AD_____

GRANT NUMBER: DAMD17-94-J-4175

TITLE: Mapping of a Breast Carcinoma Tumor Suppressor Gene to Chromosome 11p15.5

PRINCIPAL INVESTIGATOR: Tracy Moore, Ph.D.

CONTRACTING ORGANIZATION: University of North Carolina
at Chapel Hill
Chapel Hill, North Carolina 27599-4100

REPORT DATE: July 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
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
	July 1996	Annual (1 Jul 95 - 30 Jun 96)

4. TITLE AND SUBTITLE
Mapping of a Breast Carcinoma Tumor Suppressor Gene to Chromosome 11p15.5

5. FUNDING NUMBERS
DAMD17-94-J-4175

6. AUTHOR(S)
Tracy Moore, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of North Carolina
at Chapel Hill
Chapel Hill, North Carolina 27599-4100

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, 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*

Loss of heterozygosity (LOH) on the short arm of chromosome 11 has been demonstrated in many cancers, suggesting the presence of a tumor suppressor gene. A number of studies have narrowed the region showing LOH in breast tumors to 11p15.5. The technical objectives of this study are: to screen breast tumor samples for LOH in the 11p15.5 region using markers in the region exhibiting the greatest LOH to further resolve the location of the putative tumor suppressor gene; and to screen candidate genes for abnormal expression in breast tumor samples.

In the three months since reactivation of the fellowship, the collection of breast tumor DNA samples has been expanded. We have used these samples to begin our candidate gene analysis. Currently, we are working with p57/KIP2, NAP2, and TAPA1. Following our candidate gene analysis, we plan to study the region of interest using polymorphic markers to look for LOH in the breast tumor DNA. We have selected markers which fall near the contig map that has been constructed in our laboratory. We will start with existing markers to define the area exhibiting LOH and then further delineate the region with new markers.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast cancer Tumor suppressor genes	9 pages
	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

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

JM In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

JM In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Tony Moore 7-19-96

PI - Signature Date

(4) TABLE OF CONTENTS

page 1 Front Cover

page 2 Report Documentation Page

page 3 Foreword

page 4 Table of Contents

page 5 Introduction

page 6 Body

page 7 Conclusions

page 8 References

(5) INTRODUCTION

Breast cancer is the most frequent cause of cancer death among North American and European women. An understanding of the basic mechanisms underlying the initiation and progression is imperative to both reducing the incidence of and improving the prognosis for the disease. Like the majority of human neoplasms, mammary carcinomas arise from epithelial cells, which suggests that insights gained from the study of breast cancer may shed light on the development and progression of various other cancers as well.

Cellular transformation can result from overabundance of oncogene product and subsequent overwhelming of normal cellular control mechanisms. Two well documented mechanisms include mutation of oncogenes or amplification of normal genes. In addition, numerous studies have suggested that the loss of a gene or genes involved in growth control may convert normal cells to their neoplastic counterparts. Demonstration of loss of heterozygosity (LOH) at specific chromosomal locations in a variety of familial cancers including retinoblastoma, Wilms' tumor and Recklinghausen's neurofibromatosis have led to the identification of tumor suppressor genes for these diseases (1). Some other cancers that have been associated with LOH are neuroblastoma, colorectal cancers, and carcinomas of the kidney, lung, and breast (1, 2). The identification of these genes by positional cloning techniques supports the same strategy for mapping putative breast carcinoma genes, particularly if LOH data have already indicated a likely location to within a 6-9 megabase pair (mb) region.

Loss of heterozygosity on the short arm of chromosome 11 has been demonstrated in many cancers, including rhabdomyosarcoma, Wilms' and other embryonal tumors, as well as tumors of the brain, bladder, lung, ovary, liver, adrenals, colon, and breast (3-16). In addition, functional studies have demonstrated loss of tumorigenicity by MCF-7 breast carcinoma cells after transfer of a whole chromosome 11 (17) and LOH at 11p15.5 by human milk epithelial cells immortalized by microinjection of SV40 DNA (18). A number of studies have narrowed the region showing LOH in breast tumors to 11p15 (19, 20) or even further, to 11p15.5 (7, 21-23).

We plan to narrow the region of LOH chromosome 11p15.5 in breast cancer to a small area amenable to positional cloning approaches. This will include the development of a long-range physical map of the region, the identification of new polymorphic markers in that region and the application of these markers to over 100 matched sets of normal and breast tumor material. This will eventually lead to the isolation of candidate genes for tumor suppressor activity in breast cancer. In addition to furthering our understanding of the basic mechanisms of oncogenic processes, identification of tumor suppressor genes and elucidation of their contribution to tumor formation and progression will aid in both diagnosis and treatment of cancers. For example, the loss of a gene product presents a situation that may be particularly amenable to nonsurgical interventions, such as gene replacement and/or drug therapy. The missing substance provided by the lost tumor suppressor gene may regulate oncogene expression, so replacement or enhancement of a tumor suppressor gene product may be a particularly valuable tool in a variety of situations.

(6) **BODY**

Since reactivating this fellowship three months ago, we have concentrated on the isolation of DNA samples and the screening of candidate genes by Southern analysis. The previously isolated samples of normal tissue and breast tumor DNA were found to be of insufficient quantity for Southern hybridization. We have received cDNA for several candidate genes in the region. Our lab has constructed a 500 kb contig of P1/PAC/BAC clones which includes the 11p15.5 region. Our future plans include screening the matched pairs of normal and tumor DNA with polymorphic markers to further localize the region of LOH in breast tumor cells.

A. Collection of breast tumor samples

We have isolated genomic DNA from 26 primary breast tumor samples in sufficient quantities for Southern analysis. The breast tumor material is available in greater numbers and yields more DNA than the matched tumor/normal breast pairs. Normal breast tissue has a high number of adipocytes and so the yield of DNA is low from even medium size samples. The breast cancer bank has given us 6 matched normal/tumor pairs to date. Of the 6 pairs only 3 of the normal breast tissue samples were able to give enough DNA for Southern analysis. We plan to continue isolating DNA from the matched tissue pairs as they become available. However, we have also isolated normal human DNA from tissue culture cell lines constructed from members of the CEPH families. Although the normal human DNA does not substitute for the matched normal tissue from breast cancer patients it will help us with our analysis.

B. Analysis of candidate genes

We have hybridized the cDNA for the human p57 gene to nine breast tumor samples (24). p57 is a good candidate because not only is it located in our region of interest, but also because it encodes a protein involved in the negative regulation of cell cycle progression. There were no differences in the migration of bands, the number of bands, in either EcoRI or HindIII digested genomic DNA (10 kb EcoRI band or the 11.5kb HindIII bands expected). The p57 probe will be used to screen more primary tumor DNA samples in the future, but our preliminary data suggest that this gene is not altered in breast samples.

Our lab and others have isolated a gene, NAP2, with homology to the NAP/SET family of proteins (25). This gene is an interesting candidate because our collaborators have found homozygous deletions in the NAP2 gene in Wilm's tumors and it is related to a family of proteins involved in cell cycle control. Since NAP2 resides in our region of interest we have been looking for changes in this gene in breast tumor DNA. The NAP2 analysis was more difficult than anticipated due to the fragmentation of the gene into 14 small exons. Initially we hybridized the entire cDNA to our tumor samples, but two of the EcoRI fragments recognised by the cDNA were the same size so we subcloned the cDNA in two fragments which would separate the similar sized bands recognized by the probe. The resulting fragments have been hybridized to nine tumor samples, none of which has shown a deletion or difference in the hybridization pattern. We plan to continue the Southern analysis using these probes on more tumor samples.

We have recently received another candidate gene that is located in our region of interest, TAPA1, which is related to KAI-I, a metastisis supressor (26, 27). The human cDNA for the TAPA1 gene has been hybridized to normal human DNA isolated from the CEPH family cell lines. We will now use this probe to analyze our tumor DNA on a Southern blot.

(7) CONCLUSIONS

The collection of breast tumor DNA samples has been expanded. We have used these samples to begin our candidate gene analysis. Currently we are working with p57/KIP2, NAP2, and TAPA1. We have collected DNA from CEPH family cell lines to use as normal controls and we plan to aquire more matched tumor/normal DNA pairs.

Following our candidate gene analysis, we plan to study the region of interest using polymorphic markers to look for LOH in the breast tumor DNA. We have selected markers which fall near the contig map that has been constructed in our laboratory. We will start with existing markers to define the area exhibiting LOH and then further delineate the region with new markers.

(8) REFERENCES

1. Evans, H. J., Prosser, J. Tumor-suppressor genes: Cardinal factors in inherited predisposition to human cancers. *Environ. Health Perspect.* 98: 25-37 (1992).
2. Stanbridge, E. J. Human tumor suppressor genes. *Annu. Rev. Genet.* 24: 615-657 (1990).
3. Weston, A., Willey, J. C., Modali, R., Sugimura, H., McDowell, E. M., Resau, J., Light, B., Haugen, A., Mann, D. L., Trump, B. F., Harris, C. C. Differential DNA sequence deletions from chromosomes 3, 11, 13, and 17 in squamous-cell carcinoma, large-cell carcinoma, and adenocarcinoma of the human lung. *Proc. Natl. Acad. Sci. USA* 86: 5099-5103 (1989).
4. Fults, D., Petronio, J., Noblett, B. D., Pedone, C. A. Chromosome 11p15 deletions in human malignant astrocytomas and primitive neuroectodermal tumors. *Genomics* 14: 799-801 (1992).
5. Fults, D., Pedone, C. A., Thomas, G. A., White, R. Allelotype of human malignant astrocytoma. *Cancer Res.* 50: 5784-5789 (1990).
6. Byrne, J. A., Simms, L. A., Little, M. H., Algar, E. M., Smith, P. J. Three non-overlapping regions of chromosome arm 11p allele loss identified in infantile tumors of the adrenal and liver. *Genes Chrom. Cancer* 8: 104-111 (1993).
7. Devilee, P., Van Den Broek, M., Mannens, M., Slater, R., Cornelisse, C. J., Westerveld, A., Meera Khan, P. Differences in patterns of allelic loss between two common types of adult cancer, breast and colon carcinoma, and Wilms' tumor of childhood. *Int. J. Cancer* 47: 817-821 (1991).
8. Mannens, M., Slater, R. M., Heyting, C., Bliek, J., de Kraker, J., Coad, N., de Pagter-Holthuizen, P., Pearson, P. L. Molecular nature of genetic changes resulting in loss of heterozygosity of chromosome 11 in Wilms' tumors. *Hum. Gen.* 81: 41-48 (1988).
9. Scrable, H. J., Witte, D. P., Lampkin, B. C., Cavenee, W. K. Chromosomal localization of the human rhabdomyosarcoma locus by mitotic recombination mapping. *Nature* 329: 645 (1987).
10. Wadey, R. B., Pal, N., Buckle, B., Yeomans, E., Pritchard, J., Cowell, J. K. Loss of heterozygosity in Wilms' tumour involves two distinct regions of chromosome 11. *Oncogene* 5: 901-907 (1990).
11. Reeve, A. E., Sih, S. A., Raizis, A. M., Feinberg, A. P. Loss of allelic heterozygosity at a second locus on chromosome 11 in sporadic Wilms' tumor cells. *Mol. Cell. Bio.* 9: 1799-1803 (1989).
12. Skinner, M. A., Vollmer, R., Hyuper, G., Abbott, P., Inglehart, J. D. Loss of heterozygosity for genes on 11p and the clinical course of patients with lung carcinoma. *Cancer Res.* 50: 2303-2306 (1990).
13. Fujimori, M., Tokino, T., Hino, O., Kitagawa, T., T., I., Okamoto, E., al, e. Allelotpe study of primary hepatocellular carcinoma. *Cancer Res.* 51: 89-93 (1991).
14. Ehlen, T., Dubeau, L. Loss of heterozygosity on chromosomal segments 3p, 6q and 11p in human ovarian carcinomas. *Oncogene* 5: 219-223 (1990).
15. Fearon, E. R., Feinberg, A. P., Hamilton, S. H., Vogelstein, B. Loss of genes on the short arm of chromosome 11 in bladder cancer. *Nature* 318: 377-380 (1985).
16. Dowdy, S. F., Fasching, C. L., Scanlon, D. J., Araujo, D., Livanos, E., Lai, K.-M., Weissman, B. E., Stanbridge, E. J. Suppression of tumorigenicity in Wilms' tumor by the p14:p15 region of chromosome 11. *Science* 254: 293-295 (1991).
17. Negrini, M., Castagnoli, A., Sabbioni, S., Recanatini, E., Giovannini, G., Possati, L., Stanbridge, E. J., Nenci, I., Barbanti-Brodano, G. Suppression of tumorigenesis by the breast cancer cell line MCF-7 following transfer of normal human chromosome 11. *Oncogene* 7: 2013-2018 (1992).

18. Garcia, I., Brandt, D., Weintraub, J., Zhou, W., Aapro, M. Loss of heterozygosity for the short arm of chromosome 11 (11p15) in human milk epithelial cells immortalized by microinjection of SV40 DNA. *Cancer Res.* 51: 294-300 (1991).
19. Ferti-Passantonopoulou, A., Panani, A. D., Raptis, S. Preferential involvement of 11q23-24 and 11p15 in breast cancer. *Cancer Genet. Cytogenet.* 51: 183-188 (1991).
20. Takita, K., Sato, T., Miyuagi, M., Watatani, M., Akiyuama, F., Sakamoto, G., Kasumi, F., Abe, R., Nakamura, Y. Correlation of loss of alleles on the short arms of chromosomes 11 nad 17 with metastasis of primary breast cancer to lymph nodes. *Cancer Res.* 52: 3914-3917 (1992).
21. Theillet, C., Lidereau, R., Escot, C., Hutzell, P., Brunet, M., Gest, J., Schlom, J., Callahan, R. Loss of an H-ras-1 allele and aggressive human primary breast carcinomas. *Cancer Res.* 46: 4776-4781 (1986).
22. Ali, I. U., Lidereau, R., Theillet, C., Callahan, R. Reduction to homozygosity of genes on chromsome 11 in human breast neoplasia. *Science* 238: 185-188 (1987).
23. Winquist, R., Mannermaa, A., Alavaikko, M., Blanco, G., Taskinen, P. J., Kiviniemi, H., Newsham, I., Cavenee, W. Refinement of regional loss of heterozygosity for chromosome 11p15.5 in human breast tumors. *Cancer Res.* 53: 4486-4488 (1993).
24. Reid, L. H., Crider-Miller, S. J., West, A., Lee, M., Massague, J., and Weissman, B. E. Genomic organization of the human *p57*^{KIP2} gene and its analysis in the G401 Wilm's Tumor assay, *Cancer Res.*56: 1214-1218, 1996.
25. Kellogg, D. R., Kikuchi, A., Fujii-Nakata, T., Turch, C. W., and Murray, A. W. Members of the NAP/SET family of proteins interact specifically with B-type cyclins, *J. Cell. Biol.* 130: 661-673, 1995.
26. Dong, J. T., Lamg, P. W., Rinker-Schaeffer, C. W., Vukanovic, J., Ichikawa, T., Isaacs, J. T., and Barrett, J. C. KAI1, a metastasisi suppressor gene for prostate cancer on human chromosome 11p11.2, *Science.* 268: 884-886, 1995.
27. Virtaneva, K. I., Emi, N., Marken, J. S., Aruffo, A., Jones, C., Spurr, N. K., and Schroder, J. P. Chromosomal localization of three human genes coding for A15, L6, and S5.7(TAPA1): all members of the transmembrane 4 superfamily of proteins, *Immunogenetics.* 39: 329-334, 1994.