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Loss of heterozygosity (LOH) on the short arm of chromosome 11 has been demonstrated in many cancers, suggesting the presence of a tumor suppressor gene. A number of studies have narrowed the region showing LOH in breast tumors to 11p15.5. The objectives of this study are: to screen breast tumor samples for LOH to further resolve the location of the putative tumor suppressor gene: and to screen candidate genes from this region.

We are examining LOH in our region of interest using polymorphic markers. Samples from heterozygous individuals are examined from both normal and tumor tissue. We have isolated genomic DNA from 112 tissue samples, constituting 56 matched tumor and normal pairs. These samples are being analyzed for loss of heterozygosity (LOH) at three separate polymorphic markers, D11S1318, D11S4088, and D11S860. The analysis of marker D11S1318 is complete and is comparable to results which have been previously been reported at 6% LOH. To date D11S4088 is showing 12% LOH and D11S860 is incomplete.

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FOREWORD

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(5) INTRODUCTION

Breast cancer is the most frequent cause of cancer death among North American and European women. An understanding of the basic mechanisms underlying the initiation and progression is imperative to both reducing the incidence of and improving the prognosis for the disease. Like the majority of human neoplasms, mammary carcinomas arise from epithelial cells, which suggests that insights gained from the study of breast cancer may shed light on the development and progression of various other cancers as well.

Cellular transformation can result from overabundance of oncogene product and subsequent overwhelming of normal cellular control mechanisms. Two well documented mechanisms include mutation of oncogenes or amplification of normal genes. In addition, numerous studies have suggested that the loss of a gene or genes involved in growth control may convert normal cells to their neoplastic counterparts. Demonstration of loss of heterozygosity (LOH) at specific chromosomal locations in a variety of familial cancers including retinoblastoma, Wilms' tumor and Recklinghausen's neurofibromatosis have led to the identification of tumor suppressor genes for these diseases (1). Some other cancers that have been associated with LOH are neuroblastoma, colorectal cancers, and carcinomas of the kidney, lung, and breast (1, 2). The identification of these genes by positional cloning techniques supports the same strategy for mapping putative breast carcinoma genes, particularly if LOH data have already indicated a likely location to within a 6-9 megabase pair (mb) region.

Loss of heterozygosity on the short arm of chromosome 11 has been demonstrated in many cancers, including rhabdomyosarcoma, Wilms' and other embryonal tumors, as well as tumors of the brain, bladder, lung, ovary, liver, adrenals, colon, and breast (3-16). In addition, functional studies have demonstrated loss of tumorigenicity by MCF-7 breast carcinoma cells after transfer of a whole chromosome 11 (17) and LOH at 11p15.5 by human milk epithelial cells immortalized by microinjection of SV40 DNA (18). A number of studies have narrowed the region showing LOH in breast tumors to 11p15 (19, 20) or even further, to 11p15.5 (7, 21-23).

We plan to narrow the region of LOH chromosome 11p15.5 in breast cancer to a small area amenable to positional cloning approaches. This will include the development of a physical map of the region, the use of new polymorphic markers in that region and the application of these markers to matched sets of normal and breast tumor material. This will eventually lead to the isolation of candidate genes for tumor suppressor activity in breast cancer. In addition to furthering our understanding of the basic mechanisms of oncogenic processes, identification of tumor suppressor genes and elucidation of their contribution to tumor formation and progression will aid in both diagnosis and treatment of cancers. For example,

the loss of a gene product presents a situation that may be particularly amenable to nonsurgical interventions, such as gene replacement and/or drug therapy. The missing substance provided by the lost tumor suppressor gene may regulate oncogene expression, so replacement or enhancement of a tumor suppressor gene product may be a particularly valuable tool in a variety of situations.

(6) **BODY**

During the past year we have focused our efforts on LOH analysis of the short arm of chromosome 11. We have isolated DNA from 112 tissue samples constituting 56 matched pairs of tumor and normal breast tissue. The DNA from these samples was analyzed with three polymorphic markers in our region of interest, 11p15.5. The three markers chosen to start the analysis are D11S1318, D11S4088, and D11S860. All three have a high maximum heterogeneity. These three markers have non-overlapping allele sizes to facilitate multiplex PCR.

Table 1

	Maximum heterogeneity	Allele number and size
D11S1318	78%	10 (127-145)
D11S4088	92%	20 (204-242)
D11S860	91%	12 (154-196)

A. Collection of breast tumor samples for LOH analysis

As of our last report the UNC breast cancer bank had given us 6 matched normal/tumor pairs. Using the standard proteinase K method for DNA isolation from tissue samples we now have DNA from 56 matched pairs for our LOH analysis. At the time this fellowship was written the breast cancer bank stated that they would be able to supply us with 100 matched tumor and normal tissue pairs each year. The amount of tissue available has not been at the anticipated level and at present we have samples from every matched pair available from the facility which totals 56 pairs. We plan to continue isolating DNA from the matched tissue pairs as they become available from the breast cancer bank. We are also investigating other sources of breast tissue samples and have now been provided with slides with breast tumor slices with areas of pure tumor circled.

B. LOH analysis

We would like to narrow the region of LOH on chromosome 11p15.5 in breast cancer to a small area amenable to positional cloning techniques. This entails the use of polymorphic markers in that region to help narrow the location of a tumor suppressor gene. The polymorphic markers are used to analyze the matched sets of normal and breast tumor material to detect the minimal region which is lost in tumor samples. In this way we plan to isolate candidate genes for tumor suppressor activity in breast cancer.

To date we are analyzing 56 matched pairs with three separate polymorphic markers, D11S1318, D11S4088, and D11S860. Two of these markers have been used to analyze the LOH pattern of 11p15.5 before. D11S860 has been shown to be inside the region of LOH while D11S1318 is thought by many groups to be outside the region on the telomeric border. The results of LOH analysis with D11S4088 have not been reported to date, and we wanted to see if this marker is inside or outside our region of interest since it falls within the genomic contig map constructed by our laboratory. Our results to date are summarized in Table 2. The analysis is complete with D11S1318, and almost complete with D11S4088. Analysis of D11S860 is not yet complete and we have encountered a problem in that approximately 50% of our samples seem to be homozygous with this marker.

Table 2

A.	D11S1318		
	heterozygous samples	47/56	84%
	MSI	9/56	16%
	LOH	3/47	6%
B.	D11S4088		
	heterozygous samples	36/45	80%
	MSI	3/45	7%
	LOH	4/33	12%
	non informative	3/45	na

(7) CONCLUSIONS

The collection of breast tumor DNA samples has been expanded. We have used these samples to start our LOH analysis. Analysis is nearly finished with two of the three markers. We have results with D11S1318 which are similar to already published findings and this marker is thought to be outside the 3 Mb area of LOH. We have found that a marker which is centromeric to D11S1318, D11S4088, is also probably outside the region of interest. The telomeric boundary for the area of LOH has been moved centromeric with these results. We will move centromeric with the LOH analysis and then begin to isolate candidate genes from the area.

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