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Mapping of a Breast Cancer Tumor Suppressor Gene Locus to a 4-cM Interval on Chromosome 18q21

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***DPC4* and *DCC*, putative tumor suppressor genes implicated in the genesis of several types of human cancer, lie on the long arm of human chromosome 18. We examined 200 primary breast cancers for allelic losses on chromosome 18, using 15 microsatellite markers distributed along the long arm. Allelic loss was detected most frequently (29–30%) at loci mapped to 18q21. Deletion mapping of the 34 tumors showing partial or interstitial deletions identified a commonly deleted region within the 4-cM interval flanked by D18S474 and D18S487 at 18q21.1–q21.3. Although this interval included the *DPC4* and *DCC* genes, we excluded *DPC4* from candidacy when polymerase chain reaction-single-strand conformation polymorphism analysis of each exon failed to detect abnormalities in any of the 54 breast cancers that exhibited loss of heterozygosity involving 18q. Allelic loss on 18q was found more frequently in tumors of the solid tubular histological type (24 of 55, 44%) than in other types (24 of 113, 21%) ($P=0.0049$). The results suggest that a tumor suppressor gene located within the 4-cM region at 18q21, either *DCC* or another gene not yet identified, may play a role in the development of some sporadic breast cancers, particularly those of the solid tubular type.**

Key words: Breast cancer — Loss of heterozygosity — Tumor suppressor gene — Chromosome 18q

Carcinogenesis and progression of solid tumors in adults is considered to require a series of genetic alterations involving both dominant oncogenes and tumor suppressor genes. Loss of heterozygosity (LOH) is a common observation in regard to such tumors, and detection of frequent LOH in specific chromosomal regions has aided efforts to map and ultimately to identify several tumor suppressor genes including *RB, APC, VHL* and *NF2*.

The frequent LOH on chromosome arm 18q seen in a variety of human cancers suggests the presence of tumor suppressor genes on this chromosome arm. Observation of allelic deletions in a specific region of chromosome 18q in more than 70% of colorectal cancers[1] led to the isolation of a candidate tumor suppressor gene, "deleted in colorectal carcinoma" (*DCC*), from the 18q21.3 region. LOH in the portion of chromosome 18q that includes *DCC* has been reported in cancers of other types as well.[2–8] Recently, Hahn *et al.*[9] isolated a novel candidate tumor suppressor gene, *DPC4* ("deleted in pancreatic carcinoma locus 4"), from a region within 18q21.1 that was homozygously deleted in 30% of pancreatic cancers they examined. Among breast cancers the prevalence of LOH of chromosome 18q has been reported to range from 2.5% to 69%.[10–13] The differences among these reports are likely to be due to the fact that various markers from different regions of 18q were used to test LOH in each of these studies. To examine more closely the incidence of allelic loss on 18q in breast cancers and to determine the region(s) commonly deleted, we performed LOH analysis in 20 cases of breast cancer, using 15 microsatellite markers distributed along this chromosome arm. We also investigated the possible involvement of the *DPC4* gene in breast carcinogenesis, through mutational analysis in our panel of tumors.

MATERIALS AND METHODS

Samples and DNA preparation Tumors and corresponding noncancerous tissues were obtained at surgery from 200 patients being treated for primary breast cancers at the Cancer Institute Hospital. Tissues were frozen immediately and stored at −80°C. DNAs were extracted from frozen tissues according to methods described previously.[14]

LOH analysis DNAs were examined for LOH using the 15 polymorphic microsatellite markers along 18q reported in the CEPH/Généthon linkage map.[15] Their linear order on the chromosome is (cen.) –D18S56–D18S57–D18S450–D18S479–D18S474–DCC–D18S484–D18S487–D18S69–D18S64–D18S60–D18S55–D18S465–D18S61–D18S486–(tel.).[15,16]

Microsatellite polymorphisms were amplified by polymerase chain reaction (PCR) using 100 ng of genomic

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DNA, 30 m*M* Tris HCl (pH 8.8), 50 m*M* KCl, 2 m*M* $MgCl_2$, 5 m*M* 2-mercaptoethanol, 100 μM dNTPs, 1.6 pmol each of [γ-^{32}P]ATP-end-labeled primer and non-labeled primer, and 0.25 units 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 25–30 cycles of 94°C for 30 s, 57–62°C for 30 s, and 72°C for 30 s, with a final extension step of 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 2000 volts for 2–4 h. Gels were transferred to filter papers, dried at 80°C, and exposed to autoradiographic film at room temperature for 8 to 20 h.

PCR-single-strand conformation polymorphism (SSCP) analysis of the *DPC4* gene The *DPC4* gene was examined for sequence variations in all 54 tumors that showed

Table I. Frequencies of LOH at 15 Loci

Name	Total	Informative	LOH	LOH/Informative (%)
D18S56	94	58	12	21
D18S57	200	157	32	20
D18S450	200	129	29	23
D18S479	200	128	26	20
D18S474	200	69	14	20
DCC	200	139	41	30
D18S484	200	73	21	29
D18S487	200	133	36	27
D18S69	200	139	32	23
D18S64	200	143	29	20
D18S60	200	98	20	20
D18S55	148	109	18	17
D18S465	121	88	13	15
D18S61	121	88	8	9
D18S486	89	31	5	16
Total	200	200	54	27

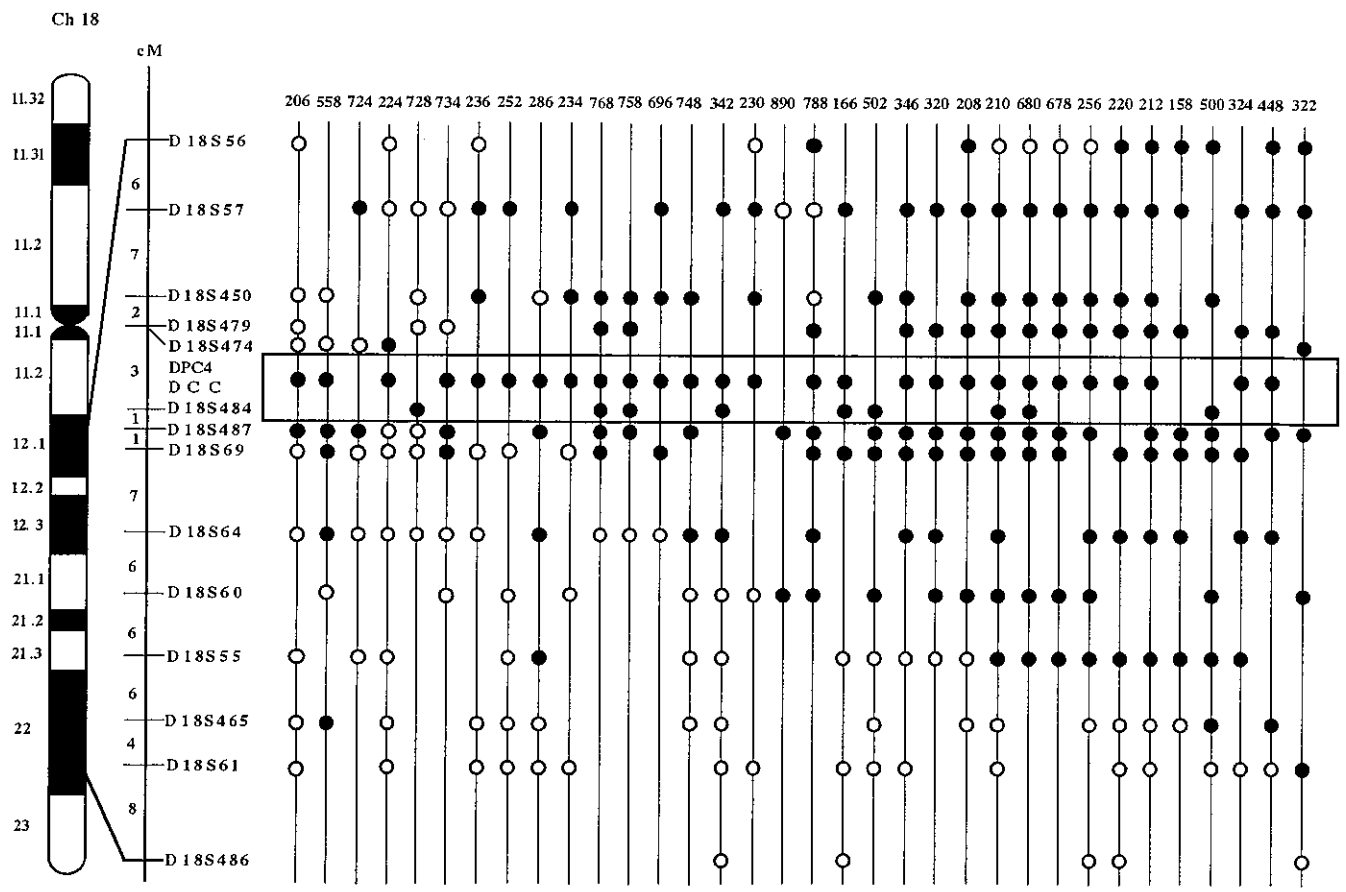

Fig. 1. Schematic representation of deletion mapping in 34 tumors that showed partial or interstitial deletions on chromosome 18q in primary breast cancers. At the far left is a diagram of chromosome 18 with the locations of the microsatellite markers. Numbers at the top of each map identify the individual tumors. Open circles, retention of heterozygosity; closed circles, LOH; no circle, uninformative locus. The region of deletion common to all 34 tumors is indicated by an enclosing rectangle. The location and genetic distance of the markers shown in the figure is based on the genetic map described by Eppert and others.[27]

LOH on 18q. PCR-SSCP analysis was performed for all coding exons (exon 1 through exon 10; codons 1 through 552). Our procedures for PCR-SSCP analysis have been described previously.[17]

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-tissue DNAs were compared to those of corresponding normal-tissue 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 samples were analyzed with markers for loci on other chromosomes.

Clinicopathological parameters The panel of parameters studied included the following: histologic type, tumor stage, tumor size, the presence of lymph node metastasis, and the number of lymph node metastases. Tumors were classified by pathologists into the following types, according to the histologic regional lymph nodes and distant metastases (TNM) classification and the histologic typing scheme of the Japanese Breast Cancer Society[18]: noninvasive tubular (1a), invasive papillotubular (a1), invasive solid tubular (a2), invasive scirrhous (a3); and other specific types of carcinoma (b group). This classification is essentially the same as the World Health Organization scheme for typing breast tumors. Estrogen receptor (ER) and progesterone (PgR) were measured by radioreceptor assay according to a standard dextran-coated charcoal method, using [^{125}I]estradiol as the labeled ligand on homogenates of fresh-frozen tissue (Otsuka Pharmaceutical, Tokushima). All samples containing >5 fmol of ER or PgR per mg protein were considered receptor positive. The χ^2 test and Fisher's exact test were used for statistical analysis of the results. One-tailed P values of less than 0.05 were considered statistically significant.

RESULTS

Our panel of 15 polymorphic markers on the long arm of chromosome 18 detected LOH in 54 (27%) of 200 breast tumors that were informative with at least one marker. The marker loci and their frequencies of LOH are listed in Table I in descending order from centromere to telomere, as determined by genetic linkage mapping.[15,16] Allelic loss was detected most frequently at DCC (30%, 41/139 cases) and D18S484 (29%, 21/73 cases), both of them located on 18q21. Among the 54 tumors with LOH, 20 showed LOH at all informative loci whereas the other 34 showed partial or interstitial deletions; Fig. 1 schematically shows the patterns of LOH in those tumors.

Representative autoradiograms of cases having deletions around the 18q21 region are shown in Fig. 2. Tumor 728 showed LOH at D18S484, but retention of alleles at D18S479 and D18S487. Tumors 206 and 558 retained alleles at D18S474, but showed LOH at *DCC* and D18S487. Analysis of LOH patterns in the 34 tumors shown in Fig. 1 revealed a single commonly deleted region in these tumors within the 4-cM interval flanked by markers D18S474 and D18S487, which lie in the 18q21.1–21.3 region.

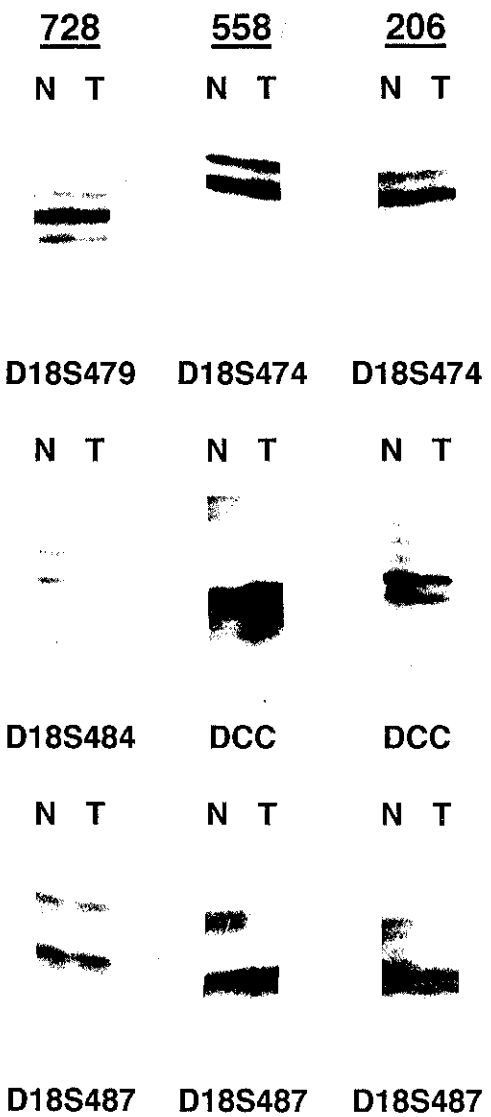

Fig. 2. Representative autoradiograms of breast tumors showing partial deletions on 18q21. Tumor numbers are shown above. N and T indicate matched DNA samples isolated from normal and tumor tissues, respectively.

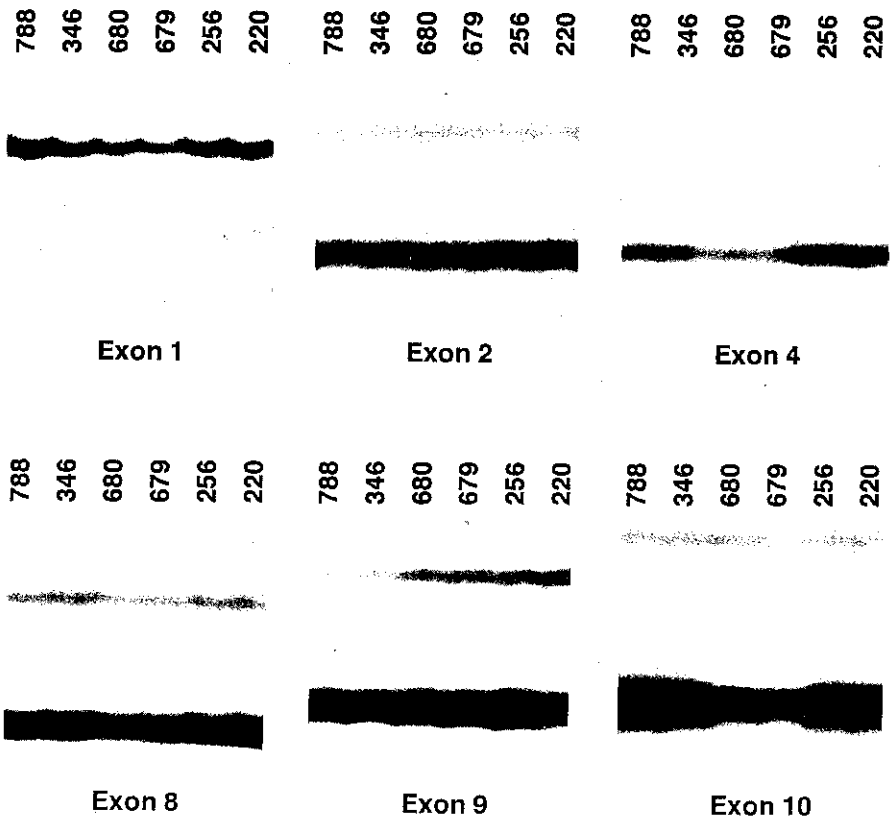

Fig. 3. Representative autoradiograms of PCR-SSCP analysis of all coding exons of the *DPC4* gene. SSCP patterns of exons 1, 2, 4, 8, 9 and 10 for each of six cases (788, 346, 680, 679, 256, 220) are shown. Tumor numbers are shown above. No bands indicating mobility shifts are present.

Table II. Correlation between LOH at 18q and Histologic Types in Sporadic Breast Cancers

		1a[a] intraductal carcinoma	a1[a] papillotubular carcinoma	a2[a] solid tubular carcinoma	a3[a] scirrhous carcinoma	Total
18q[b]	LOH	0	5	24	18	47
	Retention	6	34	31	55	126

a) Histologic classification of breast cancers according to criteria of the Japanese Breast Cancer Society.
b) a2 vs. others: $P=0.0049$ ($\chi^2=7.925$).
Of the total of 200 tumors we analyzed for 18q LOH, information on histologic types was available in 173 cases.

Since this map interval contains the *DPC4* locus, we examined by PCR-SSCP analysis all coding exons (exons 1–10) of *DPC4* in the 54 tumors that had shown LOH on 18q. We detected no abnormality of *DPC4* in any of these tumors; representative SSCP patterns are shown in Fig. 3.

Correlations between 18q allelic status and histological type among the breast cancers are shown in Table II. Allelic loss on 18q was found more frequently in tumors of the solid tubular histological type (24 of 55, 44%) than in other types (23 of 118, 20%) ($P=0.0049$). No significant correlation was observed between 18q loss and tumor stage, lymph node metastasis, or hormone-receptor status.

DISCUSSION

In this study, 54 of 200 (27%) sporadic breast cancers were found to have allelic loss on 18q at one or more of 15 polymorphic microsatellite loci. We constructed a detailed deletion map of 18q in the 34 tumors showing partial or interstitial deletions and identified a commonly deleted region 4-cM in length at 18q21.1–q21.3.

LOH on 18q21 has been reported in a variety of cancers, including 70% of colorectal carcinomas,[19] 60% of ovarian cancers,[2] 61% of gastric cancers,[20] 45% of prostatic cancers[4] and 60% of osteosarcomas examined.[8] Fearon *et al.* isolated the *DCC* gene on chromosome 18q21.3 as a candidate tumor suppressor gene for colorectal cancer.[1] Several deletion-mapping studies have indicated that this locus may be involved in other types of tumors as well.[4, 20–22] However, to date few point mutations in coding regions of *DCC* have been reported.[1, 23] Because the human *DCC* gene has at least 29 exons which span more than 1350 kb of genomic DNA, it is difficult to carry out a precise mutational analysis of this gene. Some investigators have observed frequent LOH at the *DCC* locus in breast cancers, but others have not confirmed such a finding.[11, 13, 14, 22] Recently, the *DPC4* gene was mapped to 18q21, in close proximity to *DCC*, and was identified as a candidate tumor suppressor for pancreatic cancer.[9] *DPC4* was homozygously deleted in about 30% of pancreatic cancers examined, and inactivating mutations accompanying the LOH were found in this gene. Although *DPC4* inactivation therefore appears to be prevalent among pancreatic cancers, it is uncommon in other tumors,[24–27] and Eppert *et al.* have identified yet another candidate tumor suppressor gene for colorectal cancers, *MADR2*, near *DPC4* at 18q21.[27]

The 4-cM region of 18q21 identified in the present study as being commonly deleted in breast cancers corresponds to the interval that contains *DCC* and *DPC4*, but the *MADR2* gene lies outside it. Our results indicated that *DCC* or *DPC4*, or both, might be targets of allelic loss and that their inactivation could be involved in the carcinogenesis of some sporadic breast cancers. However, we found no mutations in the *DPC4* gene by PCR-SSCP analysis of all coding exons. Thus, it is unlikely that *DPC4* plays any significant role in breast carcinogenesis. Detailed analysis of the *DCC* gene has been hampered by its huge size and complexity.

In the present study, LOH on 18q was observed equally frequently in small tumors and tumors without lymph node metastasis, as well as in tumors of more advanced phenotype. These data are consistent with the notion that LOH on 18q is an early event in breast carcinogenesis. We did not observe a significant association between 18q LOH and 16q LOH in our panel of breast cancers in a χ^2 analysis ($P > 0.5$). We identified a significant association between LOH at 18q and breast tumors of the solid tubular histologic type. Previously, we had shown an association of LOH at 17q with breast tumors of this type.[28] The association of allelic losses at 18q and 17q with solid tubular breast tumors indicates that loss or inactivation of tumor suppressor genes present on these two chromosomal arms may affect carcinogenetic mechanisms in a manner specific to histologic type.

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