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Deletion Mapping on the Short Arm of Chromosome 3 in Squamous Cell Carcinoma and Adenocarcinoma of the Lung[1]

Shiro Yokoyama, Kazuhiro Yamakawa, Eiju Tsuchiya, Motoi Murata, Shigeru Sakiyama, and Yusuke Nakamura[2]

Departments of Biochemistry [S. Y., K. Y., Y. N.] and Pathology [E. T.], Cancer Institute, Kami-ikebukuro, Toshima-ku, Tokyo 170, Japan; The Second Department of Surgery, Shinshu University, School of Medicine, Asahi, Matsumoto City, Nagano 390, Japan [S. Y.]; and Divisions of Epidemiology [M. M.] and Biochemistry [S. S.], Chiba Cancer Center, Nitona-cho, Chiba City, Chiba 280, Japan

ABSTRACT

We examined loss of heterozygosity in 49 adenocarcinomas and 18 squamous cell carcinomas of the lung with 19 RFLP markers on the short arm of chromosome 3. Although no interstitial deletions were observed in any squamous cell carcinomas, interstitial or partial deletions were detected in 23 adenocarcinomas. Identification of two common regions of deletion in adenocarcinomas, at 3p21.3 and 3p14.1-21.1, suggested the presence of at least two tumor suppressor genes on 3p within the same regions commonly deleted in renal cell carcinomas. Correlation between the frequency of loss of heterozygosity on 3p and histopathological grade of adenocarcinoma also was observed. These results imply an etiological difference between two major types of non-small cell lung cancers, adenocarcinoma and squamous cell carcinoma.

INTRODUCTION

Genetic changes associated with lung cancer have been investigated intensively because mortality among patients is very high and because the incidence of this disease has increased significantly in recent years. Studies have identified many genetic changes in lung cancer, including the activation of protooncogenes, such as H-*ras*, K-*ras*, c-*myc* (1-10), as well as the inactivation of tumor suppressor genes by point mutations and chromosomal losses. Among the tumor suppressor genes that are candidates for lung carcinoma, the p53 and RB genes had already been identified by their mutations in tumors (11-16). However, although one or more tumor suppressor gene(s) are thought to exist on the short arm of chromosome 3 on the basis of frequent loss of heterozygosity in lung tumors (17), no such gene has yet been identified. Because lung tumors are usually categorized as either small cell carcinoma or non-small cell carcinoma, squamous cell carcinoma and adenocarcinoma have been studied together for analysis of LOH[3] in many studies.

To investigate the short arm of chromosome 3 for the location of the putative tumor suppressor gene(s) and to examine the possibility of a different etiology for the two major types of non-small cell carcinomas, we tested 67 lung carcinomas (49 adenocarcinomas and 18 squamous cell carcinomas) for LOH with 19 RFLP markers. The detailed deletion maps constructed in this study point to etiological differences between squamous cell carcinoma and adenocarcinoma and identify two regions on chromosome 3p that are commonly deleted in adenocarcinoma.

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[2] To whom requests for reprints should be addressed, at Department of Biochemistry, Cancer Institute, 1-37-1, Kami-ikebukuro, Toshima-ku, Tokyo 170, Japan.

[3] The abbreviations used are: LOH, loss of heterozygosity; RFLP, restriction fragment length polymorphism; 3p, short arm of chromosome 3.

MATERIALS AND METHODS

Samples. Tumors and corresponding normal tissue (lung or peripheral blood) were obtained from each of 67 patients with primary lung carcinoma. No patient had been treated with chemotherapy or radiation therapy prior to surgery. Tissues from 47 patients were obtained at the Cancer Institute Hospital, Tokyo, Japan, and tissues from 20 other patients were obtained from the Chiba Cancer Center, Chiba, Japan. All tissues were frozen in liquid nitrogen immediately after surgery and stored at −80°C until extraction of DNA. Samples of peripheral blood were also stored at −80°C until use. Clinicopathological data concerning each of these patients are summarized in Table 1.

DNA Extraction and Southern Blotting. Frozen tissues were powdered in liquid nitrogen with a mortar and pestle and transferred into lysis buffer. After proteinase treatment, DNAs were extracted according to the method described by Blin and Stafford (18). Each DNA sample (5 μg) was digested with appropriate restriction enzymes (Boehringer Mannheim, Tokyo, Japan), electrophoresed in an 1.0% agarose gel, and transferred onto a nylon membrane (Biodyne B; Pall, Tokyo, Japan) in transfer buffer (0.1 N NaOH and 0.1 M NaCl).

Probes and Hybridization. Probes used in this study are listed in Table 2. All 19 have been reported previously (19, 20). Membranes were hybridized with probes labeled by the random priming procedure (21). Washing, autoradiography, and stripping were performed under conditions described previously (22).

Definition of Loss of Heterozygosity. The signal intensity of the polymorphic alleles was quantified by a densitometer to ascertain loss of the hybridization signal. After DNA loading differences were corrected, the signal intensity of alleles of tumor tissue was compared to that of normal tissue. When signal reduction was >50%, it was counted as loss of heterozygosity.

RESULTS

Deletion Mapping in Adenocarcinoma. Adenocarcinomas from 49 patients with lung cancer were examined for LOH with 19 RFLP markers on the short arm of chromosome 3. All patients showed constitutional heterozygosity for at least one of these loci. In 33 cases (67.3%), LOH was detected at one or more loci, and 23 of these tumors showed partial or interstitial deletions on chromosome 3p. The Southern blot in Fig. 1 reveals an interstitial deletion in each of four adenocarcinomas where loss of heterozygosity or significant reduction of intensity at one allele was clearly seen at a locus between two loci that retained heterozygosity. The allelic losses at 19 loci in each tumor are summarized in Fig. 2. Two common regions of deletion were identified; one is between cCI3-524 and cCI3-9 (Fig. 2, *bar* labeled A) and the other is between cCI3-721 and cCI3-528 (*bar* labeled B). Probes 524 and 9 have been mapped to 3p21.3, and the estimated genetic distance between them is 5 cM. The other region, 3p14.1-21.1, is estimated to be 12 cM (20).

Deletion Mapping in Squamous Cell Carcinoma. The results of LOH studies in 18 squamous cell carcinomas at the same 19 loci are summarized in Fig. 3. Allelic losses were found at more than one locus in all 18 tumors of this type; this incidence is significantly higher than that observed in adenocarcinomas (*P*

Table 1 *Clinicopathological features of 67 lung cancer patients*

Patient	Age (yr)	Sex	Smoking index[a]	Pathological stage[b] pT	pN	pM	Stage
Adenocarcinoma							
Poorly differentiated							
3	73	M	675–900	2	1	0	II
36	81	F	1000	3	0	0	IIIa
2	57	M	1110–1480	1	2	0	IIIa
122	61	M	Unknown	3	2	0	IIIa
50	70	M	510	2	2	0	IIIa
83	85	M	900	2	0	0	I
28	47	M	520–780	3	2	0	IIIa
Moderately differentiated							
6	48	M	None	1	2	0	IIIa
18	73	F	None	2	2	0	IIIa
25	68	F	None	1	2	0	IIIa
33	37	F	30	1	0	0	I
34	59	M	480	1	0	0	I
53	70	F	None	1	0	0	I
13	46	F	None	2	0	0	I
8	46	M	1080	4	2	0	IIIb
19	57	F	None	2	2	0	IIIa
15	64	M	660	1	0	0	I
17	69	M	846	1	0	0	I
81	67	F	None	2	2	0	IIIa
118	53	F	None	2	0	0	I
24	65	F	None	1	0	0	I
43	52	M	224	1	0	0	I
116	43	F	None	1	2	0	IIIa
37	81	M	1800	1	0	0	I
21	49	F	None	2	0	0	I
46	66	F	None	1	0	0	I
22	72	M	800	2	2	0	IIIa
Well differentiated							
11	72	F	None	2	2	0	IIIa
38	77	F	None	2	2	0	IIIa
93	59	M	820	1	0	0	I
42	52	F	None	1	0	0	I
51	76	M	210–315	1	0	0	I
91	63	M	1080	2	2	0	IIIa
27	49	M	150	1	0	0	I
35	70	M	1200–1600	1	2	0	IIIa
92	62	F	None	1	0	0	I
10	58	M	None	1	0	0	I
39	57	F	None	2	2	0	IIIa
119	74	M	2000	2	0	0	I
115	65	M	720	2	0	0	I
12	73	F	None	1	2	0	IIIa
112	60	F	None	2	0	0	I
14	46	F	None	1	0	0	I
23	58	M	25	1	0	0	I
30	61	F	None	1	0	0	I
48	72	M	750	2	1	0	II
82	42	F	None	1	1	0	II
49	49	F	None	1	0	0	I
94	59	M	290	2	0	0	I
Squamous cell carcinoma							
Poorly differentiated							
40	79	M	1890–2520	1	X	X	X
109	65	M	900	2	0	0	I
120	65	M	1350	2	0	0	I
Moderately differentiated							
4	61	M	Unknown	3	2	0	IIIa
7	71	M	1320	1	0	0	I
20	70	F	1000	3	0	0	IIIa
29	76	M	600	2	0	0	I
41	76	M	1650	2	0	0	I
54	70	M	1000–1500	3	2	0	IIIa
98	68	M	1160	3	0	0	IIIa
100	53	M	825	2	0	0	I
121	68	M	250	2	0	0	I
110	71	M	765	2	2	0	IIIa
44	64	M	None	3	0	0	IIIa
32	63	M	2040–2720	3	2	0	IIIa
Well differentiated							
52	65	M	None	3	2	0	IIIa
56	59	M	1260	2	2	0	IIIa
123	72	M	2000	2	2	0	IIIa

[a] Numbers of cigarettes/day × years.
[b] Determined according to the Japanese Lung Cancer Society (39).

< 0.01; Fisher's exact test). In contrast to the pattern of chromosomal losses in adenocarcinomas, no squamous cell carcinoma revealed an interstitial deletion. Therefore, the commonly deleted region of this group covers almost the entire short arm of chromosome 3.

Relationship between Clinicopathological Factors and LOH on Chromosome 3p in Adenocarcinoma. Statistical analysis was performed to determine an association between clinicopathological features and LOH on chromosome 3p. As shown in Table 3, the incidence of LOH on chromosome 3p was significantly higher in tumors of stage III than those of stage I or II ($P = 0.043$; Fisher's exact test). Moreover, the incidence of LOH on 3p was also correlated with the pathological grade of differentiation among the adenocarcinomas ($P = 0.032$; Fisher's exact test) (Table 4). However, no significant correlation was observed between LOH on 3p and other clinicopathological factors, including age, sex, smoking status, tumor size, node status, or distant metastasis.

DISCUSSION

We have demonstrated the different patterns of allelic losses on chromosome 3p between adenocarcinoma and squamous cell carcinoma of the lung. In adenocarcinomas, interstitial or partial deletions were detected relatively frequently, and two commonly deleted regions were identified; no interstitial chromosomal losses were observed in squamous cell carcinomas. These results indicate the presence of at least two putative tumor suppressor genes on chromosome 3p for adenocarcinoma of the lung. One of them is located in a region between two markers (cCI3–524 and cCI3–9) that are tightly linked to the D3F15S2 locus (23). Kok *et al.* (17) reported LOH at D3F15S2 in all 31 non-small cell carcinomas tested (16 squamous cell carcinomas, 14 adenocarcinomas, and 1 large cell carcinoma). The other putative tumor suppressor lies between cCI3–721 and cCI3–528, at 3p14.1–21.1. This region includes the break

Table 2 *Polymorphic DNA markers used for deletion mapping*

Probe[a]	Locus symbol	Chromosomal location	Enzyme detecting polymorphism	
cCI3-312	D3S651	3p25	*Pvu*II	↑
cCI3-417	D3S669 (erbAβ)[b]	3p25	*Taq*I	Telomere
cCI3-878	D3S1020	3p21.3-22	*Taq*I	
cCI3-830	D3S1002	3p21.3-22	*Taq*I	
cCI3-377	D3S867	3p21.3-22	*Taq*I	
cCI3-515[c]	D3S685	3p21.3-22	*Msp*I	
cCI3-524	D3S686 (D3F15S2)	3p21.3	*Taq*I	
cCI3-9[c]	D3S643	3p21.3	*Pvu*II	
cCI3-769	D3S965	3p21.1-21.3	*Msp*I	
cCI3-382	D3S660	3p21.2-21.3	*Pvu*II	
cCI3-652	D3S717 (D3S2)	3p21.2-21.3	*Pvu*II	
pEFD 145	D3S32	3p21.2-21.3	*Taq*I	
cCI3-721	D3S936	3p21.1	*Msp*I	
cCI3-528	D3S687 (D3S3)	3p14.1-14.2	*Msp*I	
pYNZ86.1	D3S30	3p13	*Pvu*II	↓
cCI3-373	D3S659	3p13	*Pvu*II	
cCI3-570	D3S693	3p13	*Taq*I	Centromere
cCI3-637	D3S714	3p13-14.1	*Pvu*II	
cCI3-315[c]	D3S654	3p12	*Msp*I	

[a] Markers are ordered from 3pter to 3cen.
[b] Symbol in parentheses, markers used in previous LOH studies. The localization of them was determined by linkage analysis.[4]
[c] VNTR marker (40).

[4] S. Yokoyama, K. Yamakawa, E. Tsuchiya, M. Murata, S. Sakiyama, and Y. Nakamura, unpublished data.

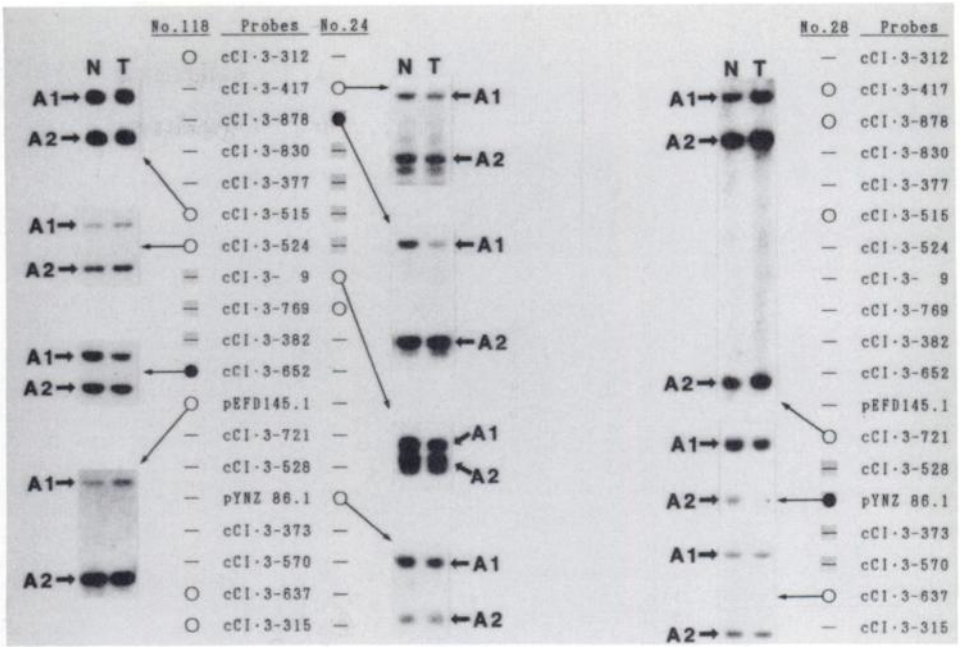

Fig. 1. Southern blots for patients 118, 24 and 28, demonstrating a typical pattern of interstitial deletion. *Left lanes (N)*, from normal tissue; *right lanes (T)*, DNA from cancer tissue. *A1* and *A2*, larger and smaller alleles, respectively. For each of the 19 markers: ●, LOH in the tumor; ○, retention of heterozygosity in the tumor; –, marker was not informative. In each case, LOH is observed at one locus, although the two flanking loci retained heterozygosity.

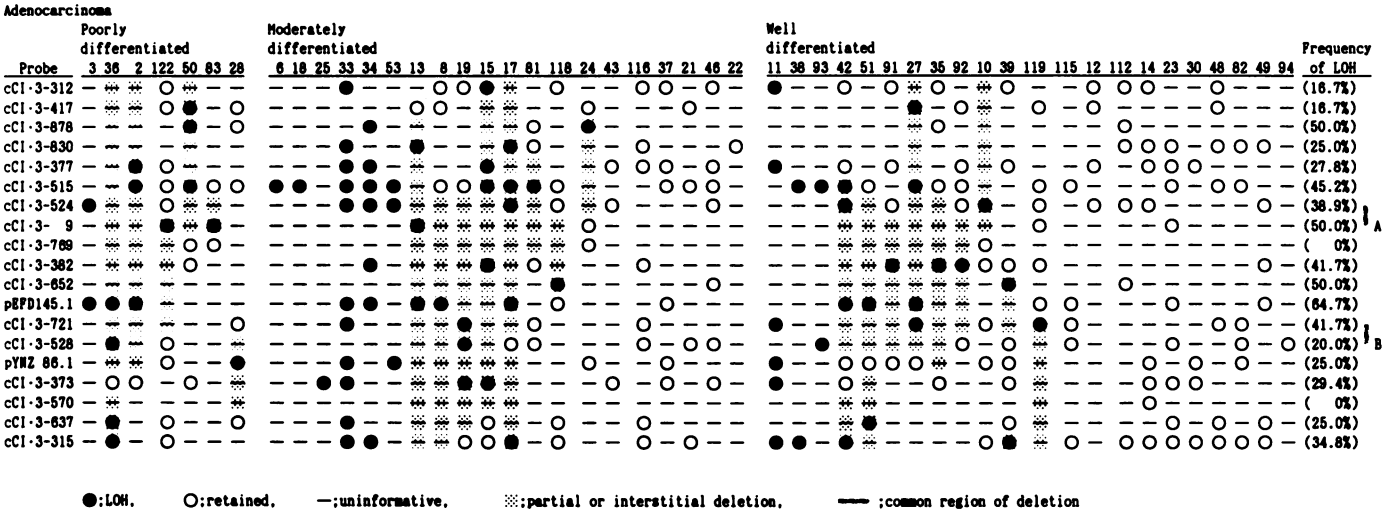

Fig. 2. RFLP analysis at 19 loci on chromosome 3p in 49 adenocarcinomas. *Top of each lane*, tumor number; *shaded region*, maximum estimation of chromosomal loss in each tumor. *Far right lane*, two common regions of deletion (*A* and *B*).

Table 3 *Correlation between LOH on 3p and pathological stage in adenocarcinoma*[a]

Stage	LOH on 3p: Present	LOH on 3p: Absent	Total
I–II	17	13	30
III	16	3	19
Total	33	16	49

[a] $P = 0.043$; Fisher's exact test.

Table 4 *Correlation between LOH on 3p and histopathological grade of differentiation in adenocarcinoma*

Differentiation	LOH on 3p: Present	LOH on 3p: Absent	Total	
Well	12	10	22[a]	[b]
Moderate	14	6	20[a]	[b]
Poor	7	0	7	[b]
Total	33	16	49	

[a] Not significant.
[b] $P = 0.032$; Fisher's exact test (well *versus* poor).

point of a t(3;8) translocation associated with hereditary renal cell carcinoma (23, 24) but is more telomeric to D3S3, where a homozygous deletion has been detected in the U2020 cell line (25). The similarity of these regions to those commonly deleted in renal cell carcinoma (23) suggests the possibility that tumor suppressor genes common to more than one type of cancer exist on the short arm of chromosome 3 (26–28).

Cytogenetic and RFLP analyses have shown 3p deletion in all small cell carcinomas tested (17, 29–34). On the other hand, 3p deletion occurs in a majority of cases of non-small cell carcinoma (30, 31, 34–38). The incidence of LOH in all cases tested in our study was 76% (51 of 67), which is similar to the frequency reported in previous studies. However, we found a significant difference in the frequency of LOH between squamous cell carcinoma and adenocarcinoma. Weston *et al.* (36) also reported more frequent LOH in squamous cell carcinoma than in adenocarcinoma. Furthermore, in adenocarcinoma, we observed more frequent LOH in poorly or undifferentiated types than in well-differentiated types. These results suggest

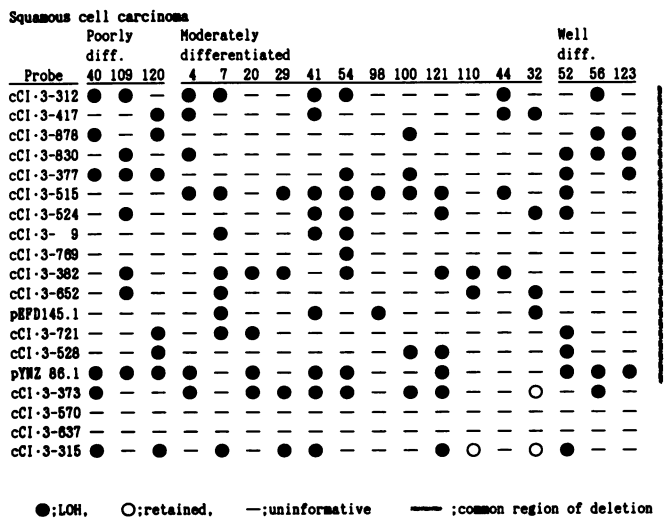

Fig. 3. Deletion maps of chromosome 3p in 18 squamous cell carcinomas. *Top of each lane,* tumor number; *vertical bar on right,* common region of deletion covers almost the entire short arm.

that tumor suppressor genes on the short arm of chromosome 3 may play a significant role in malignant transformation of small cell carcinoma and squamous cell carcinoma, both of which are thought to be associated with smoking. However, in adenocarcinomas, these genes might be associated with progression of the tumor.

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