[CANCER RESEARCH 49, 3598-3601, July 1, 1989]

Loss of Heterozygosity on the Short Arm of Chromosome 3 in Carcinoma of the Uterine Cervix[1]

Jun Yokota,[2] Yuka Tsukada, Takashi Nakajima, Masahiro Gotoh, Yukio Shimosato, Naoki Mori, Youko Tsunokawa, Takashi Sugimura, and Masaaki Terada

National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan

ABSTRACT

Loss of genes at specific chromosomal loci is a common genetic alteration in human tumors and is thought to be critical for unmasking the recessive genetic changes for tumorigenesis. To learn whether such recessive mutations are involved in the development of carcinoma of the uterine cervix, 18 fresh tumors were analyzed by Southern blot hybridization using 34 polymorphic DNA markers covering 19 different chromosomes. We found loss of heterozygosity at the *D3S2* locus on chromosome 3p in all nine patients who could be evaluated. Human papillomavirus type 16 and type 18 were present in seven and three of 18 tumors, respectively, while no amplification of 13 oncogenes, including c-*myc* and H-*ras*, was detected in these tumors. These results suggest that recessive genetic changes on chromosome 3p are one of the important genetic alterations for the development of carcinoma of the uterine cervix. Since this locus is also lost commonly in lung cancer and in renal cell carcinoma, it is possible that these three different types of adult tumors result from mutations of the same recessive gene on chromosome 3p.

INTRODUCTION

Carcinoma of the uterine cervix is a common malignancy in adult females, and 2 different types of genetic alterations, HPV[3] infection and alterations of the c-*myc* and H-*ras* oncogenes, are thought to be involved in the development of this cancer (1–3). However, several lines of evidence suggest that HPV infection alone does not lead to the development of a malignant tumor (1), and that oncogene activation may be involved in tumor progression rather than being critical for tumorigenesis (2, 3).

Recent molecular genetic studies have shown that loss of genes on specific chromosomal loci occurs frequently in certain types of tumor, suggesting that recessive genetic changes are involved in the development of a wide variety of human cancers (4). At present, only limited cytogenetic information is available, and specific chromosomal changes have not yet been identified in carcinoma of the uterine cervix (5–7). However, it has been reported that tumorigenicity of HeLa cells, derived from carcinoma of the uterine cervix, was suppressed by introduction of normal chromosome 11, suggesting the presence of a tumor suppressor gene(s) on chromosome 11 (8–10). In addition, loss of heterozygosity for chromosome 11 in HeLa cells was suggested by an RFLP analysis (11). In order to identify the chromosomal loci commonly deleted in carcinoma of the uterine cervix, we undertook an RFLP analysis.

Received 11/29/88; revised 3/2/89; accepted 3/29/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by a Grant-in-Aid for a Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health and Welfare of Japan, and Grants-in-Aid from the Ministry of Health and Welfare and the Ministry of Education, Science, and Culture of Japan. N. M. is an awardee of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research.

[2] To whom requests for reprints should be addressed, at Section of Studies on Metastasis, National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan.

[3] The abbreviations used are: HPV, human papillomavirus; RFLP, restriction fragment length polymorphism.

MATERIALS AND METHODS

Human Tissue Samples. Eighteen tumors and the adjacent noncancerous tissues were obtained from patients with carcinoma of the uterine cervix at surgery. Stages and histological types of these tumors are summarized in Table 1. All 18 cases belonged to Stage I or II and had not received any chemotherapy or radiotherapy before the surgical removal of the tumors.

DNA Isolation and Southern Blot Analysis. High-molecular-weight DNA was prepared by proteinase K digestion and phenol-chloroform extraction as described (12). Approximately 10 μg of DNA were digested to completion with appropriate restriction enzymes, subjected to electrophoresis on 0.8% agarose gel, transferred to nitrocellulose filters, and hybridized to ^{32}P-labeled probes. The filters were hybridized repeatedly with RFLP probes, oncogene probes, and HPV probes to confirm the amount of DNA on the filters.

DNA Probes. The 34 polymorphic DNA probes used in this study are listed in Table 2 (13–17). These probes are the DNA segments homologous to 33 different loci on 19 different chromosomes and recognize RFLPs at each locus. The length of each allelic fragment observed was identical to that published previously except for the *RAF1* locus (14) (Fig. 1), although the frequency of constitutional heterozygosity in the patients examined here at certain loci, such as *DNF15S2* and *D3S2*, was markedly different from that previously reported. DNA probes used for evaluation of 13 oncogenes included a 1.5-kilobase pair *Cla*I-*Eco*RI fragment for c-*myc*, a 1.8-kilobase pair *Sma*I-*Eco*RI fragment for L-*myc*, a 1.0-kilobase pair *Eco*RI-*Bam*HI fragment for N-*myc*, a 3.0-kilobase pair *Sst*I-*Sst*I fragment for H-*ras*, a 0.64-kilobase pair *Eco*RI-*Hin*dIII fragment for K-*ras*, a 1.0-kilobase pair *Pvu*II-*Pvu*II fragment for N-*ras*, a 2.4-kilobase pair *Cla*I-*Cla*I fragment for *erb*B-1/*EGFR*, a 4.68-kilobase pair *Dra*I-*Dra*I fragment for *erb*B-2, a 0.59-kilobase pair *Ava*II-*Ava*II fragment for *hst*-1, a 0.9-kilobase pair *Sst*I-*Sst*I fragment for *int*-2, a 0.75-kilobase pair *Sst*I-*Hha*I fragment for *myb*, a 2.2-kilobase pair *Eco*RI-*Eco*RI fragment for *raf*-1, and a 1.5-kilobase pair *Eco*RI-*Eco*RI fragment for *erb*Aβ (18–21). The cloned HPV16 (a 7.9-kilobase pair *Bam*HI-*Bam*HI fragment) and HPV 18 (a 7.8-kilobase pair *Eco*RI-*Eco*RI fragment) sequences were used as probes to detect HPV sequences in tumor DNA samples (22).

RESULTS

Loss of Chromosomal Heterozygosity. Southern blot analysis was performed to determine whether loss of heterozygosity occurs at specific chromosomal loci in carcinoma of the uterine cervix using 34 polymorphic DNA markers which detect RFLPs at loci on 19 different chromosomes, except chromosomes 4, 8, and 21. Loss of heterozygosity was observed at 13 loci on 10 different chromosomes: chromosomes 1; 2; 3; 5; 7; 10; 13; 14; 16; and 17 (Table 2). However, the incidence of such loss was less than 33% at all loci examined except the *D3S2* locus on chromosome 3p14-21 (13) and the *ERBAβ* locus on chromosome 3p22-24.1 (17). Although it has been suggested that the tumor suppressor gene(s) for carcinoma of the uterine cervix is located on chromosome 11 (8–11), no loss of heterozygosity was observed at 3 loci on chromosome 11: 0 of 6 at the *HRAS1* locus; 0 of 3 at the *INS* locus; and 0 of 15 at the *INT2* locus (Table 2).

Loss of Heterozygosity on Chromosome 3. Nine of 18 patients were constitutionally heterozygous at the *D3S2* locus on chro-

Table 1 *Loss of heterozygosity on chromosome 3 in carcinomas of the uterine cervix*

Case	Stage[a]	Type of tumor	HPV[b]	Locus[c]							
				RAF1 3p24-25	*ERBAβ* 3p22-24.1		*DNF15S2* 3p21	*D3S2* 3p14-21	*D3S3* 3p14	*SST* 3q28	
				*Taq*I	*Bam*HI	*Hin*dIII	*Hin*dIII	*Msp*I	*Msp*I	*Eco*RI	*Bam*HI
1	Ia	S[d]	16	–	–	1,2	–	–	–	–	1,1,2
2	Ib	S	(–)	–	–	–	–	–	–	–	–
3	Ib	S	(–)	–	–	–	–	2	–	–	–
4	Ib	S	16	1,2	–	1,2	1,2	–	–	–	–
5	Ib	A	(–)	–	–	2	–	–	–	–	1,2
6	Ib	AS	(–)	–	–	–	1,2	–	–	–	–
7	Ib	AS	(–)	–	1,2	–	–	–	–	–	–
8	IIb	S	18	–	–	1	–	1	–	1,2	–
9	IIb	S	(–)	–	–	–	–	1	–	–	–
10	IIb	S	16	–	–	–	–	2	–	–	1,1,2
11	IIb	S	18	2	–	2	–	1	–	–	1,2
12	IIb	S	16	–	–	–	–	1	–	–	–
13	IIb	S	16	–	–	–	–	–	–	–	–
14	IIb	S	(–)	–	–	–	–	1	–	–	–
15	IIb	S	16	–	1,2	–	–	1	–	1,2	–
16	IIb	S	(–)	–	–	–	–	2	–	–	–
17	IIb	S	16	1,2	–	–	–	–	–	–	–
18	IIb	A	18	–	–	–	–	–	–	–	–

[a] The tumors were staged and histologically classified according to criteria outlined in Refs. 31 and 32.

[b] HPV type 16 or type 18 present in the tumor is shown as 16 or 18, respectively. (–) indicates that neither HPV type 16 nor type 18 was detected by Southern blot analysis.

[c] The restriction fragment alleles present in tumor tissue at loci that were constitutionally heterozygous are indicated by 1 and 2; 1 indicates loss of the smaller sized constitutional allele, 2 indicates loss of the larger sized constitutional allele, 1,2 indicates that heterozygosity remained in the tumor, and a minus sign indicates constitutional homozygosity.

[d] S, squamous cell carcinoma; A, adenocarcinoma; AS, adenosquamous carcinoma.

Table 2 *Loss of heterozygosity at chromosomal loci in carcinomas of the uterine cervix*

Marker locus	Chromosome location	Enzyme	No. of cases	Heterozygosity	
				Constitutional	Loss in tumor
MYCL	1p	*Eco*RI	18	10	1
REN	1q	*Hin*dIII	18	12	2
CRYG	2q	*Taq*I	18	9	1
D3S2	3p	*Msp*I	18	9	9
DNF15S2	3p	*Hin*dIII	18	2	0
ERBAβ	3p	*Bam*HI	18	2	0
		*Hin*dIII	18	5	3
RAF1	3p	*Taq*I	18	3	1
D3S3	3p	*Msp*I	18	0	0
SST	3q	*Eco*RI	18	2	0
		*Bam*HI	18	4	0
D5S2	5q	*Msp*I	18	3	1
MYB	6q	*Eco*RI	18	10	0
EGFR	7p	*Hin*dIII	18	8	1
COLIA2	7q	*Eco*RI	18	6	0
D9S1	9p	*Taq*I	5	2	0
PLAU	10q	*Bam*HI	18	4	1
HRAS	11p	*Bam*HI	18	6	0
INS	11p	*Taq*I	18	3	0
D11S12	11p	*Msp*I	18	0	0
D11S24	11q	*Bam*HI	16	0	0
INT2	11q	*Bam*HI	18	15	0
		*Pst*I	18	0	0
D12S4	12	*Msp*I	18	0	0
D13S1	13q	*Msp*I	18	7	2
D13S2	13q	*Msp*I	18	2	0
D13S3	13q	*Msp*I	18	8	1
		*Hin*dIII	18	10	3
D13S4	13q	*Msp*I	18	10	0
D14S1	14q	*Eco*RI	18	10	2
D15S1	15q	*Msp*I	18	6	0
HP	16q	*Eco*RI	18	9	1
D17S1	17p	*Msp*I	18	7	2
D18S5	18	*Taq*I	16	7	0
D19S9	19q	*Eco*RI	18	1	0
D20S6	20p	*Taq*I	12	2	0
D22S1	22q	*Taq*I	17	0	0

mosome 3p14-21 and were informative for determining whether or not loss of heterozygosity occurred in the respective tumors. Loss of heterozygosity at the *D3S2* locus was observed in all of these 9 patients (100%). The probe p12-32 for *D3S2* detects two allelic fragments of 2.9 kilobase pairs (allele 1) and 1.3 kilobase pairs (allele 2) in *Msp*I digests (13), and the intensity of one of these two fragments in the tumors was always less than that in the normal tissue from the same patients. The residual faint signal probably originates from contaminating normal cells in tumor specimens, because inflammatory cells are often present in cervical tumors. Representative results of the constitutional and tumor genotypes of 3 patients are shown in Fig. 1*a*. Patient 10 showed loss of allele 1; Patients 11 and 12 showed loss of allele 2.

To determine the commonly deleted region on chromosome 3 in carcinoma of the uterine cervix, these samples were also examined for loss of heterozygosity at 5 other loci on chromosome 3: *RAF1*(3p24-25) (14); *ERBAβ*(3p22-24.1) (17); *DNF15S2* (3p21) (13); *D3S3*(3p14) (13); and *SST*(3q28) (13). Loss of heterozygosity at the *RAF1* locus and the *ERBAβ* locus was observed in one of 3 patients and 3 of 7 patients (Fig. 1, *b c*), respectively, but no loss was observed at 2 other loci, *DNF15S2* and *SST*, whereas duplication of one of two alleles was observed in 2 of 6 patients at *SST* (Table 1). Analysis of loss of heterozygosity at the *ERBAβ* locus was performed using 2 different DNA probes: a human *ERBAβ* complementary DNA clone, pheA4; and a genomic *ERBAβ* DNA clone, pBH302 (15–17). The pheA4 and pBH302 probes detect *Bam*HI and *Hin*dIII RFLP, respectively, and loss of heterozygosity at this locus was observed in none of 2 patients by using the pheA4 probe and in 3 of 5 patients by the pBH302 probe (Table 1; Fig. 1*b*). No information was available on loss of heterozygosity at the *D3S3* locus, because none of the 18 patients was heterozygous in normal tissue at this locus. Therefore, the commonly deleted chromosomal region in carcinoma of the uterine cervix is probably a small part of the short arm of chromosome 3, including the *D3S2* locus. These results strongly suggest that recessive genetic changes on chromosome 3p are involved in the development of carcinoma of the uterine cervix.

HPV Infection and Oncogene Alterations. Since there has been increasing evidence that HPV infection plays an important role in the development of carcinoma of the uterine cervix (1), we also examined these 18 tumors for the presence of HPV

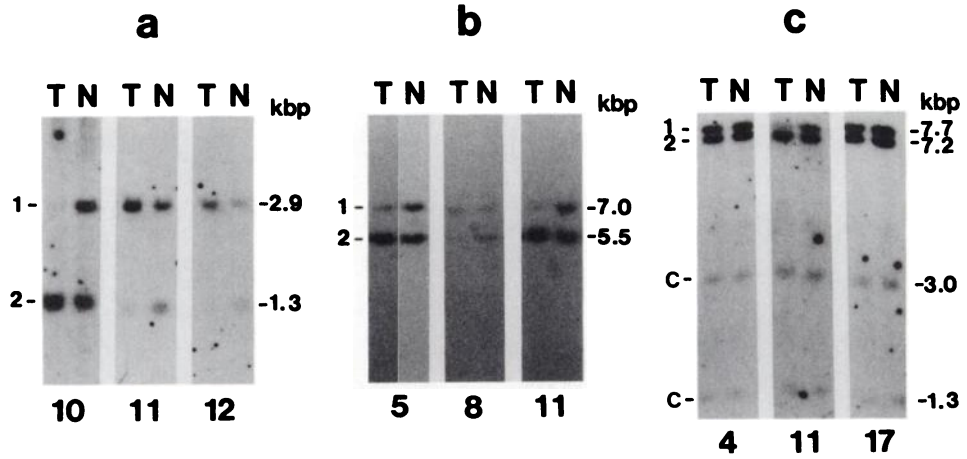

Fig. 1. Southern blot analysis of DNA from carcinoma of the uterine cervix (*T*) and corresponding normal tissue (*N*) with probes for chromosome 3. *Patient numbers* are shown below the blots, and the size of each hybridizing band in kilobases (*kbp*) is shown on the *right*. Alleles at each locus are designated *1* or *2* according to decreasing length and are shown on the *left*; *C* indicates a constant invariant band. *a*, *Msp*I digests hybridized to probe for *D3S2*; *b*, *Hin*dIII digests hybridized to probe for *ERBAβ*; *c*, *Taq*I digests hybridized to probe for *RAF1*. DNA from each sample (10 μg) was digested and hybridized as described previously (27). After hybridization, filters were washed and exposed to Kodak XAR-5 films. The following probes were used: a 1.1-kilobase pair *Sal*I-*Hin*dIII fragment derived from the recombinant plasmid p12-32 (*D3S2*) (13); a 2.0-kilobase pair *Bam*HI-*Bam*HI fragment derived from the recombinant plasmid pBH302 (*ERBAβ*) (16); and a 2.2-kilobase pair *Eco*RI-*Eco*RI fragment derived from the recombinant plasmid LibA21 (*RAF1*) containing complementary DNA sequences (exons 1 to 11) for the human *RAF1* oncogene (obtained from Dr. J. Ghysdael of Institut Pasteur, Lille, France). Our estimates of the sizes of hybridizing fragments to the probe for *RAF1* are slightly different from those published previously (14).

type 16 and type 18 DNA sequences by Southern blot analysis. HPV type 16 and type 18 were present in 7 and 3 of 18 tumors, respectively (Table 1). Alterations of 13 different oncogenes (c-*myc*, L-*myc*, N-*myc*, H-*ras*, K-*ras*, N-*ras*, *erb*B-1, *erb*B-2, *hst*-1, *int*-2, *myb*, *raf*-1, and *erbAβ*) in these tumors were also analyzed by Southern blot hybridization. No amplification or rearrangement of 13 oncogenes, including c-*myc* and H-*ras*, was detected in these 18 tumors.

DISCUSSION

Carcinogenesis is considered to be a multistep process involving more than one genetic change. Several lines of evidence suggest that HPV infection alone does not lead to the development of a malignant tumor and that additional factors are involved (1). In this study, we demonstrated that loss of genes on chromosome 3p is a common event in carcinoma of the uterine cervix, suggesting that recessive genetic changes on chromosome 3p are one of the cellular factors for the tumor development. It is possible that both 3p deletion and HPV integration are necessary to convert normal cervical epithelial cells into malignant cells. The relationship between 3p deletion and HPV integration remains to be determined. However, it is unlikely that HPV integration is causally related to 3p deletion, because previous studies have shown that there are no specific chromosomal regions for HPV integration (23, 24), although in one case of carcinoma of the uterine cervix the HPV integration site was reported to be localized to chromosome 3p21 (25).

No amplification or rearrangement of either c-*myc* or H-*ras* was observed in this study. These results are in marked contrast to the previous reports from other laboratories, showing a very high frequency of amplification of c-*myc* and H-*ras* oncogenes in carcinoma of the uterine cervix (2, 3). The fact that all cases examined here belonged to Stage I or II might be the reason for the absence of alteration of c-*myc* and H-*ras* in the present study. Our results demonstrating 3p deletion without c-*myc* and H-*ras* alteration in the Stage I and II tumors suggest that alteration of c-*myc* and H-*ras* may not be directly involved in tumorigenesis but may be a secondary event during tumor progression.

The commonly deleted region on chromosome 3p in carcinoma of the uterine cervix is exactly the same as the region deleted in 100% of small cell carcinoma of the lung as previously reported (26–28). This locus is also lost frequently in renal cell carcinoma (29, 30). Thus, loss of genes on chromosome 3p is a common genetic change in 3 different types of tumors: carcinoma of the uterine cervix; small cell carcinoma of the lung; and renal cell carcinoma. It is highly possible that mutation of the same recessive gene is involved in the development of these 3 types of tumors. It is also possible that 3 different genes for each type of tumor are located on the same region of chromosome 3p; allele loss at the *DNF15S2* locus occurs more frequently than that at the *D3S2* locus in renal cell carcinoma (30), and loss at both loci occurs frequently in small cell lung cancer (26, 27) while loss was observed only at the *D3S2* locus and not at the *DNF15S2* locus in carcinoma of the uterine cervix in this study. Isolation and characterization of gene(s) from the commonly deleted chromosomal region in these 3 types of tumors will clarify whether or not 3 different types of tumors arise from the same molecular genetic mechanism(s), and will lead us to an understanding of the biological significance of these gene(s).

ACKNOWLEDGMENTS

We thank the following scientists for providing DNA probes: Dr. W. Cavenee; Dr. G. Bell; Dr. R. White; Dr. T. Glaser; Dr. N. Maeda; Dr. A. Roses; Dr. W. E. C. Bradley; Dr. J. Minna; Dr. I. Pastan; Dr. J. Ghysdael; Dr. L.-C. Tsui; Dr. R. Evans; Dr. J. Chirgwin; Dr. C. Dickson, Dr. F. Ramirez; Dr. D. Slamon; Dr. T. Yamamoto; and Dr. H. zur Hauzen. DNA probes were also obtained from the American Type Culture Collection, Rockville, MD, and from the Japanese Cancer Research Resources Bank, Tokyo, Japan.

REFERENCES

1. Steinberg, B. M., Brandsma, J. L., and Taichman, L. B. Papillomaviruses. *In:* Cancer Cells, Chapter 5, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1987.
2. Ocadiz, R., Sauceda, R., Cruz, M., Graef, A. M., and Gariglio, P. High correlation between molecular alterations of the c-*myc* oncogene and carcinoma of the uterine cervix. Cancer Res., *47:* 4173–4177, 1987.

3. Riou, G. F., Barrois, M., Dutronquay, V., and Orth, G. Presence of papillomavirus DNA sequences, amplification of c-*myc* and c-H-*ras* oncogenes, and enhanced expression of c-*myc* in carcinomas of the uterine cervix. *In:* P. M. Howley, and T. R. Broker (eds.), Papillomaviruses: Molecular and Clinical Aspects, pp. 47–56. New York: Alan R. Liss, Inc., 1985.
4. Ponder, B. Gene losses in human tumours. Nature (Lond.), *335:* 400–402, 1988.
5. Atkin, N. B., and Baker, M. C. Nonrandom chromosome changes in carcinoma of the cervix uteri. Acta Cytol., *26:* 568–569, 1982.
6. Atkin, N. B., and Baker, M. C. Nonrandom chromosome changes in carcinoma of the cervix uteri. I. Nine near-diploid tumors. Cancer Genet. Cytogenet., *7:* 209–222, 1982.
7. Atkin, N. B., and Baker, M. C. Nonrandom chromosome changes in carcinoma of the cervix uteri. II. Ten tumors in the triploid-tetraploid range. Cancer Genet. Cytogenet., *13:* 189–207, 1984.
8. Saxon, P. J., Srivatsan, E. S., and Stanbridge, E. J. Introduction of human chromosome 11 via microcell transfer controls tumorigenic expression of HeLa cells. EMBO J., *5:* 3461–3466, 1986.
9. Srivatsan, E. S., Benedict, W. F., and Stanbridge, E. J. Implication of chromosome 11 in the suppression of neoplastic expression in human cell hybrids. Cancer Res., *46:* 6174–6179, 1986.
10. Kaelbling, M., and Klinger, H. P. Suppression of tumorigenicity in somatic cell hybrids. Cytogenet. Cell Genet., *41:* 65–70, 1986.
11. Kaelbling, M., Roginski, R. S., and Klinger, H. P. DNA polymorphisms indicate loss of heterozygosity for chromosome 11 of D98AH2 cells. Cytogenet. Cell Genet., *41:* 240–244, 1986.
12. Sakamoto, H., Mori, M., Taira, M., Yoshida, T., Matsukawa, S., Shimizu, K., Sekiguchi, M., Terada, M., and Sugimura, T. Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. Proc. Natl. Acad. Sci. USA, *83:* 3997–4001, 1986.
13. Pearson, P. L., Kidd, K. K., and Willard, H. F. Human gene mapping by recombinant DNA techniques. Cytogenet. Cell Genet., *46:* 390–566, 1987.
14. Seizinger, B. R., Rouleau, G. A., Ozelius, L. J., Lane, 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. I., 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 carcinoma. Nature (Lond.), *332:* 268–269, 1988.
15. Wyllie, F. S., Lazarou, L. P., and Wynford-Thomas, D. RFLP for the human *erbAβ* locus. Nucleic Acids Res., *16:* 5224, 1988.
16. Gareau, J.-H. P., Houle, B., Leduc, F., Bradley, W. E. C., and Dobrovic, A. A frequent *Hin*dIII RFLP on chromosome 3p21-25 detected by a genomic *erbAβ* sequence. Nucleic Acids Res., *16:* 1223, 1988.
17. Drabkin, H., Kao, F.-T., Hartz, J., Hart, I., Gazdar, A., Weinberger, C., Evans, R., and Gerber, M. Localization of human *ERBA2* to 3p22-3p24.1 region of chromosome 3 and variable deletion in small cell lung cancer. Proc. Natl. Acad. Sci. USA, *85:* 9258–9262, 1988.
18. Yokota, J., Tsunetsugu-Yokota, Y., Battifora, H., Le Fevre, C., and Cline, M. J. Alterations of *myc*, *myb*, and *ras*^{Ha} proto-oncogenes in cancers are frequent and show clinical correlation. Science (Wash. DC), *231:* 261–265, 1986.
19. Yoshida, M. C., Wada, M., Satoh, H., Yoshida, T., Sakamoto, H., Miyagawa, K., Yokota, J., Koda, T., Kakinuma, M., Sugimura, T., and Terada, M. Human *HST1(HSTF1)* gene maps to chromosome band 11q13 and coamplifies with the *INT2* gene in human cancer. Proc. Natl. Acad. Sci. USA, *85:* 4861–4864, 1988.
20. Yokota, J., Yamamoto, T., Miyajima, N., Toyoshima, K., Nomura, N., Sakamoto, H., Yoshida, T., Terada, M., and Sugimura, T. Genetic alterations of the c-*erb*B-2 oncogene occur frequently in tubular adenocarcinoma of the stomach and are often accompanied by amplification of the v-*erb*A homologue. Oncogene, *2:* 283–287, 1988.
21. Yokota, J., Wada, M., Yoshida, T., Noguchi, M., Terasaki, T., Shimosato, Y., Sugimura, T., and Terada, M. Heterogeneity of lung cancer cells with respect to the amplification and rearrangement of *myc* family oncogenes. Oncogene, *2:* 607–611, 1988.
22. Takebe, N., Tsunokawa, Y., Nozawa, S., Terada, M., and Sigimura, T. Conservation of E6 and E7 regions of human papillomavirus types 16 and 18 present in cervical cancers. Biochem. Biophys. Res. Commun., *143:* 837–844, 1987.
23. Durst, M., Croce, C. M., Gissmann, L., Schwarz, E., and Huebner, K. Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. Proc. Natl. Acad. Sci. USA, *84:* 1070–1074, 1987.
24. Inagaki, Y., Tsunokawa, Y., Takebe, N., Nawa, H., Nakanishi, S., Terada, M., and Sugimura, T. Nucleotide sequences of cDNAs for human papillomavirus type 18 transcripts in HeLa cells. J. Virol., *62:* 1640–1646, 1988.
25. Cannizzaro, L. A., Durst, M., Huebner, K., Croce, C., Hecht, B. K., and Hecht, F. Chromosome sublocalization of cervical carcinoma papillomavirus sites near cellular protooncogenes and fragile sites. Cytogenet. Cell Genet., *46* (Suppl.): 590, 1987.
26. 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.
27. Yokota, J., Wada, M., Shimosato, Y., Terada, M., and Sugimura, T. Loss of heterozygosity on 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.
28. Kok, K., Osinga, J., Carritt, B., Davis, M. B., van der Hout, A. H., van der Veen, A. Y., Landsvater, R. M., de Leij, L. F. M. H., Berendsen, H. H., Postmus, P. E., Poppema, S., and Buys, C. H. C. M. Deletion of a DNA sequence of the chromosomal region 3p21 in all major types of lung cancer. Nature (Lond.), *330:* 578–581, 1987.
29. Zbar, B., Braugh, H., Talmadge, C., and Linehan, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature (Lond.), *327:* 721–724, 1987.
30. Kovacs, G., Erlandsson, R., Boldog, F., Ingvarsson, S., Muller-Brechlin, R., Klein, G., and Sumegi, J. Consistent chromosome 3p deletion and loss heterozygosity in renal cell carcinoma. Proc. Natl. Acad. Sci. USA, *85:* 1571–1575, 1988.
31. Harmer, M. H. TNM Classification of Malignant Tumours, Ed. 3. Geneva: WHO, 1978.
32. Poulsen, H. E., Taylor, C. W., and Sobin, L. H. Histological typing of female genital tract tumours. *In:* International Histological Classification of Tumours No. 13. Geneva: WHO, 1975.