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Definition of Regions of the Human Genome Affected by Loss of Heterozygosity in Primary Human Breast Tumors

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Abstract We have undertaken a systematic study of primary human breast tumor DNA to identify and characterize frequently occurring somatic mutations. Loss of heterozygosity (LOH) has been the most frequent mutation in our panels of primary breast tumor DNA. It is currently thought that LOH reveals recessive mutations within the affected region of the genome. One goal of our studies has been to physically define the target genes revealed by LOH in primary breast tumors. We have focused our efforts on chromosome 17, finding five regions of the chromosome which are independently affected by LOH in breast tumors. Two apparent target loci are on chromosome 17p; one is the TP53 gene. The other is an as-yet undefined locus telomeric to the TP53 gene. Loss of expression of the *nme*1 gene on chromosome 17q in tumors was linked to patients with a poor prognosis ($p = 0.018$). Although a significant trend ($p = 0.05$) was found between LOH of the *nme*1 gene and loss of *nme*1 expression, no point mutations were found within the coding region of the *nme*1 gene by single strand conformational polymorphism (SSCP) or nucleotide sequence analysis. These and other results suggest to us that there may be potential tumor suppressor genes both centromeric and telomeric to the *nme*1 locus on chromosome 17q.

Key words: Breast cancer, loss of heterozygosity, *nme*1, TP53

The heterogeneity of factors necessary for most epithelial tumors to develop suggests that multiple somatic mutations are required, probably acting in concert to produce an invasive carcinoma that can metastasize to distant organ sites. In this scenario, carcinomas are a consequence of accumulated somatic mutations which either uncouple normal growth regulatory signals or provide the tumor cell with some growth advantage. Consistent with this view, studies of mouse models of mammary carcinogenesis have shown that activation of multiple genes is associated with the development of preneoplastic mammary gland lesions and tumor development [reviewed in 1]. These studies suggest at least two distinguishable classes of genes which, when qualitatively or quantitatively activated, contribute to mammary tumorigenesis. Mutations in one group of genes deregulate normal growth controls, but alone are not sufficient to induce malignant growth of the mammary epithelium. The other group of genes has no apparent effect on normal mammary gland development, but contributes to mammary tumorigenesis after additional mutations or hormonal stimulation. Based

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on these results, a minimum of at least three, or more likely several, collaborating mutations are required to develop malignant mammary carcinoma in mice.

In human breast cancer, the etiological complexity is compounded by the diversity of potential factors in the patient's history which could provide a selective environment for the clonal outgrowth of cells containing either particular or sets of somatic mutation(s). Some factors are menstrual and reproductive history, family history, long-term treatment with estrogens, diet, and previous atypical benign breast disease [2–4]. To gain insight into somatic genetic damage incurred during malignant progression, we and others have surveyed the human genome for frequently occurring mutations in panels of primary human breast tumor DNA [reviewed in 5]. In general, the initial goal of these studies was to determine whether particular mutations have a significant association with specific clinical parameters in the patient's history, characteristics of the tumor, or the patient's prognosis. Our results indicated that at least 12 regions of the human genome are either amplified or affected by loss of heterozygosity (LOH). The most frequent type of mutation is LOH, which we detected on nine different chromosomal arms (1p, 1q, 3p, 7q, 11p, 13q, 17p, 17q, and 18q). Most of these mutations are primarily associated with more aggressive tumors. LOH is thought to reveal recessive mutations in tumor suppressor genes located within the affected region [6].

SUBSETS OF TUMORS DEFINED BY THE MUTATIONS THEY CONTAIN

Is the apparent high frequency of mutations, particularly LOH, a consequence or a contributing factor in malignant breast tumor progression [7]? Among candidate targets for gene amplification (*myc*, *fgf3*/*int*-2, *erb*B-2/HER-2), the quantitative activation of expression of each of these genes has been shown to increase the frequency of mammary tumors in the transgenic mouse model system [reviewed in 1,5]. A role in tumor progression for regions of the genome affected by LOH is less compelling, primarily because most of the putative target genes have not been identified. However, if the observed frequency of LOH at different chromosomal locations was simply a consequence of tumor progression, it seems unlikely that subsets of tumors could be defined by the particular regions of the genome affected by LOH. Yet we and others have found this in the tumor panels examined [reviewed in 5]. These studies have consistently shown that common subsets of tumors can be defined by the mutations they contain; however, none of the studies presently have the statistical power to make a "global" statement of reproducibility between tumor panels.

DEFINITION OF TARGET GENES WITHIN REGIONS AFFECTED BY LOH

Since LOH is the most common type of mutation found in primary human breast tumors, defining the target genes should be the next priority. Most studies to date have used a limited number of marker loci per chromosome arm to define the regions containing the putative target genes [5]. The rapid advances of the Human Genome Project in developing probes which detect polymorphic loci throughout the human genome have significantly advanced the experimental probability of determining the location and identity of the putative target genes with great precision [8,9]. These considerations, together with the recent localization of the familial breast and breast/ovarian cancer locus to chromosome 17q21 [10,11], and the Li-Fraumeni locus to the TP53 gene on chromosome 17p13.1 [12], have focused our efforts on the regions of chromosome 17 affected by LOH in our panels of sporadic breast tumor DNAs.

CHROMOSOME 17p

Two regions of chromosome 17p were found to be independently affected by LOH in a panel of 121 primary invasive ductal breast carcinomas previously typed for their proliferative index [13–15]. One region is located between the D17S34 and D17S28/D17S30 loci on 17p13.3 (Fig. 1). Although the putative target gene has not yet been identified, it seems likely that it is close to D17S28/D17S30. We base this prediction on the highly significant association between LOH at these loci and tumors having a high proliferative index; this association was not found in tumors having LOH at the D17S34 locus. The second region (Fig. 1) affected by LOH is located at 17p13.1 and contains the TP53

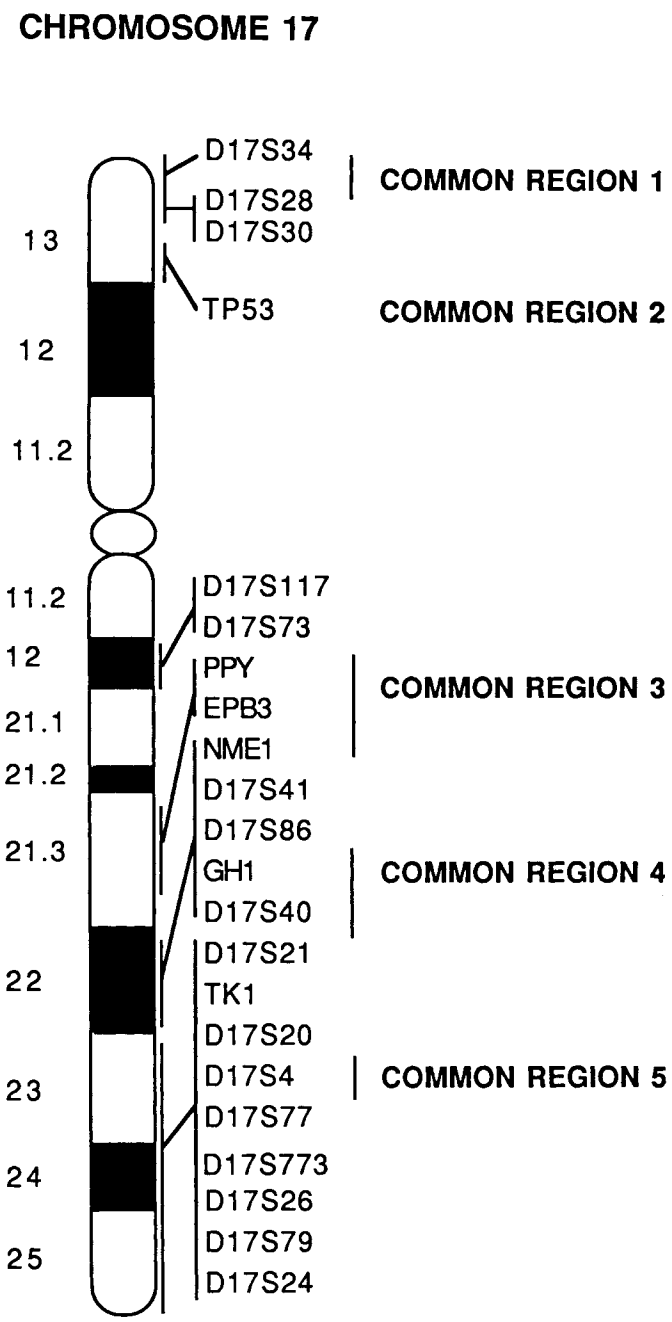

Fig. 1. Human chromosome 17 map of polymorphic loci. The regions of the chromosome affected by LOH are indicated.

gene [14,15]. Polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis was performed on TP53 exons 5 through 8 in the same panel of 121 tumor DNAs. TP53 point mutations were found in 29% of the tumors. Although the mutations were evenly distributed among the four exons, only tumors with a point mutation in exons 5 or 6 had a significant association with a high proliferative index.

Very few *in vivo* studies have been undertaken

to determine whether mutations in specific regions of TP53 are associated with particular biological characteristics of the tumor. Our results establish a link between loss of TP53 suppressor function due to mutation of a specific region of the gene, and the proliferative activity of the tumor *in vivo*. Since a high proliferative index is an independent indicator of poor prognosis [16,17], our results are consistent with other studies demonstrating that TP53 protein accumulation in invasive ductal carcinomas predicts a poor patient outcome [18,19]. Davidoff *et al.* [20] have shown that the tumors of patients who raise a humoral response to the TP53 protein host tumors which primarily have mutations in TP53 exons 5 and 6, but not exons 7 and 8. Although these experiments should be repeated on larger panels of tumors, the collective results to date suggest that it may be possible to identify the fraction of lymph node-negative breast cancer patients at high risk for relapse and decreased survival. Secondly, these results provide the rationale for future experiments to determine whether patients whose breast tumors have mutations in TP53 exons 5 and 6 can be stimulated to mount a cytotoxic T cell response to the tumor.

CHROMOSOME 17q

We have examined chromosome 17q for LOH in separate panels of primarily invasive ductal breast carcinomas from those used for 17p analysis [21,22]. Early in these studies the *nme*1 gene, located at chromosome 17q21.3, represented a potential target gene for LOH on chromosome 17q [23]. Loss or decreased levels of *nme*1 protein in primary human breast tumors and other experimental systems is associated with an increased probability of metastatic spread of the tumor to distant organ sites [24–26]. This observation, together with transfection data [27], has suggested that *nme*1 could be considered a metastasis suppressor gene. We observed a significant trend between loss of *nme*1 expression and LOH of the *nme*1 gene (Cropp *et al.*, manuscript in preparation). However, exceptions existed in which *nme*1 LOH did not result in reduced protein expression, and was not significantly associated with poor patient survival. Nucleotide sequence analysis of seven of these tumors failed to reveal any point mutations within the coding region of the gene. Similarly, PCR-SSCP analysis of 20 additional breast tumor DNAs, as well as 9 breast tumor cell lines, also failed to reveal mutations in the coding region. In contrast to the LOH data, *nme*1 expression data in this cohort showed a significant correlation between loss of *nme*1 expression and decreased time to metastasis, confirming earlier studies. These results suggest that loss of expression of the *nme*1 protein in primary breast tumors must reflect mechanisms other than the combined effect of LOH and point mutations within the coding region of the gene.

More recently, we expanded the study of chromosome 17q in primary breast tumor DNAs to include 18 polymorphic loci in this region of the genome [22]. At least three distinct regions located at 17q21.1–q21.3 (Region 3), 17q22-qter (Region 4), and 17q23–q25 (Region 5) could be identified which are independently affected by LOH (Fig. 1). Based on the current recombination linkage map of chromosome 17q [28], a proximal region is located within a 22 cM region as defined by D17S73 and *nme*1 (Region 3), and thus is similar in location to the region thought to contain the BRCA1 locus associated with familial breast and breast/ovarian cancer. The central region (Region 4) is bordered by the D17S86 and D17S21 loci, which are about 28 cM apart. The distal region (Region 5) is bordered by D17S20 and D17S77 loci, which are 11 cM apart.

FUTURE DIRECTIONS

Although the effort to identify the target genes in the regions of chromosome 17 affected by LOH is continuing in our own as well as other laboratories, the apparent complexity of putative target genes on this chromosome alone was unexpected. Most of the "alleletyping" studies of primary breast tumor DNAs have examined one or two polymorphic loci per chromosome arm. Moreover, the location of some of these loci is imprecisely mapped relative to other loci on the particular chromosome arm. As high density maps of the other chromosomes (both those which are known to be affected and those which currently appear to be unaffected by LOH) in breast tumor DNAs are developed, it is probable that additional regions affected by LOH will be uncovered. The increasing availability of highly polymorphic loci that have been either physically

mapped or mapped by linkage analysis on all chromosome arms suggests that identification of the target genes for LOH is not only feasible in the near future but should have the highest priority in the effort to solve the puzzle of breast cancer genetic pathology. Answers from these studies will provide a sound foundation to determine the linkage between particular mutations and clinical parameters of the disease, as well as whether particular subsets of tumors can be defined by the mutations they contain and the clinical parameters with which they are associated.

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