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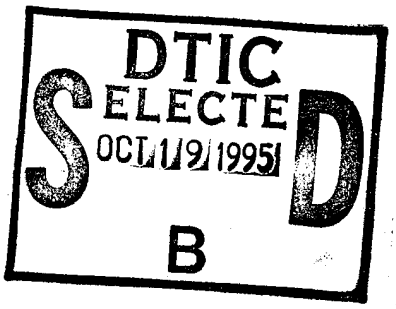

TITLE: Genetic Analysis of Human Breast Cancer

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REPORT DATE: August 14, 1995

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for public release;
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19951018 031

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REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	Aug. 14, 1995	Annual July 15, 1994 - July 14, 1995

4. TITLE AND SUBTITLE
Genetic Analysis of Human Breast Cancer

5. FUNDING NUMBERS
DAMD17-94-J-4247

6. AUTHOR(S)
Dr. Michael H. Wigler/Dr. Nikolia Lisitsyn

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

8. PERFORMING ORGANIZATION REPORT NUMBER
705100

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, distibution unlimited

12b. DISTRIBUTION CODE

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Cancer is a disorder brought upon by the accumulation of specific mutations in the cancerous cells. Our understanding of the disease, and potentially its diagnosis and therapeutic treatment, is enhanced by defining the genetic lesions that cause it. Representational difference analysis, or RDA, was developed to do just this. RDA is a DNA subtraction methodology that finds sequences present in one DNA population, the tester, that is absent or reduced in a second, the driver (1, 2). RDA has been used to discover sequences lost or amplified in the genomic DNA of the cancerous cells (3). Genetic loss and gene amplification are hallmarks of tumor suppressor genes and oncogenes, respectively. We have been applying RDA to the discovery of sequences lost in breast cancer. At least three loci undergoing loss in breast cancer have been identified. The transcriptional potential of these loci is being explored. Characterization of many other RDA probes is in progress. We expect that the continued execution of our stated plan will accomplish our stated goal, the identification of tumor suppressor genes that are commonly involved in breast cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Oncogenes, Tumor suppressors, DNA,difference analysis, Genetic Lesions	7
	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 1	Unclassified	Unlimited

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INTRODUCTION

Cancer is a disorder brought upon by the accumulation of specific mutations in the cancerous cells. Our understanding of the disease, and potentially its diagnosis and therapeutic treatment, is enhanced by defining the genetic lesions that cause it. Representational difference analysis, or RDA, was developed to do just this. RDA is a DNA subtraction methodology that finds sequences present in one DNA population, the tester, that is absent or reduced in a second, the driver (1, 2). RDA has been used to discover sequences lost or amplified in the genomic DNA of the cancerous cells (3). Genetic loss and gene amplification are hallmarks of tumor suppressor genes and oncogenes, respectively. We have been applying RDA to the discovery of sequences lost in breast cancer.

RESULTS

The application of RDA to cancer requires the availability of matching tumor and normal DNA from the same individual, as otherwise the cloning of DNA polymorphisms results. The vast majority of available tumor material is not provided with accompanying normal cell samples. However, all tumors contain normal stroma. Since many tumors are aneuploid, we have chosen to apply RDA to tumor biopsies that have been sorted by flow cytometry into aneuploid (tumor) and diploid (normal) nuclei. Our studies have confirmed the utility of samples prepared in this way (3).

To date a total of 250 human breast cancer biopsy samples have been obtained from collaborating hospitals. These include 123 from Sloan-Kettering Memorial Hospital, 58 from the Cooperative Human Tissue Network, 65 from North Shore University Hospital, and 4 from Nassau County Medical Center. DNA content analysis by flow cytometric techniques have been performed on 198 of these samples. Of the samples analyzed a total of 51 have been sorted into diploid and aneuploid fractions. From these sorted fractions DNA has been prepared for RDA. An additional 17 samples have been identified for sorting from the initial 198 analyzed samples.

Seven pairs of normal and tumor DNAs have been analyzed by RDA using DNA samples isolated from aneuploid nuclei, fractionated by fluorescence-activated cell sorter from breast cancer biopsies. Many candidate probes have been isolated. Two probes have been characterized to date. One detected loss of heterozygosity of a polymorphic marker. Another nonpolymorphic probe presumably detected homozygous loss.

The last probe has been used to screen a P1 phage human genomic library. The ends of P1 clones have been cloned and their sequences as well as

the sequence of the original probe have been used for synthesis of three pairs of PCR primers. No additional homozygous losses have been detected with this probe in 150 tumor DNAs isolated from our collection of cell lines.

The P1 clones described above have been located by flourescent in situ hybridization to the long arm of chromosome 22. This region has been shown to be frequently deleted in breast tumors. In collaboration with Human Genome Center for chromosome 22 (The Children's Hospital of Philadelphia) these probes have been placed on a YAC contig in a region 22q11.12 positioned three megabases apart from the centromere, and several megabases apart from the site of constitutional reciprocal translocation t(11q;22q) found to be associated with increased risk of breast cancer.

In addition to these studies, we have identified loci that undergo deletion in colon and kidney cancers. Probes from these loci detect deletion in one breast cancer cell line. In particular two probes located on chromosomes 3 (band p21) and 20 (band p11) generated in studies of DNA losses in colorectal tumors, have been found to be simultaneously missing in breast cancer cell line MDA-MB-436, indicating that potential tumor supressor genes, which are encoded in these regions, are involved in different pathways. The original two probes have been used for screening YAC libraries and several additional sequences from the same genomic region have been subcloned from each YAC, using new subtraction technology which we developed for this purpose.

Frequent homozygous losses of these sequences have been detected by the polymerase chain reaction in a collection of DNAs isolated from >200 cancer cell lines of different origin and the regions of common loss on chromosomes 3 and 20 have been identified. The probes from these regions have been found to be homozygously lost with remarkable frequency (14.9% and 7% correspondingly) in cell lines established from tumors of the gastrointestinal tract (stomach, duodenum, colon, rectum). We have focused our efforts on positional cloning of the candidate genes from these loci.

To make a physical map of the chromosome 3 region, four P1 and nine cosmid clones have been isolated. We applied an exon-trapping system (4) to the clones and 12 exon candidates were identified. Further analysis revealed that two of them are evolutionarily conserved and that three are expressed in brain and kidney. Full length cDNA is being cloned. As for chromosome 20, two P1 clones have been isolated and were analyzed by the exon-trapping system. Three exon candidates have been isolated. Screening of cDNA liraries is being carried out.

CONCLUSIONS

RDA is an effective way to identify regions of genetic change in cancers, and flow cytometry is an effective way to obtain material for analysis. At least three loci undergoing loss in breast cancer have been identified. The transcriptional potential of these loci is being explored. Characterization of many other RDA probes is in progress. We expect that the continued execution of our stated plan will accomplish our stated goal, the identification of tumor suppressor genes that are commonly involved in breast cancer.

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PERSONNEL

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