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Ductal carcinoma *in situ* (DCIS) is a pre-invasive stage of breast cancer in which the malignant cells have not penetrated the basement membrane. From our initial allelotyping study of DCIS we determined that the background rate of loss of heterozygosity (LOH) is low (5%). The chromosomal regions showing LOH significantly above background were 8p, 13q, 16q, 17p and 17q. In the past year we have concentrated on defining the smallest region of loss on 8p. To do this a fine-structure map of 8p was required. Twenty three markers on 8p were genotyped and uniquely placed to generate the map. The map spans from the telomere to just beyond the centromere, a distance of 80.8cM. 18 markers on 8p and two on 8q were used for LOH analysis on 65 examples of microdissected DCIS. Of 60 informative specimens, LOH was seen for at least one marker on 8p in 18 (30%). In 10 samples the region of loss includes a large portion of the short arm. Several other samples refine the smallest region of LOH to a 1.4cM region from D8S265 to D8SD520. Our data are consistent with the presence of at least two tumor suppressor genes involved in breast oncogenesis located on 8p.

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Tumor Suppressor Genes in Early Breast Cancer and its Progression

Diane M. Radford MD

Table of Contents

Introduction

A. Nature of the problem

An increasing percentage of breast cancer is being detected at a pre-invasive stage: ductal carcinoma *in situ* (DCIS). DCIS is a form of breast cancer in which malignant cells have not penetrated the basement membrane (1). The histopathological diagnosis encompasses a heterogeneous group of subtypes, including comedo, cribriform, solid, papillary and micropapillary, some of which may differ in biological behavior. The potential for associated micro invasion and likelihood of recurrence after breast conservation therapy are higher with the comedo subtype (2, 3). As with invasive breast cancer, DCIS can also be stratified by nuclear grade: high, intermediate, and low. Circumstantial evidence that DCIS is a precursor lesion to invasive ductal carcinoma is based on three observations: the frequent co-existence of DCIS and invasive cancer in the same breast (4); the greatly increased risk of subsequent invasive breast cancer in women with biopsy-proven DCIS (5); and the finding that when a local recurrence is seen after breast-conserving treatment of DCIS there is a 50% chance that the recurrence will be of the invasive variety (6). DCIS is not an obligate precursor however, and other possible pathways to invasion may exist such as the de novo transition to malignancy of normal epithelium without an intervening non-invasive stage. For many years the standard treatment for DCIS has been total mastectomy, though lumpectomy with adjuvant radiation is being utilized currently for small, well localized areas of DCIS.

Lobular carcinoma *in situ* (LCIS), on the other hand, is not thought to be a pre-invasive cancer but rather an indicator of increased risk of breast cancer. Interestingly, the risk is the same in both breasts regardless of the side in which the LCIS was detected. That the LCIS cells do not inevitably progress to invasive breast cancer is evidenced by the fact that, of those cancers which do develop, half are of the invasive ductal variety (7).

Atypical lobular hyperplasia (ALH) and atypical ductal hyperplasia (ADH) are considered to be high-risk lesions both associated with an increase of 4-5 fold compared to the general female population. If a strong family history of breast cancer exists, the risk is doubled to 8 to 9 fold (5).

Our studies have concentrated on the genetic changes which occur in DCIS and the transition from DCIS to invasive breast cancer. A better understanding of the oncogenesis of breast cancer at the molecular level, and the correlation of this information with clinical data, may aid in treatment choices.

B. Background of Previous Work

Most solid tumors arise due to the inactivation of tumor suppressor genes and activation of oncogenes. The accumulation of genetic changes is believed to result in the invasive followed by the metastatic phenotypes. Loss of heterozygosity (LOH) of one of a pair of alleles in tumor tissue compared to matched normal control can reveal areas of chromosome deletion which are likely to contain putative tumor suppressor genes. A large number of articles have been published on LOH in invasive breast cancer, and virtually every human chromosome has been shown to exhibit allelic loss (8,9). The most frequent losses in invasive breast cancer are seen on chromosome 7q (0 -83%) (8,10,11), 16q (32-63%) (8,9,12-14), 17p (31-75%) (8,9,15-18), 17q (24-79%) (8,9,19-25), and 18q (24-69%) (8,22,26,27). Less frequent losses are found on 1p (3-47%) (8,9,28), 1q (16-32%) (8,9,29,30), 3p (11-47%) (8,9,17), 6q (9-48%) (8,9), 8p (27-33%) (8,16) 11p (10-41%) (8,31) and 13q (16-40%) (9,17).

Several investigators have reported two distinct regions of loss on 8p in breast cancer, located at 8p21 and 8p22. Yaremko et. al. studied 20 examples of invasive ductal cancer and found the overall rate of LOH on 8p to be 55% with loss at 8p22 observed more frequently than at 8p21 (32). On the other hand, Aldaz et. al. found loss on 8p in only one

of 15 informative samples of DCIS (7%) (33). At the time of our progress report last year we had assayed for LOH using 8 markers on 8p. Of 55 informative samples, LOH was found for at least one 8p marker in 15 tumors (27.3%) (34).

Because of the multiple putative tumor suppressor loci which exhibit LOH in invasive breast cancer, it is not clear which loci are involved in oncogenesis and which are lost randomly due to the instability conferred by the malignant state of the genome.

Allelotyping involves the comprehensive screen of the genome for LOH in a particular cancer. Generally an initial screen will involve assay with at least one marker from each non-acrocentric chromosomal arm. Thereby the average or baseline level of LOH can be determined. This may vary from 5 to 20% depending on the type of cancer. A significant level of LOH, indicating the site of possible tumor suppressor genes involved in oncogenesis, can be ascertained once the background level is known. Regions which show significant LOH can then be analyzed with additional markers to refine the smallest deleted region which may contain the tumor suppressor gene. The analysis of tumors with a number of markers also permits calculation of the fractional allelic loss (FAL) for each tumor. This has been defined as the total number of chromosomal arms which show LOH divided by the total number of informative arms for that tumor (35,36). FAL has been correlated with patient outcome in colon cancer (35), and may correlate with clinical information in other tumor types.

Fewer reports exist on the molecular changes in DCIS than can be found pertaining to invasive breast cancer. Davidoff et. al. (37) studied 6 examples of synchronous DCIS and invasive cancer for expression of p53 and found the same levels of protein expression in each tissue type. Expression of the oncogenes c-erbB-2 and c-myc is also consistent between coexisting pre-invasive and invasive breast cancer (38,39). Zhuang et. al. studied allelic loss for two loci on 11q13 (INT2 and PYGM). They found that for every case of DCIS which showed LOH (N=15), loss of the same allele was seen in the corresponding invasive tumor (40). O'Connell et. al. (41) studied four loci [TPO (2pter), D4S192 (4q25-34), D16S265 (16q21) and D17S579 (17q21)] and found that 8 of 10 cases of DCIS shared LOH patterns with more advanced lesions for at least one of the 4 loci.

During the first year of this project we completed the allelotyping of DCIS. Ours was the first laboratory to allelotype DCIS. Our findings were as follows: A total of 61 samples of DCIS were assayed. The average number of informative tumors examined for each marker was 19 (range 8 to 48). The median fractional allelic loss (FAL) was 0.037. The highest % of LOH was shown for loci on 8p (18.7%), 13q (18%), 16q (28.6%), 17p (37.5%) and 17q (15.9%). LOH on 18q was found in 10.7% of informative tumors. FAL was associated with LOH on 17p, with high nuclear grade and with the comedo subtype of DCIS. LOH on 17p correlated with LOH on 17q and on 13q. Additional markers were employed for 16q and 17p to determine the smallest common region of deletion and maps of 17p and 16q were generated (42-44). Aldaz et. al. also studied allelic loss in a total of 23 examples of DCIS. they found the most frequent sites of loss to be on chromosomes 7p, 16q, 17p and 17q (33).

To study genetic changes and the evolution of breast cancer we have assayed for loss of heterozygosity (LOH) in twelve sets of synchronous carcinoma *in situ* (CIS) and invasive cancer, compared to normal control DNA. Microsatellite markers were used which map to each non-acrocentric autosomal arm. Eight tumor sets demonstrated LOH of the same allele in both concurrent invasive cancer and DCIS, for a total of eighteen chromosomal loci. Three of nine tumor sets showed LOH on 11p. In two of these sets LOH was seen on 11p only in the invasive tumor, not the corresponding CIS. One of these tumors also exhibited allelic loss in the invasive tumor for 4 loci, all of which were retained in the non-invasive tumor. For two tumor sets LOH was mirrored in matched DCIS, invasive tumor and lymph node metastasis. The maintenance of LOH for certain loci throughout the stages of breast cancer suggests clonality of the cancer cells. Tumor suppressor loci on 11p may be involved in the invasive phenotype (45).

Over the past year we have concentrated our efforts on the refinement of the area of loss on 8p. Simultaneously with that study we have generated a fine structure map of 8p.

C. Purpose of the Present Work

Statement of Work:

Task 1: The identification and characterization of the extent of chromosomal deletions in DCIS. Months 1-12.

a. The different subtypes of DCIS will be allelotyped with a panel of microsatellite markers.

b. The smallest common region of deletion will be refined.

Task 2: The study of chromosomal deletions in hyperproliferative breast conditions. Months 12-24.

Task 3: The study of chromosomal deletions in the progression of DCIS to invasive and metastatic phenotypes. Months 1-36.

a. Simultaneous DCIS, invasive cancer and lymph node metastases will be assayed for chromosomal deletions.

Task 4: Cloning a tumor suppressor gene involved in breast cancer. Months 24-48.

a. The smallest common region of deletion will be encompassed with overlapping YAC contigs.

b. Candidate genes will be identified.

D. Methods of approach

a) Accumulation of specimens.

Collaborations have been established with pathologists in St. Louis area hospitals. Archival paraffin embedded material is collected from several hospitals in St. Louis (Barnes-Jewish, Deaconess, St. Louis University, St. Luke's Hospital and the Outpatient Surgery Center). Either matched archival normal lymph node DNA or leukocyte DNA is used as control. When it is necessary to draw blood for normal control, informed consent is obtained following Institutional Review Board approval. A total of 89 examples of DCIS have been accumulated and assayed for LOH with various markers.

b) Microdissection.

For LOH analysis it is necessary to have a relatively pure tumor sample with little if any contaminating normal stroma. We have been using a microdissection technique to enrich for tumor cells in which an unstained 20 micron thick section from a particular block is overlaid on a stained 5 micron thick section. Landmarks such as blood vessels are aligned and the tumor dissected from the unstained section using a scalpel blade.

This year 46 dissections and DNA extractions were performed on new tumors and to replenish stocks of previously acquired tumors.

c) DNA extraction and LOH analysis.

Following separation of tumor and normal tissue DNA is extracted by digestion with proteinase K, purified with phenol/ chloroform and precipitated with alcohol. DNA is quantified with a fluorimeter. For assay of LOH we have used a panel of highly polymorphic microsatellite markers. Polymerase chain reaction (PCR) is performed in the tumor/normal pairs and the products separated on acrylamide denaturing gels. Reactions have been optimized for 5 to 10 ng of template DNA in order to maximize the number of reactions possible with each tumor. On autoradiography, absence or greatly reduced intensity of one allele in the tumor compared to the heterozygous normal control indicates LOH.

Several samples contain insufficient tumor cells to permit the extraction technique described above. New methods have been developed in order to consistently amplify via PCR these low quantities of DNA. Following microdissection the tumor tissue is digested in a small volume (10-20 microliters) of lysis buffer containing proteinase K. After complete digestion has been determined, samples are phenol extracted once to destroy the proteinase K and chloroform extracted once to remove the phenol. Aliquots of this material are then used directly as a template for PCR amplification. A disadvantage of this method is that only a limited number (10-20) of reactions can be done, and therefore this technique would not be suitable for an allelotyping study.

d) Generation of genetic linkage maps.

Once a region of chromosomal deletion has been identified it can be narrowed down using a panel of closely linked markers which map to that area. Since new microsatellite markers are becoming available daily, they often do not appear on currently published maps. In order to determine the deletion map in the tumors, it is necessary to know the precise location of the markers being used. A fine structure map can be generated using genotypic data from a number of families made available through the Centre d'Etude Polymorphisme Humaine (CEPH). Having identified a small region of deletion (preferably no larger than 1cM) positional cloning techniques can be undertaken to clone the putative tumor suppressor gene contained within the region.

Body: Experimental Methods Used and Results Obtained.

Task 1: The identification and characterization of the extent of chromosomal deletions in DCIS. Months 1-12.

The allelotyping study was completed in the first year of the project and the results were described above.

a) Construction of genetic linkage map of 8p

Simply stated, map construction utilizes patterns of inheritance to determine the order of markers on a chromosome. Differences in the patterns of inheritance from marker to marker represent recombinations and are used to determine the distance between markers measured in centimorgans (cM). Deletion data cannot be interpreted unless a strong unique order exists for the markers used. Genethon utilizes eight of the 61 CEPH reference families (102, 884, 1331, 1332, 1347, 1362, 1413, and 1416) for the construction of framework maps of the human chromosomes (46). The CEPH collaborative mapping groups have made these primary data publicly available. To further strengthen the unambiguous positioning of markers an additional 8 CEPH families (66, 1333, 1334, 1340, 1341, 1345, 1375, 1377) are typed and the data are merged with the available

primary data from CEPH V7. Merged data is then processed through the mapping program CRIMAP (P. Green, unpublished) resulting in genetic map with at least 1000:1 odds on order. New markers are typed on the 16 families. Primer sequences and reactions conditions for these markers are available from GDB (genome data base, http://gdbwww.gdb.org). Primers are synthesized locally (H. Donis-Keller), or ordered from Research Genetics, Inc. Program permutation options "flips2" "flips3" and "flips4" determine the most likely order with the data from the "build" analysis.

The PCR conditions for genotyping are: initial denaturing @ 94° for 30 seconds one cycle, denaturing @ 94° for 30 seconds annealing @ 50-60° for 30 seconds and extension @ 72° for 30 seconds for 25 cycles. After amplification the samples are separated by electrophoresis on a standard 8% polyacrylamide denaturing gel, dried, and exposed to film for 12-24 hours.

We have genotyped a total of 23 markers which uniquely place with odds of 1000:1. The map spans from the telomere of 8p to just beyond the centromere (80.8cM). The average distance between markers is 3.5cM (Figure 1). These data are unpublished. We plan to submit a manuscript by the end of 1996.

Once a marker is uniquely placed on a chromosomal map, that marker will then taken through the DCIS tumor/normal pair panel to determine the percentage of LOH and refine the smallest common region of deletion.

b) Deletion on 8p in DCIS

Preliminary data on the extent of deletion on 8p were published in *Surgical Forum* (34). A total of 66 cases of DCIS have now been assayed for LOH using 18 markers on 8p and 2 on 8q. Information on marker placement was obtained from the map we had generated. PCR and product separation were done as described in D (c). LOH was determined by a combination of naked-eye assessment and scanning densitometry. Densitometry was performed by scanning autoradiographs with a UMAX UC630 color scanner and with the use of the software program Adobe Photoshop 2.5.1 (Adobe Systems, Inc.). The densitometry histograms were analyzed on a Power Macintosh 6100/60 with the use of the public domain NIH image program (Wayne Rasband, NIH). A 3 fold difference in the relative allele intensity ratios between tumor and normal DNA in an informative tumor/normal pair was scored as LOH (allele 1/allele 2 in tumor compared to allele 1/allele 2 in normal). To maintain a conservative scoring approach, marginal allele reduction by inspection was not scored as LOH. All LOH designations were scored by two independent scientists and repeated to verify the result. Figure 2 shows an example of LOH in DCIS.

Of 60 informative samples, LOH was found for at least one 8p marker in 18 tumors (30%). Ten tumors have lost most of the short arm. The smallest common region of deletion localizes to a 1.4cM region at 8p22-23, between the markers D8S520 and D8S265 (figure 3). No correlation has been found between LOH on 8p and histologic parameters such as subtype, nuclear grade or presence of microinvasion. Our data are consistent with two regions of loss on 8p. The other region is more centromeric in the area of the marker LPL (see tumor 79). Similarly, these data are also unpublished.

Task 2: The study of chromosomal deletions in hyperproliferative breast conditions. Months 12-24.

To date we have assayed 3 examples of LCIS using several markers on 8p. No LOH has been seen. Additional samples of LCIS have been obtained from Barnes-Jewish and St. Luke's Hospitals. 11 matched tumor normal pairs have been microdissected and DNA extracted.

Task 3: The study of chromosomal deletions in the progression of DCIS to invasive and metastatic phenotypes. Months 1-36.

Our initial study of simultaneous DCIS and invasive breast cancer is described above. Samples containing LCIS and invasive lobular cancer are also being accrued. 5 examples have been obtained and are being microdissected.

Task 4: Cloning a tumor suppressor gene involved in breast cancer. Months 24-48.

These experiments will be carried out in Dr. Helen Donis-Keller's laboratory as she is the PI on the project as of October 1st 1996.

The plan is to construct a radiation hybrid map of a substantail region of 8p which will enable the identificatoin of large insert genomic clones (YAC, BAC, P1) that will comprise a physical map for the regions containing breast cancer tumor suppressor genes. Additional data regarding expressed genes will also become available from the construction of these maps and they can in turn be tested as candidate genes. Dr. Donis-Keller will concentrate on gene cloning activities on the region we have defined on 8p that contains a gene important for the development of DCIS.

Conclusions

Numerous tumor suppressor loci are involved in DCIS. The most common sites of allelic loss are on chromosomes 8p, 13q, 16q, 17p and 17q. . A fine structure map of 8p was genetated in order to define the region of deletion. New genotypic data were obtained on 23 markers. These markers could be uniquely mapped with odds of 1000:1. LOH on 8p was found in 18 of 60 informative examples of DCIS (30%). Data are consistent with the presence of at least two tumor suppressor genes on 8p involved in breast oncogenesis. The smallest region of deletion found to date spans 1.4cM at 8p22.

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Appendices

Figure 1

Ideogram of 8p map. 23 markers uniquely map with odds greater than 1000:1. (UNPUBLISHED)

Figure 2

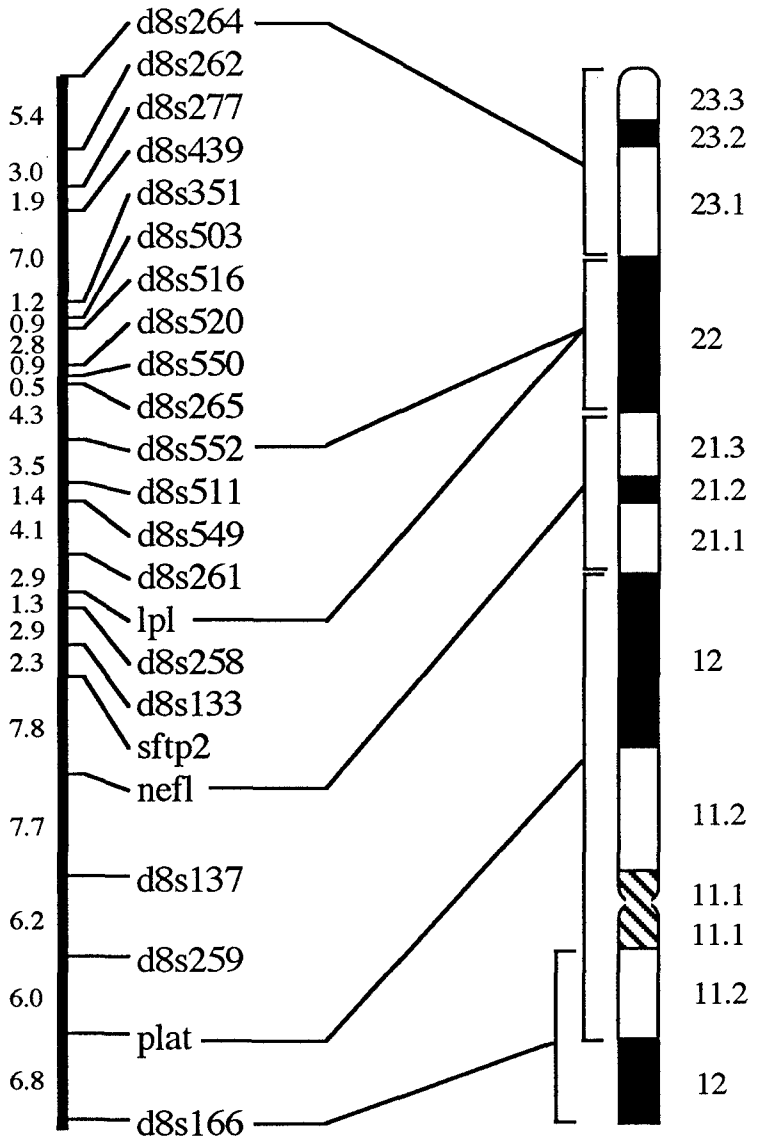
Example of LOH in DCIS on 8p in tumor #61. T = tumor lane, N = normal tissue from the same patient. An allele is lost in the tumor specimen.

Figure 3

Cumulative data (UNPUBLISHED) for LOH on 8p. A filled oval indicates no LOH, an open oval indicates LOH and a hatched oval indicates the reaction was not informative. Of 60 informative samples, LOH was seen in 18. The smallest common region of deletion spans from D8S265-D8S520. Data from tumor 79 indicate the likely presence of another tumor suppressor gene on 8p located in the area containing LPL. The reactions for tumor 56 using D8S520 and 550 are being repeated. All other reactions have been verified with repeat reactions.

Copy of letter transferring the project to Dr. Helen Donis-Keller

Human Chromosome 8p



80.8 cM

Figure 1

Figure 2

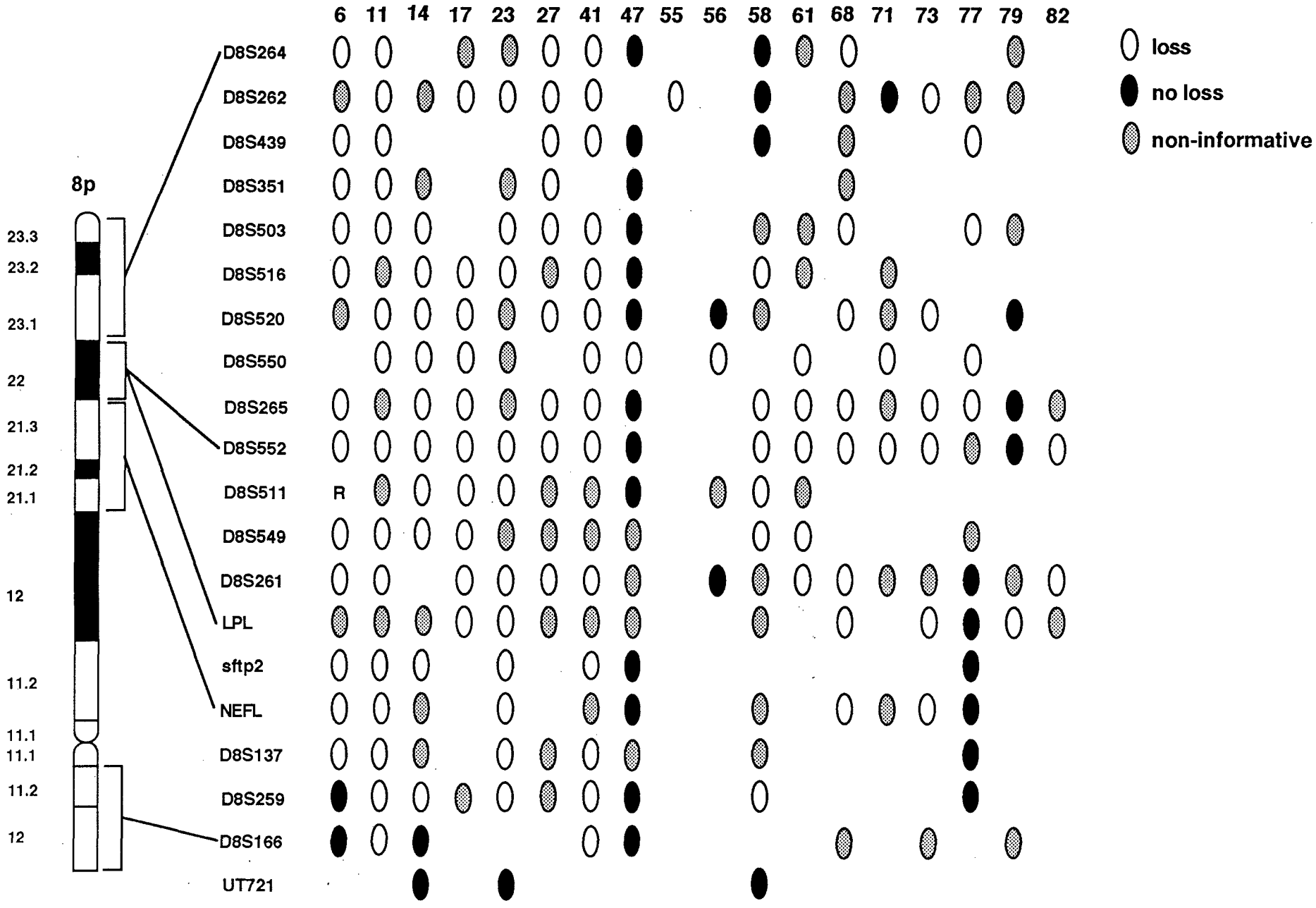

6 11 14 17 23 27 41 47 55 56 58 61 68 71 73 77 79 82
loss
no loss
non-informative
8p
23.3
23.2
23.1
22
21.3
21.2
21.1
12
11.2
11.1
11.1
11.2
12
D8S264
D8S262
D8S439
D8S351
D8S503
D8S516
D8S520
D8S550
D8S265
D8S552
D8S511
D8S549
D8S261
LPL
sftp2
NEFL
D8S137
D8S259
D8S166
UT721
R

Figure 3

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August 30, 1996

Mr. Daniel R. Signore
Contract Specialist
United States Army Medical Research and Development Command
Fort Detrick
Frederick, MD 21702-5014

Re: DAMD17-94-J-4293 "Tumor Supressor Genes in Early Breast Cancer and Its Progression" D. Radford, MD, FRCSEd, Principal Investigator
US Army Medical Research and Development Command

Dear Mr. Signore:

As I discussed with you in a recent telephone conversation, I plan to leave academic surgery and shall not have a Washington University appointment after September 30, 1996. With this letter I formally request transfer of my research grant (DAMD17-94-J-4293) On October 1, 1996, to Helen Donis-Keller, Ph.D., who has been my long-standing collaborator on this project. I believe that she is the most appropriate person to continue the work that we have begun. There should be no interruption in the progress of the project since she is willing to transfer the project and staff immediately. The research technician currently employed on the project, Mr. Matthew Holt, formerly trained with Dr. Donis-Keller, and he is enthusiastic about returning to her lab to continue the project. I will continue as a collaborator, assisting with overall study design and evaluation of results. In addition I will continue to provide breast tissue samples and relevant clinical information.

Dr. Donis-Keller is well qualified to continue the project. She is Professor of Surgery at Washington University and holds joint appointments as Professor of Genetics and Professor of Genetics in Psychiatry in the Departments of Genetics and Psychiatry, respectively (see enclosed CV). She supported my original grant application (see appended letter from the application submitted in June of 1994) and she has continued to be a close collaborator on the project by regularly reviewing and evaluating primary data from my laboratory, contributing mapping data from her laboratory, and by assisting in the writing of manuscripts. She is the senior author of five published breast cancer molecular genetics articles for which I am the first author (Radford et al., 1993a,b, 1995 a,b,c), the latter two of which (Radford et al., 1995b,c) were supported by this grant. In addition we have collaborated in the past on another breast cancer molecular biology project with Dr. Peter Milner. Two publications (Milner et al., 1992; Garver et al., 1994) resulted from our efforts. When Dr. Milner moved from Washington University to a position in industry the ACS grant supporting this work was transferred by Dr. Milner to her so that the research

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could be completed. She has demonstrated a continuing commitment to breast cancer research and is currently one of four principal investigators of an NCI sponsored initiative to develop a tissue repository, the Collaborative Breast Cancer Tissue Registry (CBCTR) (NIH-U01-CA62773-04, "St. Louis Breast Tissue Registry," H. Donis-Keller, PI). In addition, a current project in Dr. Donis-Keller's laboratory, to map a tumor supressor gene that lies within a region of chromosome 8p, is complementary to our current breast cancer mapping work on another portion of 8p and will provide a synergistic effect as reagents, expertise, and data can be shared to generate a more complete map of the region and enable a smooth transition to the next (gene cloning) phase of the breast cancer project.

Dr. Donis-Keller and I plan to continue the project as outlined in my previous progress report (submitted in October of 1995). Briefly, under the supervision of Dr. Donis-Keller, Mr. Holt and staff will continue the ductal carcinoma *in situ* (DCIS) 8p LOH analysis and linkage mapping as proposed (Task 1). We expect to submit a manuscript describing this work by the end of 1996. A major effort during the coming year will be to carry out the lobular carcinoma *in situ* (LCIS) study as we now have a substantial number of matched tumor/normal DNA samples ready for LOH analysis (Task 2). The work described in Task 3: Study of chromosomal deletions in the progression of DCIS to invasive and metastatic phenotypes, will proceed as previously described. During the coming year we should be able to make substantial progress on the final task proposed, Task 4: Cloning a tumor supressor gene involved in breast cancer. We plan to construct a radiation hybrid (RH) map of a substantial region of 8p which will enable the identification of large insert genomic clones (YAC, BAC, P1) that will comprise a physical map for the regions containing breast cancer tumor suppressor genes. Additional data regarding expressed genes will also become available from the construction of these maps and they can, in turn, be tested as candidate genes. We will first concentrate our gene cloning activities on the region we have defined on 8p that contains a gene important in the development of DCIS. The relevant YAC and P1 clone libraries are resident in Dr. Donis-Keller's laboratory, as is the expertise for identifying and characterizing gene candidates. In summary, Dr. Donis-Keller should be able to make substantial progress on the project goals as originally defined (Tasks 1-3) and continue to carry the project forward employing state-of the-art technology as we move into the gene analysis phase outlined in Task 4.

A revised budget for the remainder of the grant period prepared by Dr. Donis-Keller is appended. We would like to make use of the grant funds that previously supported my salary to now support a portion of Dr. Donis-Keller's salary (5%) and to fund additional laboratory studies that are vital to the continuation of our goals, e.g. the RH mapping and gene cloning studies. Mr. Matt Holt (100% effort) will continue the LOH studies and genotyping associated with chromosome mapping using the CEPH reference family DNAs. This is a continuation of his full-time effort on the project. Ms. Suzanne Cole (50% effort), a well qualified full-time laboratory technician who has been a member of the Donis-Keller laboratory for more than 4 years, will perform the RH mapping and other physical mapping laboratory studies. Ms. Cindy Helms (15% effort), the most senior research assistant in Dr. Donis-Keller's laboratory who has been a co-author on numerous publications and a member of Dr. Donis-Keller's laboratory for more than 12 years, will undertake the statistical analysis for linkage mapping and assist in the evaluation of the RH mapping results. Ms. Helms will assemble the physical maps and participate in the identification and characterization of gene candidates (e.g. YAC and P1 contig assembly, sequence analysis, cDNA analysis).

I am confident that the project will be in good hands in the laboratory of Dr. Donis-Keller and I look forward to continuing my involvement in the research as a collaborator. I deeply appreciate the opportunity this grant has provided to advance the research into the molecular events that are responsible for breast cancer. As I continue to care for patients afflicted with breast cancer, which will be the complete focus of my practice, I look forward to continuing my association with Dr. Donis-Keller and to doing whatever I can to help cure this devastating disease. Your approval of my request for transfer of the remaining grant funds to Dr. Donis-Keller will enable continuation of the work in the most optimal of circumstances. Dr. Donis-Keller and I have developed this plan together and she signs this letter in agreement with our wishes as outlined above.

Sincerely,

Diane M. Radford

Diane M. Radford, M.D., FRCSEd, FACS,
Assistant Professor of Surgery

Literature Citations:

Milner, P., D. Shah, R. Veile, H. Donis-Keller, and B. V. Kumar (1992). Cloning, nucleotide sequence and chromosome localization of the human pleiotropin (PTN) gene. Biochemistry 31: 12023-12028.

Garver, R. I., Jr., D. M. Radford, H. Donis-Keller, M. R. Wick, and P. G. Milner (1994). Midkine and pleiotropin expression in normal and malignant breast tissue. Cancer 74:1584-1590.

Radford, D. M., K. L. Fair, A. M. Thompson, J. H. Ritter, M. Holt, S. A. Wells, Jr., and H. Donis-Keller (1993a). Chromosomal regions implicated in the development of breast cancer. Surgical Forum 44: 502-504.

Radford, D. M., K. Fair, A. M. Thompson, T. Steinbrueck, M. Holt, J. H. Ritter, M. Wallace, D. Patterson, S. A. Wells, Jr., and H. Donis-Keller (1993b). Allelic loss on chromosome 17 in ductal carcinoma *in situ* of the breast. Cancer Research 53: 2947-2950.

Radford, D. M., K. L. Fair, N. J. Phillips, J. H. Ritter, T. Steinbrueck, M. S. Holt, and H. Donis-Keller (1995a). Allotyping of ductal carcinoma *in situ* (DCIS) of the breast: deletion of loci on 8p, 13q, 16q, 17p, and 17q. Cancer Research 55: 3399-3405.

Radford, D. M., N. J. Phillips, K. L. Fair, J. H. Ritter, M. Holt, and H. Donis-Keller (1995b). Allelic loss and the progression of breast cancer. Cancer Research, 55:5180-5183.

Radford, D. M., M. S. Holt, J. H. Ritter, N. J. Phillips, K. L. Fair, K. DeSchryver, M. E. Schuh, and H. Donis-Keller (1995c). Allelic Loss on chromosome 8p occurs early in the development of breast carcinoma. Surgical Forum 46:553-535.

Agreement to proposal by Dr. Radford:

Helen Donis-Keller 8/30/96

Proposed Principal Investigator, Helen Donis-Keller, Ph.D. Date

[signature] 9/3/96

Department Chairman Approval, Samuel A. Wells, Jr., M.D. Date

Rebecca S. Evans 9/3/96

Director, Gifts, Grants and Contracts Office, Rebecca S. Evans Date

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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FORT DETRICK, MARYLAND 21702-5012

Received 7/23/2001

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports. Request the limited distribution statement for reports on the enclosed list be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Encl