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Smoking, Gender, and Survival Association with Allele Loss for the *LOH11B* Lung Cancer Region on Chromosome 11[1]

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Abstract

We have reported frequent allele loss for the marker *HRAS* on chromosome 11p in human lung cancer and defined the smallest common region of deletion (designated *LOH11B*) to approximately 500 kb. Here, we investigated the association of allele loss for *LOH11B* with epidemiological, pathological, and clinical parameters. Analysis of allele loss was performed using Southern blotting on a cohort of 200 patients with lung cancer, and data were interpreted with the use of a phosphorimager. Results were statistically compared with retrospectively collected variables. *LOH11B* allele loss was significantly associated with cigarette consumption ($P = 0.009$), gender ($P = 0.02$), and survival ($P = 0.04$). None of the nonsmokers had allele loss as compared with 28% of the patients with low and 43% with high cigarette consumption. Allele loss was more frequent in men (43%) than in women (11%). The median survival of patients without allele loss was 42 months compared with 25 months for patients with allele loss. These results suggest that the *LOH11B* region contains a gene responsible for a more malignant phenotype independent of the metastatic potential of lung cancer. They also suggest that alterations in this gene are associated with cigarette consumption and are more frequent in men than in women.

Introduction

Lung cancer is the third most common cancer in the United States and causes more death annually than breast, colon, and prostate cancer combined (1). Despite progress in the short-term survival, the overall 5-year survival for patients has only marginally improved from 13 to 15% (2). Randomized early detection trials showed that current diagnostic and imaging procedures are inefficacious in the screening setting (3). Major risk factors for lung cancer are active and passive cigarette smoking (4), radon exposure (5), asbestos exposure (6), and dietary factors (7). Recent chemoprevention trials have shown an increased incidence and mortality for lung cancer with β-carotene in male smokers (8–10) and no effect for vitamin E (8).

We and others have reported frequent LOH[3] for the *LOH11B* region on the telomeric portion of chromosome segment 11p15.5 in lung cancer (11–13). This region was identified by the polymorphic marker *HRAS*. The smallest common region of deletion is confined by the tetranucleotide repeat marker *TH* centromeric and the dinucleotide repeat marker *D11S1363* telomeric and may harbor a tumor suppressor gene involved in lung cancer pathogenesis. In addition, this region is frequently deleted in breast cancer (12) and appears to be associated with shortened survival for patients with breast cancer (14). The four major histological lung cancer types are squamous cell, small cell, adenocarcinoma, and large cell carcinoma which account for 25%, 20%, 30%, and 10% of the cases, respectively. Important variables predictive of survival are tumor stage, performance status, weight loss, and gender (15–17). Here, we investigated the association of allele loss for *LOH11B* with epidemiological, pathological, and clinical data using the polymorphic marker *HRAS*, chart review, and telephone contacts.

Materials and Methods

Specimen Collection and DNA Extraction. Four hundred fifty-four specimens were collected from 223 patients in four hospitals. One hundred seventeen patients were seen at The Prince Charles Hospital (Brisbane, Australia), 54 patients at the Duke University Medical Center (Durham, NC), 24 patients at the Veterans Administration Medical Center (Durham, NC), and 28 patients at the National Naval Medical Center (Bethesda, MD). Tumor and corresponding normal tissue, obtained at surgery and stored in liquid nitrogen, were collected from patients undergoing surgery for lung cancer in Brisbane and Durham. At the National Naval Medical Center tumor and normal tissues were collected at autopsy from 21 patients. Lung cancer and corresponding B-lymphoblastoid cell lines were established from 10 patients prior to entry into treatment trials. Tissue samples were pulverized in liquid nitrogen. DNA was extracted using a phenol extraction method (18), an affinity resin column (midi column; Qiagen), or a guanidium-isothiocyanate/phenol extraction method (19). Of the 200 patients with primary lung cancer and complete retrospective data, DNA of

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[3] The abbreviations used are: LOH, loss of heterozygosity; ECOG, Eastern Cooperative Oncology Group; ATS, American Thoracic Society.

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adequate quantity and quality for Southern blot analysis with *HRAS* was available from 151.

Data Collection. Patients were followed up every 3–12 months, and epidemiological, pathological, and clinical data were collected retrospectively. Data collected in reference to the sample collection date were age, gender, smoking history, tumor type, tumor stage, performance status using the ECOG scale, weight loss (more or less than 5% in 3 months), last follow-up date, and survival status. Pathological staging of patients with non-small cell carcinomas (because of similar clinical properties lung cancers other than small cell are frequently referred to as non-small cell carcinomas) was done according to the ATS system (15). In this system, patients with stages II and IV have metastatic disease to hilar lymph nodes (stage II) or other sites (stage IV), and patients with stage I have no metastases. Patients with stage III have either metastatic disease to ipsilateral (stage IIIA) and contralateral (stage IIIB) mediastinal lymph nodes or large primary tumors invading surrounding structures without lymph node metastases (stage IIIA). Small cell carcinoma is staged into limited disease, defined as carcinoma confined to one hemithorax, including metastatic disease to regional lymph nodes, and extensive disease, defined as carcinoma metastatic outside of these borders (20). Limited disease thus encompasses the ATS stages I–III and extensive disease is equivalent to stage IV. Of the 223 patients, 7 were inaccessible for review and 16 had cancers of other sites metastatic to the lung. Thus, our primary lung cancer data base consisted of 200 patients.

Southern Blot Analysis. DNA was digested with *MspI*/*HpaII*, resolved in an agarose gel, and transferred to nylon filters (MS-Nytran; Schleicher & Schuell or Hybond N; Amersham). The human genomic *EcoRI* insert of plasmid pbc-N1 (American Type Culture Collection), which detects a *MspI* polymorphism at the *HRAS* locus, was used as a probe. Visualization of bands was done by exposure to X-ray film and phosphorimager screens (Molecular Dynamics). For quantification of signals on phosphorimager screens, the integrated signal volume over local background was determined on a phosphorimager (Molecular Dynamics). The formula used to calculate the relative signal intensity in tumor compared to normal specimens, *i.e.*, the allele locus dosage was

$$\frac{\text{Allele 1 (normal tissue)} \times \text{Allele 1 (tumor specimen)}}{\text{Allele 2 (normal tissue)} \times \text{Allele 2 (tumor specimen)}}$$

For results greater than 1.0, the formula was reversed (product of allele 2 values divided by the product of allele 1 values). Thus, values for signal intensity ratios ranged from 0.0 to 1.0, *i.e.*, total absence of one allele in the tumor specimen to equal presence of both alleles in the tumor and normal specimen. For instance, integrated signal volumes for the normal (*N*) and tumor (*T*) alleles of patient 55 in Fig. 1 were 113,033, 32,991, 162,900, and 66,702, respectively, which resulted in a calculated signal intensity ratio of 0.71. The formula corrects for aneuploidy of tumors and unequal loading (21). Seventy-four patients (49%) were heterozygous for *HRAS*.

Statistical Analysis. Epidemiological, pathological, and clinical correlates of allele loss were evaluated using Fisher's exact test and the Wilcoxon rank sum test. The continuous covariates examined were age, cigarette consumption, and performance status. The categorical variables examined were gender, weight loss, tumor type, tumor stage, and presence or absence of metastatic spread. The survival status for patients with and without allele loss was described with Kaplan-Meier estimation

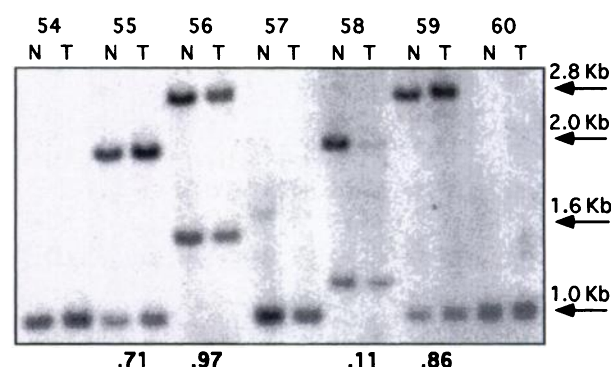

Fig. 1. Phosphorimage of a Southern blot of *MspI/HpaII*-digested genomic DNA from seven consecutive patients (patients 54–60) probed with pbc-N1 for *HRAS*. *N*, DNA from normal tissue; *T*, DNA from tumor tissue. *Numbers below the blot*, allele ratios. The tumor of patient 58 was interpreted as LOH. The tumors of patients 55, 56, and 59 had retained heterozygosity. *Arrows*, allele sizes.

(22), and the groups were compared with the log rank test (23). *HRAS* was evaluated for its association with nine covariates. We used a two-tailed significance level of 0.05 to select associations of greatest interest.

Results

Lung Cancer Population. There were 52 women and 148 men, with a mean age of 61 (range, 26–82) years. Nineteen patients were nonsmokers. In the 170 smokers, cigarette consumption ranged from 1 to 150 pack-years (mean 52, median 43). Sixty-nine patients had squamous cell, 23 patients had small cell, 76 patients had adenocarcinoma, 9 patients had large cell, 17 patients had adenosquamous carcinomas, and 6 patients had carcinoids. Three small cell carcinoma patients had limited disease (one was in stage I) and 20 patients had extensive disease (stage IV). Of the patients with non-small cell carcinomas, 97 had stage I, 37 had stage II, 37 had stage III (8 without lymph node involvement, 24 with ipsilateral mediastinal lymph node involvement, and 5 with stage IIIB), and 6 had stage IV. The ECOG performance status was recorded in 176 patients and was 0 (no symptoms) in 104 patients, 1 (minor symptoms) in 47 patients, 2 (symptoms present but able to care for personal needs) in 22 patients, 3 (needing assistance for personal needs) in 1 patient, and 4 (terminally ill) in 2 patients. Thirty-one patients had lost weight, and 133 had not. Survival time ranged from 0 to 95 months (median, 17) for the 92 deceased patients; follow-up time for the 108 patients still alive was 0.1–99 months (median, 19). Disease stage ($P = 0.0002$), metastatic tumor spread ($P = 0.0007$), gender ($P = 0.03$), weight loss ($P = 0.0009$), and performance status ($P = 0.02$) correlated significantly with survival in univariate analyses using the proportional hazards model. Independent predictors of survival were disease stage and weight loss. These results were consistent with other published retrospective and prospective studies (16, 17) and suggested that our lung cancer data base was representative of a general lung cancer population undergoing treatment in a tertiary referral center.

Assessment of Allele Loss. Surgical tumor specimens contain variable amounts of normal cells which make an accurate assessment of allele loss by visual inspection difficult. To avoid subjectivity in the assessment, we quantified signal intensities with a phosphorimager. The value used as cutoff for *HRAS* LOH was 0.6. We arrived at this value by examining the

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distribution of relative signal intensity values among heterozygous normal specimens. The patient closest to the mean signal intensity value was used as the reference, and every other specimen was compared with this specimen. For ratios greater than 1.0, the reciprocal was used. The 5th, 10th, and 25th percentile of the distribution of relative signal intensity values for *HRAS* was 0.6, 0.6, and 0.7, respectively. We selected the 10th percentile as cutoff point for distinguishing LOH from retained heterozygosity. A representative Southern blot of specimens from seven consecutive patients (patients 54–60) is shown in Fig. 1. In this example, patients 54, 57, and 60 were homozygous and thus uninformative for *HRAS* allele loss, and patients 55, 56, 58, and 59 were heterozygous. Allele signal intensity ratios for these patients were 0.71, 0.97, 0.11, and 0.86. Of these specimens, only patient 58 was interpreted as having allele loss. Using this analysis, of all 74 patients heterozygous for *HRAS*, 26 (35%) had LOH.

Association of Allele Loss with Epidemiological, Pathological, and Clinical Variables. *HRAS* allele loss correlated with cigarette consumption ($P = 0.009$), gender ($P = 0.02$), and survival ($P = 0.04$). Of the six heterozygous patients who had never smoked, none had LOH. Heterozygous patients with equal or less than 43 pack-years cigarette consumption (median in this population) had LOH in 28% (8/29) of the cases and those with more than 43 pack-years had LOH in 43% (15/35) of the cases (Table 1). Men had more frequent LOH (24/56, 43%) than women (2/18, 11%). *HRAS* allele loss was significantly related to survival (Fig. 2) even when controlling for stage ($P = 0.049$), although it was not significant when controlling for weight loss ($P = 0.20$). The median survival for patients without LOH was 42 months compared with 25 months for patients with LOH (Table 2). No other associations for *HRAS* LOH and patients' characteristics were observed (Tables 1 and 2).

Table 1 Association of patient characteristics and *HRAS* allele loss

Variable	*HRAS*		
	LOH (%)	Sample size	*P*
Age (yr)			
≤63	36	13/36	0.65
>63	31	11/36	
Unknown		2/2	
Gender			
Male	43	24/56	0.02
Female	11	2/18	
Smoking [pack-years]			
0	0	0/6	0.009
≤43	28	8/29	
>43	43	15/35	
Unknown		3/4	
Tumor type			
Squamous	29	8/28	0.14
Small cell	58	7/12	
Adenocarcinoma	26	6/23	
Other[a]		5/11	
Stage			
I	29	10/35	0.27
II	50	8/16	
III	18	2/11	
IV	50	6/12	
Metastatic spread			
No	28	10/36	0.33
Yes	41	15/37	
Unknown		1/1	
Performance status (ECOG)			
0	28	11/39	0.57
1–4	39	13/33	
Unknown		2/2	
Weight Loss (over 3 mo)			
≥5%	44	7/16	0.12
<5%	22	10/45	
Unknown		9/13	

[a] LOH was present in three of four adenosquamous carcinomas, one of four large cell carcinomas, and one of three carcinoids.

Discussion

The risk of developing lung cancer is not uniformly distributed among the population. In part, this lack of uniformity may be explained by different environmental exposure histories, particularly to cigarette smoke. However, only a minority of exposed individuals develop lung cancer. Moreover, the risk among individuals with similar exposures is unevenly distributed. Such interindividual susceptibility differences may result from variations in the activity of metabolizing enzymes responsible for the conversion of procarcinogens to carcinogens and a wide spectrum of DNA repair capability (24). Analysis of lung cancer families suggested that, after allowing for an individual's pack-years of tobacco use, the pattern of disease was best explained by Mendelian codominant inheritance of an allele responsible for earlier age of onset (25). These observations suggest that the risk of lung cancer may in part be determined by genetic factors.

Over the past decade, several regions for genes potentially involved in the pathogenesis of lung cancer have been identified. In addition to chromosomes 5q (*APC/MCC* gene cluster), 9p (*MTS*1 gene), 13q (*rb* gene), and 17p (*p53* gene) with already known tumor suppressor genes, other chromosomes including chromosome 11p may harbor genes important in lung cancer development and progression (11–13, 26–30). The objective of this study was to investigate the association of allele loss for the *LOH11B* region on chromosome segment 11p15.5 which encompasses the H-*ras* gene with epidemiological, pathological, and clinical variables to gain knowledge on the potential use of *LOH11B* allele loss for intervention trials and on the potential function of a novel putative tumor suppressor gene located in this region.

HRAS allele loss, present in 35% of the cases, was significantly associated with smoking, gender, and survival. The strong association of cigarette consumption with *HRAS* allele loss suggests that this environmental carcinogen may contribute to the loss of a small part of the telomeric portion of chromosome segment 11p15.5, and that this may result in turn in the loss of function of a tumor suppressor gene. The deleted region is approximately 500 kb (12, 13, 31). One mechanism by which carcinogens in tobacco smoke cause such deletions could be the induction of telomere loss, since telomere loss makes adjacent chromosome portions more susceptible to rearrangements (32). This mechanism can, however, be excluded because allele loss for *LOH11B* is mainly an interstitial deletion and only rarely a terminal deletion (13). A second mechanism could be carcinogen-induced DNA methylation changes (33, 34) which are known to be associated with allele loss (35). Inactivation of a putative tumor suppressor gene in the nondeleted allelic chromosome region could result from coding region mutations through germ-line transmission or somatic events (36), from CpG island hypermethylation (37, 38), or from imprinting (39, 40). The early detection of an inactivating mutation of a tumor suppressor gene in this region would be of extreme importance for interventions directed at lung cancer prevention predomi-

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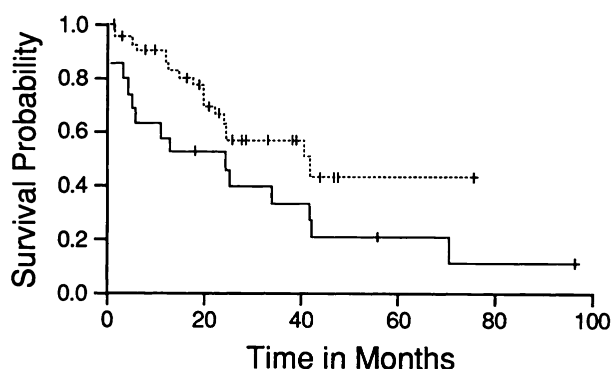

Fig. 2. Survival of patients with (——) and without (- - - - -) allele loss for *HRAS*. The probability of survival for patients within each group as a function of time measured in months was calculated according to the method of Kaplan and Meier. The number of patients in the group with allele loss was 21 and in the group without allele loss 45.

Table 2 Association of patient survival and *HRAS* allele loss

	HRAS	
	LOH	No LOH
Median survival (mo)	25	42
Sample size[a]	21	45
No. of deaths	15	18
P	0.04	

[a] Tissue specimens from eight patients with small cell lung cancer were obtained at autopsy. These patients were excluded from the survival analysis.

nantly in smokers. An explanation for the association of *LOH11B* allele loss with gender could be that different parts of the human genome may be prone to different degrees to structural aberrations in men and women.

LOH11B allele loss was significantly associated with shortened survival but not with tumor stage and weight loss, which are the most important independent clinical predictors of survival. This association with survival suggests that a gene in this region may be responsible for a more malignant phenotype of lung cancer independent of the tumors' metastatic potential. This result may have great importance to clinicians and help guide their decision on the use of adjuvant therapies.

The *ras* family of genes (H-*ras* located on chromosome 11p15.5, K-*ras* located on chromosome 12p, and N-*ras* located on chromosome 1p) has received particular attention by lung cancer researchers. In surgical specimens K-*ras* mutations are particularly frequent in adenocarcinomas (41). Mutations have also been described for large cell carcinomas, squamous cell carcinomas, and carcinoids (in descending order of frequency; Ref. 42). K-*ras* mutations may be associated with a shorter survival when compared with the survival of patients without mutations (43). H-*ras* and N-*ras* are rarely if ever mutated in lung cancer (42). There is, however, experimental evidence for a possible role of the H-*ras* gene in lung cancer pathogenesis. Mutant H-*ras* transgene expression controlled by the SV40 early gene promoter was predominantly found in the lung of transgenic mice. These mice developed benign adenomatous tumors reminiscent of well-differentiated adenocarcinomas (44). Transfection of a small cell carcinoma cell line expressing high levels of c-*myc* with the viral H-*ras* gene resulted in a phenotypic change closely resembling large cell carcinoma (45). It is intriguing to speculate that the putative tumor suppressor gene in the *LOH11B* region may be H-*ras*. A quantitatively and qualitatively normal H-*ras* may partially suppress the function of a mutated K-*ras* gene. Allele loss for H-*ras* may then result in a more pronounced oncogenic potential of a mutated K-*ras* gene and thus a more malignant phenotype of lung cancer associated with shortened survival.

The H-*ras* gene is flanked on its 3′ region by a "minisatellite" (also referred to as variable number of tandem repeats). These minisatellites consist of repeat elements 10–100 bp in length and are predominantly located in the vicinity of genes near the telomere (46). Differences in the number of such repeats result in a variety of alleles. The H-*ras*-associated minisatellite consists of a 28-bp element which is repeated 30–100 times, resulting in 30 alleles 1–3 kb in size (47). These alleles occur in different frequencies in the general population, and four alleles (*A1*, 1.0 kb; *A2*, 1.6 kb; *A3*, 2.3 kb, and *A4*, 2.8 kb) account for 96.1% of all alleles. It has been suggested that rare alleles of the H-*ras*-associated minisatellite are associated, albeit not statistically significant, with an increased susceptibility to lung cancer (47).

Taken together, allele loss in the *LOH11B* region is associated with shortened survival, male gender, and high levels of cigarette consumption. Prospective studies, however, are needed prior to using *LOH11B* allele loss for clinical decision making.

References

1. Parker, S. L., Tong, T., Bolden, S., and Wingo, P. A. Cancer statistics 1996. Calif. Cancer J. Clin., *46:* 5–27, 1996.

2. Ihde, D. C. Chemotherapy of lung cancer. N. Engl. J. Med., *327:* 1434–1441, 1992.

3. Eddy, D. M. Screening for lung cancer. Ann. Intern. Med., *111:* 232–237, 1989.

4. Bartecchi, C. E., MacKenzie, T. D., and Schrier, R. W. The human costs of tobacco use. N. Engl. J. Med., *330:* 907–912, 1994.

5. Samet, J. M., and Nero, A. V. Indoor radon and lung cancer. N. Engl. J. Med., *320:* 591–593, 1989.

6. Mossman, B. T., and Gee, J. B. L. Asbestos-related diseases. N. Engl. J. Med., *320:* 1721–1730, 1989.

7. Omenn, G. S. What accounts for the association of vegetables and fruits with lower incidence of cancers and coronary heart disease? Ann. Epidemiol., *5:* 333–335, 1995.

8. Heinonen, O. P., Huttunen, J. K., Albanes, D., and The Alpha-Tocopherol β-Carotene Cancer Prevention Study Group. The effect of vitamin E and β carotene on the incidence of lung cancer and other cancers in male smokers. N. Engl. J. Med., *330:* 1029–1035, 1994.

9. Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., Keogh, J. P., Meyskens, F. L., Valanis, B., Williams, J. H., Barnhart, S., and Hammar, S. Effects of a combination of β carotene and vitamin A on lung cancer and cardiovascular disease. N. Engl. J. Med., *334:* 1150–1155, 1996.

10. Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R., Belanger, C., LaMotte, F., Gaziano, J. M., Ridker, P. M., Willett, W., and Peto, R. Lack of effect of long-term supplementation with β carotene on the incidence of malignant neoplasms and cardiovascular disease. N. Engl. J. Med., *334:* 1145–1149, 1996.

11. Bepler, G., and Garcia-Blanco, M. A. Three tumor-suppressor regions on chromosome 11p identified by high-resolution deletion mapping in human non-small-cell lung cancer. Proc. Natl. Acad. Sci. USA, *91:* 5513–5517, 1994.

12. Tran, Y. K., and Newsham, I., F. High-density marker analysis of 11p15.5 in non-small cell lung carcinomas reveals allelic deletion of one shared and one distinct region when compared to breast carcinomas. Cancer Res., *56:* 2916–2921, 1996.

13. O'Briant, K. C., and Bepler, G. Delineation of the centromeric and telomeric chromosome segment 11p15.5 lung cancer suppressor regions LOH11A and LOH11B. Genes Chromosomes & Cancer, *18:* 111–114, 1997.

14. Theillet, C., Lidereau, R., Escot, C., Hutzell, P., Brunet, M., Gest, J., Schlom, J., and Callahan, R. Loss of a c-H-*ras*-1 allele and aggressive human primary breast carcinomas. Cancer Res., *46:* 4776–4781, 1986.

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15. Mountain, C. Prognostic implications of the international staging system for lung cancer. Semin. Oncol., *15:* 236–245, 1988.

16. Holmes, E. C., Livingston, R., and Turrisi, A. Neoplasms of the Thorax. *In:* J. F. Holland, E. Frei, R. C. Bast, D. W. Kufe, D. L. Morton, and R. R. Weichselbaum (eds.), Cancer Medicine 2, pp. 1285–1337. Philadelphia: Lea & Febiger, 1993.

17. Ginsberg, R. J., Kris, M. G., and Armstrong, J. G. Non-small cell lung cancer. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Cancer; Principles and Practice of Oncology, Vol. 4, pp. 673–722. Philadelphia: J. B. Lippincott Co., 1993.

18. Blin, N., and Stafford, D. W. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res., *3:* 2303–2308, 1976.

19. Chomczynski, P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques, *15:* 532–536, 1993.

20. Bunn, P., Lichter, A., Makuch, R., Cohen, M., Veach, S., Matthews, M., Anderson, A., Edison, M., Glatstein, E., and Minna, J. Chemotherapy alone or chemotherapy with chest radiation therapy in limited stage small cell lung cancer. A prospective, randomized trial. Ann. Intern. Med., *106:* 655–662, 1987.

21. Center, R., Lukeis, R., Dietzsch, E., Gillespie, M., and Garson, O. M. Molecular deletion of 9p sequences in non-small cell lung cancer and malignant mesothelioma. Genes Chromosomes & Cancer, *7:* 47–53, 1993.

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

23. Peto, R., and Peto, J. Asymptotically efficient rank invariant test procedures (with discussion). J. R. Stat. Soc. Ser. A, *135:* 185–198, 1972.

24. Spitz, M. R., Fueger, J. J., Halabi, S., Schantz, S. P., Sample, D., and Hsu, T. C. Mutagen sensitivity in upper aerodigestive tract cancer: a case-control analysis. Cancer Epidemiol., Biomarkers & Prev., *2:* 329–333, 1993.

25. Sellers, T. A., Bailey-Wilson, J. E., Elston, R. C., Wilson, A. F., Elston, G. Z., Ooi, W. L., and Rothschild, H. Evidence for Mendelian inheritance in the pathogenesis of lung cancer. J. Natl. Cancer Inst., *82:* 1272–1279, 1990.

26. Shiraishi, M., Morinaga, S., Noguchi, M., Shimosato, Y., and Sekiya, T. Loss of genes on the short arm of chromosome 11 in human lung carcinomas. Jpn. J. Cancer Res., *78:* 1302–1308, 1987.

27. Weston, A., Willey, J. C., Modali, R., Sugimura, H., McDowell, E. M., Resau, J., Light, B., Haugen, A., Mann, D. L., Trump, B. F., and Harris, C. C. Differential DNA sequence deletions from chromosomes 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.

28. Skinner, M. A., Vollmer, R., Huper, G., Abbott, P., and Iglehart, J. D. Loss of heterozygosity for genes on 11p and the clinical course of patients with lung carcinoma. Cancer Res., *50:* 2303–2306, 1990.

29. Ludwig, C. U., Raefle, G., Dalquen, P., Stulz, P., Stahel, R., and Obrecht, J. P. Allelic loss on the short arm of chromosome 11 in non-small cell lung cancer. Int. J. Cancer, *49:* 661–665, 1991.

30. Fong, K. M., Zimmerman, P. V., and Smith, P. J. Correlation of loss of heterozygosity at 11p with tumour progression and survival in non-small cell lung cancer. Genes Chromosomes & Cancer, *10:* 183–189, 1994.

31. Higgins, M., Smilinich, N., Sait, S., Koenig, A., Pongratz, J., Gessler, M., Richard, C., James, M., Sanford, J., Kim, B-W., Cattelane, J., Nowak, N., Winterpacht, A., Zabel, B., Munroe, D., Eric, E., Houseman, D., Jones, C., Nakamura, Y., Gerhard, D., and Shows, T. An ordered *Not*I fragment map of human chromosome band 11p15. Genomics, *23:* 211–222, 1994.

32. Kipling, D. Telomerase: immortality enzyme or oncogene? Nat. Genet., *9:* 104–106, 1995.

33. Thomas, J., and Guernsey, D. Altered oncogenes in UV-transformed C3H 10T1/2 mouse cells: identification of mutated H-*ras* allele(s). Int. J. Radiat. Biol., *59:* 15–29, 1991.

34. Vorce, R., and Goodman, J. Hypomethylation of *ras* oncogene in chemically induced and spontaneous B6C3F1 mouse liver tumors. J. Toxicol. Environ. Health, *34:* 367–384, 1991.

35. Vachtenheim, J., Horakova, I., and Novotna, H. Hypomethylation of CCGG sites in the 3′ region of H-*ras* protooncogene is frequent and is associated with H-*ras* allele loss in non-small cell lung cancer. Cancer Res., *54:* 1145–1148, 1994.

36. Liu, B., Nicholaides, N., Markowitz, S., Willson, J., Parsons, R., Jen, J., Papadopolous, N., Peltomaeki, P., de la Chapelle, A., Hamilton, S., Kinzler, K., and Vogelstein, B. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nat. Genet., *9:* 48–55, 1995.

37. Issa, J-P. J., Ottaviano, Y., Celano, P., Hamilton, S., Davidson, N., and Baylin, S. Methylation of the estrogen receptor CpG island links aging and neoplasia in human colon. Nat. Genet., *7:* 536–540, 1994.

38. Baylin, S., Herman, J., Makos-Wales, M., Vertino, P., Wu, J., Kuerbitz, S., and Issa, J-P. Hypermethylation of CpG islands and inactivation of tumor suppressor genes. Proc. Am. Assoc. Cancer. Res., *36:* 691–692, 1995.

39. Rainier, S., Johnson, L. A., Dobry, C. J., Ping, A. J., Grundy, P. E., and Feinberg, A. P. Relaxation of imprinted genes in human cancer. Nature (Lond.), *362:* 747–749, 1993.

40. Ogawa, O., Eccles, M. R., Szeto, J., McNoe, L. A., Yun, K., Maw, M. A., Smith, P. J., and Reeve, A. E. Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. Nature (Lond.), *362:* 749–751, 1993.

41. Rodenhuis, S., van de Wetering, M. L., Mooi, W. J., Evers, S. G., van Zandwijk, N., and Bos, J. L. Mutational activation of the K-*ras* oncogene: a possible pathogenetic factor in adenocarcinoma of the lung. N. Engl. J. Med., *317:* 929–935, 1987.

42. Mitsudomi, T., Viallet, J., Mulshine, J. L., Linnoila, R. I., 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.

43. 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.

44. Suda, Y., Aizawa, S., Hirai, S., Inoue, T., Furuta, Y., Suzuki, M., Hirohashi, S., and Ikawa, Y. Driven by the same Ig enhancer and SV40 T promoter *ras* induced lung adenomatous tumors, *myc* induced pre-B cell lymphomas and SV40 large T gene a variety of tumors in transgenic mice. EMBO J., *6:* 4055–4065, 1987.

45. Mabry, M., Nakagawa, T., Nelkin, B. D., McDowell, E., M., G., Eggleston, J. C., Casero, R. A., and Baylin, S. B. v-Ha-*ras* oncogene insertion: a model for tumor progression of human small cell lung cancer. Proc. Natl. Acad. Sci. USA, *85:* 6523–6527, 1988.

46. Jeffreys, A. J., Wilson, V., and Thein, S. L. Hypervariable "minisatellite" regions in human DNA. Nature (Lond.), *314:* 67–73, 1985.

47. Krontiris, T. G., DiMartino, N. A., Colb, M., and Parkinson, D. R. Unique allelic restriction fragments of the human Ha-*ras* locus in leukocyte and tumour DNAs of cancer patients. Nature (Lond.), *313:* 369–374, 1985.

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