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Genetics

Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13

(carcinogenesis/mapping/somatic mutations/DNA polymorphisms)

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ABSTRACT **The genotypes at chromosomal loci defined by recombinant DNA probes revealing restriction fragment length polymorphisms were determined in constitutional and tumor tissue from 10 cases of ductal breast cancer: eight premenopausal females and two males. Somatic loss of constitutional heterozygosity was observed at loci on chromosome 13 in primary tumor tissue from three females and one male. In two cases, specific loss of heterozygosity at three distinct genetic loci along the length of the chromosome was observed. In another case, concurrent loss of alleles at loci on chromosomes 2, 13, 14, and 20 was detected, whereas a fourth case showed loss of heterozygosity for chromosomes 5 and 13. In each instance, the data were consistent with loss of one of the homologous chromosomes by mitotic nondisjunction. Analysis of loci on several other chromosomes showed retention of constitutional heterozygosity suggesting the relative specificity of the events. In contrast, similar analyses of other breast cancers, including comedocarcinoma, medullary carcinoma, and juvenile secretory carcinoma, showed no loss of alleles at loci on chromosome 13. These data indicate that the pathogenesis of ductal breast cancer may, in a substantial proportion of cases, involve unmasking of a recessive locus on chromosome 13 and suggest the involvement of such a locus in heritable forms of this disease.**

Breast cancer is the most frequent malignant tumor in females. Although most cases are sporadic, a familial aggregation is sometimes found; about 5% of all cases are estimated to be hereditary with an autosomal dominant mode of inheritance. These latter cases demonstrate a significantly earlier age of onset, an excess of multiple primary tumors, a longer life expectancy, and a greater proportion of affected males than the sporadic cases (1). A familial association between breast cancer and other histologically distinct tumors has been noted analogous to observations with embryonal developmental tumors such as retinoblastoma, Wilms tumor, and neuroblastoma. These embryonal tumors may also be inherited as autosomal dominant traits and exhibit early onset, multiple primary tumors, and association with other neoplasms (2–6).

Recent studies suggest that the initiation of these latter embryonal tumors involves two separate genetic events that serve to unmask a recessive locus predisposing to malignant transformation (7–12). Thus, the pathogenesis of retinoblastoma frequently involves chromosomal rearrangements that eliminate the wild-type allele of a locus on chromosome 13q14. Furthermore, osteosarcomas, the most frequently occurring secondary tumor in survivors of heritable retinoblastoma (4), also show similar rearrangements of chromosome 13 (13). Another example of the tissue pleiotropy of this class of recessive cancer genes has been provided by analysis of Wilms tumor, hepatoblastoma, and rhabdomyosarcoma, for which specific rearrangements involving the short arm of chromosome 11 were demonstrated (14). Cases of these tumors sometimes show familial clustering as one manifestation of the autosomal dominant Beckwith–Wiedemann syndrome (5), which has also been regionally mapped to 11p (15), or have been observed simultaneously as heterotropic tumors.

These studies have presented experimental evidence in support of the two-step hypothesis for tumorigenesis by Knudson (6). They indicate that sporadic and inherited forms of embryonal tumors affect the same loci and that the tumors arise as a result of two distinct events, of which the first is a germinal mutation in the heritable cases (7, 8). Since the second event specifically affects the chromosomal loci homologous to that that is defective in the heritable form of a tumor, it may be possible to localize genes predisposing to other inherited tumors by studying chromosomal rearrangements in sporadic cases of the same neoplasm.

Here we have tested the hypothesis that the pathogenesis of breast cancer in males and young females involves a chromosomal rearrangement that serves to unmask a recessive cancer gene. Genotypes have been determined in normal and breast tumor tissues from 14 patients. The data indicate that a significant fraction of ductal breast cancers arises subsequent to abnormal mitotic events that could serve to unmask a recessive mutation on chromosome 13.

MATERIALS AND METHODS

Human Tissue Samples. Human breast cancer samples were obtained from 12 premenopausal females (age, 25–43 years) and two males (age, 42 and 60 years). All tumors were removed surgically prior to irradiation and chemotherapy, and fresh specimens were frozen at −70°C for 0.5–5 years before isolation of DNA. Peripheral venous blood was obtained 0.5–5 years after surgery. Relevant clinical details (16–18) are listed in Table 1.

Southern Hybridizations. High molecular weight DNA was isolated from nuclei of peripheral blood lymphocytes and from tumor samples as described (7–9, 13, 14). Restriction endonuclease digestion of these samples, agarose gel electrophoresis, Southern transfer, prehybridization, hybridization to recombinant DNA probes radiolabeled by nick-translation, and autoradiography were as described (7, 9, 13, 14) except that DNA transfer was accomplished in 0.4 M NaOH/0.6 M NaCl to GeneScreen*Plus* nylon membranes (New England Nuclear). Quantitation of the intensity of hybridization to each restriction fragment length allele was performed with a Bio-Rad 1650 scanning densitometer. Bound probes were removed from the blotting membranes by treatment with alkali and the membranes were repeatedly rehybridized. When loss of alleles was detected in tumor

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Table 1. Clinical characteristics of patient and breast tumor DNA sources

Patient*	Sex	Diagnosis age, years	TNM†	Differentiation‡	% tumor cells§
		Ductal carcinoma			
BC1	♀	34	T1N0M0	High	90
BC2	♀	37	T1NIM0	Medium	75
BC4	♀	42	T1NIM0	Medium	75
BC6	♀	43	T2N0M0	Low	90
BC11	♀	43	T1N0M0	Medium	90
BC14	♀	40	T1N0M0	Medium	90
BC18	♀	39	T1N0M0	Medium	90
BC20	♂	42	T1N1M0	Medium	90
BC21	♀	38	T2N2M0	Medium	ND
BC27	♂	60	T1N2M0	Low	75
		Comedocarcinoma			
BC3	♀	39	T3N0M0		50
BC15	♀	38	T1N0M0		ND
		Medullary carcinoma			
BC24	♀	41	T1N0M0		50
		Juvenile secretory carcinoma			
BC29	♀	25	T3N1M0		ND

*Histopathological diagnosis was performed according to criteria outlined in ref. 16. All patients were followed clinically for 6 years after surgery, except patients BC20 (1.5 years) and BC29 (2 years), and all were alive as of September 1986.
†The clinical description of each case was according to ref. 17.
‡The degree of differentiation of each tumor was determined histologically according to the criteria in ref. 18.
§The percentage of each tumor mass that was composed of neoplastic cells was determined by histopathological analysis. ND, not determined.

Table 3. Specificity of loss of heterozygosity

Case no.	Chromosomes remaining heterozygous	Chromosomes losing heterozygosity
	Ductal carcinoma	
BC1	3, 11, 12, 13	ND
BC2	2, 11, 12, 13, 18, 20	ND
BC4	2, 6, 8, 13, 16, 18, 20	ND
BC6	6, 7, 11, 16, 17, 18	2, 13, 14, 20
BC11	2, 11, 12, 14, 15, 19, 20	5, 13
BC14	2, 3, 6, 8, 11, 12, 15	13
BC18	2, 3, 13, 15, 20	ND
BC20	2, 3, 6, 7, 11, 13, 14, 17, 22	ND
BC21	2, 11, 12, 13, 14, 15, 17, 18, 20, 22	ND
BC27	2, 3, 6, 11, 12, 14, 19, 20	13
	Comedocarcinoma	
BC3	2, 3, 6, 11, 12, 13, 14, 15, 18, 20	17
BC15	2, 6, 8, 11, 13, 17, 20	ND
	Medullary carcinoma	
BC24	2, 3, 5, 6, 11, 12, 13, 14, 15, 18, 20	ND
	Juvenile secretory carcinoma	
BC29	1, 2, 5, 11, 12, 13, 14	ND

Alleles at loci on each chromosome (except 4, 9, 10, 21, X, and Y) were determined in constitutional and tumor DNA from breast cancer patients. Chromosomes not delineated above were constitutionally homozygous and so uninformative for this analysis. The loci examined (19 and 20) were D1S2, D2S1, CRYG, D3S2, D3S3, D5S1, D5S2, D6S10, MetH, CA2, HBBC, D11S12, HRAS1, INS, D12S7, D13S1, D13S2, D13S3, D13S4, D13S5, D13S6, D13S7, D14S1, D15S1, APRT, D17S1, D17S2, D18S1, D19S11, D20S4, and D22S1. ND, not detected.

DNA, the analysis was repeated with a new membrane and the same membranes were then hybridized repeatedly with probes homologous to other chromosomes to exclude the possibility of unequal loss of DNA from the membranes. The DNA segments homologous to various polymorphic human chromosomal loci used in this study are listed in Tables 2 and 3: the nomenclature is according to the currently accepted convention (19). In all cases, the allele lengths reported are identical to those published (19, 20).

Table 2. Genotypes in breast tumor DNA at loci on chromosome 13

Case no.	Locus (enzyme) D13S6 (*Xmn* I)	D13S1 (*Msp* I)	D13S1 (*Taq* I)	D13S2 (*Msp* I)	D13S2 (*Taq* I)	D13S4 (*Msp* I)	D13S5 (*Hin*dIII)	D13S5 (*Eco*RI)	D13S7 (*Bgl* II)	D13S3 (*Msp* I)	D13S3 (*Hin*dIII)
					Ductal carcinoma						
BC1		1, 2	1, 2	—	—	—	—			1, 2	
BC2		—	—	—	—	—	—			1, 2	
BC4		1, 2	—	—	1, 2	—	—			—	—
BC6	—	—	*1*	*1*	—	*1*	—			*2*	*1*
BC11	*2*	—	—	—	—	—	*1*	*1*	*1*	—	—
BC14	—	—	*1*	*1*	—	*2*	—			—	—
BC18		—	—	—	—	1, 2				1, 2	
BC20	—	—	1, 2	—	—	—	—		—	—	—
BC21	—	—	—	—	1, 2	1, 2	1, 2	—	—	—	
BC27	*2*	*2*	*2*	—	*1*	—	—		—	*1*	*1*
					Comedocarcinoma						
BC3	—	—	—	1, 2	—	—	1, 2	—	—	1, 2	—
BC15	1, 2	—	—	—	—	—	—	—	—	—	—
					Medullary carcinoma						
BC24	1, 2	1, 2	1, 2	1, 2	—	—	1, 2		—	1, 2	1, 2
					Juvenile secretory carcinoma						
BC29		—	1, 2	—	—	1, 2	—		1, 2		

Numbers indicate the restriction fragment length alleles present in tumor tissue at loci that were constitutionally heterozygous. Italicized numbers indicate loss of a constitutional allele. — indicates constitutional homozygosity.

RESULTS

Loss of Heterozygosity for Chromosome 13 in Breast Tumors. In these analyses, we determined allelic combinations at 43 loci on 18 chromosomes in normal and tumor DNA samples from unrelated patients with breast carcinoma. Losses of constitutional heterozygosity were found in four cases involving three (BC14), four (BC11), five (BC6), or six (BC27) loci on chromosome 13. Table 2 displays the alleles present at all loci on this chromosome in the tumors examined for each instance in which the normal tissue was informative—i.e., heterozygous, for this analysis.

Fig. 1*a* illustrates loss of constitutional alleles on chromosome 13 in the breast cancer tissue from case BC14. Normal tissue from this patient was constitutionally heterozygous at the D13S1 (*Taq* I) and D13S4 loci, whereas the short allele at the D13S1 (*Taq* I) and the long allele at the D13S4 locus were absent in the tumor tissue. The tumor tissue also showed loss of the short allele at the D13S2 (*Msp* I) locus (Table 2, autoradiogram not shown).

Similarly, Fig. 1*b* displays the genotypic data obtained with paired tissues from case BC11 at the D13S6, D13S5 (*Hin*dIII), and D13S7 loci on chromosome 13. This patient was constitutionally heterozygous at each of these loci, whereas only one of the alleles at each locus was apparent in the breast tumor tissue. Since loss of heterozygosity was demonstrated at all informative loci on chromosome 13, mapping from 13q12 (D13S6) to 13q22 (D13S7), these data are consistent with nondisjunctional loss of one chromosome 13 in the genesis of the tumor tissue.

Similar, but slightly different, results were obtained in two other cases, BC6 and BC27. Loss of constitutional alleles in the tumor tissue from BC6 was found at five loci on chromosome 13 (Table 2). Fig. 2*a* displays the constitutional and tumor genotypes at three of these loci. The shorter alleles at the D13S1 (*Taq* I) and D13S4 loci and the longer allele at the D13S3 (*Msp* I) locus were each dramatically diminished

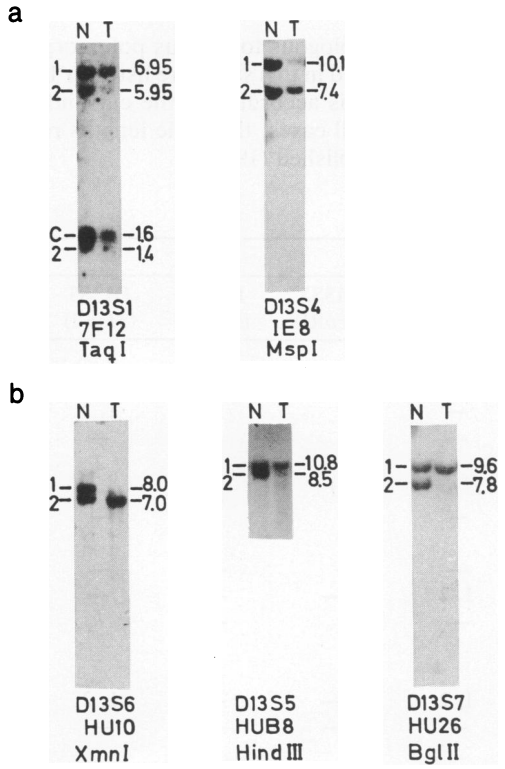

FIG. 1. Loss of constitutional heterozygosity at loci on chromosome 13 in ductal carcinoma of the breast. DNA samples from normal (N) and tumor (T) tissues obtained from patients BC14 (*a*) and BC11 (*b*) were hybridized to the indicated chromosome 13-specific probes as detailed. The allele designations are to the left of each autoradiogram (C indicates a constant band), and allele lengths in kilobase pairs are to the right.

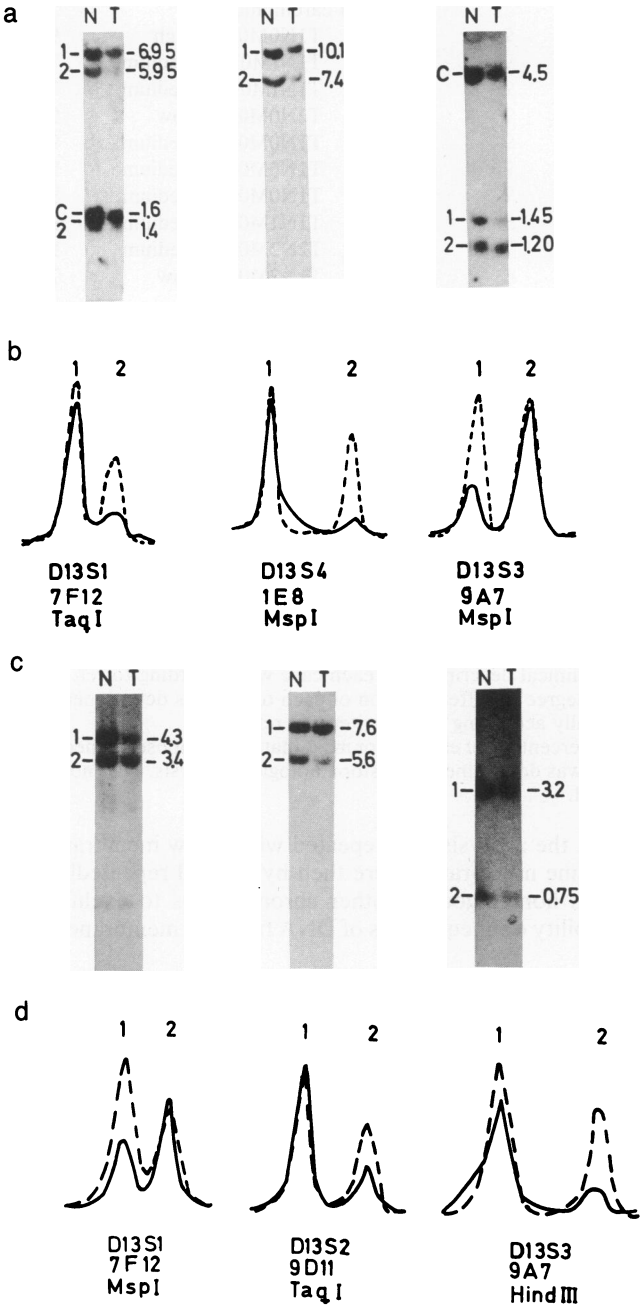

FIG. 2. Loss of constitutional heterozygosity at loci on chromosome 13 in ductal breast carcinomas that encompass normal tissue. DNA samples from normal (N) and tumor (T) tissues obtained from patients BC6 (*a* and *b*) and BC27 (*c* and *d*) were hybridized to the indicated chromosome 13-specific probes as detailed. The allele designations are to the left of each autoradiogram (C designates a constant band), and allele lengths in kilobase pairs are to the right. (*b*) Densitometric tracings of the autoradiograms in *a* showing diminished allele intensities in BC6 tumor tissue (the 1.6- and 1.4-kilobase-pair bands of the D13S2 and the 4.5-kilobase-pair band of D13S3 are not shown in the tracings). These diminutions bear an indirect relationship to the proportion of normal stromal tissue present in the tumor sample as quantified in Table 1. (*d*) Densitometric tracings of the autoradiograms in *c* showing diminished allele intensities in BC27 tumor tissue. These diminutions bear an indirect relationship to the proportion of normal stromal tissue in the tumor sample as quantified in Table 1.

in intensity. No case of retention of constitutional heterozygosity was found on chromosome 13 in this tumor (Table 2). Since all informative loci on chromosome 13, from band q12 (D13S1) to qter (D13S3), showed loss of one of the constitutional alleles, these data are consistent with a nondisjunctional loss or a large deletion involving almost the entire chromosome 13. Fig. 2*b* shows densitometric tracings made of the autoradiographic data in Fig. 2*a*. These data show that the remaining hybridization signals for each of the diminished alleles at each locus are in direct proportion to the small percentage of normal stromal tissue in the tumor sample (Table 1).

The constitutional and tumor genotypes at three loci on chromosome 13 in case BC27 are shown in Fig. 2*c*: although both alleles were clearly detectable, decreased hybridization intensity of one allele at each locus was visible in the tumor tissue. The longer allele of the D13S1 (*Msp* I) locus was fainter in the tumor tissue as was the shorter allele at the D13S2 (*Taq* I) and D13S3 (*Hin*dIII) loci. Quantitative analysis of these loci showed a 50–60% decreased intensity of one allele in the tumor DNA that is apparent in the densitometric tracings of the autoradiographic data shown in Fig. 2*d*. In contrast, similar analysis of other loci determined with the same blots as in Fig. 2*c* showed equal quantitative relationships between all bands in normal and tumor tissue (data not shown). Furthermore, tumor tissue from case BC27 showed a similar loss at three other loci on chromosome 13 (Table 2, autoradiograms not shown). Since all informative loci from 13q12 to 13qter showed partial loss of the constitutional alleles, these data are consistent with a loss of one chromosome 13 complement in 50–60% of the cells in this tumor. The most likely explanations for the partial loss of chromosome 13 in this case are (*i*) the tumor tissue was composed of two different types of neoplastic cells or (*ii*) only one population of cancer cells was present containing a single chromosome 13 complement and this population was contaminated with normal stromal cells. Independent histological analyses of the tumor in case BC27 showed that the fraction of the tumor that was comprised of constitutional cells was directly proportional to the retained hybridization (Table 1), in support of the second explanation.

After the analyses described above were complete, the clinical and histopathological characteristics of each case were reviewed; relevant details are shown in Table 1. The tumors in this study could be clearly divided into four groups: ductal carcinoma, comedocarcinoma, medullary carcinoma, and juvenile secretory carcinoma. Each of the tumors that lost constitutional heterozygosity for loci on chromosome 13 (BC14, BC11, BC6, BC27) was classified as a ductal carcinoma of low to medium differentiation. None of the samples classified as medium to high differentiated ductal carcinoma, medullary carcinoma, or juvenile secretory carcinoma behaved similarly (Table 2).

Specificity of the Allele Losses. To determine whether the tumor-specific losses of alleles at loci on chromosome 13 were unique events in the pathogenesis of human ductal breast carcinoma and whether similar events had occurred for other chromosomes in the other breast tumor types, the analyses summarized in Table 3 were performed. The same Southern blots from which the data in Figs. 1 and 2 and Table 2 were derived were alkali treated to remove the chromosome 13-specific probes; they then were hybridized to the probes listed in the legend to Table 3, which are homologous to 31 loci on various other human chromosomes. Analyses of tumor DNA from patient BC14, which had lost constitutional heterozygosity at 3 loci on chromosome 13, showed a maintenance of the constitutional genotype at each informative locus on chromosomes 2, 3, 6, 8, 11, 12, and 15. Similarly, analysis of tumor DNA from patient BC27, which had lost constitutional heterozygosity at 6 loci on chromosome 13, retained the constitutional genotype at each informative locus on chromosomes 2, 3, 6, 11, 12, 14, 19, and 20.

Analysis of tumor DNA from patient BC11, which had lost constitutional heterozygosity at four loci on chromosome 13 and retained the constitutional genotype on chromosomes 2, 11, 12, 14, 15, 19, and 20, showed that this sample also lost constitutional heterozygosity at one locus [D5S1 (*Bql* II)] on chromosome 5. Analysis of tumor DNA from patient BC6, which had lost constitutional heterozygosity at five loci on chromosome 13, retained the constitutional genotype at loci on chromosomes 6, 7, 11, and 16–18. However, this sample also lost constitutional heterozygosity at loci on chromosomes 2 [D2S1 (*Msp* I) and CRYG], 14 [D14S1 (*Eco*RI)], and 20 (D20S4).

None of the other six ductal carcinomas showed detectable losses of constitutional heterozygosity at loci on chromosomes 2, 3, 6–8, 11–18, 20, or 22. Analyses of medullary breast carcinoma and juvenile secretory carcinoma also showed no detectable losses of constitutional heterozygosity at any locus, although only one sample of each tumor type was available for examination. Determination of constitutional and tumor genotypes in two cases of comedocarcinoma (BC3, BC15) showed maintenance of heterozygosity at loci on chromosomes 2, 3, 6, 8, 11–15, 18, and 20. One of the two cases (BC3) showed loss of heterozygosity at two loci [D17S1 (*Msp* I) and D17S2 (*Bgl* II)] on chromosome 17. Whether this loss is specific to comedocarcinoma will require the analysis of a larger set of tumors of this type, but it suggests the possibility of different recessive mutations in the etiology of ductal tumors and comedocarcinomas.

As illustrated in Table 2, analyses of 41 informative loci on 18 different chromosomes indicate that a loss of constitutional heterozygosity in breast carcinomas is a relatively specific event. The only chromosome in common to those ductal tumors that lost constitutional alleles was chromosome 13, suggesting that this was a significant event in these tumors. Furthermore, the tumor and constitutional genotypes were always consistent at loci where the patients were homozygous (data not shown). Therefore, elimination of both chromosomes (nullizygosity) could be excluded at all loci studied in these tumors. Since the constitutional and tumor genotypes were identical for all loci studied, with the exception of those described above, the possibility of mismatches of tissue samples could also be excluded.

DISCUSSION

In the present study, we have analyzed tumors from sporadic cases of breast cancer chosen for the similarity of their clinical characteristics to the familial forms of this disease. Twelve of the tumors were obtained from males or premenopausal females who had survived at least 6 years after the initial diagnosis. Four histological types of breast carcinoma were examined; loss of constitutional heterozygosity for loci on chromosome 13 was observed only in ductal carcinomas. These data suggest that mitotic nondisjunction for this chromosome was involved in the pathogenesis of this tumor type. Chromosomal events similar to those described here have been reported for retinoblastomas (7, 8), osteosarcomas (13), Wilms tumor (9–12), hepatoblastomas (14), and rhabdomyosarcomas (14). The relative specificities of these events suggest, by analogy to these latter studies of embryonal cancers, that the unmasking of a predisposing recessive mutation at a locus on human chromosome 13 may be intimately involved in the pathogenesis of human ductal breast carcinoma. An obvious extension of this analogy is that the heritable forms of this disease may involve the same cancer locus with the initial predisposing mutation in these cases being inherited through the germ line as has been shown for retinoblastomas (8). The genomic location of such a locus can be inferred through mitotic recombination mapping (7, 8),

through analysis of chromosomal translocations or other types of rearrangements (13), and by the determination of the frequency of cosegregation of the disease with allelic forms of the polymorphic loci on chromosome 13 homologous to the cloned DNA segments used herein. The determination of such a locus will be of obvious utility in the genetic analysis of this disease and may be useful in the ascertainment of risk for disease development in families, in a manner recently described for retinoblastoma (21).

Cytogenetic investigation of breast tumors has been hampered by major inherent technical problems. In primary cell cultures of breast tumors, only a minority of cells is mitotic and few of these mitoses could be analyzed (22, 23). This problem was further confounded by the observation that the few highly selected cells that could be analyzed karyologically differ greatly among even those from the same tumor (22, 23). Virtually every published breast tumor karyotype has shown multiple complex rearrangements, including loss of both chromosome homologs in some cases (22–24). Thus, no consistent cytogenetically detectable chromosome abnormality has been clearly associated with breast tumors to date. In contrast, cytogenetic analyses of retinoblastomas have revealed abnormalities involving 13q (2, 25), and the localization of the retinoblastoma locus to chromosome 13q14 has been independently confirmed by several methods (2, 7, 8, 25–27). Analysis of restriction fragment length alleles in retinoblastoma has shown specific loss of chromosome 13 alleles in about 70% of all cases investigated (7, 8), although karyotypes of the same tumors sometimes have shown multiple abnormalities and several marker chromosomes (7). Karyotypes of Wilms tumors, rhabdomyosarcomas, and osteosarcomas also have shown multiple chromosomal rearrangements (24), whereas molecular analysis has indicated the specific involvement of a locus in the oncogenesis of these tumor types (9–14).

The results described here with breast carcinomas dramatically underscore the enhanced resolution of the molecular cytogenetic approach to the question of the chromosomal basis of neoplasia. Somatic losses of constitutional heterozygosity were shown to be relatively specific, nonrandom, and few in number. The retention of the constitutional genotype at a large number of loci in all of the tumors examined indicates that little widespread loss of genetic material occurred during the oncogenesis of these breast tumors. No instance of nullizygosity was observed at any of the 43 loci examined in the set of 14 samples. It was also evident that the random massive losses of chromosomes reported for malignant melanomas (28) did not occur in the breast tumors examined here.

Finally, these results point at the possibility of using molecular cytogenetics as an adjunct to histopathology in the diagnosis of breast tumors. A comparison of the clinical data in Table 1 and the results in Table 3 suggests a possible relationship between the histological type of the breast tumors and the chromosomal rearrangements found in tumor tissue. The four cases showing chromosome 13 rearrangements (BC6, BC11, BC14, BC27) were each among the 10 tumors classified as ductal cancer, whereas the single case (BC3) showing chromosome 17 rearrangements was classified as a comedocarcinoma. This may suggest the involvement of distinct chromosomal loci in the pathogenesis of these forms of breast cancer. Support for this notion is provided by a study of affected twins that indicates that, within a single family segregating for breast cancer, the affected individuals develop tumors of the same histological type (1).

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