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Distal Deletion of Chromosome 1p in Ductal Carcinoma of the Breast

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Summary

By use of recombinant DNA probes that correspond to genetic loci residing on human chromosome 1, DNA samples from 37 ductal breast carcinomas and constitutional DNA from the same individuals were tested for loss of heterozygosity. A high frequency (41%) of reduction to homozygosity was detected with the probe p1–79, which recognizes the highly polymorphic locus D1Z2, localized on 1p36. Loss of heterozygosity at other chromosome 1 loci was much less common, not exceeding a frequency of 10%, and was never observed in the absence of the D1Z2 loss. Somatic loss of heterozygosity at D1Z2 was more frequent in patients with a strong family history of breast cancer (60%), in patients with early diagnosis (before 45 years of age) (70%), and in those with multiple tumors or tumor foci (50%) than in patients with none of the characteristics of hereditary tumors (21%). No associations were observed between loss of heterozygosity and prognostic factors. These results suggest that inactivation of a tumor suppressor gene located on the distal portion of chromosome 1p, alone or combined with other genetic changes, may represent a fundamental step in the pathogenesis of ductal carcinoma of the breast.

Introduction

The inactivation of tumor suppressor genes has been implicated in the development of several kinds of cancers (Nordenskjold and Cavenee 1988), whose prototype is retinoblastoma. This rare pediatric tumor can occur in either a hereditary or a sporadic form. In the hereditary form, all cells in the affected individual carry the same constitutional mutation of the RB1 gene, which maps to human chromosome 13q14; in the sporadic form two or more mutational events take place in somatic cells. In both situations, two hits are thought to be required for tumor development, as originally proposed by Knudson (1971). On the basis of knowledge of its location, the RB1 gene has been cloned (Friend et al. 1987; Lee et al. 1987), thus allowing direct investigation of its structure and function in retinoblastoma and other tumors (Fung et al. 1987; Lee et al. 1988; Dunn et al. 1988; T'Ang et al. 1988; Weichselbaum et al. 1988). Because the sequence of other tumor suppressor genes is unknown, the identification of chromosomal regions where these are located relies on the detection of somatic allele loss in the tumors. This approach, combined with linkage analysis, has contributed to the localization of other cancer-predisposing genes, such as those responsible for multiple endocrine neoplasia type 1 (MEN-1) (Larsson et al. 1988) and type 2A (MEN-2A) (Mathew et al. 1987*a;* Simpson et al. 1987), familial polyposis of the colon (Bodmer et al. 1987), von Hippel-Lindau disease (Seizinger et al. 1988), and neurofibromatosis type 2 (Rouleau et al. 1987).

The model of two-step tumorigenesis could also apply to most common adult tumors, especially to those showing a genetic component. Breast cancer represents a good candidate for such investigation, as approximately 4%–9% of the total cases appear to be inherited via an autosomal dominant mechanism (Lynch and Lynch 1986; Newman et al. 1988). The hereditary form

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of breast cancer shares several features with other kinds of hereditary tumors. Tumors are frequently bilateral and multifocal; they tend to occur in premenopausal women (while the overall incidence of breast cancer shows a peak in postmenopausal age); and male relatives in high-risk families are more often affected than are males in the general population (Anderson 1977; Grossbart Schwartz et al. 1985; Lynch and Lynch 1986). Like embryonal tumors, the sporadic forms of breast cancer might be related to somatic inactivation of the same loci involved in hereditary predisposition.

Loss of heterozygosity for sequences lying on chromosomes 13q and 11p has been detected in breast cancer. Although loss of 11p appears to be related to tumor progression (Ali et al. 1987), loss of 13q alleles was found in 40% of premenopausal ductal carcinomas (Lundberg et al. 1987). Subsequent studies have identified deletions of the RB1 gene or absence of its product in approximately 20% of unselected breast carcinomas (Lee et al. 1988; T'Ang et al. 1988). Mutations in a gene residing on chromosome 13, a gene that possibly is the RB1 gene itself, could thus account for at least a portion of breast cancers.

Cytogenic studies have shown that structural rearrangements, including deletions and duplications, of chromosome 1 are the most common aberrations in breast carcinoma (Kovacs 1978; Pathak 1980; Rodgers et al. 1984). Preliminary results obtained by linkage analysis have provided weak but suggestive evidence of linkage between a hereditary form of breast cancer and the RH locus on chromosome 1p (maximum lod score 2.28 at $\theta = 0$) (Anderson et al. 1985). On the basis of this preliminary evidence, we have tested a set of breast carcinomas for loss of heterozygosity at loci on chromosome 1. Here we present evidence for the presence of a suppressor gene for ductal carcinoma in the proximity of the telomere of 1p.

Material and Methods

Breast-tumor and normal breast tissue samples were obtained from 37 patients undergoing surgery. The samples were either processed immediately or after storage at −70°C for 1 wk–1 year. Whenever normal breast tissue was unavailable or the amount of DNA extracted was insufficient, peripheral blood leukocytes were used as a source of constitutional DNA. High-molecular-weight DNA was isolated according to a method described elsewhere (Dao et al. 1987). After digestion with the appropriate restriction endonucleases and fractionation on 0.7% agarose gels, DNA was transferred to nylon membranes (Zetabind;™ Biorad) following the manufacturer's recommendations and then was hybridized to DNA probes labeled with ^{32}P according to the random primer technique (Feinberg and Vogelstein 1983).

The following chromosome 1–specific DNA probes were used for this study: Probe pAT3, corresponding to the antithrombin III locus (AT3) on 1q23, recognizes a *Pst*I RFLP (Prochownick et al. 1983). Probe phβN8C6, recognizing the beta-nerve growth factor locus (NGFB) on 1p13–1p22.1, identifies a *Bgl*II polymorphism (Ullrich et al. 1983; Darby et al. 1985). Probe pLmyc10 identifies the locus coding for the L-*myc* proto-oncogene (MYCL) on 1p32 and is polymorphic for *Eco*RI (Nau et al. 1985). Probe AF3 (Lowler et al. 1986) recognizes a *Pvu*II polymorphism pertaining to the alpha-L-fucosidase gene (locus FUCA1) mapped to 1p34. Probe p1–79 provides a fingerprint pattern, with the number of bands varying between 25 and 40, after digestion with several restriction enzymes (*Msp*I, *Pst*I, *Pvu*II, and *Taq*I were used for this study). The high interindividual variability of the corresponding locus D1Z2, which is located on 1p36, is related to the presence of different copy numbers of a core sequence made of 39–40 bp (Buroker et al. 1987; Nakamura et al. 1987). In situ hybridization of p1-79 showed that the loci of hybridization are clustered on human chromosome band 1p36; localization of all *Taq*I fragments to chromosome 1 was confirmed with a human-rodent somatic cell hybrid panel (Buroker et al. 1987).

Genetic linkage maps of human chromosome 1 (O'Connell et al. 1989) and 1p (Cracopoli et al. 1988) were recently published. The availability of these linkage maps should facilitate isolation of genes involved in autosomal genetic diseases.

The mechanisms underlying the loss of specific alleles (simple loss or loss associated with reduplication of the other allele) were investigated by scanning densitometry. First, normalization for DNA content of each lane was obtained by comparing the relative signal intensity produced by constant bands at D1Z2 and a control locus. In those cases in which allele loss was confined to D1Z2, controls were represented by other chromosome 1 loci that had maintained heterozygosity. Alternatively, the filters were rehybridized to the nonpolymorphic probe p7–2D-1446 for the HLA class II–associated invariant chain (locus DHLAG), which maps to 5q32 (Genuardi and Saunders 1988). Then, the ratio between normalized signals in tumor and constitutional DNA was calculated.

The 5q32 probe served as a control in these experi-

ments in that its copy number did not vary in any of the samples examined here. Further, the AT3 probe provided a good marker for the long arm of chromosome 1, thus restricting the chromosomal changes involved to chromosome 1p.

Results

Molecular Studies

Genotype analysis at loci on chromosome 1 was performed on DNA from 37 ductal breast carcinomas and their normal counterparts. The results are displayed in table 1. Thirty-one samples were informative for at least one of the loci tested for classical RFLPs (AT3, NGFB, MYCL, and AF3). Among these, three (9.7%) showed loss of heterozygosity at one or more loci.

Probe p1–79 identifies a deletion/insertion-type polymorphism at locus D1Z2. When using this probe to test DNA from 91 individuals, we never found the same pattern of autoradiographic bands in unrelated subjects. However, in two siblings we found the same haplotypes as in their parents. We therefore considered every sample as informative at locus D1Z2. In the absence of family data it is virtually impossible to distinguish among subloci defined by allelic fragments at D1Z2, even though some bands can be scored as homozygous, because they appear to be invariant. Loss of heterozygosity at D1Z2 thus involves multiple bands, corresponding to the loss of a whole haplotype. As expected, a reduction in signal intensity in the tumor DNA relative to constitutional DNA, indicative of loss of heterozygosity, always concerned at least two bands. Haplotype loss, most commonly displayed by loss of three to five bands (fig. 1), occurred in 15/37 (41%) of the samples.

To confirm that the lower intensity of the autoradiographic signal was caused by the absence of DNA sequences in the tumor rather than by an artifact consequent to unequal loss of DNA from the filters, hybridization to p1–79 was repeated in each case after digestion with a different restriction endonuclease. The results were reproducible in that a comparable signal reduction was observed when a different enzyme was used to restrict DNA. In no instance did the bands disappear completely; a faint residual signal was observed, presumably the result of presence of contaminating normal DNA from stromal cells and/or infiltrating leukocytes. However, the possibility of tumor heterogeneity, with presence of a neoplastic population carrying normal chromosome 1 content, cannot be ruled out.

Table 1

Loss of Heterozygosity at Loci on Chromosome 1 in Ductal Breast Carcinoma

	Locus				
Patient	AT3	NGFB	MYCL	AF3	D1Z2
DA-2	NI	NI	NI	NI	LH
DA-3	NI	RH	NI	RH	LH
DA-4	NI	LH	LH	NI	LH
DA-5	NI	NI	NI	NI	LH
DA-6	NI	NI	RH	RH	LH
DA-7	NI	RH	RH	RH	RH
DA-8	NI	RH	NI	NI	LH
DA-9	NI	RH	RH	NI	RH
DA-10	NI	NI	NI	RH	RH
DA-11	RH	LH	LH	NI	LH
DA-12	NI	NI	NI	RH	LH
DA-13	NI	NI	RH	NI	LH
DA-14	NI	RH	NI	NI	RH
DA-15	RH	NI	NI	NI	RH
DA-16	NI	NI	NI	RH	RH
DA-17	RH	RH	NI	NI	RH
DA-19	NI	RH	NI	NI	RH
DA-20	RH	RH	NI	NI	RH
DA-21	RH	NI	RH	RH	RH
DA-22	RH	NI	NI	NI	LH
DA-23	RH	NI	NI	NI	RH
DA-24	RH	NI	NI	NI	LH
DA-25	RH	RH	RH	NI	RH
DA-26	NI	NI	RH	NI	RH
DA-27	RH	NI	RH	NI	RH
DA-28	NI	RH	RH	NI	LH
DA-29	RH	NI	RH	NI	LH
DA-30	LH	NI	RH	NI	LH
DA-31	NI	NI	NI	NI	RH
DA-32	NI	NI	RH	RH	RH
DA-33	NI	NI	NI	NI	RH
DA-34	RH	NI	RH	NI	RH
DA-36	NI	RH	NI	RH	RH
DA-37	RH	NI	NI	NI	LH
DA-38	NI	NI	RH	RH	RH
DA-39	NI	NI	RH	NI	RH
DA-41	NI	NI	NI	NI	RH

Note.—NI = not informative; RH = retention of heterozygosity; LH = loss of heterozygosity.

Estimation of allele copy number by quantitative densitometry showed that in every case the loss of heterozygosity on chromosome 1p was caused by a simple deletion, and only one copy of the remaining allele was present in tumor DNA (table 2). In patient DA-30 separate portions of chromosome 1 had undergone distinct rearrangements. A D1Z2 haplotype was deleted in the tumor, and the same locus on the other chromosome was not duplicated. On the other hand, loss of

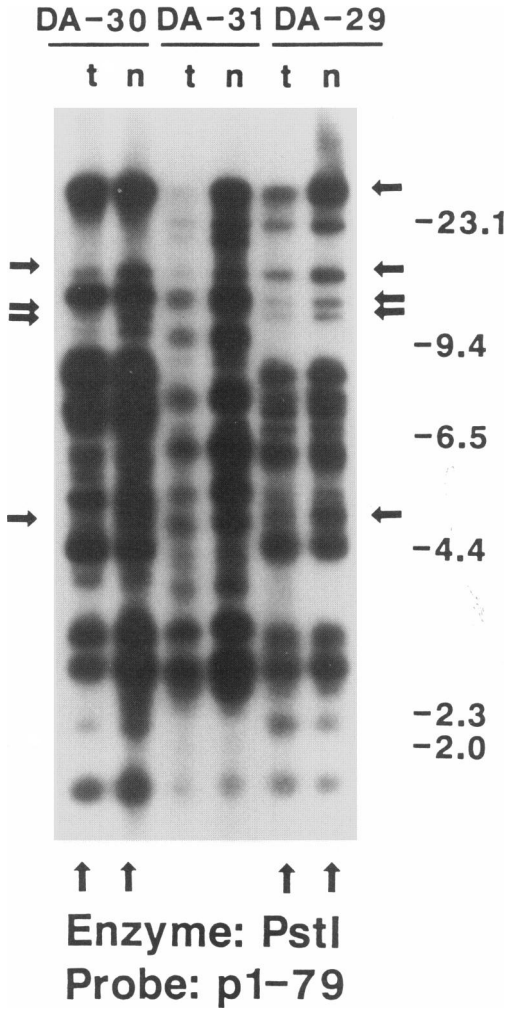

Figure 1 Autoradiogram of genomic DNA samples hybridized to probe p1–79 after digestion with *Pst*I. Molecular-weight-standard fragments are shown by the position of the numbers on the right. Lower arrows indicate lanes (n = normal; t = tumor) containing the two samples showing loss of heterozygosity. Arrows on the right point to the bands lost in patient DA-29, while arrows on the left indicate bands lost in patient DA-30.

the 5- and 5.5-kb bands at locus AT3 (fig. 2) was accompanied by duplication of the 10.5-kb band, although loss of heterozygosity was not found at locus MYCL. Thus, the long arm of chromosome 1—or at least of band 1q23, where AT3 is localized—was present in two identical copies, whereas the tip of the short arm was deleted from one homologue and the intermediate portion that comprises the locus MYCL was unmodified.

In two other cases (DA-4 and DA-11) loss of heterozygosity at multiple loci (NGFB, MYCL, and D1Z2)

Table 2

Quantitative Densitometry of Autoradiographic Signals from Constitutional (C) and Tumor (T) DNA

Patient	Deleted Locus/Central Locus	T	C	T/C
DA-2	D1Z2/FUCA1	1.46	2.53	.58
DA-3	D1Z2/FUCA1	1.81	3.07	.59
DA-4	D1Z2/DHLAG	1.12	2.08	.54
DA-5	D1Z2/FUCA1	1.70	2.93	.58
DA-6	D1Z2/FUCA1	2.05	3.67	.56
DA-8	D1Z2/FUCA1	1.63	2.54	.64
DA-11	D1Z2/DHLAG	2.11	3.98	.53
DA-12	D1Z2/FUCA1	1.17	2.36	.50
DA-13	D1Z2/FUCA1	1.59	2.89	.55
DA-14	D1Z2/FUCA1	1.29	2.64	.48
DA-22	D1Z2/AT3	2.25	3.97	.57
DA-28	D1Z2/AT3	1.99	3.57	.56
DA-29	D1Z2/AT3	2.57	4.82	.53
DA-30	D1Z2/DHLAG	2.11	3.98	.53
DA-30	AT3/DHLAG	1.15	1.03	1.12
DA-37	D1Z2/AT3	1.59	3.43	.46

on chromosome 1 was not associated with reduplication. An entire chromosome 1 homologue was presumably lost in patient DA-4, while in patient DA-11 the deletion spanned a considerable portion of 1p.

Correlations with Features of Hereditary Breast Cancer

Data concerning age at onset, bilaterality and tumor multiplicity, and familial occurrence of breast cancer are summarized in table 3. An impressive familial clustering of breast cancer was found in three cases. Breast cancer occurred in five relatives (one sister and four paternal relatives) of patient DA-5, in four relatives (one sister and three paternal relatives) of patient DA-8, and in three relatives (one sister and two paternal relatives) of patient DA-13, suggesting the segregation of a cancer-predisposing mutation in these three families. Hereditary transmission through an unaffected carrier father is likely for patient DA-37 because, in this case, two features of the hereditary form—i.e., early age at onset (33 years) and breast cancer in two paternal aunts—were combined.

Other familial occurrences of breast cancer involving one first-degree relative and/or more distantly related individuals were observed in eight additional patients; however, no evidence was available to establish whether these occurrences were random or due to environmental or genetic factors. It has been estimated that more than 8% of breast cancer patients have one affected first-degree relative (Newman et al. 1988) and that 23% have one or more first- or second-degree affected rela-

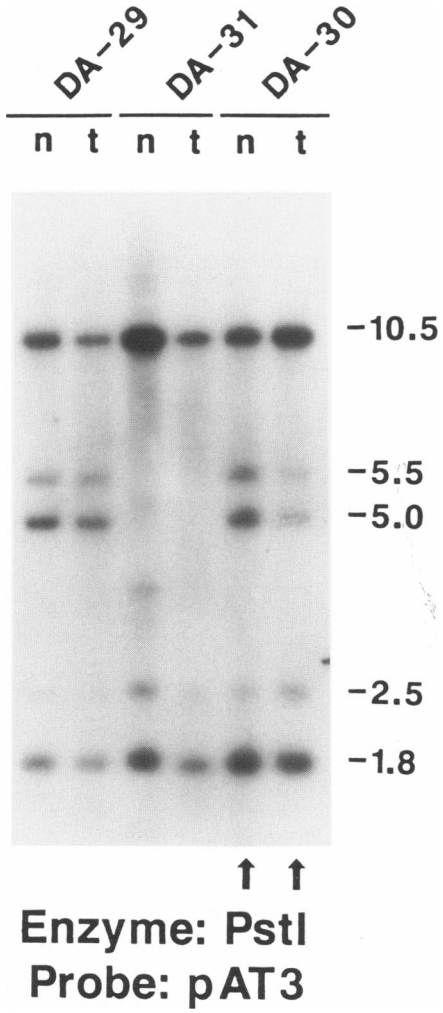

Figure 2 Filter shown in fig. 1, washed to remove residual signal and then rehybridized to probe pAT3. Polymorphic alleles correspond to the 10.5-kb band and to the 5.0- and 5.5-kb bands because of the presence of an internal *PstI* restriction site in the 10.5-kb fragment. Patient DA-30 shows loss of heterozygosity, as documented by the reduced intensity of the 5.0- and 5.5-kb bands.

tives who do not show a clear vertical transmission pattern (Lynch and Lynch 1986). Chance is the most likely explanation for these types of familial breast cancer occurrences.

Loss of heterozygosity at D1Z2 was found in three (60%) of five patients with a family history in a first-degree or in a first- and second-degree relative, in two (28%) of seven patients with a family history in a second-degree relative, and in 10 (40%) of 25 patients with no family history. Tumors in 10 patients (DA-3, DA-5, DA-6, DA-9, DA-11, DA-19, DA-24, DA-29, DA-37, and DA-38) arose prior to age 45 years. Loss of heterozygosity was present in seven (70%) of these samples, in three (43%) of seven with diagnoses between 46 and 49 years of age, and in five (25%) of 20 with diagnoses at 50 years of age or older. Thus, loss of heterozygosity was more than twice as frequent for tumors developing before age 50 years as it was for tumors developing after age 50 years, although this difference was not statistically significant ($P > .05$). With regard to tumor multiplicity, loss of heterozygosity was observed in four (50%) of eight cases with bilateral or multifocal cancer, compared with 11 (38%) of 29 cases with no evidence of multiplicity. Samples were also divided into two groups, sporadic and presumptively hereditary, the latter defined by the presence of at least one hereditary characteristic, e.g., a strong family history, diagnosis before age 45 years, or multiple tumors. The incidence of 1p deletion was higher in the hereditary category (61%, vs. 21% in the sporadic group), a significant difference ($P < .05$).

Prognostic Factors

Loss of heterozygosity at D1Z2 was also evaluated for associations with prognostic factors, including TNM stage, tumor grade, number of positive nodes, and estrogen and progesterone receptor status. No significant associations were detected for any of these.

Discussion

The data reported in this study suggest that a critical region located in proximity to the telomere of the short arm of chromosome 1 contains a DNA sequence that contributes to the development of ductal breast carcinoma. That reduction to homozygosity on band 1p36 is a specific event is confirmed by the low frequency of allele loss at other loci on chromosome 1. Moreover, deletions affecting these other loci were always concomitant with absence of a D1Z2 haplotype, which suggests that the putative suppressor gene lies close to the telomere of 1p and distal to 1p34. Furthermore, we have recently found 65% loss of heterozygosity for a variable-number-of-tandem-repeats probe (pYNZ2) in this region (Mars et al., in press).

The genomic organization of locus D1Z2 is similar to that of minisatellites (Jeffreys et al. 1985). Both contain a variable number of short tandem repeats, which are responsible for the high degree of polymorphism of these loci. As D1Z2 spans a considerably larger portion (250–500 kb) of DNA, the term *midisatellite* has been coined for this kind of chromosome-specific re-

Table 3

Features of Hereditary Breast Cancer in the Present Series

Patient	Age (years)	Multiple Tumors[a]	Relatives with Breast Cancer
DA-2	48	Y	...
DA-3	39	Y	...
DA-4	46	N	...
DA-5	34	Y	Sister, paternal aunt, paternal grandmother, two paternal cousins
DA-6	40	N	Maternal great-aunt
DA-7	57	N	...
DA-8	46	N	Sister, paternal aunt, two paternal cousins
DA-9	41	N	...
DA-10	56	N	...
DA-11	43	N	...
DA-12	51	N	...
DA-13	52	N	Sister, paternal niece, paternal cousin
DA-14	69	N	...
DA-15	48	Y	Maternal aunt
DA-16	46	N	...
DA-17	59	N	...
DA-19	37	N	...
DA-20	64	N	...
DA-21	62	Y	...
DA-22	54	N	...
DA-23	68	Y	Sister
DA-24	42	N	...
DA-25	54	N	...
DA-26	46	N	Mother, cousin
DA-27	58	N	Aunt
DA-28	50	Y	...
DA-29	43	N	...
DA-30	54	N	...
DA-31	67	N	...
DA-32	61	N	...
DA-33	61	N	Maternal grandmother
DA-34	55	N	...
DA-36	56	N	Paternal aunt
DA-37	32	N	Two paternal aunts
DA-38	36	Y	...
DA-39	52	N	...
DA-41	48	N	Maternal grandmother

[a] Y = presence of bilateral, multiple tumors or multiple tumor foci; N = no multiple tumors or tumor foci.

peat (Nakamura et al. 1987). The function of repetitive DNA, if any is present, is unknown. The structural organization of minisatellites suggests that these might act as recombinatory signals. This hypothesis is supported by their sequence similarity to the lambda "χ" sequence (Jeffreys et al. 1985), which promotes recombination in phage. In fact, a higher than expected rate of meiotic recombination at minisatellite loci has been found in both mouse and human species (Jeffreys et al. 1987, 1988). Whether the increased recombination frequency is part of the intrinsic properties of these "χ-like" sequences or random evolutionary events that have favored the clustering of tandem repeats, the final effect is the same; that is, there are hot spots of recombination in the genome. As midisatellites contain a higher number of repeat units than do minisatellites, they are conceivably more liable to undergo nonhomologous pairing leading to deletion/duplication events,

which could take place in somatic as well as in germinal cells. Somatic recombination events occurring in the surroundings of specific chromosome regions have been documented in several tumors, and they have contributed to the precise localization of cancer recessive genes, as in the case of rhabdomyosarcoma (Scrable et al. 1987). The presence of a midisatellite-type sequence in proximity to a tumor suppressor gene could increase the chances of unequal recombination and thus be partly responsible for tumorigenesis, as it would predispose to those rearrangements postulated by the two-hit hypothesis. Stoler and Bouck (1985) obtained evidence for the presence of a tumor suppressor gene on chromosome 1.

Deletions in band 1p36 were found both in sporadic tumors and in tumors showing some characteristics of hereditary breast cancer, with a higher incidence in the latter group. An analogous situation has been observed for chromosome 13. Lundberg et al. (1987) found that, for breast tumors developing at premenopausal ages, a 40% frequency of reduction to homozygosity at chromosome 13 loci occurred. Other investigators have analyzed the structure of the RB1 locus in unselected breast tumor specimens (Lee et al. 1988; T'Ang et al. 1988). The frequency of deletions and other rearrangements did not exceed 20% in these reports. A higher incidence of structural changes was observed only in breast carcinoma cell lines (T'Ang et al. 1988), which suggests that inactivation of the RB1 gene product can represent, at least in some cases, a secondary event favoring the establishment of cell lines.

The findings of specific loss of heterozygosity on both chromosome 1 and chromosome 13 are only apparently contradictory. Breast cancer is not a homogeneous disease, and, presumably, the maturation of breast epithelium is not under the control of a single gene. Different forms of ductal carcinoma could be caused by separate genetic mechanisms or, alternatively, by a combination of changes at different chromosomal loci, including, but not necessarily in all cases, those residing on chromosomes 1 and 13.

Structural rearrangements and amplification of dominantly acting proto-oncogenes are frequent in breast cancer (Escot et al. 1986; Slamon et al. 1987; Morse et al. 1988) and have been implicated more commonly in the progression to more advanced stages of disease. However, it cannot be ruled out that these alterations play a primary role when associated with other genetic lesions. Since structural changes affecting the proto-oncogenes take place only in somatic cells, one would expect a higher frequency of this kind of genetic lesion in sporadic tumors, while tumor suppressor genes are more likely to be involved in the hereditary forms, thus explaining the apparent discrepancy between the findings for hereditary and sporadic tumors.

The distal portion of chromosome 1p has been implicated in the pathogenesis of neuroblastoma (Gilbert et al. 1984), familial melanoma (Greene et al. 1983), and MEN2 (Mathew et al. 1987*b*). Deletions of this chromosome region have been detected with high (50%) frequency in two tumor types—medullary carcinoma of the thyroid and pheochromocytoma—which arise in individuals affected by MEN2 (Mathew et al. 1987*b*); the MEN2 tumor–predisposing locus has been mapped to chromosome 10 by linkage analysis (Mathew et al. 1987*a;* Simpson et al. 1987). These observations suggest that mutations affecting tumor suppressor genes can have pleiotropic effects, depending on the target tissue and on the concomitance of other DNA alterations. Likewise, recent data provide evidence against linkage between hereditary Wilms tumor (WT) and loci residing on 11p13 (Grundy et al. 1988; Huff et al. 1988), the band at which the WT gene had been located on the basis of cytogenetic and molecular data. Loss of heterozygosity in colorectal carcinoma often occurs concomitantly at loci on different chromosomes (Law et al. 1988); however, the gene for familial polyposis of the colon appears to reside on the long arm of chromosome 5 (Bodmer et al. 1987). Loss of chromosomal heterozygosity has also been observed for chromosomes 6, 13, 15, and 17 in osteosarcoma (Toguchida et al. 1988) and for chromosomes 3, 13, and 17 in small-cell lung carcinoma (Yokota et al. 1987), whereas the genes primarily involved in the pathogenesis of these tumors had been mapped to chromosomes 13 and 3, respectively.

Whether a similar situation applies to breast cancer has yet to be demonstrated, since no convincing evidence of linkage between a hereditary breast cancer and a chromosomal locus has been found. The evidence collected to date is weak (Anderson et al. 1985) or inconclusive (Bishop et al. 1988). A further complication could derive from genetic heterogeneity between high-risk families. This problem can only be solved by studying extended pedigrees. Molecular detection of chromosome deletions can help in this regard by pointing to candidate regions for analysis and thus prevent a random, time-consuming search performed with markers covering much of the genome.

Finally, for the detection of cancer recessive genes, molecular genetic analysis applied to fresh tumor specimens appears to carry several advantages over cyto-

genetic investigation. Owing to the low mitotic index and to the poor quality of the methaphase spreads, cytogenetic studies have often been performed on established cell lines or after medium-term culture. This may not reflect the in vivo situation. In fact, discrepancies between molecular and cytogenetic data have been observed (Seizinger et al. 1987). On the contrary, as the data here reported concern only uncultured specimens, the specific 1p deletion cannot be attributed to the influence of in vitro conditions. Therefore, a comparative search for similar lesions in fresh and cultured tumor cells will contribute to the definition of the sequence of genetic events involved in the multistep process of breast carcinogenesis. Such an approach could provide hints for the identification of the loci responsible for hereditary predisposition and, consequently, useful grounds for primary prevention and early diagnosis.

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