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GRANT NUMBER: DAMD17-94-J-4129

TITe: Identification of BRCA1 and 2 Other Tumor Suppressor Genes on Chromosome 17 Through Positional Cloning

PRINCIPAL INVESTIGATOR(S): Dr. Raymond L. White

CONTRACTING ORGANIZATION: University of Utah
Salt Lake City, Utah 84102

REPORT DATE: October 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1995	Annual 1 Jul 94 - 30 Jun 95

4. TITLE AND SUBTITLE
Identification of BRCA1 and 2 Other Tumor Suppressor Genes on Chromosome 17 Through Positional Cloning

5. FUNDING NUMBERS
DAMD17-94-J-4129

6. AUTHOR(S)
Dr. Raymond L. White

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Utah
Salt Lake City, Utah 84102

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

After the identification of BRCA1, it became evident that some breast and prostate tumors show allelic loss on chromosome 17q12-21 very close to, but not including, the BRCA1 locus. These observations strongly suggests the presence of important tumor suppressor genes at these two locations. Therefore, we have analyzed two regions approximately 1Mb proximal and 1Mb distal to BRCA1, which show well defined loss of heterozygosity in sporadic breast tumors and sporadic prostate tumors respectively, to identify additional candidate tumor suppressor genes. Transcripts from these regions were identified using genomic reagents from our 4.5-Mb physical contig that contains the BRCA1 gene. Two novel members of the dlg2 and dlg3 disc-large family of genes, and a gene encoding an ADP-ribosylation factor (ARF4L) were identified in the distal region. In the proximal region we identified the plakoglobin gene, which previously had been thought to be located on chromosome 7. Plakoglobin, dlg2 and dlg3 are believed to be molecular components of cell-cell junctions, whereas ARF4L is believed to be involved in protein secretion and signal transduction. Several prominent tumor suppressor genes including the adenomatous polyposis coli gene (APC) and the Drosophila discs-large gene (Dlg) are components of cell-cell junctions, making three of the novel genes we identified and/or placed in the genomic region surrounding BRCA1 particularly interesting candidates for further investigation.

14. SUBJECT TERMS
breast cancer, tumor suppressor genes, LOH mapping, Chromosome 17, genetic mapping, CDNA screening

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Introduction

The initial objectives of this project were to identify a hereditary breast cancer gene, BRCA1, located on the long arm of chromosome 17, and two other tumor suppressor genes located near the extremities of chromosome 17. During the first three months of the grant period all our efforts were directed towards the characterization of candidate genes for BRCA1. The DNA sequences of several genes were obtained and screened for mutations during this effort to extend the development of our map of expressed sequences in the BRCA1 region (Albertsen et al., 1994). This was all done according to Task 1 of our original grant proposal. However, our emphasis on detecting BRCA1 changed after the appearance of two articles in Science (Miki et al. (1994), Futreal et al. (1994)) where a strong candidate gene for BRCA1 was presented. Evidence confirming that the candidate gene indeed is BRCA1 was subsequently found by several independent groups (Castilla et al.(1994), Friedman et al.(1994), Simard et al.(1994)). Based on these results, Task 1 in our proposal had been reached. Task 2 in our proposal is divided into two sections; a. genetic mapping, and b. physical mapping of the regions harboring two additional tumor suppressor loci. Great progress has been made with respect to the genetic mapping of these two chromosomal regions at each extremity of chromosome 17. The current resolution of the genetic map is now at a resolution of approximately 1 genetic marker per cM, -largely sufficient to start the development of physical maps-, and has been described in detail in three manuscripts from our laboratory (Murray et al. (1994), Gerken et al. (1995), The Utah Marker Development Group (1995). The development of physical maps as originally proposed have been put on hold for reasons described below. With the exact location of BRCA1 known, it became evident that a narrowly delimited region of allelic loss could be detected less than 1Mb distal to BRCA1 in certain sporadic prostate tumors (Brothman et al. in press, Williams et al. submitted); 12 unrelated specimens showed narrow allelic losses distal to BRCA1, as observed either by loss of heterozygosity (LOH) centered around D17S902 or by fluorescent in-situ hybridization using a collection of P1 clones isolated from a 400-1000kb region surrounding D17S902. Similarly, we and others reported a narrow region of loss of heterozygosity less than 1Mb proximal to BRCA1 in sporadic breast tumors (Cropp et al. 1994, Aberle et al. 1995); ten unrelated specimens showed LOH of a region with a minimal overlap of less than 120kb centered around D17S846. Because neither of these regions included the BRCA1 locus itself, the question was raised as to whether additional tumor suppressor genes were located in the immediate vicinity of BRCA1. Based on this observation and the observation that BRCA1 mutations have been identified in only a

small fraction of sporadic breast tumors that show LOH in this region of the long arm of chromosome 17 (Futreal et al., 1995), we concluded that the larger BRCA1 region (4.5Mb) was the likely home for additional tumor suppressor genes. Therefore, several of the expressed sequences we had identified in these two regions have been developed into full-length cDNA sequences and their predicted protein structure have been analyzed for recognizable domains and homologies. Of the four genes we identified, three are novel; the fourth gene, plakoglobin, has previously been extensively characterized for its biological function. However, its original genomic localization to chromosome 7 appears to have been based on a mis-interpretation. Plakoglobin's true location on chromosome 17, its genomic structure and possible implication in tumorgenesis has been discussed (Aberle et al.,1995; Callahan et al. in preparation). Brief descriptions of each gene will be given below. Manuscripts from our laboratory which have been accepted for publication and are related to our grant are included with this report and describes both which methods have been used and which results have been obtained.

Body

During the past twelve months several discoveries have been made with respect to breast cancer. Most significant was the discovery of Miki et al. (1994) and Futreal et al. (1994) of the BRCA1 gene itself; however, the genomic environment of BRCA1 has also revealed several interesting features. Dr. Solomon's group showed that the 5-prime end of BRCA1 is in very close proximity to the 5-prime end of the 1A1-3B gene (Brown et al., 1995), raising the possibility of studying transcriptional control (transcriptional dominance, positive or negative interference) among these two genes. Our group has found that the L21 riboprotein is located within the BRCA1 locus, in an intron flanking BRCA1 exon 14. We have made other potentially very interesting observations by analyzing primary tumors for allelic loss; those results have suggested the presence of two additional tumor suppressor genes in the immediate vicinity of BRCA1.

This new information placed us in a position where the resources available through our army grant (DAMD17-94-J-4126) were adequate to pursue either the physical mapping of the two distal tumor supperssor loci, as originally proposed, or to identify the two potential tumor suppressor genes immediately flanking BRCA1 and located within our existing physical map of the BRCA1 region. Based on the fact that a physical map of the entire human genome is under construction at the Centre d'Etude des Polymorphisme Humain (CEPH) in Paris, France (Chumakov I.M. et al., 1995), we

realized that the component from our original proposal involving the construction of a physical map surrounding the two distal tumor suppressor regions would be redundant to the physical mapping of the entire human genome currently undertaken at the CEPH. Because the establishment of a physical map of the distal regions of chromosome 17 is NOT our primary goal, but rather a necessary tool to identifying the two distal tumor suppressor loci described in our original proposal, we decided to postpone our search for the two distal tumor suppressor genes for one or two years, until physical maps and genomic reagents from these regions become available from the CEPH. In the interim period we propose to concentrate our efforts on investigating the two potential tumor suppressor loci located next to BRCA1. Four possible candidate genes from these regions have been identified over the past 12 months: two Drosophila discs-large homologs, plakoglobin, and an ADP-ribosylation factor.

Protein components of cell-cell junctions.

Interaction among cells is critically important for maintaining proper cellular polarization and organization of the epithelial sheet. Generally described, intra-cellular orientation and inter-cellular communication and organization of epithelial cells are mediated via protein components of cell-cell junctions. Mutations in these protein components can lead to partial or complete loss of function of the cell-cell junctions, which would lead to misorganization of the tissue and subsequently to tumorgenesis. (For a literature review describing cell-cell junctions and their protein components see Kirkpatrick and Peifer 1995). On the basis of the predicted structure of two human Drosophila discs-large homologs we have identified in the BRCA1 region, dlg2 and dlg3, we believe that the products of the genes, along with Plakoglobin, are protein components of cell-cell junctions.

Common to all members of the Drosophila discs-large family of genes are three distinct structural domains. At the N-terminal are 1-3 somewhat degenerate Drosophila homology regions (DHRs). DHR motifs are approximately 90 amino acids long and have been shown in vitro to bind cytoskeletal proteins of the band 4.1 family. A region with homology to src oncogene motif 3 (SH3) is found in the central part of the discs-large proteins. This motif, approximately 60 amino acids in length, is known to be a site of protein-protein interactions. Finally, a guanylate kinase domain (GK) is found at the C-termini of the Dlg proteins. The function of this domain is the catalytic transfer of phosphate from ATP to GMP, forming GDP. In Drosophila, Dlg has been shown to be a tumor suppressor (Woods et al., 1989).

Plakoglobin (also known as γ-catenin) contains several armadillo (Arm) repeats (Aberle et al., 1994). Plakoglobin is believed to interact directly with cadherins, α-catenin and the product of the well known colon-cancer gene APC (Su et al., 1993). It has also been shown that human breast cancer cells show reduced expression of plakoglobin and, in a subset of these breast cancer cells, complete absence of this gene product has been demonstrated (Sommers et al., 1994).

ADP-ribosylation factor (ARF4L) is, based on its predicted protein structure, believed to be involved in membrane trafficking and protein secretion. Six protein domains have been identified, three of which are involved with phosphate/magnesium binding, while the remaining three are involved with guanine nucelotide binding.

Genes from the critical regions.

Additional expressed sequences from the two regions in question exist, and if it becomes necessary to develop these reagents further it will be straightforward to do so. However, our present focus is to characterize the four genes described above. We intend to do this in several ways, one of which will involve screening for mutations in these genes in sporadic tumors which reveal LOH. Alternatively, the genes will be tested for their biological roles in tissue culture systems, either through DNA oligo antisense-mediated suppression of normal gene expression or by transfecting breast epithelial cells with conditionally induceible expression vectors carrying the genes.

Conclusions

Although it was not our group that identified the BRCA1 gene, the past 12 months have been quite successful in our opinion. With respect to BRCA1, we developed and published the largest and most comprehensive physical map of the region and we are confident that it would have been a matter of short time before our strategy would have led us to the identification of BRCA1. With the precise localization of BRCA1 known, two narrow regions flanking BRCA1 on either side have emerged as candidate sites for additional tumor suppressor loci. We are presently investigating four genes from these regions for their possible roles as novel tumor suppressor genes.

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List of salaried personnel:

Name:	Position:	Percent contribution to salary:
Hans M. Albertsen,	Research Instructor,	67%
Jeff Stevens,	Research associsiate,	100%
Ray White,	P.I., Professor,	10%