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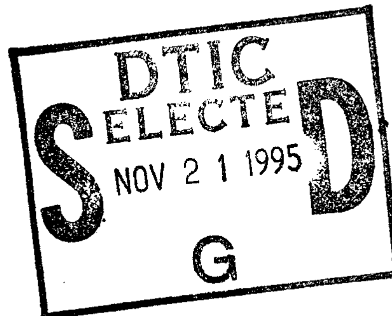

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This proposal seeks to determine if the ataxia-telangiectasia (AT) gene acts as a tumor suppressor gene in a subset of breast cancers by looking for tumor loss of constitutional heterozygosity at chromosomal region 11q22-q23, the map position of the AT gene(s). The technical aims are to analyze 300 matched pairs of tumor and normal tissue from breast cancer patients for tumor loss of constitutional heterozygosity using a panel of amplification polymorphisms mapping to 11q22-q23 and, when loss of heterozygosity is detected, to map the extent of that loss.

Over the past year, 37 sets of tumor and normal tissue in pathology storage as paraffin embedded samples were screened for 14 polymorphic loci based on simple sequence repeats (SSRs) using the polymerase chain reaction (PCR). Seventeen of these tumors were found to have lost heterozygosity either in the region of the AT gene or an adjacent region that might overlap. All but one of these deletions appear to be interstitial.

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Michael M. Weil 9/13/95

PI - Signature Date

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Introduction

The work proposed in this application is designed to determine if the gene responsible for ataxia-telangiectasia (AT) acts as a tumor suppressor gene in the development of breast cancer. The approach is to survey breast cancers for tumor loss of constitutional heterozygosity in 11q22-q23, the chromosomal region where AT maps. A conceptual framework for the project is provided by epidemiological studies showing increased breast cancer risk in women who are obligate AT carriers.

The frequency of AT carriers in the U. S. general population is estimated to be 1.4% and they may compose 9 to 18% of breast cancer patients. Their increased breast cancer risk is based on studies of women who have AT affected children and are thus obligate carriers and blood relatives of AT patients who have an increased probability of being carriers.

Deletion of genetic material is a frequent occurrence in tumors. Generally, the loss is detected by surveying normal and tumor tissue from the same individual for a selected, mapped restriction fragment length polymorphism by Southern hybridization analysis. A finding that normal tissue has two different alleles and the tumor has lost one of these indicates a loss of genetic material and is referred to as tumor loss of constitutional heterozygosity (LOH) or reduction to hemizygosity. Consistent LOH for a chromosomal region in a tumor type implies the existence of a tumor suppressor gene at that location. At the time this proposal was made there were no comprehensive LOH studies focusing on 11q22-23 and involving large numbers of breast cancer samples reported in the literature.

This project was designed to determine if the AT gene functions as a tumor suppressor gene in breast cancer by screening large numbers of breast cancer DNA samples for loss of constitutional heterozygosity at 11q22-q23, the chromosomal region where the AT gene maps. The specific aims are:

1. To analyze 300 matched pairs of tumor and normal tissue from breast cancer patients for tumor loss of constitutional heterozygosity using a panel of amplification polymorphisms mapping to 11q22-q23.

2. To map the extent of the loss in those tumors where it is detected through the use of markers spanning 11q22-q23 as well as several markers in proximal and distal 11q.

Body

Assay

Paraffin blocks and hematoxylin and eosin (H&E) stained slides composing paired samples of normal (tumor negative lymph nodes) and tumor tissues from the same patient were obtained from storage. The H&E slides were reviewed and sections containing large areas of invasive tumor identified. Histological parameters (tumor size, presence of multifocality, tumor subtype/classification and nuclear grade) and clinical parameters (patient's age, medical history,

family medical history etc.) were documented. DNA was extracted from the tissues following the protocol of Schibata *et al* (1). Six 10 μm serial sections were cut from each block and mounted on thin, flexible plastic slides. The sections were stained with H&E and screened by a pathologist who placed a dot of ink from a laboratory marker over nests of tumor cells and left normal tissues unmarked. The sections were then UV irradiated which crosslinks the DNA and prevents it from being used as a substrate in the PCR reaction. DNA in cells covered with a layer of ink is protected from the UV irradiation. Extraction of the DNA was accomplished by cutting sections of the slide into pieces that were placed into microfuge tubes and boiled in the presence of chelex 100 resin. Normal tissue uncontaminated by tumor cells was usually available and could be used for DNA preparation without the UV irradiation step.

The SSR genotyping using these DNA samples was done using the method described by Dietrich *et al* (2). Aliquots of the DNA were amplified in PCR reactions in which one of the primers had been ^{32}P labeled. The labeled PCR products were then separated on a polyacrylamide gel and visualized by autoradiography or storage phosphor imaging.

Markers

The normal and tumor DNAs were assayed with fourteen PCR markers that spanned chromosome 11. Each sample pair was evaluated for tumor loss of heterozygosity at each SSR locus.

Results

The results are summarized in Table 1 (appendix). The critical region containing the AT gene is defined by the markers D11S876 and D11S1391 and is immediately flanked by D11S901 (proximal) and D11S976 (distal). Of the 50 tumors examined, 17 lost heterozygosity for a marker in the critical region or for one of the flanking markers. No examples of nullizygosity were seen. Most of the loss of heterozygosity appears to be interstitial, only a single tumor lost most of the distal markers.

Conclusions

About 45% of breast cancers lose heterozygosity in 11q22-23 and this loss is interstitial. The same finding has now been reported by two groups (3,4).

The work in the upcoming year will not be an expansion of the work described here to additional tumors and markers as originally proposed. The gene which, when mutated, results in AT has recently been cloned and given the designation ATM (AT mutated) (5). The premise that ATM acts as a tumor suppressor gene can now be tested directly.

Development of a DNA-based assay of ATM is complicated by the large size of the ATM transcript (12 kb) which has not yet been completely cloned. Also, of the dozen mutations that have been characterized in ATM, seven are small deletions or insertions of only a few nucleotides and these vary from patient to patient. Therefore, we will generate antisera to the protein

encoded by ATM and use this antisera to develop quantitative immunoprecititation and immunoblotting assays for ATM protein and a kinase assay to quantitate levels of functional ATM protein. If a quanitative immunohistology assay can be developed for this antisera that will work in paraffin-embedded sections, we will screen paraffin-embedded tumor samples, including the 17 already known to have lost heterozygosity at 11q22-23, to detect ATM antigen loss. Since 7 of the 12 ATM mutations described lead to truncated proteins this approach should have a reasonable chance of success, particularly using antisera raised against the COOH-terminal region of the protein.

Thus far, we have cloned the ATM transcript in four overlapping fragments after amplifying it with RT-PCR. We have confirmed that the cloned amplification products are derived from the ATM transcript by sequence analysis. Currently, we are subcloning the fragments into a vector that allows the production of recombinant proteins with short polyhisidine leader sequences that can be purified by affinity chromatography on a nickel-chelating resin.

Antisera to the recombinant proteins will be produced in rabbits at the UTMDACC Science Park facility. Test bleeds will be titered by ELISA. Antisera will be characterized by immunoblotting and by immunoprecipitation to determine the number of protein species recognized and if native protein can be recognized. Serum collected from the same animal prior to immunization will be used as a control. Immunoblotting, immunopercipitation, ELISA and immunohistology will all be done using standard methodologies (6).

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Sample Number	Locus	D11S 902	D11S 904	D11S 916	D11S 901	D11S 876	D11S 1391	D11S 976	drd2	D11S 29	D11S 614	cd3d	D11S 968	D11S 872	D11S 874
1		H	NI	NI	H	NI	H	NI	H	NI	H	H	L	H	H
2		H	H	H	H		H		H	H	H	H	H	NI	NI
3		L	H	H	H	H	H	L	H		H	H	H		H
4		H	L	H	H	H	H	H	H		H	H	H	NI	
5		H	NI	H	H	H	H	H	H	H	H	H	H	H	H
6		L	NI	H	H		H	L	H	H	L	L	H		
7			NI	H	L		H	H	H	H	H	H	H		H
8			NI	H	NI	NI	L	H	H	H	H	H	H	L	H
9		H	H	H	L			H	H	H	L	L	H		NI
10		H	H	H			L		H	H	H	H	H	NI	NI
11		NI	H	H	NI	L	L	H	H	H	H	L	H		H
12		H	NI	L	H	L	H	L	H	H	NI	H	H	H	
13		H	L	H	H	H	H		H		H	H	H	H	NI
14		H	H	L	NI			H	H			H	H		
15		H		H		NI		H	H		H	H	H	NI	NI
16		H							H		H	H	H		
17		H		H	H	NI		H	H		H	H	H	NI	
18		H	L	L	H	NI	L	H	H	H	H	H	H	H	H
19		H	H	H	H	NI		H	H	H	H	H	H	H	H
20		H	H	H	H	H	H	H	H	H	H	H	H	H	NI
21		H	H	H	H	L	L	H	H	L	L	H	H	H	
22		H	H	H	H	H	H	H	H	H	H	H	H	NI	H
23		H	H	H	H	H	H	H	H	H	H	H	H	H	H
24		L	H	H	L	L	L	L	L	L	H	L	L	H	L
25				H	L			H	H		H	H	H	H	H
26		H		H	H	H		H	H	H	H	H	H	H	NI
27		H	H	H	H	H		H	H	H	H	H	H	H	NI
28		H	H	H	NI	L	H		H	L	L	H	H	H	L
29		H	H	H	NI	NI	H	H	H	L	H	H	H	H	H
30			NI	H	NI	L	H	H	H	H	H	H	H	H	L
31		H	L	H	H	H	H	H	H	H	H	H	H	H	L
32		H	H	H	H	H	H	H	H	H	H	H	H	H	NI
33		H	L	H		L	H	H	H	H	H	H	H	NI	H
34		H		H		H	H	H	H			H	H	NI	H
35		H	L	H		H	H	H	H			H	H	NI	H
36		H	L	H	NI	H	L	H	H			H	H	H	NI
37		H	H	H	H	L	L	H	H			H	H	NI	H

Table 1 Loci are ordered from p(ter) to q(ter). Tumor samples retain constitutional heterozygosity (H), lose heterozygosity (L), or are non-informative (NI).