancer: new targets for intervention. Nat. Med., *1:* 309–320, 1995.

3. Minna, J. D. The molecular biology of lung cancer pathogenesis. Chest, *103:* 445S–456S, 1993.

4. Sato, S., Nakamura, Y., and Tsuchiya, E. Differences of allelotype between squamous cell carcinoma and adenocarcinoma of the lung. Cancer Res., *54:* 5652–5655, 1994.

5. Shiseki, M., Kohno, T., Nishikawa, R., Sameshima, Y., Mizoguchi, H. and Yokota, J. Frequent allelic losses on chromosomes 2q, 18q, and 22q in advanced non-small cell lung carcinoma. Cancer Res., *54:* 5643–5648, 1994.

6. Whang-Peng, J., Kao-Shan, C., Lee, E., Bunn, P., Jr., Carney, D., Gazdar, A., and Minna, J. A specific chromosome defect associated with human small-cell lung cancer: deletion 3p(14–23). Science (Washington DC), *215:* 181–182, 1982.

7. Hung, J., Kishimoto, Y., Sugio, K., Virmani, A., McIntire, D. D., Minna, J. D., and Gazdar, A. F. Allele-specific chromosome 3p deletions occur at an early stage in the pathogenesis of lung carcinoma. J. Am. Med. Assoc., *273:* 558–563, 1995.

8. Kishimoto, Y., Sugio, K., Hung, J. Y., Virmani, A., McIntire, D. D., Minna, J. D., and Gazdar, A. F. Allele-specific loss of chromosome 9p in preneoplastic lesions accompanying non-small cell lung cancers. J. Natl. Cancer Inst., *87:* 1224–1229, 1995.

9. Brauch, H., Johnson, B., Hovis, J., Yano, T., Gazdar, A., Pettingill, O. S., Graziano, S., Sorenson, G. D., Poiesz, B., Minna, J., Linehan, M., and Zbar, B. Molecular analysis of the short arm of chromosome 3 in small-cell and non-small-cell carcinoma of the lung. N. Engl. J. Med., *317:* 1109–1113, 1987.

10. Kok, K., Osinga, J., Carritt, B., Davis, M., vanderHout, A., vanderVeen, A., Landsvater, R., deLeiji, L., Berendsen, H., Postmus, P., Poppema, S., and Buys, C. Deletion of a DNA sequence at the chromosomal region 3p21 in all major types of lung cancer. Nature (Lond.), *330:* 578–581, 1987.

11. Yokota, J., Wada, M., Shimosato, Y., Terada, M., and Sugimura, T. Loss of heterozygosity of chromosome 3, 13, and 17 in small-cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. Proc. Natl. Acad. Sci. USA, *84:* 9252–9256, 1987.

12. Naylor, S. L., Johnson, B. E., Minna, J. D., and Sakaguchi, A. Loss of heterozygosity of chromosome 3p markers in small cell lung cancer. Nature (Lond.), *329:* 451–454, 1987.

13. Rabbits, P., Douglas, J., Daly, M., Sundaresan, V., Fox, B., Haselton, P., Wells, F., Albertson, D., Waters, J., and Bergh, J. Frequency and extent of allelic loss in the short arm of chromosome 3 in non-small cell lung cancer. Genes Chromosomes & Cancer, *1:* 95–105, 1989.

14. Hibi, K., Takahashi, T., Yamakawa, K., Ueda, R., Sekido, Y., Ariyoshi, Y., Suyama, M., Takagi, H., Nakamura, Y., and Takahashi, T. Three distinct regions involved in 3p deletion in human lung cancer. Oncogene, *7:* 445–449, 1992.

15. Yokoyama, S., Yamakawa, K., Tsuchiya, E., Murata, M., Sakiyama, S., and Nakamura, Y. Deletion mapping on the short arm of chromosome 3 in squamous cell carcinoma and adenocarcinoma of the lung. Cancer Res., *52:* 873–877, 1992.

16. Horio, Y., Takahashi, T., Kuroishi, T., Hibi, K., Suyama, M., Niimi, T., Shimokata, K., Yamakawa, K., Nakamura, Y., Ueda, R., and

Takahashi, T. Prognostic significance of *p53* mutations and 3p deletions in primary resected non-small cell lung cancer. Cancer Res., *53:* 1–4, 1993.

17. Kishimoto, Y., Sugio, K., Mitsudomi, T., Oyama, T., Virmani, A. K., McIntire, D. D., and Gazdar, A. F. Frequent loss of the short arm of chromosome 9 in resected non-small cell lung cancers from Japanese patients and its association with squamous cell carcinoma. J. Cancer Res. Clin. Oncol., *121:* 291–296, 1995.

18. Mitsudomi, T., Steinberg, S. M., Oie, H. K., Mulshine, J. L., Phelps, R., Viallet, J., Pass, H., Minna, J. D., and Gazdar, A. F. *ras* gene mutations in non-small cell lung cancers are associated with shortened survival irrespective of treatment intent. Cancer Res., *51:* 4999–5002, 1991.

19. Mitsudomi, T., Oyama, T., Kusano, T., Osaki, T., Nakanishi, R., and Shirakusa, T. *p53* gene mutations as a predictor of poor prognosis in patients with non-small cell lung cancer. J. Natl. Cancer Inst., *85:* 2018–2023, 1993.

20. Mitsudomi, T., Oyama, T., Nishida, K., Ogami, A., Osaki, T., Nakanishi, R., Sugio, K., Yasumoto, K., and Sugimachi, K. p53 nuclear immunostaining and gene mutations in non-small cell lung cancer and their effect on patient survival. Ann. Oncol., *10:* S9–S13, 1995.

21. Jones, M. H., Yamakawa, K., and Nakamura, Y. Isolation and characterization of 19 dinucleotide repeat polymorphisms on chromosome 3p. Hum. Mol. Genet., *1:* 131–133, 1992.

22. Kaplan, E., and Meier, P. Non-parametric estimation from incomplete observation. J. Am. Stat. Assoc., *53:* 457–481, 1958.

23. Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother. Rep., *50:* 163–170, 1966.

24. Cox, D. R. Regression models and life tables. J. R. Stat. Soc. Ser. B, *34:* 187–220, 1972.

25. Ganly, P. S., Jarad, N., Rudd, R. M., and Rabbits, P. H. PCR-based RFLP analysis allows genotyping of the short arm of chromosome 3 in small biopsies from patients with lung cancer. Genomics, *12:* 221–228, 1992.

26. Weston, A., Willey, J., Modali, R., Sugimura, H., McDowell, E., Resau, J., Light, B., Haugen, A., Mann, D., Trump, B., and Harris, C. Differential DNA sequence deletions from chromosome 3, 11, 13 and 17 in squamous-cell carcinoma, large cell carcinoma, and adenocarcinoma of the human lung. Proc. Natl. Acad. Sci. USA, *86:* 5099–5103, 1989.

27. Rodenhuis, S., Slebos, R., Boot, A., Evers, S., Mooi, W., Wagenaar, S., van Bodegom, P., and Bos, J. Incidence and possible clinical significance of K-*ras* oncogene activation in adenocarcinoma of the human lung. Cancer Res., *48:* 5738–5741, 1988.

28. Chiba, I., Takahashi, T., Nau, M. M., D'Amico, D., Curiel, D. T., Mitsudomi, T., Buchhagen, D. L., Carbone, D., Piantadosi, S., Koga, H., Reissman, P. T., Slamon, D. J., Holmes, E. C., Minna, J. D., and Lung Cancer Study Group. Mutations in the *p53* gene are frequent in primary, resected non-small cell lung cancer. Oncogene, *5:* 1603–1610, 1990.

29. Harbour, J. W., Lai, S-L., Whang-Peng, J., Gazdar, A. F., Minna, J. D., and Kaye, F. J. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. Science (Washington DC), *241:* 353–357, 1988.

30. Yokota, J., Akiyama, T., Fung, Y-K., Benedict, W., Namba, Y., Hanaoka, M., Wada, M., Terasaki, T., Shimosato, Y., Sugimura, T., and Terada, M. Altered expression of the retinoblastoma (*RB*) gene in small-cell carcinoma of the lung. Oncogene, *3:* 471–475, 1988.

31. Takahashi, T., Takahashi, T., Suzuki, H., Hida, T., Sekido, Y., Ariyoshi, Y., and Ueda, R. The *p53* gene is very frequently mutated in small-cell lung cancer with a distinct nucleotide substitution pattern. Oncogene, *6:* 1775–1778, 1991.

32. D'Amico, D., Carbone, D., Mitsudomi, T., Nau, M., Fedorko, J., Russell, E., Johnson, B., Buchhagen, D., Bodner, S., Phelps, R., Gazdar, A., and Minna, J. D. High frequency of somatically acquired *p53* mutations in small-cell lung cancer cell lines and tumors. Oncogene, *7:* 339–346, 1992.

33. Mitsudomi, T., Viallet, J., Linnoila, R. I., Mulshine, J. L., Minna, J. D., and Gazdar, A. F. Mutations of ras genes distinguish a subset of non-small cell lung cancer cell lines from small cell lung cancer cell lines. Oncogene, *6:* 1353–1362, 1991.

34. Nishio, M., Koshikawa, T., Kuroishi, T., Suyama, M., Uchida, K., Takagi, Y., Washimi, O., Sugiura, T., Ariyoshi, Y., Takahashi, T., Ueda, R., and Takahashi, T. Prognostic significance of abnormal p53 accumulation in primary, resected non-small-cell lung cancers. J. Clin. Oncol., *14:* 497–502, 1996.

35. Kern, J. A., Schwartz, D. A., Nordberg, J. E., Weiner, D. B., Greene, M. I., Torney, L., and Robinson, R. A. $p185^{neu}$ expression in human lung adenocarcinomas predicts shortened survival. Cancer Res., *50:* 5184–5191, 1990.

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