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Common Regions of Deletion on Chromosomes 5q, 6q, and 10q in Renal Cell Carcinoma

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ABSTRACT

Relatively frequent losses of heterozygosity on chromosomes 5q, 6q, and 10q, in addition to loss of heterozygosity on the short arm of chromosome 3, have been observed in renal cell carcinomas. As the first step toward isolation of tumor suppressor genes on these three chromosomal arms, we used six restriction fragment length polymorphism markers for 5q, nine for 6q, and eight for 10q to identify regions commonly deleted in a panel of 64 renal cell carcinomas. Allelic losses were common at chromosome 5q21, the region where the MCC (mutated in colorectal cancer) gene was recently identified; at chromosome 6q27; and at chromosome 10q21-23. Furthermore, an association was observed between accumulation of allelic losses on these three chromosomal arms and progression of tumors. Loss of heterozygosity on chromosome 5 showed a correlation with the histopathological grade of a given tumor and the incidence of distant metastasis.

INTRODUCTION

Numerous studies using RFLP[2] markers to investigate the molecular biology of carcinogenesis have suggested that an accumulation of genetic alterations at specific chromosomal loci contributes to tumor development and/or progression. Several genes, including oncogenes and tumor suppressor genes, appear to be involved in tumorigenesis of colon and breast cancers (1–3). In RCC, several lines of evidence point to the existence of a tumor suppressor gene(s) on the short arm of chromosome 3: somatic chromosomal aberrations involving this chromosome (4, 5); t(3;8) balanced translocations in germline cells of some patients with hereditary renal cell carcinoma (6); and frequent LOH on chromosome 3p (7–10). Our earlier study suggested that, in addition to the gene on chromosome 3p, tumor suppressor genes associated with RCC may exist on chromosomes 5q, 6q, 10q, 11q, and 17p (11). However, the frequency of LOH on these chromosomal arms was not as high as that on chromosome 3p. To examine further whether these chromosomal arms contain tumor suppressor genes, and if they do, to map the loci precisely, we have analyzed these candidate loci in detail with a large number of RFLP markers. Additionally, we have expanded the number of tumors investigated to 64 and correlated the accumulation of events causing LOH on various chromosomal arms with clinical and histopathological parameters.

MATERIALS AND METHODS

Materials. Normal and cancerous tissues from 64 patients with primary sporadic RCC were obtained during radical nephrectomy at the Kobe National Hospital, the School of Medicine, Kobe University, or the Department of Urology, Kyoto University. The tissues were frozen immediately after nephrectomy and stored at −70°C until extraction of DNA.

DNA Extraction and Probes. The extraction of DNA from tissues was carried out according to methods described previously (3). All probes used in this study are listed in Table 1. The probes in the cCI6 series were isolated from a human/mouse hybrid cell line which contained only chromosome 6 as human-derived material (12) and were mapped by fluorescent *in situ* hybridization.[3]

Southern Blotting and Hybridization. All DNA samples were transferred to nylon membrane (Pall Biodyne) with 0.1 N NaOH/0.1 M NaCl. Neutralization, fixation, and hybridization were done according to methods described previously (3).

RESULTS

Chromosomal localization, the enzyme detecting RFLP, and frequency of LOH for each probe are summarized in Table 1. Fig. 1 presents examples of deletion maps and Southern blots which show loss or retention of heterozygosity in tumors. A significant reduction of signal intensity was observed in one of two polymorphic alleles in blots probed with CI6-39, CI6-49, and OS-2. On the other hand, polymorphic alleles in blots with CI6-4, THH54, and MCT122.2 were retained in tumors. The results of LOH studies on four chromosomal arms (3p, 5q, 6q, and 10q) and the histopathological features of each tumor are summarized in Table 2. LOH was observed on chromosome 3 in 52 of 58 informative cases (90%). LOH also occurred frequently on chromosomes 5q (13 of 39, 33%), 6q (17 of 44, 39%), and 10q (25 of 61, 41%). However, LOH on chromosomes 11q (3 of 26, 12%), 17p (13 of 63, 21%), and 19p (8 of 55, 15%) was not observed as frequently as in our previous study.

In view of these results, we constructed deletion maps of chromosomes 5q, 6q, and 10q. On chromosome 5q, Tumor 5 showed LOH with RFLP marker pL5.71-3, but retained both alleles at proximal loci defined by E5.55 and LS5.3. Tumors 35 and 94 lost one allele at the L5.71 locus but retained heterozygosity at the distal YN5.48 locus. From these results, a common region of deletion was identified at chromosome 5q21, where the gene responsible for adenomatous polyposis coli is located (Fig. 2*a*). On chromosome 6q (Fig. 2*b*), a commonly deleted region was identified at q27: the tumor of Patient 22 lost an allele in the region distal to cCI6-52; Tumor 33 showed LOH in the region distal to cCI6-91; and Tumor 104 lost a part distal to cCI6-4. In Fig. 2*c*, a deletion map of chromosome 10q shows that Tumor 6 lost an allele at cTBQ16 but retained both alleles at pEFD75, Tumor 90 showed LOH at THH54 but retained both OS-2 alleles, and Tumor 104 showed LOH at OS-2 but retained heterozygosity at THH54 and MCT122.2. Therefore, a common region of deletion was

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[2] The abbreviations used are: RFLP, restriction fragment length polymorphism; RCC, renal cell carcinoma; LOH, loss of heterozygosity.

[3] S. Saito *et al.*, unpublished data.

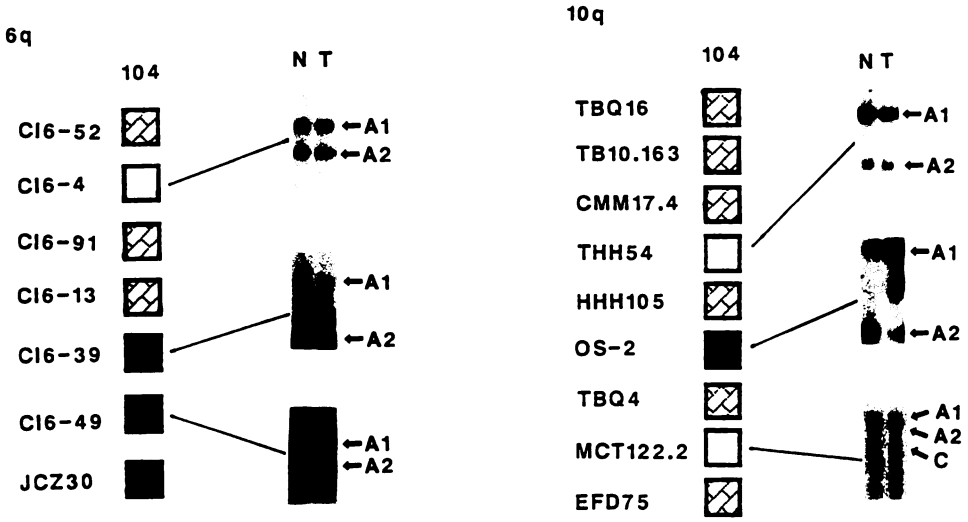

Fig. 1. Deletion maps of Tumor 104 on chromosomes 6q and 10q and typical Southern blots demonstrating loss or retention of heterozygosity in DNAs from normal kidney (*N*) and from renal cell carcinoma (*T*). *A1*, *A2*, and *C* indicate allele 1 (larger fragment), allele 2 (smaller fragment), and invariant band, respectively. ■, LOH; □, retained; ▨, uninformative.

Table 1 *Probes tested for loss of heterozygosity*

Probe	Symbol	Location	Enzyme	LOH/ informative cases
Chromosome 5				
E5.55	D5S133	q21	*Taq*I	1/6 (17)[a]
L5.71-3	D5S141	q21	*Msp*I	8/26 (31)
YN5.48-4	D5S81	q21	*Msp*I	5/28 (18)
LS5.3-4	D5S140	q21	*Msp*I	3/16 (19)
MC5.61	D5S84	q21	*Taq*I	2/5 (40)
KK5.19		q32	*Rsa*I	2/10 (20)
Total (chromosome 5q)				13/39 (33)
Chromosome 6				
HHH157	D6S29	p21	*Bam*HI	3/22 (14)
CI6-7	D6S138	p21.3	*Pst*I	6/34 (18)
CI6-47	D6S160	p21.2	*Bgl*II	5/23 (22)
CI6-37	D6S154	q21	*Taq*I	3/15 (20)
CI6-52	D6S164	q23	*Msp*I	1/11 (9)
CI6-3	D6S135	q24	*Msp*I	5/9 (56)
CI6-4	D6S136	q25	*Msp*I	2/16 (13)
CI6-91	D6S186	q26	*Msp*I	7/34 (21)
CI6-13	D6S142	q27	*Msp*I	8/30 (27)
CI6-39	D6S156	q27	*Taq*I	7/27 (26)
CI6-49	D6S161	q27	*Taq*I	6/27 (22)
JCZ30	D6S37	q27	*Taq*I	9/25 (36)
Total (chromosome 6q)				17/44 (39)
Chromosome 10				
TBQ7	D10S28	pter-p13	*Msp*I	3/31 (10)
MHZ15	D10S17	pter-p13	*Msp*I	1/11 (9)
TBQ16	D10S30	q11	*Msp*I	2/13 (15)
TB10.163	D10S22	q21	*Msp*I	3/10 (30)
CMM17.4	D10S23	q21	*Pvu*II	3/15 (20)
THH54	D10S14	q21	*Msp*I	5/17 (29)
HHH105	D10S13	q22-q25	*Bgl*II	9/32 (28)
OS-2	D10S20	q22-q25	*Hind*III	8/25 (32)
TBQ4	D10S27	q22-q25	*Msp*I	3/26 (12)
MCT122.2	D10S36	q26	*Taq*I	5/27 (19)
EFD75	D10S25	q26	*Taq*I	10/30 (33)
Total (chromosome 10q)				25/61 (41)
Chromosome 11				
MCMP1	PYGM	q12-q13.2	*Msp*I	2/15 (13)
HBI59	D11S146	q12-q13.2	*Taq*I	1/15 (7)
SS6	INT2	q13	*Taq*I	3/9 (33)
Total (chromosome 11q)				3/26 (12)
Chromosome 17				
YNZ22	D17S5	p13.3	*Taq*I	8/48 (17)
MCT35.1	D17S31	p13.1-p11.2	*Msp*I	5/28 (18)
BHP53	P53	p13	*Bam*HI	4/20 (20)
Total (chromosome 17p)				13/63 (21)
Chromosome 19				
MCOB5	D19S21	p13.3	*Pst*I	3/30 (10)
JCZ3.1	D19S20	p13.3	*Taq*I	8/40 (20)
YNZ21		p13.3	*Msp*I	7/48 (15)
Total (chromosome 19p)				8/55 (15)

[a] Numbers in parentheses, percentage.

assigned between THH54 and OS-2 at the q21-q23 band of chromosome 10.

We attempted to find correlations between chromosomal alterations and histopathological features. As shown in Fig. 3, the accumulated number of LOHs on chromosomes 3p, 5q, 6q, and 10q in each tumor showed a correlation with its histopathological grade. In the clear cell type of RCC, LOH was detected at two or more of these chromosomal arms tested in 82% of moderately differentiated tumors, although LOH was detected at only one chromosomal arm or not at all in nearly half of the well-differentiated types. The results suggest that an accumulation of events causing LOH (probably reflecting loss of function of tumor suppressor genes) is associated with tumor progression. In particular, LOH on 5q showed a significant correlation with tumor grade (Fisher's test, $P = 0.015$) and a weak association with distant metastasis ($P = 0.062$) (Table 3). However, stage and tumor size were not correlated with the number of chromosomal arms showing LOHs.

DISCUSSION

Many lines of evidence have suggested that one or more genes on the short arm of chromosome 3 are associated with development and/or progression of renal cell carcinomas: loss of heterozygosity (7–10); cytogenetic aberrations (4, 5) and hereditary t(3;8) translocations (6); or von Hippel-Lindau disease (13, 14). In a previous study, we detected two commonly deleted regions at chromosomes 3p14 and 3p21.3 in RCCs (10). However, studies on colorectal carcinoma (1, 2), lung cancer (15, 16), and breast cancer (Ref. 3; Footnote 4) have shown that alterations in oncogenes and tumor suppressor genes accumulate to transform a normal cell to a malignant cell. In the present study, we have investigated additional loci on five chromosomal arms (5q, 6q, 10q, 17p, and 19q) that were implicated by LOH in our previous studies (11) to examine the significance of the allelic losses at these loci. Although chromosomes 17p and 19q did not show the frequency of LOH reported previously, the other three regions revealed 33% to 41% LOH, and commonly deleted regions were identified at 5q21, 6q27, and 10q21-q23.

Kovacs and Kung (17) and Presti *et al.* (18) reported cytogenetic abnormalities on chromosome 5 due to unbalanced trans-

[4] T. Sato *et al.*, unpublished data.

Table 2 *Genotype and histopathological features of tumors*

Patient	Genotype of each arm 3p	5q	6q	10q	Grade[a]	Cell type[b]
3	●[c]	—[d]	○[e]	○	1	C
11	●	○	○	●	1	C
14	●	—	—	○	1	C
15	●	○	○	○	1	C
19	●	○	—	○	1	C
21	●	—	○	●	1	C
22	●	○	●	○	1	C
26	●	—	○	○	1	C
29	●	○	—	○	1	C
32	●	○	—	●	1	C
34	●	○	—	—	1	C
37	●	●	○	○	1	C
51	●	○	○	●	1	C
53	●	○	●	○	1	C
54	●	●	○	●	1	C
57	●	—	●	●	1	C
86	●	●	○	●	1	C
87	●	—	—	—	1	C
88	●	○	○	●	1	C
91	●	○	○	○	1	C
92	●	○	●	○	1	C
94	●	●	●	○	1	C
95	●	○	○	○	1	C
97	●	—	—	○	1	C
100	●	○	○	●	1	C
104	●	○	●	●	1	C
105	●	—	○	○	1	C
5	●	●	○	○	2	C
6	●	○	—	●	2	C
7	●	○	—	○	2	C
16	●	—	○	●	2	C
17	○	●	●	●	2	C
18	●	●	—	○	2	C
20	●	—	—	○	2	C
23	●	●	—	○	2	C
24	●	—	—	●	2	C
25	○	●	●	●	2	C
33	●	○	●	○	2	C
35	●	●	—	○	2	C
38	●	○	—	○	2	C
39	○	●	●	●	2	C
43	●	●	○	—	2	C
83	●	—	—	●	2	C
41	●	—	—	●	3	C
55	●	—	●	○	X	C
89	—	○	○	○	1	G
96	—	—	○	○	1	G
101	○	●	●	●	1	G
36	●	○	—	●	2	G
40	○	○	●	○	2	G
82	●	—	●	○	2	G
84	—	—	●	●	2	G
85	●	○	●	○	2	G
2	●	○	○	○	3	S
81	○	—	—	○	1	C/G
103	●	—	○	○	1	C/G
102	●	—	○	○	2	C/G
90	—	○	—	●	1	C/G
99	—	—	●	●	2	C/G
98	●	—	○	○	3	G/S
52	●	—	○	●	X	X
56	—	—	○	○	X	X
58	●	—	○	○	X	X
59	●	○	○	●	X	X
% of LOH	90	33	39	41		

[a] Grade of each tumor was determined according to the criteria of the Japanese Pathological Society (20).
[b] C, clear cell type; G, granular cell type; S, spindle cell type; C/G, mixed type of clear and granular; G/S, mixed type of granular and spindle; X, not determined.
[c] LOH was observed for at least one locus on this arm.
[d] Uninformative at every locus on this arm.
[e] Retention of heterozygosity at every locus on this arm.

location between chromosomes 3p and 5q with the breakpoints at Bands 3p13 and 5q22, resulting in an extra copy of the 5q22-qter region. It is likely that some of our cases may have partial trisomy of 5q. However, it was very hard to judge such partial trisomy due to difficulties of distinguishing gain of allele from loss of allele on Southern blots. We have indicated that a gene on chromosome 5q may be associated with progression of RCCs. As the commonly deleted region at chromosome 5q includes the L5.71 locus, which is a genomic clone of the recently ascertained MCC (mutated in colorectal cancer) gene (19), the MCC gene might be a candidate for a role in RCC.

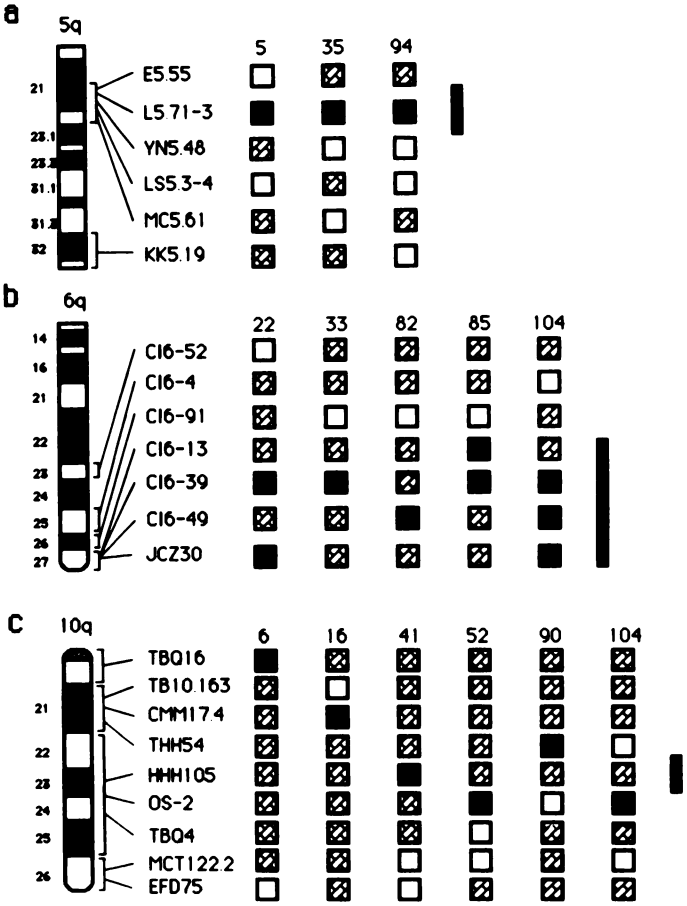

Fig. 2. Deletion maps of 5q (*a*), 6q (*b*), and 10q (*c*) in sporadic renal cell carcinoma. Common regions of deletion are indicated by a *vertical bar* on the *right*. ■, LOH; □, retained; ⊡ uninformative.

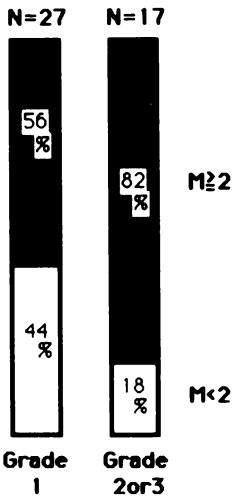

Fig. 3. Correlation between tumor grade and the number of accumulated mutations detected on four specific chromosomal arms (3p, 5q, 6q, and 10q) in clear cell carcinomas. *M*, number of mutations on these arms. *Grade 1*, well differentiated; *Grades 2 or 3*, moderately differentiated.

Table 3 *Correlation between mutation on 5q and tumor grade or distant metastasis*

	Histopathological grade[a]		Distant metastasis[b]	
	1	2 or 3	−	+
Retained	15	4	16	1
LOH	4	8	7	4

[a] $P = 0.015$ (Fisher's test).
[b] $P = 0.062$ (Fisher's test).

Furthermore, as in the case of colorectal carcinoma (1, 2) and breast cancer (Ref. 3; Footnote 4), accumulation of genetic alterations was correlated with the grade of primary renal carcinomas. Our results suggested that alterations on 6q and 10q also are important to progression of RCC. Tumors from Patients 17, 25, 39, 40, and 101 retained heterozygosity at loci on chromosome 3p, but they did show LOH on other chromosomal arms. These tumors may contain a very small interstitial deletion on chromosome 3p which was not detectable by our panel of probes; it is also possible that an alternative pathway exists for development of RCC, without association of genes on 3p.

REFERENCES

1. Vogelstein, B., Fearon, E. R., Kern, S. E., Hamilton, S. R., Preisinger, A. C., Nakamura, Y., and White, R. Allelotype of colorectal carcinomas. Science (Washington DC), *244:* 207–211, 1989.
2. Fearon, E. R., Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.
3. Sato, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G., and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res., *50:* 7184–7189, 1990.
4. Yoshida, M. A., Ohyashiki, K., Ochi, H., Gibas, Z., Pontes, J. E., Prout, G. R., Jr., and Sandberg, A. A. Cytogenetic studies of tumor tissue from a patient with nonfamilial renal cell carcinoma. Cancer Res., *46:* 2139–2147, 1986.
5. Kovacs, G., Szucs, S., DeRiese, W., and Baumgartel, H. Specific chromosome aberration in human renal cell carcinoma. Cancer Res., *40:* 171–178, 1987.
6. Cohen, A. J., Li, F. P., Berg, S., Marchetto, D. T., Tsai, S., Jacobs, S. C., and Brown, R. S. Hereditary renal cell carcinoma associated with a chromosomal translocation. N. Engl. J. Med., *301:* 592–595, 1979.
7. Zbar, B., Brauch, H., Talmadge, C., and Lineham, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature (Lond.), *327:* 721–724, 1987.
8. Kovacs, G., Erlandsson, R., Boldog, F., Ingvarsson, S., Muller-Brechlin, R., Klein, G., and Sumegi, J. Consistent chromosome 3p deletion and loss of heterozygosity in renal cell carcinoma. Proc. Natl. Acad. Sci. USA, *85:* 1571–1575, 1988.
9. Bergerheim, U., Nordenskjold, M., and Collins, V. P. Deletion mapping in renal cell carcinoma. Cancer Res., *49:* 1390–1396, 1989.
10. Yamakawa, K., Morita, R., Takahashi, E., Hori, T., Ishikawa, J., and Nakamura, Y. A detailed deletion mapping of the short arm of chromosome 3 in sporadic renal cell carcinoma. Cancer Res., *51:* 4707–4711, 1991.
11. Morita, R., Ishikawa, J., Tsutsumi, M., Hikiji, K., Tsukada, Y., Kamidono, S., Maeda, S., and Nakamura, Y. Allelotype of renal cell carcinoma. Cancer Res., *51:* 820–823, 1991.
12. Koi, M., Shimizu, M., Morita, H., Yamada, H., and Oshimura, M. Construction of mouse A9 clones containing a single human chromosome tagged with neomycin-resistance gene via microcell fusion. Jpn. J. Cancer Res., *80:* 413–418, 1989.
13. King, C. R., Schimke, R. N., Arthur, T., Davoren, B., and Collins, D. Proximal 3p deletion in renal cell carcinoma cells from a patient with von Hippel-Lindau disease. Cancer Genet. Cytogenet., *27:* 345–348, 1987.
14. Seizinger, B. R., Rouleau, G. A., Ozelius, L. J., Laue, A. H., Farmer, G. E., Lamiell, J. M., Haines, J., Yuen, J. W. M., Collins, D., Majoor-Krakauer, D., Bonner, T., Mathew, C., Rubenstein, A., Halperin, J., McConkie-Rosell, A., Green, J. S., Trofatter, J. A., Ponder, B. A., Eierman, L., Bowmer, M. S., Schimke, R., Oostra, B., Aronin, N., Smith, D. I., Drabkin, H., Waziri, M. H., Hobbs, W. J., Martuza, R. L., Conneally, P. M., Hsia, Y. E., and Gusella, J. F. Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinomas. Nature (Lond.), *332:* 268–269, 1988.
15. Naylor, S. L., Johnson, B. E., Minna, J. D., and Sakaguchi, A. Y. Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer. Nature (Lond.), *329:* 451–454, 1987.
16. Kok, K., Osigna, J., Carritt, B., Davis, M. B., van der Hout, A. H., van der Veen, A. Y., Landsvater, R. M., de liji, L. F. M. H., Berendsen, H. H., Postmus, P. E., Poppema, S., and Buys, C. H. M. Deletion of a DNA sequence at chromosomal region 3p21 in all major types of lung cancer. Nature (Lond.), *330:* 578–581, 1987.
17. Kovacs, G., and Kung, H. Nonhomologous chromatid exchange in hereditary and sporadic renal cell carcinomas. Proc. Natl. Acad. Sci. USA, *88:* 194–198, 1991.
18. Presti, J. C., Rao, P. H., Chen, Q., Reuter, V. E., Li, F. P., Fair, W. R., and Jhanwar, S. C. Histopathological, cytogenetic, and molecular characterization of renal cortical tumors. Cancer Res., *51:* 1544–1552, 1991.
19. Kinzler, K. W., Nilbert, M. C., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hamilton, S. R., Hedge, P., Markhan, A., Carlson, M., Joslyn, G., Groden, J., White, R., Miki, Y., Miyoshi, Y., Nishisho, I., and Nakamura, Y. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. Science (Washington DC), *251:* 1366–1370, 1991.
20. Japanese Urological Association, the Japanese Pathological Society, and Japan Radiological Society. General Rules for Clinical and Pathological Studies on Renal Cell Carcinoma, Ed. 1, pp. 68–73. Tokyo: Kanehara, 1983.