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PRINCIPAL INVESTIGATOR(S):  Dr. Michael H. Wigler
                             Dr. Nikolia Lisitsyn

CONTRACTING ORGANIZATION:  Cold Spring Harbor Laboratory
                             Cold Spring Harbor, New York 11724

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Genetic Analysis of Human Breast Cancer

Dr. Michael H. Wigler/Dr. Nikolia Lisitsyn

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

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

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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.

Oncogenes, Tumor suppressors, DNA, difference analysis, Genetic Lesions

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Michael Hughey, Aug 12, 1995

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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 subraction 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 libraries
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.

REFERENCES


PERSONNEL

Drs. Nikolai Lisitsyn, Hao Peng Xu, Peter Barker, Robert Lucito, Masaaki Hamaguchi, Linda Rodgers, Wen Wei.