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TITLE: Defining the Smallest Common Region of Chromosome 17p that is Deleted in Sporadic Breast Tumors

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Germline mutations in breast cancer susceptibility genes account for 8% of all breast cancer cases. BCRA1 on 17q12 and BCRA2 on 13q12-13 are implicated in 50%-60% of breast cancer families, and they are both likely to also have an impact in sporadic breast cancer cases. Recently defined loci on chromosome 8p 12-22 are frequently lost in sporadic breast cancers and there is evidence for families with linkage to these loci. We propose to ascertain 8 ethnically diverse (non-Caucasian) families linked to putative BCRA3 loci on chromosome 8p, and to investigate whether a recently isolated prostate cancer metastases suppressor gene, KAI2, is involved in the genetic alterations of inflammatory breast cancer, a particularly aggressive form of breast cancer which accounts for 15% of all cases.
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Introduction

Hereditary breast cancer accounts for 5-8% of breast cancer cases (1). Loci on 8p12-22 are frequently lost in breast tumors and may thus harbor one or more tumor suppressor genes (2). Almost all of breast cancer families studied so far in North America and Western Europe are of Caucasian origin. We hypothesize that more ethnically diverse families with evidence of autosomal dominant transmission of breast cancer are needed to study loci on 8p12-22. As current data indicate that BRCA2 families are rarer than previously predicted, we hypothesize that a proportion of families found to be unlinked to both BRCA1 and BRCA2 will be linked to recently defined loci on chromosome 8p12-22; as a corollary, the characterization of the 8p-linked familial tumors will help define the limits of the candidate regions on this chromosome. The specific aims proposed in this application are:

1. To ascertain at least 5 site-specific breast cancer families of diverse ethnic background who are linked to loci on chromosome 8p12-22.

2. To analyze the 8p-linked tumors for LOH at closely spaced markers to help refine the breast cancer candidate region(s).

3. To test families unlinked to BRCA1, BRCA2, and loci on 8p12-22 for linkage to the KAI1 gene, a metastases suppressor gene, and to analyze sporadic breast tumors for mutations in KAI1.

Significance and Preliminary Studies

Family collection.

Breast cancer will affect 1,600,000 women world-wide in 1995. In the US alone, 48,000 will die of this disease in 1996 (3). The discovery of breast cancer susceptibility genes has allowed the study of a relatively simple and unique model system for human mammary epithelial carcinogenesis. The tumors suffered by the women belonging to these families are clinically indistinguishable from sporadic tumors. Our laboratory has recently proven that at least 10% of sporadic ovarian cancers undergo inactivation of both BRCA1 alleles.

The scarcity of African American and other minority families in the study of breast cancer susceptibility genes provides the impetus for this application which combines basic research in molecular genetics of breast cancer with a rational attempt at a minority family collection (5-8). The study of the etiology of the widening gap in survival (3) between Caucasian and African American populations requires that future experiments on cancer susceptibility genes be done on an ethnically balanced population of samples. The lack of availability of these samples and the long lag-time for their acquisition have invariably been the limiting factors. Our proposal intends to contribute shared resources. The Breast Cancer Tissue Core at the University of Michigan (DOD-funded; S. Ethier, Principal Investigator; S. Merajver, co-investigator) provides an independently funded mechanism to freely share these resources for the next 4 years.

Epidemiological data on family history and breast cancer incidence on Hispanic, Japanese, and Arab women suggests that familial clustering of breast cancer also appears in these populations (9-11). We have been involved for 5 years in an extensive breast cancer family collection and counseling which was at first dedicated to the positional cloning effort of the breast cancer susceptibility gene, BRCA1 (12,13). Since 7/1/94, under the direction of Dr. Sofia Merajver, the Breast Cancer Genetics Project has ascertained 114 new breast cancer families. Thirty of those families are in active collection, and 7 have been completely collected. The principal sources of referrals are: the University of Michigan Breast Care Center (BCC) (450 new breast cancer patients a year), community surgeons, oncologists, and general practitioners; physicians at other research institutions. We have established a successful collaboration with researchers at Henry Ford Hospital (HFH) in Detroit, MI, where a large number (over 150/year) of African American and Hispanic breast cancer patients with a family history are diagnosed and treated. The family collection at the University of Michigan has proven invaluable in the localization of BRCA1 and in the assessment of familial mutations. The collection provides a renewable source of RNA and DNA for functional studies after a tumor suppressor gene is cloned. The families ascertained through the research have the option of gaining knowledge of their risk if they so wish under approved protocols.

Linkage studies to 8p12-22 and other loci.

Fueled by the observation in several laboratories that fewer (10-20%) breast cancer families than previously anticipated (40-50%) are linked to BRCA2, other candidate loci such as 8p12-22 are being actively tested, where LOH has consistently been observed above background levels. Forty-seven percent of tumor
samples have LOH here and at least one breast cancer family with a LOD (logarithm base 10 of the odds favoring linkage) score of 3.70 for markers in this locus has been described. The study of specific breast cancer families linked to the different loci will provide key information and materials for the understanding of these pathways. The preliminary studies described here show that there is enough statistical evidence to pursue other tumor suppressor loci as putative familial cancer susceptibility genes on chromosomes 8p12-22 and 11p.

**Loss of heterozygosity (LOH)**

Following Knudsen's "two-hit hypothesis" (19), loss of heterozygosity (LOH) is generally a marker of the presence of a tumor suppressor gene. S. Merajver has studied LOH in familial and sporadic breast and ovarian tumors for the last 4 years (4,12). These experiments demonstrate that LOH at closely spaced, ordered markers on a tumor suppressor candidate region can define a minimum region of overlap and help localize the desired transcript. This experimental design can be extended to other tumor suppressor regions, such as 8p12-22.

**Mutation analyses of familial and sporadic specimens.**

Advances in familial cancer syndromes are also relevant to sporadic carcinogenesis. For example, the PI and others have recently shown that BRCA1 is implicated in sporadic carcinogenesis (4). Dr. Merajver's laboratory was the first to report 4 somatic mutations (12). The initial screen for mutations was performed by a modification of single-strand conformation polymorphism (SSCP) technique. These experiments show that even for a fairly large gene such as BRCA1, thorough, semi-automated, batch-mode mutation analyses can be performed efficiently and rapidly with a combination of SSCP and direct sequencing of familial and sporadic tumors. Even in the complicated situation posed by BRCA1, these methods have proven effective; we believe that it is not overly optimistic to predict that they will serve us well for tumor suppressor genes on 8p12-22.

**SUMMARY OF PRELIMINARY STUDIES.**

The PI has performed family collection studies (13), directs a high-risk breast cancer clinic where family members are counseled, has conducted detailed mapping studies of sporadic tumors by LOH (12), and has ascertained that the mutations of the first described familial breast cancer gene (BRCA1) are present in sporadic tumors (4). Research in all these areas has been published in the peer-reviewed literature. (4,12,13)

**Body**

The work performed in Dr. Merajver’s laboratory under this grant is part of a multifaceted collaborative effort to understand the molecular genetics of familial and sporadic breast cancers. As can be seen below, we have proceeded with our studies of families with potential linkage to BRCA3 putatively assigned to chromosomal region 8p12-22. In addition, the much welcomed isolation of the BRCA2 gene in the period covered by this report provoked a transitory change in direction, due to the urgency and timeliness of ascertaining the role of BRCA2 in sporadic tumors and the sequencing of the full length cDNA. Our laboratory collaborated in efforts towards solving these problems; peer-reviewed publications reporting findings on the BRCA2 gene have already appeared, and more are being prepared at the time of this writing. In addition, the quest for understanding both the molecular genetics and the clinical course of familial and sporadic breast cancer has led to publications in the clinical breast cancer literature, supported in part by this grant. A complete list of publications which have appeared or are in press for the period 10/1/95-9/31/96, are listed at the end of this section.

The work on 8p involving families and tumors has progressed as follows, in accordance to the specific aims of the project.

**Family ascertainment.** We assembled an information network by obtaining the names and addresses of charitable organizations and predominantly minority (mainly African American and Hispanic) churches in the metropolitan Detroit area and by an ongoing collaboration with HPH. We will be sending a mailing outlining the project to these individuals and organizations in the next few weeks. We are seeking families with 3 or more cases of female breast cancer in 2 or more generations within a lineage, average age of onset < 60 years, bilateral disease, or male breast cancer. Because of our success in obtaining and using tumor blocks, we are not limited by the number of living affected individuals, as long as we are able to ascertain that the tumor blocks are indeed available. We expect to ascertain 3-4 8p12-22 families through the screening of new
minority cases at UM and HFH. The remaining 2-3 BRCA2 families will be ascertained through the community-based effort we have begun with African American church leaders in the Detroit metropolitan area, which has a 40% minority population communication. We aim to complete the family ascertainment and analyses of pedigrees during the next 6 months even if the community-oriented efforts are only half as successful as our expectations. Of the 23 non-BRCA1 non-BRCA2 families we have screened so far, 4 have shown to share a common haplotype at 8p12-22, but the LOD scores are small due to the small number of affected individuals whose specimens are available. We are yet to fully utilize the community resources we have gathered, but we expect to accomplish that in the next 6 months, approximately as planned in the SOW. Familial specimen collection.

The collection of blood specimens has been undertaken in the manner in which we have proceeded so far, under an IRB-approved protocol. In brief, after signing informed consent, we draw 3 lavender-top tubes (5 cc each) for DNA and 2 green-top tubes (for lymphocyte immortalization). The DNA extraction and immortalization protocols are described below. Pre-paid mailing kits or travel to the family’s domicile are used for remote specimens. This latter method is very cost-effective, allows for the establishment of close rapport with the family, and provides for an opportunity to answer questions regarding the research and breast cancer. The familial specimen collection is proceeding at an extremely fast pace, with over 50 tumor specimens, many of them quite old and from remote sites, already received and processed at the University of Michigan. We have extracted the DNA and have started the LOH experiments on 8p. We are also poised to proceed in an expedient manner were the actual BRCA3 gene to be isolated by other labs in the near future. This portion of aim 1 is ahead of schedule as per the SOW.

Linkage analyses.

Linkage analysis is performed using the method of LOD scores (14-16). Calculations are carried out with the programs MENDEL (15) and LINKAGE (16). All families will be screened for linkage to BRCA1 with D17S855 and D17S1323, two intragenic polymorphic markers, and BRCA2, the latter with D13S221, D13S260, D13S267, and D13S263. Following Kerangueven et al (2), we will use the following panel of markers on 8p12-22 (Table 1).

For each family not linked to either BRCA1, or BRCA2, or 8p12-22, rapid SSCP analyses of 2 affected individuals with age of onset of cancer under 50 will be conducted on the KAI1 gene on 11p11.2 (17). The primers for this gene have been designed and synthesized, and tested on DNA from lymphocytes and archival materials. we are planning to proceed to "run" the KAI1 gene through these tumors in the next 6-12 months.

TABLE 1. 8p markers to be used in this study ordered from centromere to telomere

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The 8p markers have all been synthesized, tested on trial paraffin specimens (we are always extremely frugal with our valuable familial specimen resources), and optimized for amplification of paraffin-embedded material. The analyses of 50 familial and over 40 sporadic specimens along the 8p12-22 region will continue for the next 1.2 years, as per the SOW.

Additional Projects and Publications. (Completed under this grant due to auspicious circumstances which modified and expanded the course of the research).

BRCA2. Cloning and sequencing of the full-length cDNA, and analyses of mutations in families, as well as the investigation of the role of BRCA2 in familial and sporadic male breast cancers.

Miscellaneous. The use of mammography in locally advanced breast cancer to determine which patients are amenable to breast conservation. Although seemingly unrelated, this work actually ties in beautifully with our work on the KAI1 gene, because it is these aggressive tumors which we are targeting for studying the KAI1 gene on.

List of Publications.


Conclusions

It is still premature to draw definitive conclusions from the research described above, except in those cases where the material has already been published. Our preliminary work on 8p encourages us even more than when the grant was written, to aggressively pursue this line of research, as we expect this region will be crucial in our understanding of breast carcinogenesis.

References