Jpn. J. Cancer Res. **89**, 916–922, September 1998

Allelic Loss on Chromosome 9q Is Associated with Lymph Node Metastasis of Primary Breast Cancer

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Frequent allelic losses on chromosome 9 are seen in a wide variety of human tumors; moreover, two genes (*P16* and *PTC*) whose mutant alleles confer predispositions to some inherited cancer syndromes have been identified on this chromosome. Using 15 highly polymorphic microsatellite markers distributed on both arms of chromosome 9, we tested 96 primary breast carcinomas for allelic loss in order to define the locations of genes that might be involved in this type of tumor. Allelic loss was observed in 37 of the tumors (39%) and detailed deletion mapping identified target regions at 9p21, 9q22.3 and 9q33. Losses at 9q22.3 and 9q33 were correlated with the presence of lymph node metastasis, and allelic loss at 9q22.3 was observed more frequently in scirrhous tumors than in less aggressive histologic types. Therefore, inactivation of tumor suppressor genes in 9q22.3 and 9q33 regions might play a role in progression of breast cancers, especially in metastasis to lymph nodes and in development of scirrhous tumors.

Key words: Breast cancer — Loss of heterozygosity — Tumor suppressor gene — Chromosome 9

Breast carcinogenesis in humans is considered to require a series of genetic alterations involving dominant oncogenes and tumor suppressor genes.[1–4] Inactivation of tumor-suppressor functions usually occurs as a consequence of deletion of one allele followed by mutation of the other. Frequent allelic losses (loss of heterozygosity, LOH) observed at specific chromosomal loci in several types of human cancers have implied the presence of putative tumor suppressor genes in the regions where deletions were detected. In breast carcinomas LOH has been reported on chromosomes 1p,[5] 3p,[6] 7p,[7] 11p,[8] 13q,[9] 16q,[10] 17,[11–13] 18q,[14] and 22q[15] and putative tumor suppressor genes in these chromosomal regions are postulated to be targets of those cancer-associated events.

Germline mutations of tumor suppressor genes confer predispositions to several dominantly inherited cancer syndromes. Two genes in this category have been localized to chromosome 9. On the short arm, *P16*, a gene associated with familial malignant melanoma[16] was isolated recently from 9p21 near *P15*. Homozygous or hemizygous deletions of these genes are also common in cell lines or primary tissues derived from various types of cancer[17, 18] including esophageal squamous cell carcinoma,[19] pancreatic adenocarcinoma,[20] and squamous cell carcinoma of the bladder.[21] On the long arm, a predisposing gene for nevoid basal cell carcinoma (Gorlin) syndrome, *PTC*, was recently isolated from 9q22.[22] Somatic mutations and losses of this gene have also been found in medulloblastoma and meningioma.[23] LOH is also frequent in cancers of the urinary bladder at yet another region, 9q32-q33.[24]

To determine the role of genetic alterations on chromosome 9 in the development and/or progression of breast tumors, we performed LOH analysis of 96 primary breast cancers using 15 microsatellite markers from both arms of chromosome 9, and looked for correlations between LOH and certain clinicopathological parameters.

MATERIALS AND METHODS

Specimens Tumor tissues and corresponding normal tissues were obtained from 96 patients with primary breast cancers during mastectomy at the Cancer Institute Hospital in Japan. Tumors were diagnosed according to the histological typing scheme of the Japanese Breast Cancer Society.[25] None of the patients had distant metastasis at the time of surgery. The tumor tissues and non-cancerous tissues were dissected and stored at −80°C until DNA extraction.

DNA extraction Frozen tissue samples were powdered, suspended in lysis buffer, treated with proteinase K and extracted with phenol-chloroform-isoamyl alcohol as described by Sato *et al.*[26] Before polymerase chain reaction (PCR) amplification, paired genomic DNAs were diluted and adjusted individually to 10 ng/μl.

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LOH analysis DNAs were examined for LOH using 15 highly polymorphic microsatellite markers distributed over the entire length of chromosome 9. The linear order of the markers is (ptel)-D9S178-D9S157-D9S736-(cen)-D9S153-D9S180-D9S176-D9S1690-D9S127-D9S299-D9S58-D9S302-D9S61-D9S113-D9S66-D9S158-(qtel), according to the Généthon and CHLC maps. The microsatellites were amplified from each sample by means of PCR using 20 ng of genomic DNA, 10 m*M* Tris-HCl (pH 8.4), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 200 m*M* each dNTP, 2.5 pmol each of [γ-^{32}P]ATP-end-labeled primer and non-labeled primer, and 0.25 unit of *Taq* polymerase in a total volume of 10 μl. Cycling conditions in the GeneAmp PCR 9600 System (Perkin Elmer Cetus, Norwalk, CT) were 94°C for 4 min, then 30 cycles of 94°C for 30 s, 55–62°C for 30 s, and 72°C for 30 s, with a final extension for 10 min at 72°C. PCR products were electrophoresed in 0.3-mm-thick denaturing 6% polyacrylamide gels containing 36% formamide and 8 *M* urea, at 1800 volts for 2–4 h. The separated products were transferred to blotting paper, which was dried at 80°C and exposed to autoradiographic film at room temperature for 8 to 20 h.

Definition of LOH Signal intensities of polymorphic alleles were quantified by a Hoefer GS-300 scanning densitometer; peak areas corresponding to each signal were calculated by electronic integration using a GS-370 electrophoresis data system (Hoefer Scientific Instruments, San Francisco, CA). The signal intensities of alleles of tumor DNAs were compared to those of the corresponding normal DNAs. We judged a reduction in signal intensity >50% to be allelic loss, after normalizing each signal to the signal obtained when the same DNA sample was analyzed with markers for loci on other chromosomes.

Clinicopathological parameters The following parameters were studied: histological type, tumor size and infiltration (t-factor), lymph node metastasis status (n_0 or n_1), and estrogen receptor (ER) and progesterone receptor (PgR) status (negative or positive). Tumors were classified by pathologists, according to the histological typing scheme of the Japanese Breast Cancer Society,[25] into the following types: non invasive tubular (1a), invasive papillotubular (a1), invasive solid tubular (a2), invasive scirrhous carcinoma (a3), and other specific types. This classification is essentially the same as the World Health Organization scheme for typing breast tumors. The t-factor was classified according to the histologic tnm classification, into the following types: tumors of 2 cm or less in diameter (t1), tumors more than 2 cm in diameter without invasion to skin or pectoral muscle (t2), and those with invasion to skin or pectoral muscle (t3). The χ^2 test and Fisher's exact test were used for statistical analysis. One-tailed *P* values of less than 0.05 were considered statistically significant.

RESULTS

LOH was detected on chromosome 9 in 37 (39%) of 96 breast tumors examined, all of which were informative with at least one of the 15 polymorphic microsatellite markers. The marker loci and their frequencies of LOH in our panel of patients are listed in Table I, in descending order from 9pter to 9qter according to the Généthon human linkage map and comprehensive human linkage map. Among the 23 tumors with LOH on 9p, 12 showed LOH at all informative loci but 11 showed partial or interstitial deletions. Representative autoradiograms for these cases are shown in Fig. 1A, where Tumor 66 showed retention of alleles at D9S157 but LOH at D9S736, and Tumor 25 showed LOH at D9S157 but retention of alleles at D9S736. The deletion map of 9p illustrated in Fig. 2 showed that eight tumors with partial or interstitial deletions were critical in defining a commonly deleted region at 9p21, within a 9-cM interval flanked by D9S157 and D9S736. This interval contains the p16 locus.

On the long arm of chromosome 9, the observed frequencies of LOH ranged from 10% at D9S61 to 29% at D9S66 (Table I). Among the 32 tumors with LOH on 9q, four showed LOH at all informative loci but the other 28 showed partial or interstitial deletions. For example, Tumor 618 showed LOH at D9S127 but retention of alleles at D9S176 and D9S58, whereas Tumor 1038 showed LOH at D9S1690 but retention of alleles at D9S176 and D9S58 (Fig. 1B). Similarly, Tumor 2170 showed LOH at D9S66 but retention of alleles at D9S113 and D9S158. Tumor 1038 showed LOH at D9S66 but

Table I. Frequencies of LOH at 15 Loci on Chromosome 9

Marker	Informative cases	LOH	LOH/informative cases (%)
D9S178	89	10	20.8
D9S157	90	15	21.1
D9S736	90	6	13.6
D9S153	92	13	18.3
D9S180	93	11	20.8
D9S176	90	16	24.2
D9S1690	88	16	26.7
D9S127	85	15	26.8
D9S299	78	12	24.0
D9S58	90	17	21.3
D9S302	89	9	12.3
D9S61	92	8	10.3
D9S113	93	16	21.3
D9S66	90	19	28.8
D9S158	88	8	13.3
Total	96	37	38.5

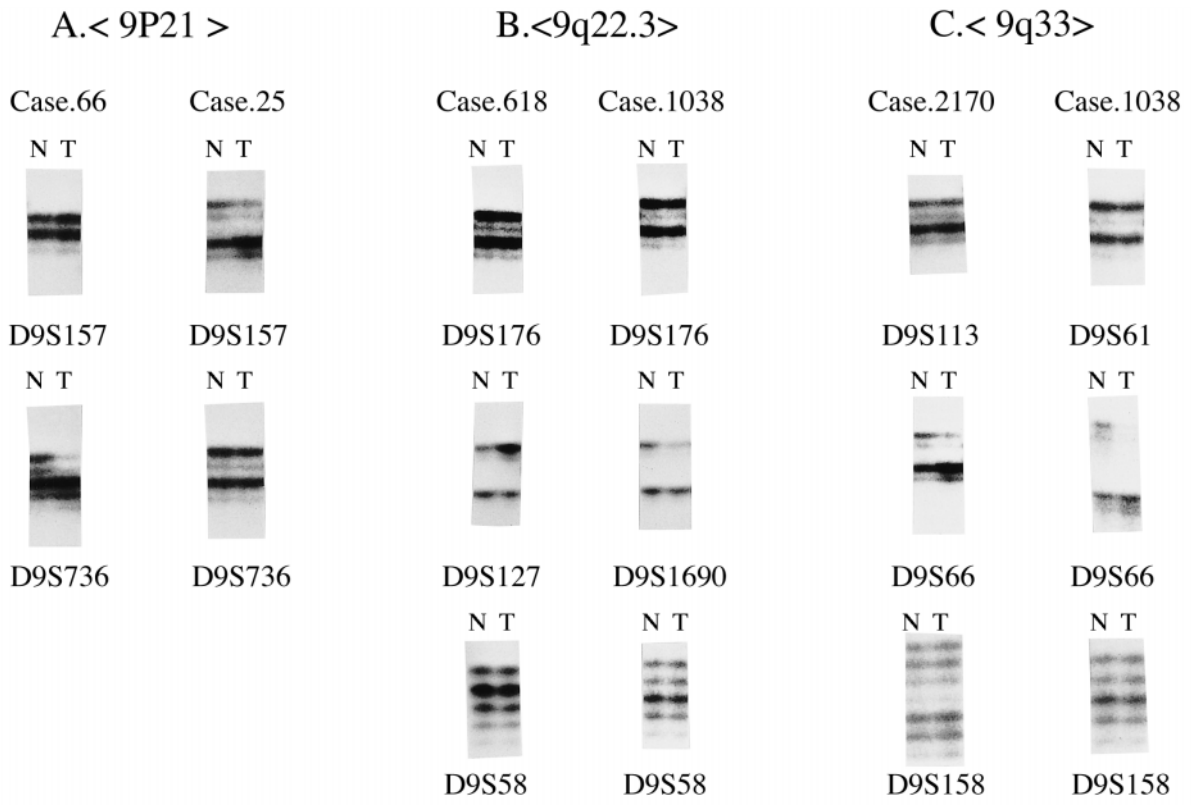

Fig. 1. Representative autoradiograms of LOH analysis. Markers are indicated at the bottom of each panel. T and N; paired DNA samples isolated from tumor and normal tissues, respectively. Critical cases that represent each commonly deleted region are shown.

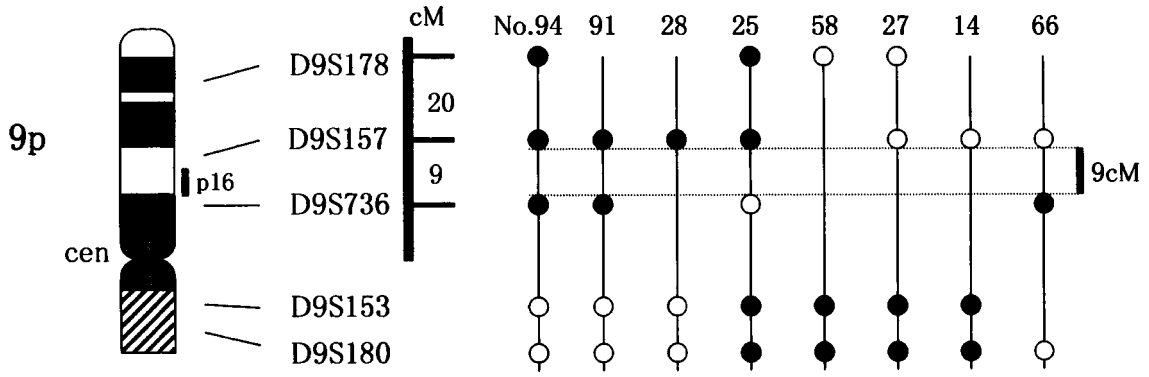

Fig. 2. Deletion map showing partial or interstitial deletions of chromosome 9p in breast cancers. Locations and order of the microsatellite markers were derived from published linkage information; distances between loci are indicated in female centimorgans. Tumor numbers are at the top of each column. White circles indicate retention of heterozygosity, black circles indicate LOH, and gaps reflect uninformativeness. The commonly deleted region that contains the *p16* gene is indicated by a vertical bar on the far right, between the dotted lines.

retention of alleles at D9S61 and D9S158 (Fig. 1B). Fig. 3 illustrates, as a deletion map, the seven tumors with partial or interstitial deletions that were critical in defining a new commonly deleted region at 9q22.3, within a 17-cM interval flanked by D9S176 and D9S58; this interval contains the *PTC* locus. Eleven tumors, including five of

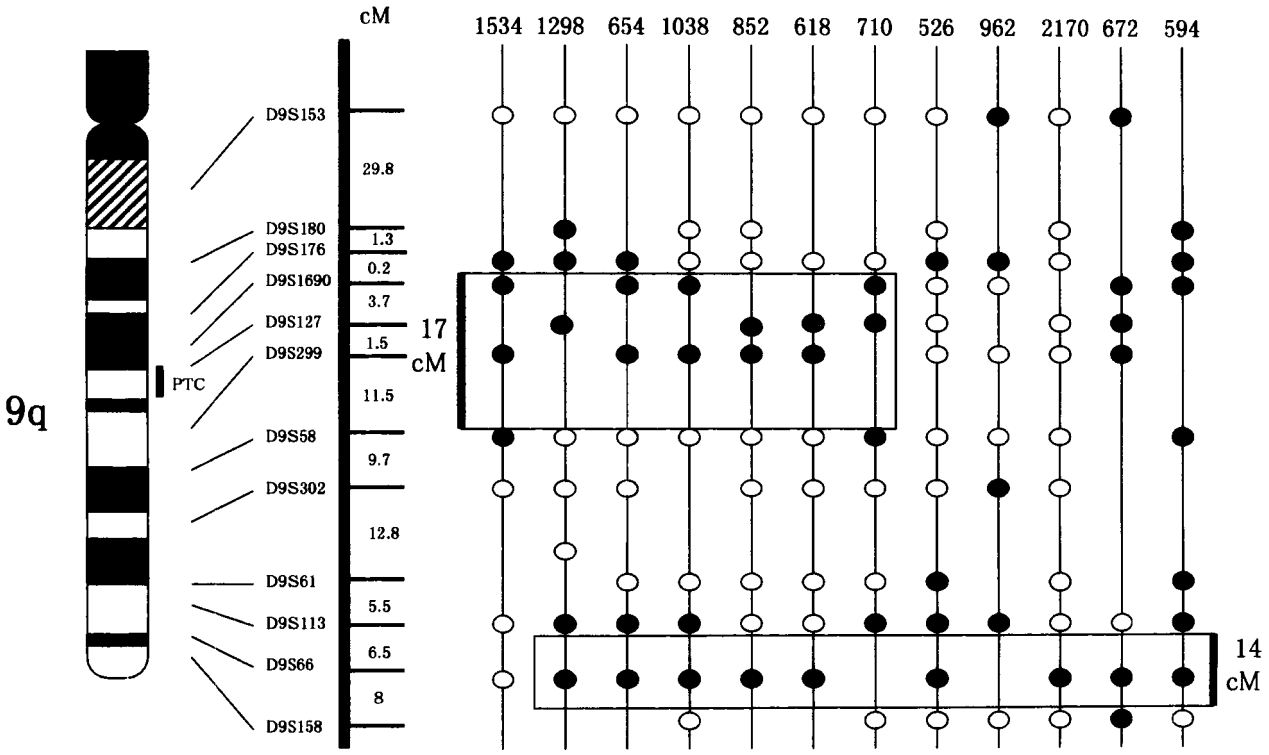

Fig. 3. Deletion map showing partial or interstitial deletions of chromosome 9q in breast cancers. Explanations for symbols are the same as in Fig. 2. Two commonly deleted regions were determined, each outlined by a rectangle.

Table II. Correlation of LOH on Chromosome 9 with Clinicopathological Parameters

	No. of samples	9p21 LOH(+)	9p21 LOH(−)	Statistical significance	9q22.3 LOH(+)	9q22.3 LOH(−)	Statistical significance	9q33 LOH(+)	9q33 LOH(−)	Statistical significance
t-factor										
t1	9	1	7		2	5		2	7	
t2	66	10	36	NS	15	43	NS	19	43	NS
t3	15	3	8		4	9		3	12	
Lymph node metastasis										
n (−)	35	2	21	NS	2	26	P=0.0032	4	29	P=0.0123
n (+)	55	13	32		19	31		20	33	
ER										
positive	34	7	21	NS	11	21	NS	10	21	NS
negative	35	4	20		6	23		10	24	
PgR										
positive	46	4	31	P=0.0196	12	30	NS	11	31	NS
negative	24	7	11		7	14		9	15	
Histological type										
1a; intraductal carcinoma	1	0	1		0	1		1	0	
a1; papillotubular carcinoma	15	2	9	NS	2	10	P=0.0343[a)]	3	12	NS
a2; solid-tubular carcinoma	34	5	19		6	24		7	26	
a3; scirrhous carcinoma	32	7	19		12	17		11	19	
	(Total 96)									

ER, estrogen receptor; PgR, progesterone receptor; NS, not significant.
a) a3 vs. others.

those seven, were critical in defining a different commonly deleted region at 9q33 within a 14-cM interval flanked by D9S113 and D9S158.

We attempted to correlate LOH at loci on chromosome 9 with clinical parameters (histological diagnosis, tnm classification, lymph node metastasis, and hormone receptor status) in all tumors for which clinicopathological data were available. Table II summarizes the results of these comparisons. While chromosome 9 loss had no particular association with tumor size, it did exhibit a significant association with lymph node metastasis. Of the 21 tumors that showed LOH at 9q22.3, 19 (90%) had metastasized to lymph nodes, whereas only 31 (54%) tumors of the 57 tumors that showed retention of both alleles at 9q22.3 had metastasized (P=0.0032). Similarly, of the 24 tumors that showed LOH at 9q33, 20 (83%) had metastasized to lymph nodes whereas only 33 (53%) of the 62 tumors that showed retention of both alleles at 9q33 had metastasized (P=0.012). We also identified a significant correlation between 9q allelic status and histological type; i.e., LOH at 9q22.3 was observed more frequently in scirrhous type (12/29, 41%) than in other types (8/43, 18%) (P=0.034). Also, a significant correlation was identified between 9p allelic status and hormonal status: of the 11 tumors that showed LOH at 9p21, 7 (63%) were PgR-negative, whereas only 11 (26%) tumors of the 42 tumors that showed retention of both alleles at 9p21 were PgR-negative (P=0.0196). No other regions on chromosome 9 showed a significant correlation with any other clinicopathological parameter examined.

DISCUSSION

In the present study we detected frequent LOH (39%) on chromosome 9 in a large panel of primary breast cancers, though LOH frequencies of less than 10% (3–8%) had been observed when the same panel was studied with markers from 1q21-32, 2q13-21, 19q13, 20p12-13, and other sites (unpublished data). Our observations with regard to chromosome 9 therefore reflect non-random genetic alterations associated with breast carcinogenesis. We constructed a high-resolution deletion map using 15 microsatellite markers along the entire length of this chromosome, and identified three distinct commonly deleted regions; 9p21, 9q22.3 and 9q33. When a particular type of cancer exhibits frequent LOH in a specific chromosomal region, one can infer that a tumor suppressor gene important in the genesis of that tumor is likely to be present there. This idea received experimental support when LOH studies revealed 5q21 and 17p13 as targets of frequent LOH in colon cancers[27] and further investigations led to identification of the *APC*[28] and *p53*[29] genes, respectively, as the mutated tumor suppressors in the regions indicated. The frequent allelic losses at multiple sites on chromosome 9 (9p21, 9q22.3 and 9q33) and on other chromosomes in studies of breast cancers reported by us and others[6–12, 26] have suggested that numerous mutant genes can participate in breast carcinogenesis.

The commonly deleted region we defined in a 9-cM interval at 9p21 contains the *p16* gene, a known tumor suppressor. LOH at 9p21-22 has been observed in 38–58% of breast cancers examined,[30–33] but is also frequent in cancers originating in a variety of tissues: nasopharyngeal carcinoma,[34] malignant mesothelioma,[35] bladder cancer,[36] renal cell carcinoma,[37] non-small cell lung cancer[38] and head and neck cancer.[39] Homozygous deletion of the *p16* gene has been observed in esophageal squamous cell carcinoma,[19] pancreatic adenocarcinoma,[20] squamous cell carcinoma of the bladder[21] and a small proportion of breast-cancer cell lines.[32] However, specific mutations of *p16* have been documented in only a few cases of sporadic breast cancer.[30, 40]

The commonly deleted region at the 17-cM interval on 9q22.3 contains the *PTC* gene. *PTC* was recently isolated as a predisposing gene for nevoid basal cell carcinoma (Gorlin) syndrome.[22] Somatic mutations and loss of this gene have also been found in some cutaneous basal cell carcinomas, medulloblastomas, and meningiomas, but seldom in breast cancers.[23]

Frequent LOH on 9q33 (14-cM) has not been described previously in breast cancers, although it has been observed in bladder cancer.[24] We believe this chromosomal region to be another locus containing a candidate gene for breast cancer. We further suggest that *p16*, *PTC*, and the locus on 9q33 are independent targets of allelic loss in breast cancer, and that these genes might play a role in the genesis of some proportion of breast cancers. However, since somatic mutation seldom occurs on the remaining copy of these genes, the classical "two hit" theory of Knudson defining tumor suppressor genes does not apply in these situations. Elucidation of mechanisms whereby these genetic alterations contribute to breast carcinogenesis awaits further investigation.

Ito *et al.*[12] previously showed associations of LOH at 17q21 and 17p13.3 with the loss of ER and/or PgR. In the present study, we detected an association between LOH at 9p21 and PgR-negative status. The presence of ER and PgR in breast cancers is a recognized indicator for responsiveness to hormone therapy: absence of these receptors usually predicts non-responsiveness or loss of hormone dependency.[41]

We identified a significant association of LOH at both 9q22.3 and 9q33 with lymph node metastasis, as well as an association between LOH at 9q22.3 and scirrhous histologic type. These data imply that alterations of one or more tumor suppressor genes at the 9q22.3 and 9q33 region play significant roles in the development and/or progression of breast cancer.

ACKNOWLEDGMENTS

This work was supported by a Special Grant-in-Aid for "Cancer Research" and for "Genome Science" and a "Gakujutsu-Frontier" Research Grant from the Ministry of Education, Science, Sports and Culture of Japan, a Research Grant for Cancer Research from the Ministry of Health and Welfare of Japan, and by Research Grants from the Ciba-Geigy Foundation (Japan) for the Promotion of Science and the Vehicle Racing Commemorative Foundation.

(Received May 19, 1998/Revised June 23, 1998/Accepted June 29, 1998)

REFERENCES

1) Emi, M., Miki, Y. and Nakamura, Y. Genetic alterations and diagnosis in human breast cancer. *Jpn. J. Hum. Genet.*, **42**, 34–35 (1997).
2) Nakamura, Y. Multi-step carcinogenesis of colorectal cancer. *Jpn. J. Hum. Genet.*, **38**, 23–24 (1993).
3) Nakamura, Y. Application of DNA markers to clinical genetics. *Jpn. J. Hum. Genet.*, **41**, 1–14 (1996).
4) Yanase, T. Human genetics: past, present, and future. *Jpn. J. Hum. Genet.*, **42**, 265–316 (1997).
5) Tsukamoto, K., Ito, N., Yoshimoto, M., Kasumi, F., Akiyama, F., Sakamoto, G., Nakamura, Y. and Emi, M. Allelic loss on chromosome 1p is associated with progression and lymph node metastasis of primary breast carcinoma. *Cancer*, **82**, 317–322 (1998).
6) Matsumoto, S., Kasumi, F., Sakamoto, G., Onda, M., Nakamura, Y. and Emi, M. Detailed deletion mapping of chromosome 3p in breast cancers: a 2-cM region on 3p14.3-21.1 and a 5-cM region on 3p24.3-25.1 commonly deleted in tumors. *Genes Chromosom. Cancer*, **20**, 268–274 (1997).
7) Kurose, K., Iida, A., Araki, T., Sakamoto, G., Kasumi, F., Nakamura, Y. and Emi, M. Frequent allelic loss at 7p14-15 associated with aggressive histologic types of breast cancer. *Jpn. J. Cancer Res.*, **89**, 533–538 (1998).
8) Nakata, T., Yoshimoto, M., Kasumi, F., Akiyama, F., Sakamoto, G., Nakamura, Y. and Emi, M. Identification of a new commonly deleted region within a 2-cM interval of 11p11 in breast cancers. *Eur. J. Cancer*, **34**, 417–421 (1998).
9) Tsukamoto, K., Ito, N., Yoshimoto, M., Iwase, T., Tada, T., Kasumi, F., Nakamura, Y. and Emi, M. Two distinct commonly deleted regions on chromosome 13q suggest involvement of BRCA2 and retinoblastoma genes in sporadic breast carcinomas. *Cancer*, **78**, 1929–1934 (1996).
10) Iida, A., Isobe, R., Yoshimoto, M., Kasumi, F., Nakamura, Y. and Emi, M. Localization of a breast cancer tumour-suppressor gene to a 3-cM interval within chromosomal region 16q22. *Br. J. Cancer*, **75**, 264–267 (1997).
11) Takita, K., Sato, T., Miyagi, M., Watatani, M., Akiyama, F., Sakamoto, G., Kasumi, F., Abe, R. and Nakamura, Y. Correlation of loss of alleles on the short arms of chromosomes 11 and 17 with metastasis of primary breast cancer to lymph nodes. *Cancer Res.*, **52**, 3914–3917 (1992).
12) Ito, I., Yoshimoto, M., Iwase, T., Watanabe, S., Katagiri, T., Harada, Y., Kasumi, F., Yasuda, S., Mitomi, T., Emi, M. and Nakamura, Y. Association of genetic alterations on chromosome 17 and loss of hormone receptors in breast cancer. *Br. J. Cancer*, **71**, 438–441 (1995).
13) Emi, M. and Nakamura, Y. Positional cloning of breast cancer-related gene. *Jpn. J. Hum. Genet.*, **39**, 30–31 (1994).
14) Yokota, T., Matsumoto, S., Yoshimoto, M., Kasumi, F., Akiyama, F., Sakamoto, G., Nakamura, Y. and Emi, M. Mapping of a breast cancer tumor suppressor gene locus to a 4-cM interval on chromosome 18q21. *Jpn. J. Cancer Res.*, **88**, 959–964 (1997).
15) Iida, A., Kurose, K., Isobe, R., Akiyama, F., Sakamoto, G., Yoshimoto, M., Kasumi, F., Nakamura, Y. and Emi, M. Mapping of a new target region of allelic loss to a 2-cM interval at 22q13.1 in primary breast cancer. *Genes Chromosom. Cancer*, **21**, 108–112 (1998).
16) Castellano, M. C., Pollock, P. M., Walters, M. K., Sparrow, L. E., Down, L. M., Gabrielli, B. G., Parsons, P. G. and Hayward, N. K. CDKN2A/P16 is inactivated in most melanoma cell lines. *Cancer Res.*, **57**, 4868–4875 (1997).
17) Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E. and Skolnick, M. H. A cell cycle regulator potentially involved in genesis of many tumor types. *Science*, **264**, 436–440 (1994).
18) Nobori, T., Miura, K., Wu, D. J., Lois, A., Takabayashi, K. and Carson, D. A. Deletions of the cyclin-dependent kinese-4 inhibitor gene in multiple human cancers. *Nature*, **368**, 753–756 (1994).
19) Mori, T., Miura, K., Aoki, T., Nishihira, T., Mori, S. and Nakamura, Y. Frequent somatic mutation of the MTS1/CDK4I (multiple tumor suppressor/cyclin-dependent kinase 4 inhibitor) gene in esophageal squamous cell carcinoma. *Cancer Res.*, **54**, 3396–3397 (1994).
20) Caldas, C., Hahn, S. A., da Costa, L. T., Redston, M. S., Schutte, M., Seymour, A. B., Weinstein, C. L., Hruban, R. H., Yeo, C. J. and Kern, S. E. Frequent somatic mutations and homozygous deletions of the p16 (MST1) gene in pancreatic adenocarcinoma. *Nat. Genet.*, **8**, 27–32 (1994).
21) Gonzalez-Zulueta, M., Shibata, A., Ohneseit, P. F., Spruck, C. H., III, Busch, C., Shamaa, M., Elbaz, M., Nichols, P. W., Gonzalgo, M. L., Malmastrom, P.-U. and Jones, P. A. High frequency of chromosome 9p allelic loss and CDKN2 tumor suppressor gene alterations in squamous cell carcinoma of the bladder. *J. Natl. Cancer Inst.*, **87**, 1383–1393 (1995).
22) Hahn, H., Wicking, C., Zaphiropoulos, P. G., Gailani, M. R., Shanley, S., Chidambaram, A., Vorechovsky, I., Holmberg, E., Unden, A. B., Gillies, S., Negus, K., Smyth,

I., Pressman, C., Leffell, D. J., Gerrard, B., Goldstein, A. M., Dean, M., Toftgard, R., Chenevix-Trench, G., Wainwright, B. and Bale, A. E. Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. *Cell*, **85**, 841–851 (1996).

23) Xie, J., Johnson, R. I., Zhang, X., Bare, J. W., Waldman, F. M., Cogen, P. H., Menon, A. G., Warren, R. S., Chen, L.-C., Scott, M. P. and Epstein, F. H., Jr. Mutations of the PATCHED gene in several types of sporadic extracutaneous tumors. *Cancer Res.*, **57**, 2369–2372 (1997).
24) Habuchi, T., Yoshida, O. and Knowles, M. A. A novel candidate tumour suppressor locus at 9q32-33 in bladder cancer: localization of the candidate region within a single 840 kb YAC. *Hum. Mol. Genet.*, **6**, 913–919 (1997).
25) Japanese Breast Cancer Society. The general rules for clinical and pathological recording of breast cancer. *Jpn. J. Surg.*, **19**, 612–632 (1989).
26) 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).
27) Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. and Bos, J. L. Genetic alterations during colorectal-tumor development. *N. Engl. J. Med.*, **319**, 525–532 (1988).
28) Kinzler, K. W., Nilbert, M. C., Su, L. K., Vogelstein, B., Bryan, T. M., Levy, D. B., Smith, K. J., Preisinger, A. C., Hedge, P., McKechnie, D., Finniear, R., Markham, A., Groffen, J., Boguski, M. S., Altschul, S. F., Horii, A., Ando, H., Miyoshi, Y., Miki, Y., Nishisho, I. and Nakamura, Y. Identification of FAP locus genes from chromosome 5q21. *Science*, **253**, 661–665 (1991).
29) Baker, S. J., Fearon, E. R., Nigro, J. M., van Tuinen, P., Ledbetter, D. H., Baker, D. F., Nakamura, Y., White, R. and Vogelstein, B. Chromosome 17 deletions and P53 gene mutations in colorectal carcinomas. *Science*, **244**, 217–221 (1989).
30) Brenner, A. J. and Aldaz, C. M. Chromosome 9q allelic loss and p16/CDKN2 in breast cancer and evidence of *p16* inactivation in immortal breast epithelial cells. *Cancer Res.*, **55**, 2892–2895 (1995).
31) Eiriksdottir, G., Sigurdsson, A., Jonasson, J. G., Agnarsson, B. A., Sigurdsson, H., Gudmundsson, J., Bergthorsson, J. T., Barkardottir, R. B., Egilsson, V. and Ingvarsson, S. Loss of heterozygosity on chromosome 9 in human breast cancer: association with clinical variables and genetic changes at other chromosome regions. *Int. J. Cancer*, **64**, 378–382 (1995).
32) An, H.-X., Niederacher, D., Picard, F., van Roeyen, C., Bender, H. G. and Beckmann, M. W. Frequent allele loss on 9p21-22 defines a smallest common region in vicinity of the CDKN2 gene in sporadic breast cancer. *Genes Chromosom. Cancer*, **17**, 14–20 (1996).
33) Wu, Y., Barnabas, N., Russo, I. H., Yang, X. and Russo, J. Microsatellite instability and loss of heterozygosity in chromosomes 9 and 16 in human breast epithelial cells transformed by chemical carcinogens. *Carcinogenesis*, **18**, 1069–1074 (1997).
34) Heung, D. P., Lo, K.-W., van Hasselt, C. A., Woo, J. K. S., Choi, P. H. K., Leung, S.-F., Cheung, S.-T., Cairns, P., Sidransky, D. and Lee, J. C. K. A region of homozygous deletion on chromosome 9p21-22 in primary nasopharyngeal carcinoma. *Cancer Res.*, **54**, 4003–4006 (1994).
35) Bjorkqvist, A.-M., Tammilehto, L., Anttili, S., Mattson, K. and Knuutila, S. Recurrent DNA copy number changes in 1q, 4q, 6q, 9p, 13q, 14q and 22q detected by comparative genomic hybridization in malignant mesothelioma. *Br. J. Cancer*, **75**, 523–527 (1997).
36) Williamson, M. P., Elder, A. P., Shaw, M. E., Devlin, J. and Knowles, M. A. P16 (CDKN2) is a major deletion target at 9p21 in bladder cancer. *Hum. Mol. Genet.*, **4**, 1569–1577 (1995).
37) Cairns, P., Tokino, K., Eby, Y. and Sidransky, D. Localization of tumor suppressor loci on chromosome 9 in primary human renal cell carcinomas. *Cancer Res.*, **55**, 224–227 (1995).
38) Merlo, A., Gabrielson, E., Askin, F. and Sidransky, D. Frequent loss of chromosome 9p in human primary non-small cell lung cancer. *Cancer Res.*, **54**, 640–642 (1994).
39) van der Reit, P., Nawroz, H., Hruban, R. H., Corio, R., Tokino, K., Koch, W. and Sidransky, D. Frequent loss of chromosome 9p21-22 early in head and neck cancer progression. *Cancer Res.*, **54**, 1156–1158 (1994).
40) Rush, E. B., Abouezzi, Z., Borgen, P. I. and Anelli, A. Analysis of MTS1/CDK4 in female breast carcinomas. *Cancer Lett.*, **89**, 223–226 (1995).
41) Horwitz, K. B. Mechanism of hormone resistance in breast cancer. *Breast Cancer Res. Treat.*, **26**, 119–130 (1993).