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13. ABSTRACT *(Maximum 200 words)*

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 allelotyping study of DCIS, chromosomal regions of 8p, 13q, 16q, 17p and 17q showed loss of heterozygosity (LOH) significantly above background (5%). In our previous report we described the LOH mapping of a region on 8p (30% LOH) containing a putative tumor suppressor gene (TSG) which we refined to a small interval of ~1.4 cM between the markers D8S265 to D8SD520. Last year (1998) we completed the construction of a fine structure radiation hybrid map and an integrated YAC/BAC contig spanning the 1.1-cM deletion region. During the past project year, we mapped eleven expressed sequence tags (ESTs) including 4 previously reported ESTs to this contig. Twenty-three overlapping oligo (overgo) probes were generated using end sequences derived from CITB BAC clones. These overgos were used to screen RPCI-11 BAC library. A total of 299 clones including CITB BAC clones were isolated and then fingerprinted. A comprehensive sequence-ready contig that provides a useful resource for sequencing the critical region has been assembled using clones with good fingerprinting data.

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Tumor Suppressor Genes in Early Breast Cancer and its Progression

Alison M. Goate, D. Phil.

Table of Contents

Introduction

A. Response to Reviewer of 1998 Progress Report

We very much appreciate the careful consideration of our report by the reviewer. A refined radiation hybrid map for the deletion interval is shown on table 2 of the attached paper published in Genomics. This RH map contains a total of 10 markers including 6 microsatellite markers (D8S265, D8S520, D8S550, D8S1695, D8S1755 and C8S1759), one novel STS (sJCW), and 3 ESTs (A005E28, T96924, and WI-6800).

B. 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 the 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.

C. 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 from the same individual can reveal areas of chromosome deletion which are likely to contain putative tumor suppressor genes. From our previous studies, 65 cases of DCIS were assayed for LOH using 18 mapped microsatellite markers on 8p and one on 8q. Of 61 samples informative for at least one marker on 8p, LOH was found in 17 tumor DNA samples (27.8%). Our data identify the smallest common region of LOH to be located at 8p22-23 between D8S265 and D8S520.

Other studies have also reported evidence of LOH on 8p in DCIS (8, 9, 10, 11). Anbazhagan et al. (11) found the most common region of loss in 60 invasive ductal breast tumors to be at 8p21.3-p23.3 between D8S560 and D8S518. The 1.4cM region of loss we have identified lies within this region. Several studies have attempted to correlate 8p LOH and the ability of a tumor to metastasize (8, 11, 12, 13). In our data we observed no correlation between LOH on 8p and tumor grade or the presence of an invasive component.

During the second year of the project we concentrated our efforts on the refinement of the area of loss on 8p. Simultaneously with that study we have generated a fine structure linkage map of 8p. Genetic mapping efforts indicated that the deleted region (between markers D8S520 and D8S265) spanned an interval of 1.4 cM. During the third and the fourth years of the project we completed physical maps and the construction of a clone contig for the region using yeast artificial chromosome (YAC) and bacterial artificial chromosomes constructed by California Institute of Technology (CITB-BAC). We also identified and sequenced four apparently transcribed sequences that originate from the region of interest. In order to effectively sequence the deletion region, we initiated a collaboration with the Genome Sequencing Center at Washington University in St. Louis. To work efficiently with the high through-put sequencing at the center, a second BAC library (RPCI-11) was screened using the end sequences of BAC clones we isolated during previous years. All the isolated BACs were fingerprinted and a refined sequence-ready contig was assembled. In the meantime, we have mapped 7 additional ESTs to the YAC/BAC contig.

D. Purpose of the Present Work

Revised Statement of Work:

We have accomplished Task 1, The identification and characterization of the extent of chromosomal deletions in DCIS (Months 1-12). As described in the previous progress report we have focused our efforts on Task 4: Cloning a tumor suppressor gene involved in breast cancer (Months 24-48).

Task 2, The study of chromosomal deletions in hyperproliferative breast conditions. (Months 12-24) and Task 3, The study of chromosomal deletions in the progression of DCIS to invasive and metastatic phenotypes (Months 1-36) will be taken up after Task 4 has been accomplished, time and effort permitting.

E. Methods of Approach

a) Radiation hybrid maps

G3 and GB4 are the two radiation hybrid mapping panels (Research Genetics, Inc. Huntsville, AL) we used to construct the RH placement map. The G3 panel, comprised of 83 RH clones from the whole human genome, was created at the Stanford Human Genome Center and is considered a medium resolution panel (i.e. 500 Kb resolution). The GB4 panel, comprised of 93 RH clones from the whole human genome, has lower

resolution (i.e. 1000 Kb). It is a subset of the 199 clone panel developed by the laboratories of Peter Goodfellow and Jean Weissenbach.

STS markers are assayed by PCR amplification and sizing on agarose gels stained with EtBr. Each assay is performed twice, i.e. PCR products at the expected size must be observed on each of the duplicate gels in order to be scored as positive. Data generated from the GB4 panel are submitted to the Whitehead Institute Center for Genome Research (WICGR) Mapping Service Center. The program RHMAPPER at the Center is used to analyze all the submitted markers with their high-likelihood framework map and we are then forwarded a placement map with all the submitted markers including LOD score and the distance in cR between two highest-linked markers. The data generated from the G3 panel is submitted to Stanford RH server which subsequently returns the results of analysis with a list of the highest-linked mapped markers, the LOD score of the link, and the distance in cR between the submitted marker and the linked marker on the map. However, it only compares one submitted marker and the highest-linked marker at a time. In order to construct a map of higher resolution which is obtainable with the G3 panel, we used the program RHMAPPER version 1.0 from WICGR, the Stanford RH database and our own G3 data for the 6 markers of interest. We used the RH database from Stanford to build a G3 panel placement map (framework), then we integrated our RH data from the six markers covering the deletion region with the framework map.

b) YAC/BAC clone contig construction

YACs available from the CEPH library that we maintain in our laboratory were streaked on YPD plates, and 10 colonies from each clone were tested by a "whole cell PCR" assay using the STS markers to identify the positive clones. For "whole cell PCR, a small amount of cells from an isolated colony are suspended in 5 ul of deionized water and the suspension is used directly as the template in a standard PCR reaction. The presence of other markers within the YACs are also assayed by PCR assays of STS markers. The standard ligation-mediated PCR method was used to develop new STSs from YACs.

We screened a CITB-978SK-B and CITB-HSP-C BAC library (Research Genetics, Inc., Huntsville, AL) to identify BAC clones for contig construction. STS assays for relevant markers were tested using the 120 standard PCR screening reactions to survey the STS content of the entire library. As with YAC clone isolation, each identified BAC clone was then verified by "whole cell PCR" assay using 10 randomly selected colonies as candidates. After the positive BAC clones were verified, single BAC colonies were propagated in liquid medium, cells harvested and insert DNA prepared using the Plasmid Midi-Kit from Qiagen Inc. (Chatsworth, CA). Each BAC clone was partially sequenced from the insert ends using T7 and Sp6 primers. The sequence generated from ABI sequencing was analyzed for candidate PCR primer sequences using the program PRIMER 0.5 (Lincoln and Lander, MIT Center for Genome Research). STS assays were developed and then used as new entry points for chromosome walking.

c) Colony Hybridization

We used end sequences derived from CITB-978SK-B and CITB-HSP-C BAC clones to generate 23 overgos using the techniques described in Current Protocols in Human Genetics. Briefly, two 24-mer oligonucleotides with an overlap of 8 bp are designed from the target sequence and then annealed to create two 16-bp overhangs. Klenow fragment with incorporated radionucleotides is used to fill in the 16-bp overhangs to generate a double-stranded 40-mer probe with high specific activity. Overgo probes were pooled and hybridized to nylon filters containing immobilized DNA from the RPCI-11 BAC library (Research Genetics, Inc., Huntsville, AL). After

hybridization, the filter was washed to remove nonspecifically bound probe and visualized through autoradiography.

d) Fingerprinting

Two colonies for each positive BAC clone from the hybridization screening were isolated and propagated in liquid YT medium containing chloramphenicol. A modified alkaline lysis procedure (14, 15) was followed for DNA preparation. Samples were digested with restriction endonuclease Hind III and electrophoresed on 1% SeaKem LE (FMC BioProducts) agarose gels. After electrophoresis, gels were stained with 1:10,000 dilution of SYBR Green (FMC BioProducts) and then imaged using a Molecular Dynamics FluorImager SI. Fingerprinting data were collected as the distance that restriction fragments migrate on an agarose gel relative to fragments in the standard marker (Boehringer-Mannheim marker V in our study) lane using the program FPC version 3.2 (15, 16; http://www.sanger.ac.uk). The relative mobilities of restriction fragments from newly isolated RPCI clones were compared with the fingerprinting data of CITB clones to assemble the comprehensive sequence-ready contig.

Body: Experimental Methods Used and Results Obtained.

Task 4: Cloning a tumor suppressor gene involved in breast cancer. Months 24-60.

RH mapping

In order to efficiently clone and characterize a putative tumor suppressor gene involved in breast cancer we have constructed a refined radiation hybrid map (Table 2 of attached paper) for the deletion region using the Stanford G3 panel. A total of 10 markers including 6 microsatellite markers (D8S265, D8S520, D8S550, D8S1695, D8S1755 and C8S1759), one novel STS (sJCW), and 3 ESTs (A005E28, T96924, and WI-6800) that were identified through database searches were ordered on the map using our typing data and the program RHMAP, version 3.0. The order of markers D8S265, D8S520 and D8S550 confirmed our genetic linkage map order. The distance of 42.3 cR, or approximately 1565 kb (assuming 37 kb per centiray) (17), between the markers D8S265 and D8S520 demonstrated ~1:1 correspondence between genetic and physical distances for this interval.

Sequence-ready BAC contig construction and TSG candidate identification

Last year (1998) we completed the construction of an integrated YAC/BAC clone contig using the CITB-978SK-B / CITB-HSP-C library. This contig spans a total distance of ~1730 kb covering the putative tumor suppressor gene region of ~1100 kb based on sizes of BAC clones (Figure 3 of the attached paper). The publicly available databases were searched for candidate genes within the region of chromosome 8p22-23. We verified by PCR assay the locations of 11 ESTs reported to map within this region. EST A005E28 identified by the Institute for Genomic Research (TIGR) mapped to YAC 770E9. Like STS sJCW, it tested positive for BAC clones 17H6, 65L19 and 516P13. EST T90820 also tested positive for BAC clones 65L19 and 516P13, but not for YAC 770E9 (Table 5 of the attached paper). EST T96924 mapped to YACs 770E9, 915H4, 715C10, 737E5, and 729E12. It also tested positive for BAC clones 271O23 and 367I24 identified by D8S265. Two cDNA clones (T96924, 0.8 kb and W67504, 1.6 kb) were isolated and fully sequenced. The two sequences were

compiled into one continuous sequence using clustalW alignment of MacVector 6.0 program (Oxford Molecular Limited, 1997). This sequence contains $(CA)_{22}$ repeats which could be used to develop a microsatellite marker. BAC clones 271O23 and 367I24 were also partially sequenced with a primer selected from the 3' end of cDNA T96924. The sequences from these two BAC clones contain a $(CA)_{22}$ repeat and match the sequence from cDNA W67504.

A cluster of 6 ESTs that appeared to localize to the region from the UniGene (http://www.ncbi. nlm.nih.gov/UniGene) map were placed at the proximal end of our YAC/BAC contig. They all mapped to YAC 809H8. EST R01769 tested positive for BAC clones 358B10 and 435N19. ESTs AA018590, N29512, R01183 and Z45810 tested positive for clone 358B10 only. EST H16027 did not identify any BAC clone on the contig (Table 5 of the attached paper). Eight of these 11 ESTs appeared to be unique since they did not identify homologous sequences in the publicly available databases. Thus we have no clue as to the normal function of the proteins encoded by these ESTs. ESTs N29512 and AA018590 identified by the marker SGC30677 in UniGene were developed from cDNA clones with sequence homology to human Farnesyl Diphosphate Farnesyltransferase. EST H16027, which is one of the ESTs detected by the marker WI-8953, is derived from a cDNA with homology to human Cathepsin B precursor.

In addition to the previously reported ESTs from the region, both end sequences generated from BAC clones were analyzed with BLASTN. Only the Sp6-end sequence from clone 269E3 identified a homologous sequence, Stratagene's fetal retina Homo sapiens cDNA clone (AA504989). About 1.5 kb of this cDNA clone has been sequenced. Another cDNA clone (W68256, 0.6 Kb) identified by the marker WI-6800 was isolated and the insert was fully sequenced. A total of nearly 25 kb of sequence was generated from BAC insert ends and 4 cDNA inserts.

Since only 11 ESTs were mapped to the contig, most of which gave no clue to their function we decided that other gene finding approaches would be necessary. This could be accomplished by cDNA selection / exon trapping methods or by sequencing the entire contig. Given the problems associated with cDNA selection / exon trapping and the proximity of the Genome Sequencing Center at Washington University, we opted for the sequencing approach. During this project year, we have begun a collaboration with the center. In order to sequence the contig, it was necessary to increase the depth of the contig to confirm the order of the BACs.

First, we focused on fingerprinting the 27 CITB BAC clones on the contig (Figure 1). In the meantime, we generated 23 overgos using the 54 end sequences derived from CITB BAC clones to screen RPCI-11 BAC library (constructed at the Rosewell Park Cancer Institute). A total of 299 clones scored positive from colony hybridization result. Two colonies from each clone were fingerprinted and the clones showed clear bands after restriction enzyme, Hind III digest were used to assemble a comprehensive sequence-ready contig (Figure 2) using program FPC version 3.2 (15, 16). Thirty-three CITB BACs and 57 RPCI BACs were incorporated into this contig. Our current plans are to focus on DNA sequencing and gene identification from the cloned DNA that spans the region. Clones forming a minimal tiling path have been subcloned into M13 ready for shotgun sequencing.

It is anticipated that sequence data for the entire region will be available during the next few months. We will use gene finding programs to identify all genes in this region and assess the likelihood of a gene being a TSG. Putative TSGs will be evaluated on normal and tumor tissues in our patients.

Conclusion

Our STS-content mapping efforts conducted during previous years have resulted in a single, continuous, integrated YAC/BAC clone contig. This contig spans a total distance of ~1.7-Mb covering the putative TSG region of ~1100 kb based on the sizes of BAC clones. Through the second BAC (RPCI-11) library screening by hybridization and fingerprinting of isolated BAC clones, this past project year we have assembled a comprehensive sequence-ready contig covering the deletion region. The fingerprints of CITB BACs confirmed the clone position on our YAC/BAC contig. We also analyzed the fingerprints of newly identified RPCI BACs together with those on the YAC/BAC contig using the FPC version 3.2 and edited them manually. Ninety clones including 33 CITB BACs and 57 RPCI BACs were incorporated into this contig, 24 of these clones were contained entirely within other clones. Future studies will involve subcloning for sequencing of candidate genes using the contig resource and characterization of each gene.

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Appendices

Figure 1. Fingerprints of 23 CITB BAC clones placed on YAC/BAC contig.

Figure 2. An integrated CITB (RG) BACs and RPCI (NO) BACs clone contig. Only one isolate (either a or b) from each clone fingerprinted was used for contig construction. * indicates the clones containing buried clones with exact or approximate fingerprints; + indicates the clones containing buried clones with poorly matched fingerprints.

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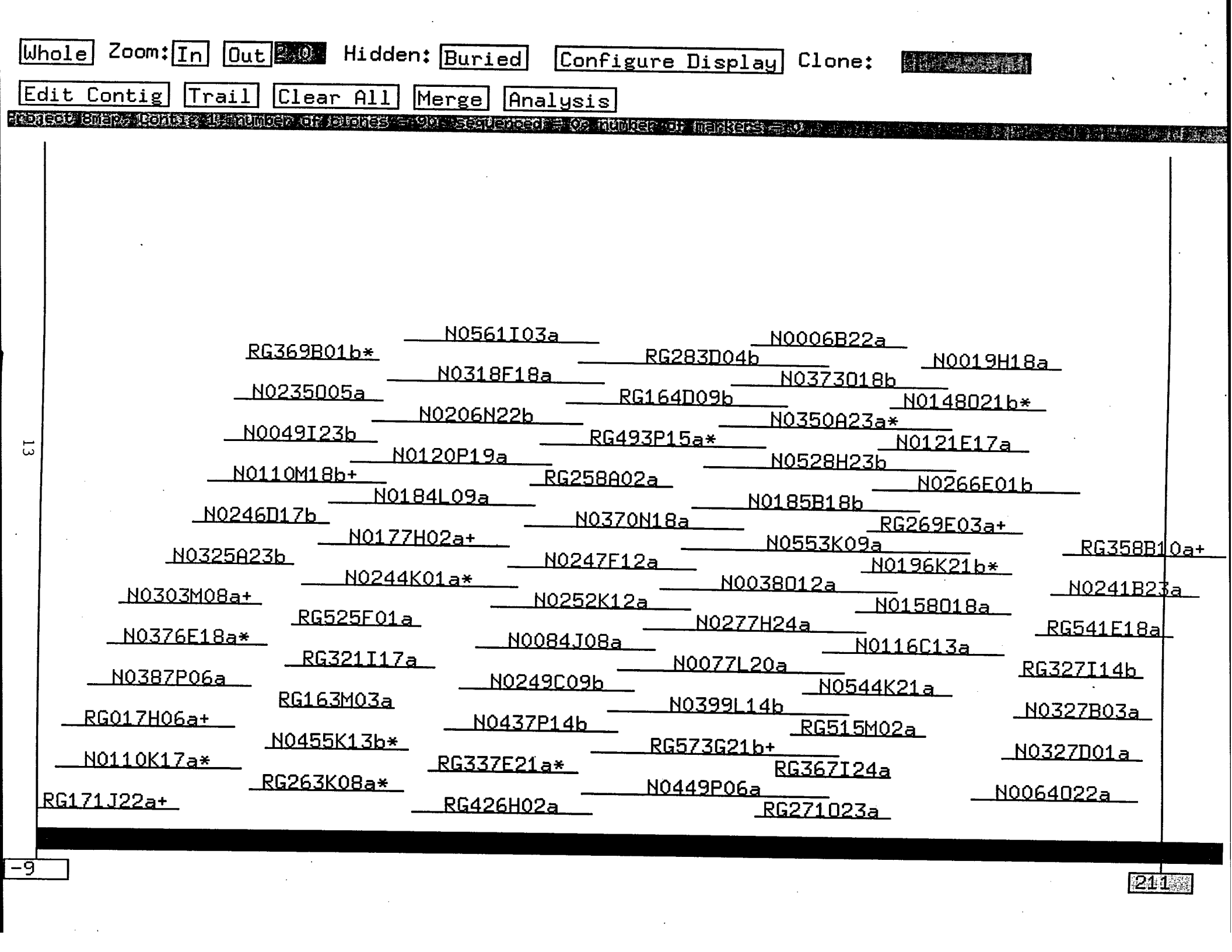
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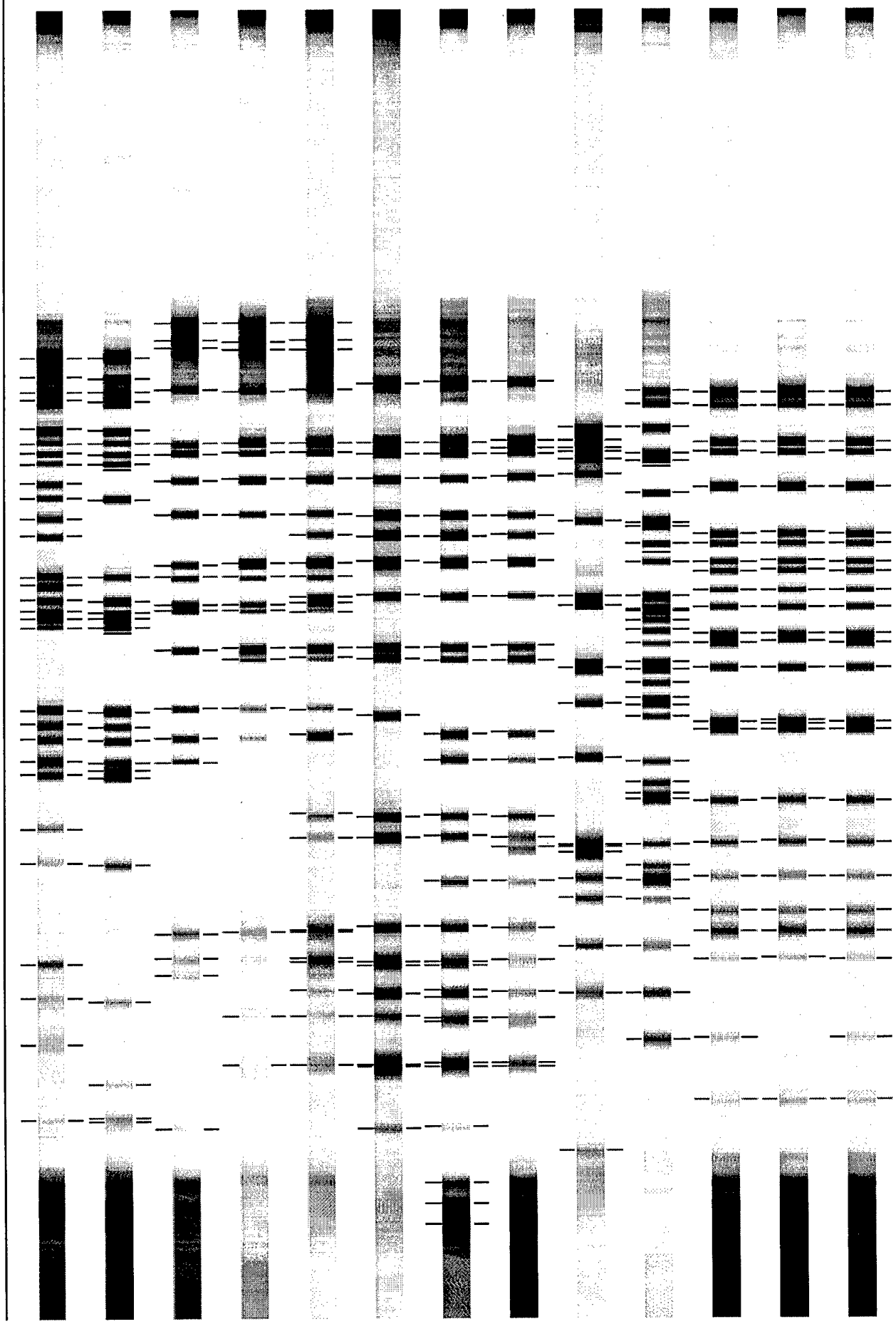

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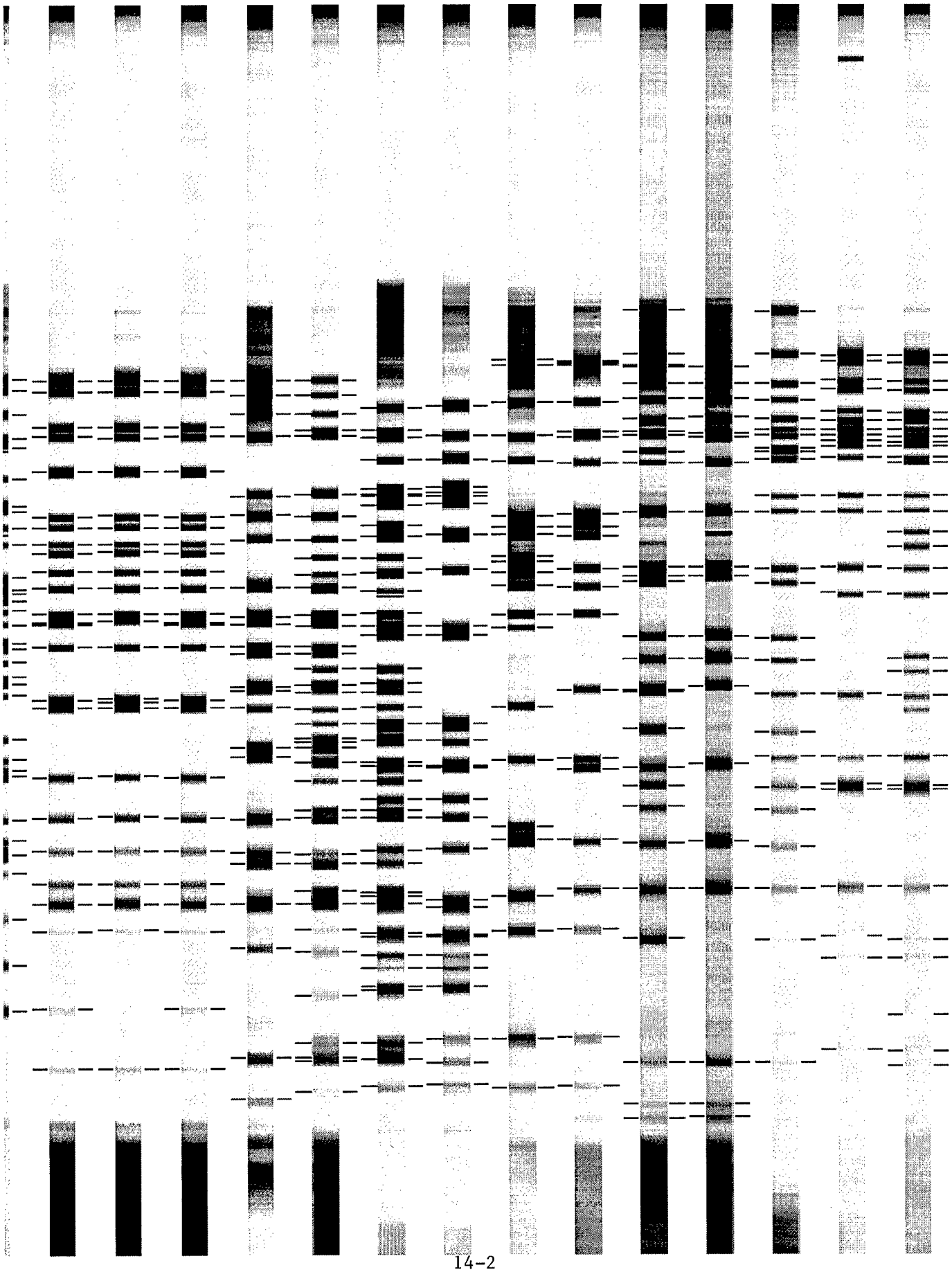

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Sequence-Ready Contig for the 1.4-cM Ductal Carcinoma *in Situ* Loss of Heterozygosity Region on Chromosome 8p22–p23

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We report the construction of an ~1.7-Mb sequence-ready YAC/BAC clone contig of 8p22–p23. This chromosomal region has been associated with frequent loss of heterozygosity (LOH) in breast, ovarian, prostate, head and neck, and liver cancer. We first constructed a meiotic linkage map for 8p to resolve previously reported conflicting map orders from the literature. The target region containing a putative tumor suppressor gene was defined by allelotyping 65 cases of sporadic ductal carcinoma *in situ* with 18 polymorphic markers from 8p. The minimal region of loss encompassed the interval between D8S520 and D8S261, and one tumor had loss of D8S550 only. We chose to begin physical mapping of this minimal LOH region by concentrating on the distal end, which includes D8S550. A fine-structure radiation hybrid map for the region that extends from D8S520 (distal) to D8S1759 (proximal) was prepared, followed by construction of a single, integrated YAC/BAC contig for the interval. The ~1730-kb contig consists of 13 YACs and 27 BACs. Fifty-four sequence-tagged sites (STSs) developed from BAC insert end-sequences and 11 expressed sequence tags were localized within the contig by STS content mapping. In addition, four unique cDNA clones from the region were isolated and fully sequenced. This integrated YAC/BAC resource provides the starting point for transcription mapping, genomic sequencing, and positional cloning of this region. © 1999 Academic Press

INTRODUCTION

Chromosome loss reveals constitutional recessive mutations that can result in tumor development (Sager, 1989; Marshall, 1991). Individuals heterozygous for a mutated tumor suppressor allele express the mutant phenotype (cancer) when the normal allele is lost. Thus, loss of heterozygosity (LOH) analysis may be used to map putative tumor suppressor genes when the DNA from normal and tumor-derived cells for the same patient are compared, using polymorphic markers flanking the locus. Frequent LOH of chromosome 8p has been reported in several types of human cancer, e.g., tumors of the prostate (Vocke *et al.*, 1996), lung (Emi *et al.*, 1992; Wood *et al.*, 1994), colon (Emi *et al.*, 1992; Wood *et al.*, 1994), larynx (Sunwoo *et al.*, 1996), liver (Emi *et al.*, 1992), and breast (Chuaqui *et al.*, 1995; Kerangueven *et al.*, 1995; Adelaide *et al.*, 1998; Dahiya *et al.*, 1998).

Studies in colorectal cancer have indicated the presence of at least two tumor suppressor genes (TSG) on 8p, at 8p11–p21.3 and 8p21–p22 (Farrington *et al.*, 1996). Similarly, three distinct regions, at 8p23, 8p22, and 8p12–p21, were defined by LOH studies for prostate cancer (Bova *et al.*, 1996; Vocke *et al.*, 1996; Perinchery *et al.*, 1999) and tumors of the head and neck (Sunwoo *et al.*, 1996; Wu *et al.*, 1997). Wright *et al.* (1998) identified three regions of loss on 8p (one in 8p22 and two in 8p23) for ovarian adenocarcinomas.

Markers in the region at 8p12–p22 have been shown by linkage analysis to have significant lod scores in several German breast cancer families, suggesting that BRCA3 may reside within that region (Seitz *et al.*,

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1997a,b). Recently Yokota *et al.* (1999) reported allelic loss in two regions (8p12–p21 and 8p22–p23.1, encompassing D8S549 and D8S1992) in advanced tumors, indicating that allelic loss on 8p may contribute to the progression of breast carcinoma.

In 1932 Broders proposed the term "*in situ*" to describe a condition in which malignant cells are present but have not yet penetrated the basement membrane. Ductal carcinoma *in situ* (DCIS) of the breast is a preinvasive form of breast cancer and can be a precursor of invasive ductal breast cancer. Several studies have described allelic losses in DCIS at multiple regions of chromosome 8p (Radford *et al.,* 1995; Yaremko *et al.,* 1995, 1996; Anbazhagan *et al.,* 1998), suggesting that these regions may be involved in early development of breast cancer.

The objective of our study was to map physically a genomic region defined by LOH assays of 65 DCIS tumor/normal pairs. We report DCIS allelic losses in 8p22–p23 and the construction of a 1.7-Mb sequence-ready contig from 8p22–p23 that may contain a tumor suppressor gene. Fifteen expressed sequence tags (ESTs) and cDNA clones from the region were also mapped to the contig, and nearly 25 kb of sequence was generated during the course of the study. This integrated YAC/BAC contig map provides a very useful resource for additional transcriptional mapping, positional cloning, and sequencing of this important region.

MATERIALS AND METHODS

LOH analysis. Sixty-five archival paraffin-embedded samples of DCIS were collected from several hospitals in St. Louis (Barnes-Jewish, Deaconess Central, St. Louis University, and the Outpatient Surgery Center). Subtypes of DCIS and nuclear grade were classified by the pathologists (N.J.P., J.H.R., K.DeS.). Either matched archival normal lymph node DNA or leukocyte DNA was used as a control. When it was necessary to draw blood for a normal control, informed consent was obtained following Institutional Review Board approval. Samples were microdissected to enrich for tumor cells, and DNA was purified as previously described (Radford *et al.,* 1993). After microdissection, tissue samples containing an insufficient number of tumor cells for the normal DNA extraction procedure were digested in small volumes (10–20 μl) of lysis buffer containing proteinase K and then phenol- and chloroform-extracted once before sodium acetate/ethanol precipitation. This material was resuspended in water, and then aliquots were used directly as a template (5–10 ng) for PCR amplification. Initial PCR conditions were obtained via the Genome Data Base (GDB, http://gdbwww.gdb.org) and then optimized in the laboratory. PCR products were separated on 7 M urea denaturing polyacrylamide sequencing gels and dried before exposure to Kodak XAR film. LOH was determined by a combination of visual inspection and scanning densitometry of the autoradiographs (Radford *et al.,* 1995). A 2.5-fold difference in the relative allele intensity ratios between tumor and normal DNA in an informative tumor/normal pair was scored as LOH (allele1/allele2 in tumor compared to allele1/allele2 in normal). To maintain a conservative scoring approach, marginal allele reduction by inspection was not scored as LOH. Tumor samples with equivocal results were redissected in an attempt to obtain a "purer" specimen, and the experiments were repeated. All LOH designations were scored by two independent scientists and repeated to verify the result.

Genetic linkage maps. Publicly available data (http://www.cephb.fr/cephdb/) of 8 Centre d'Etude Polymorphism Humaine (CEPH) reference families from CEPH version 7 were used for loci D8S264, D8S258, and D8S133. An additional 8 CEPH families (66, 1333, 1334, 1340, 1341, 1345, 1375, and 1377) were typed for markers D8S262, D8S277, D8S439, D8S351, D8S503, D8S516, D8S552, D8S261, LPL, SFTP2, NEFL, D8S137, D8S259, PLAT, and D8S166 and then merged with other family genotypes from CEPH. For loci D8S520, D8S550, D8S265, D8S511, and D8S549, an additional 12 families were genotyped (the extra 4 families being 104, 1344, 1346, and 1408). Merged data were then processed using the linkage program CRIMAP (P. Green, unpublished data) with minimum odds for order of 1000:1 during the "build." The program permutation option "flips" was used to determine the most likely marker order from the "build" results.

Radiation hybrid (RH) maps. We used the G3 radiation hybrid mapping panel (Research Genetics, Inc., Huntsville, AL) to construct the RH placement map. This 8 Krad panel, comprising 83 RH clones representing the whole human genome, was created by the Stanford Human Genome Center and has 500-kb resolution (Stewart *et al.,* 1997). Sequence-tagged site (STS) markers were assayed by PCR amplification and sized on 3% agarose gels stained with ethidium bromide. Each assay was performed twice; i.e., PCR products at the expected size were observed on each of the duplicate gels to be scored as positive. Marker data generated using the G3 panel were submitted to the Stanford RH server (http://www-shgc.stanford.edu/RH/rhserverformnew.html), which subsequently returned the results of analysis with a list of the highest-linked mapped markers, the lod score of the linkage, and the distance in centirays between the submitted marker and the linked marker on the map. However, the server compares only one submitted marker and the highest-linked marker at a time. To construct a RH placement map containing all of our markers, we also used the program RHMAP, version 3.0 (Lunetta *et al.,* 1996; http://www.sph.umich.edu/group/statgen/software) to analyze our G3 panel data together with the RH data from two flanking markers, SHGC-1955 (AFM287we5) and SHGC-13122 (D8S2061), from Stanford's RH map (http://www-shgc.stanford.edu/mapping/rh/).

YAC and BAC clone contig construction. The CEPH YAC contig WC-1195 from WICGR was used for the initial contig construction. A copy of the CEPH YAC library was maintained in our laboratory, and clones used in the mapping were propagated on YPD medium plates. Ten isolated colonies from each YAC clone were tested using a "whole-cell PCR" assay to identify colonies positive for a marker(s) from the region. For whole-cell PCR, a small amount of an isolated colony was suspended in 5 μl of deionized water, and the suspension was used directly as the template in a standard PCR. A standard ligation-mediated PCR method (Mueller and Wold, 1989; Vocero-Akbani *et al.,* 1996) was used in the development of the STS sJCW from YAC 770E9. PCR primers developed for the marker sJCW are 5′-TCAACAGCAGATTAGACACAGC-3′ and 5′-GYGGCTCTTATGCCATGTAC-3′.

For BAC contig construction, we screened the human BAC library CITB-978SK-B and CITB-HSP-C, constructed by the California Institute of Technology (obtained from Research Genetics, Inc.). The library screen involved a total of 120 PCR for each STS assay. As with YAC clone isolation, each identified BAC clone was then verified by a whole-cell PCR assay using 10 randomly selected colonies as candidates. After the positive BAC clones were verified, single BAC colonies were propagated in liquid medium, cells were harvested, and insert DNA was prepared using the Plasmid Midi-Kit from Qiagen Inc. (Chatsworth, CA). Each BAC clone was partially sequenced on an ABI 373 DNA sequencer (PE/ABI), from the insert ends using modified T7 (5′-GTAATACGACTCACTATAGGG-3′) and Sp6 (5′-CGCCAAGCTATTTAGGTGACAC-3′) primers. The sequence generated was analyzed for candidate PCR primer sequences using the program PRIMER 0.5 (Lincoln and Lander, MIT Center for Genome Research). STSs developed from BACs were tested using the NIGMS (No. 2) rodent/human hybrid panel, in which each hybrid cell contains a single human chromosome, and only chromosome 8-specific STSs were used as entry points for chromosome walking.

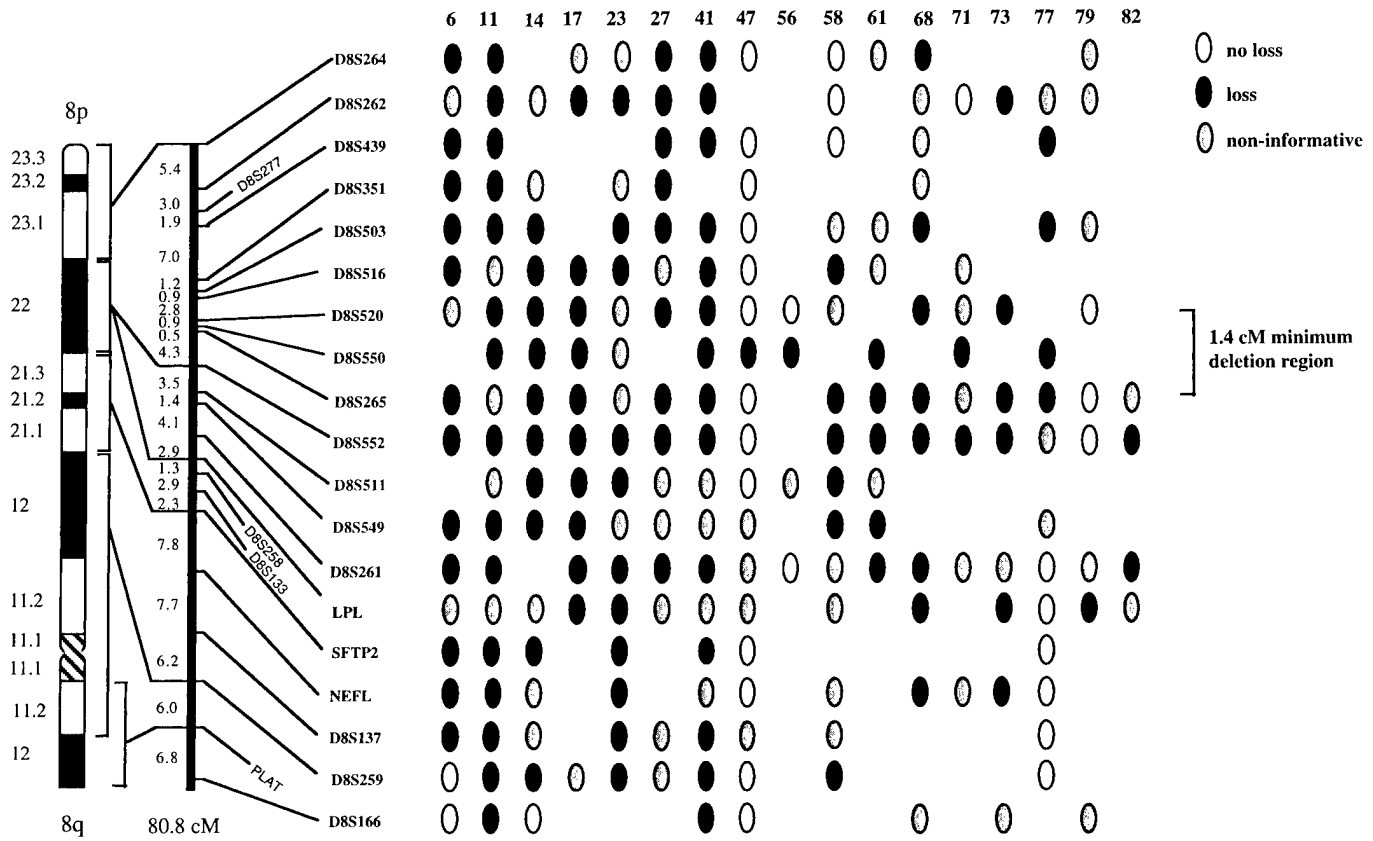

FIG. 1. LOH analysis using 18 mapped markers on 8p and one on 8q. An 80.8-cM sex-average multipoint linkage map is presented as a vertical bar to the right of the cytogenetic idiogram. Map distances are given to the left of the vertical map in cM. Genetic loci uniquely placed with odds for order of at least 1000:1 are given to the right of the map.

To estimate insert sizes, BAC clones were digested with *Not*I, and restriction fragments were separated on 1% agarose gels in 0.5× TBE buffer using MidRange II PFG markers (NE BioLabs) as size standards. Gels were electrophoresed at 6 V/cm with an initial pulse of 5 s and a final pulse of 15 s for 18 h at 14°C in a Bio-Rad CHEF DR II apparatus.

RESULTS

LOH Observed for DCIS on 8p

Sixty-five cases of DCIS were assayed for LOH using 18 mapped microsatellite markers on 8p and 1 on 8q. Of 61 samples informative for at least 1 marker on 8p, LOH was found in 17 tumor DNA samples (27.8%). Of the informative tumors, LOH was observed in 11/35 comedo, 3/14 cribriform, 1/4 solid, and 2/3 micropapillary samples. None of the 3 informative mixed and 2 informative papillary samples showed LOH. Sixteen of 44 informative samples with high nuclear grade showed LOH, 1/13 showed low nuclear grade, and 0/4 showed intermediate nuclear grade.

Ten tumor samples demonstrate loss of most of the short arm of chromosome 8 (Fig. 1). For example, tumor 6 had apparently lost an allele from all loci tested telomeric to D8S259. Tumors 56, 58, 71, and 77 contain deletions that localize the putative tumor suppressor gene between D8S520 and D8S261. Tumor 47 showed LOH for D8S550 but retained loci D8S265 and D8S520, confining the deleted region to a minimum interval of 1.4 cM at 8p22–p23, between the markers D8S520 and D8S265 (Figs. 1 and 2). Tumor 79 showed LOH for LPL at 8p22 but retained three loci that were more telomeric, suggesting a second, more centromeric deletion region at 8p22–p23. The frequency of LOH for each marker tested is listed in Table 1. The highest rates of loss were found with markers D8S550, D8S552, and D8S503. No statistical correlation was found between LOH on 8p and histologic parameters such as subtype, nuclear grade, or the presence or absence of associated invasion. Tumor 47 showed LOH for D8S550 only, suggesting that the tumor suppressor gene would be found near this locus. Thus we concentrated our physical mapping efforts on this region.

RH Mapping of the Region near D8S550

We constructed a refined radiation hybrid map (Table 2) for the deletion interval using the Stanford G3 panel. A total of 10 markers including 6 microsatellite markers (D8S265, D8S520, D8S550, D8S1695, D8S1755, and D8S1759), one novel STS (sJCW), and 3 ESTs (A005E28, T96924, and WI-6800) that were identified through database searches were ordered on the map using our typing data and the program RHMAP, version 3.0. The marker D8S520 was mapped distal to the centromere and D8S1759 proximal to the centro-

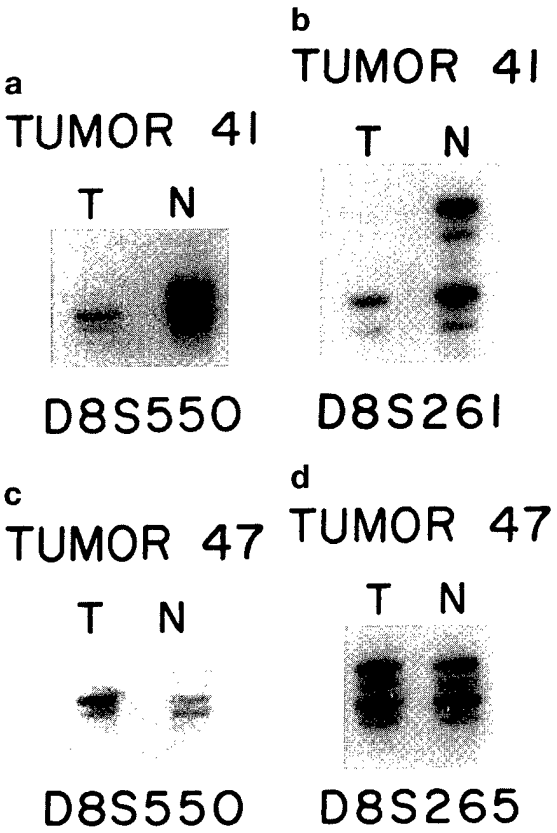

FIG. 2. Examples of LOH in DCIS samples. T indicates tumor DNA and N matched normal control. (**a**) Tumor 41 marker D8S550. The fold difference in integrated allele ratios between tumor and normal is 6.13. (**b**) Tumor 41 marker D8S261. The fold difference in integrated allele ratios between tumor and normal is 10.9. The upper allele is lost in the tumor in both cases. (**c**) Tumor 47 marker D8S550. (**d**) Tumor 47 marker D8S265. The lower allele is lost in the tumor for marker D8S550, but both alleles are retained for marker D8S265.

mere. The order of markers D8S265, D8S520, and D8S550 confirmed our genetic linkage map order. The order of other markers on the RH map also matched the position of those markers on our contig map except that of D8S1755 (Fig. 3, Table 2). When only 6 microsatellite markers were typed, D8S1755 was placed between markers D8S265 and D8S550, which corresponded to the contig map. However, when the RH data from 3 EST markers were integrated, D8S1755 mapped between D8S265 and D8S1759. Data errors such as false-positives or false-negatives or the limited resolution of the RH panel could explain this discrepancy. The distance of 42.3 cR, or approximately 1565 kb (assuming 37 kb per centiray; Stewart *et al.*, 1997), between the markers D8S265 and D8S520 demonstrated ~1:1 correspondence between genetic and physical distances for this interval.

Construction of an Integrated YAC/BAC Contig from D8S520 to D8S1759

We constructed a single, continuous, integrated YAC/BAC clone contig spanning a total distance of ~1730 kb covering the putative TSG region of ~1100 kb based on sizes of BAC clones. This sequence-ready contig comprises 13 YACs and 27 BACs including 54 STSs from BAC end sequences (Table 3).

TABLE 1

Eighteen Loci on 8p and One Locus on 8q Assayed for LOH

Locus	No. LOH[a]	No. inf.[b]	% LOH
D8S264	5	26	19.2
D8S262	7	32	21.9
D8S439	5	22	22.7
D8S351	3	18	16.6
D8S503	8	21	38
D8S516	6	26	23
D8S520	7	25	28
D8S550	8	22	36.4
D8S265	10	44	22.7
D8S552	13	32	40.6
D8S511	4	19	21
D8S549	6	19	31.6
D8S261	9	31	29
LPL	5	23	21.7
SFTP2	5	30	16.6
NEFL	5	22	22.7
D8S137	4	22	18.2
D8S259	5	26	19.2
D8S166	2	21	9.5

[a] No. LOH, number of LOH cases detected from informative tumor samples tested for the marker.
[b] No. inf., number of informative samples observed from 65 tumor cases tested.

YAC Clone Contig

Based on the publicly available contig maps from WICGR (http://carbon.wi.mit.edu), we isolated 13 YACs using the six microsatellite markers listed in RH

TABLE 2

RH Map Spanning the Deletion Interval

Marker	Distance (cR)
Telomere	
SHGC-1955 (AFM287we5)	4.7
A005E28	4.8
sJCW	4.9
D8S520	20.8
D8S550	16.2
WI-6800	0.0
T96924	5.3
D8S265	20.6
D8S1755	17.6
D8S1759	0.0
D8S1695	11.6
SHGC-13122 (D8S2061)	
Centromere	

Note. 1 cR ≅ 37 kb (Stewart *et al.*, 1997). Boldface type indicates markers we assayed using the Stanford G3 panel.

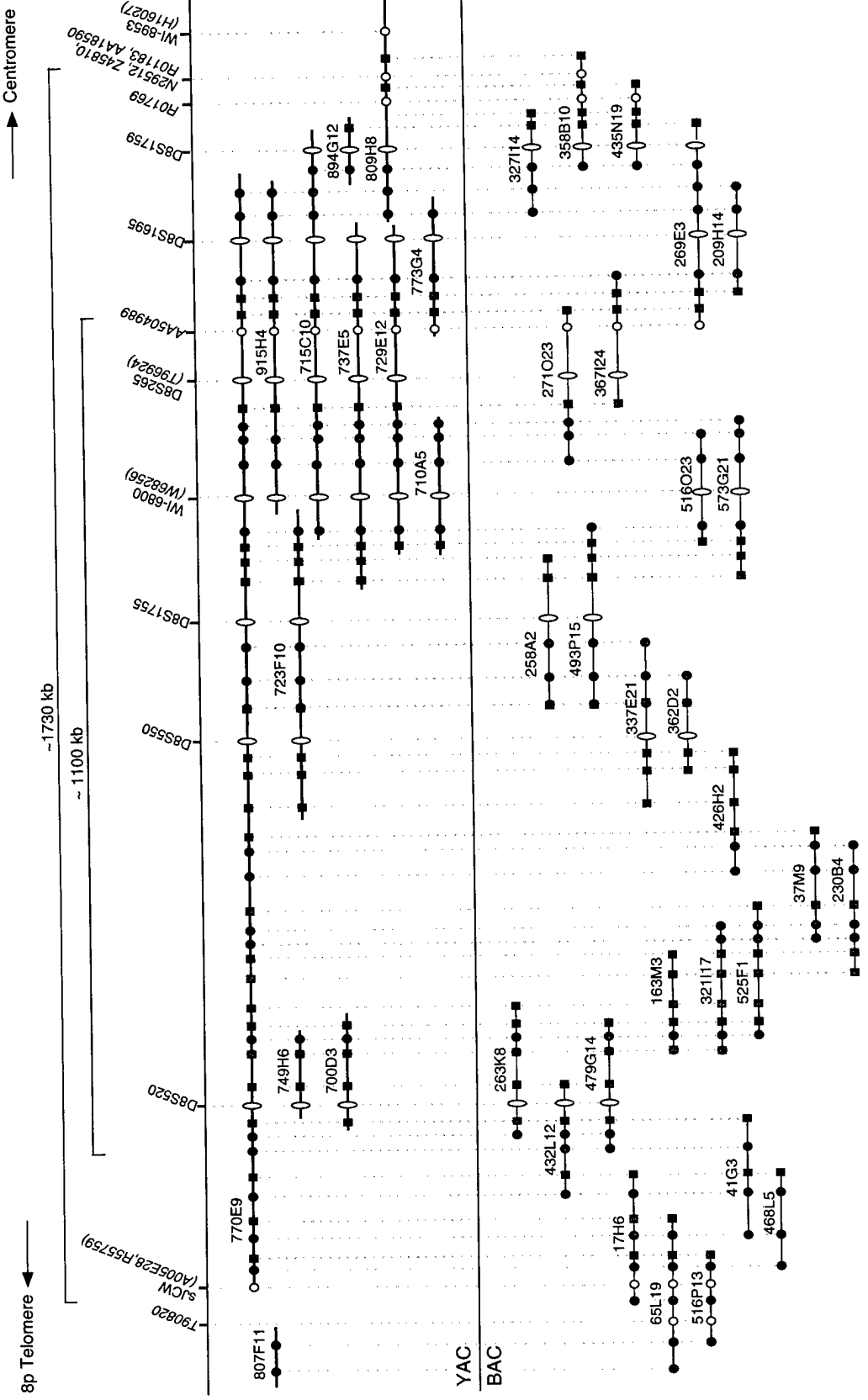

FIG. 3. An integrated YAC/BAC clone contig spanning the 1.1-cM deletion region.

TABLE 3

Primer Sequences and Annealing Temperatures for 54 Novel STS Markers on BAC Contig

STS	PCR primer	GenBank Accession No.	Product size (bp)	Annealing temperature (°C)
17H6-Sp_6	5′-CAGCCCTCAACAAAGGTAGG-3′/5′-CTCTTTCAGGTGTGGCCATT-3′	AF122927	117	56
17H6-T_7	5′-TCTTCACCTCGTGGCACTG-3′/5′-GGACAATCCATCCATCCATC-3′	AF122928	198	55
37M9-Sp_6	5′-GGCTTGTTTGTGGGCTTATA-3′/5′-GGTGAGTTTCAAGAGGGGTT-3′	AF122943	148	56
37M9-T_7	5′-TAACACTCTCCTGCACTCTGCCTC-3′/5′-AATGTCGTCTTTCCCTGCCCAAAGG-3′	AF122944	198	62
41G3-Sp_6	5′-ACAAGCTAAGGGCCATCCTT-3′/5′-GATGCCACCAGCATGTGG-3′	AF122933	126	56
41G3-T_7	5′-GGTATTTGTGAGCCTTCCCA-3′/5′-GAAACACAGCACACGATAGCA-3′	AF122934	228	59
65L19-Sp_6	5′-CCCAAAACAGTCATTGAAACG-3′/5′-CGATAAATGCTTGCTTCATCC-3′	AF122931	143	54
65L19-T_7	5′-GGGAAACAGGCACTTCACAT-3′/5′-TTTGATCATGGGGTGGATTT-3′	AF122932	163	56
163M3-Sp_6	5′-ACTCAACCTCCTGGGTTCCT-3′/5′-GTTGACAGCCTCACCCTGTT-3′	AF122937	133	58
163M3-T_7	5′-CTCCTCACTTGGGAAATGTG-3′/5′-CTCCAAGAAAATGATGACTGC-3′	AF122938	127	58
209H14-Sp_6	5′-CCAGCTTCCCTTTGAGACAG-3′/5′-AGGCAGGGCTTACTTCACCT-3′	AF124288	136	58
209H14-T_7	5′-GACTCAGTTGCTTCCAAGGC-3′/5′-TCAGCCATCAAGCTTCACAG-3′	AF124289	108	58
230B4-Sp_6	5′-TGATCCAGGGACAGAGCAAGAAG-3′/5′-GAGCCAGGAGCATGAGAATATGG-3′	AF122939	121	62
230B4-T_7	5′-CCATCTTTATTCCACAGGAAGAGG-3′/5′-GCAACAAGAGTGAAACTCCGTCTC-3′	AF122940	180	62
258A2-Sp_6	5′-CCAACCTAGCCGTGGTTAGT-3′/5′-TGTGTTCAGTCACCATGGGT-3′	AF124276	162	57
258A2-T_7	5′-CATCAGGCATTGAAGTCACA-3′/5′-TAAGCACGCACACAGCC-3′	AF124277	146	55
263K8-Sp_6	5′-TGAGCACACAGGATTTTTACG-3′/5′-CGTCATCATACCCAGAGTC-3′	AF124266	150	55
263K8-T_7	5′-TCTCGTTTGCTTATGACCTG-3′/5′-CTAACAACTAGATTGGAAGGAGC-3′	AF124267	138	57
269E3-Sp_6	5′-AGTGCTGGGGGGGATTTTTG-3′/5′-ACGTGGCAGTGGGTTAGAGTTG-3′	AF124290	148	61
269E3-T_7	5′-GCAGCTGATCTCTCTCCACC-3′/5′-CCTTTCACTCCATGTTGGCT-3′	AF124291	202	58
271O23-Sp_6	5′-GTCTTGGGGGTCAAAAATCT-3′/5′-GCTTGTGTATCCACTCCGTT-3′	AF124284	131	56
271O23-T_7	5′-TGCCCACTTTCCCTCATGG-3′/5′-TTCCTAAGCCAGTGAGTTGATGC-3′	AF124285	155	57
321I17-Sp_6	5′-TGTGCGCATAGTAGGAATGA-3′/5′-CGCAAAAAGGTCAGAGAGAG-3′	AF122941	213	57
321I17-T_7	5′-ACTCAACCTCCTGGGTTCCT-3′/5′-TCTGAGCTCCTTTTCCACGT-3′	AF122942	151	57
327I14-Sp_6	5′-TGAAGGTCTGCTCAGCACC-3′/5′-CCTGCAAAAGGCTGACCTAG-3′	AF124292	199	59
327I14-T_7	5′-CATCTGTTGATGGGCATTTG-3′/5′-TGGCAATCCCCCTTCTAAG-3′	AF124293	138	55
337E21-Sp_6	5′-ATATGTGCACCCAATGCAG-3′/5′-GAGTTCAATTACGGGATAGCC-3′	AF124272	174	56
337E21-T_7	5′-TAACCGTGACACTCTACTGCC-3′/5′-TTCATGATCTCAAGGCTGTG-3′	AF124273	203	53

TABLE 3—*Continued*

STS	PCR primer	GenBank Accession No.	Product size (bp)	Annealing temperature (°C)
358B10-Sp_6	5′-TGCTGTGCTAGCCAGTTCC-3′/5′-AACAGGTACCACAAGGCAGC-3′	AF124294	178	60
358B10-T_7	5′-GCCCTTGCTTAAGAACTCAA-3′/5′-ACCCAGGCATATTGGTGAGT-3′	AF124295	205	56
362D2-Sp_6	5′-TGCAGCCTCATTCTGTTGTC-3′/5′-CTCATCAGKGAGTCTGGAGTCG-3′	AF124274	133	57
362D2-T_7	5′-ATAGGGTTGACACTGCCACC-3′/5′-TTGGCCAGGCATGGTAGT-3′	AF124275	132	58
367I24-Sp_6	5′-AAGGAAGCGCCCAATTC-3′/5′-ACTGGCTTTGCTGGAATTTT-3′	AF124286	120	56
367I24-T_7	5′-TGCTGAGCTGGGTGAAATC-3′/5′-CCATGAAGGAAAGGACCTGA-3′	AF124287	147	56
426H2-Sp_6	5′-CCCCAAACGTATCAATCCAG-3′/5′-TCCATTTCCAGTGTGTTGGA-3′	AF122945	167	59
426H2-T_7	5′-TGACAGAAGGATCTGGGACC-3′/5′-GGAAGTTGAGGCTGCAGAGA-3′	AF122946	181	60
432L12-Sp_6	5′-AAAGGGGAGACATTCGGC-3′/5′-TGGGAGGAGAGAGCCAGATA-3′	AF124268	149	56
432L12-T_7	5′-AACCTGCACTGCCAATAACC-3′/5′-AAGGTTTTGTGACCTCGGG-3′	AF124269	147	54
435N19-Sp_6	5′-CTTTTGGTCAAAGCCCACAT-3′/5′-TTGCAGTGAAGGCTGAAATG-3′	AF124296	118	54
435N19-T_7	5′-TGTGTTAGATGATTTTGCCCA-3′/5′-ATATGCCTAGCCTACCAAACA-3′	AF124297	100	57
468L5-Sp_6	5′-ACCCAACAACGCCATAAAAC-3′/5′-GTGGGGGTGTTTTTCTTGTC-3′	AF122935	162	58
468L5-T_7	5′-TTCTCTTCCACTGGATACGC-3′/5′-TTTTCTGTGGTGGGGTAGAC-3′	AF122936	141	57
479G14-Sp_6	5′-GCACAGAGGATTTTTACGGC-3′/5′-ACACGTCATCATCACCCAGA-3′	AF124270	150	60
479G14-T_7	5′-CACCAGAGAGGTGCAAAACA-3′/5′-TTGCAGCCAAGTGGTATTTG-3′	AF124271	156	56
493P15-Sp_6	5′-ACCATCAAGCAACTWGGTTTCCAG-3′/5′-TTCATGMTGACTTGGGGTGC-3′	AF124278	145	58
493P15-T_7	5′-CCATGCTACCATTGCACTCCTC-3′/5′-TTGATGGGATGTGGGGCAAAGTGG-3′	AF124279	143	61
516O23-Sp_6	5′-TCTGCCACGTTTTGGTATCAGG-3′/5′-AAATCCCTGGACACACACACCC-3′	AF124280	257	63
516O23-T_7	5′-TCTTCAAGACCCATGCCTG-3′/5′-GGCATACATCACCATGCTCA-3′	AF124281	278	56
516P13-Sp_6	5′-TGGCTTAGTGCTAACCCTCA-3′/5′-ATGTTGGAGTCCTAAAACCTCG-3′	AF122929	101	54
516P13-T_7	5′-CATAGAGGCATTTTGGTTACG-3′/5′-ATGGAAAAGAGATCTAACAGCG-3′	AF122930	102	54
525F1-Sp_6	5′-CCTGGGTTCCTCGTGAATCTTC-3′/5′-TCCTTTTCCACGTTGACAGCC-3′	AF122947	136	62
525F1-T_7	5′-GGTACTATCCACACTCGTTGGGGT-3′/5′-AACATGCAATACCAAGGATGCG-3′	AF122948	122	60
573G21-Sp_6	5′-CTGCCTCCTGCAAAATTTGT-3′/5′-CGAGCACCAGATTTGCTTTT-3′	AF124282	196	57
573G21-T_7	5′-ACCCATCATCCCTGGAAG-3′/5′-GTAGTGCCAATTCCCTTTCA-3′	AF124283	127	56

mapping and one additional marker, WI-8953, that appeared to fall within the relevant interval. We confirmed the presence of these markers within the YAC clones 700D3, 710A5, 715C10, 723F10, 729E12, 737E5, 749H6, 770E9, 807F11, 809H8, 894G12, and 915H4 (Fig. 3).

BAC Library Screening

The initial screening of the human BAC library was conducted with 10 markers including 6 microsatellite markers (D8S520, D8S550, D8S1755, D8S265, D8S1695, and D8S1759) and 4 ESTs (T96924, WI-6800, N29512, and Z45810) anchored on the YAC contig (Fig. 3). An average of 5 BAC clones per marker were identified and isolated using PCR assays. After the positive BAC clones were verified, the insert ends of each clone were sequenced. The chromosomal origin of each newly generated STS was verified using a panel of rodent/human hybrids. Only the clones containing chromosome 8-specific STSs at both ends were used for contig construction.

Chromosome Walking

From our initial screening, we were able to build a continuous BAC clone contig from D8S550 to D8S1759, leaving a gap between the markers D8S520 and D8S550 (Fig. 3). To close the gap, STSs developed from T7-end sequences of clones 263K8 and 337E21 were used for secondary BAC library screening. Three clones, 163M3, 321I17, and 525F1, and one clone, 426H2, were identified and isolated from STSs 263K8-T7 and 337E21-T7, respectively. Both insert ends of these newly isolated BAC clones were sequenced, and six chromosome 8-specific STSs were generated. Six STSs (163M3-Sp6, 163M3-T7, 321I17-Sp6, 321I17-T7, 525F1-Sp6, and 426H2-T7) were mapped back onto the YAC/BAC contig using PCR assays, leaving a gap between 525F1-T7 and 426H2-Sp6. From another BAC library screening experiment, five clones were identified and isolated using a PCR assay from the STS 426H2-Sp6; four of them were found to be positive for the STS from 525F1-T7. Among these, four novel STSs (37M9-Sp6, 37M9-T7, 230B4-Sp6, and 230B4-T7) were developed from insert end sequences and mapped back to the contig by overlap analysis (Fig. 3). This completed construction of a continuous BAC clone-based contig of the region.

All of the 27 BAC clones used for contig construction were digested with restriction enzyme *Not*I, from which estimates of the sizes of the clones could be made. This information enabled more precise determination of the size of the region spanned by the contig and of the region containing the putative TSG. Based on the estimated sizes of the BAC clones, the contig spans approximately 1730 kb and the LOH deletion region between D8S520 and D8S265 spans ~1100 kb (Table 4).

TSG Candidate Identification and Database Homologies

First, databases were searched for candidate genes within the region of chromosome 8p22–p23. We verified by PCR assay the locations of 11 ESTs reported to map within this region. EST A005E28 identified by the Institute for Genomic Research (TIGR) mapped to YAC 770E9. Like STS sJCW, it tested positive for BAC clones 17H6, 65L19, and 516P13. EST T90820 also tested positive for BAC clones 65L19 and 516P13, but not for YAC 770E9 (Table 5). EST T96924 mapped to YACs 770E9, 915H4, 715C10, 737E5, and 729E12. It also tested positive for BAC clones 271O23 and 367I24 identified by D8S265. Two cDNA clones (T96924, 0.8 kb, and W67504, 1.6 kb) were isolated and fully sequenced. The two sequences were compiled into one continuous sequence using ClustalW alignment of MacVector 6.0 program (Oxford Molecular, http://www.oxmol.com/). This sequence contains $(CA)_{22}$ repeats that could be used to develop a microsatellite marker. BAC clones 271O23 and 367I24 were also partially sequenced with a primer selected from the 3′ end of cDNA T96924. The sequences from these two BAC clones contain a $(CA)_{22}$ repeat and match the sequence from cDNA W67504.

A cluster of 6 ESTs that appeared to localize to the region from the UniGene (http://www.ncbi.nlm.nih.gov/UniGene) map were placed at the proximal end of our YAC/BAC contig. They all mapped to YAC 809H8. EST R01769 tested positive for BAC clones 358B10 and 435N19. ESTs AA018590, N29512, R01183, and Z45810 tested positive for clone 358B10 only. EST H16027 did not identify any BAC clone on the contig

TABLE 4

BAC Clones Mapped on the Contig Spanning the 1.1-cM Deletion Interval

Clone	Size (kb)
17H6	175[a]
37M9	90[a]
41G3	—[b]
65L19	258
163M3	93
209H14	—
230B4	143
258A2	130
263K8	178
269E3	172[a]
271O23	135[a]
321I17	100
327I14	—
337E21	255
358B10	140[a]
362D2	135[a]
367I24	121
426H2	170[a]
431L12	114[a]
435N19	—
468L5	—
479G14	125[a]
493P15	170[a]
516O23	175[a]
516P13	121
525F1	127[a]
573G21	—

Note. —, clones not sized.

[a] Clones used to construct the minimal clone set for the contig spanning the region between markers of D8S520 and D8S1759.

TABLE 5

Eleven ESTs Mapped to the YAC/BAC Contig

ESTs	Accession No.	Marker name	UniGene	Mapping data
R55759	G26262	A005E28	Unidentified, Hs. 26458	BAC 17H6, 65L19, 516P13
T90820	G19346	SHGC-17264	Unidentified, Hs. 133548	BAC 65L19, 516P13
R01769	G28044	SHGC-33472	Unidentified, Hs. 119611	YAC 809H8; BAC 358B10, 435N19
R01183	R01183	stSG327	Unidentified, Hs. 4883	YAC 809H8; BAC 358B10
T96924	T96924	SGC33822	Unidentified, Hs. 35453	YAC 715C10, 729E12, 737E5, 770E9; BAC 271O23, 367I24
N29512	N29512	SGC30677	Farnesyl-diphosphate farsyltransferase, Hs. 48876	YAC 809H8; BAC 358B10
AA018590	AA018590	SGC30677	Farnesyl-diphosphate farsyltransferase, Hs. 48876	YAC 809H8; BAC 358B10
Z45810	Z45810	A005M25	Unidentified, Hs. 94151	YAC 809H8; BAC 358B10
W68256	W68256	SHGC-24302	Unidentified, Hs. 27194	YAC 710A5, 715C10, 729E12, 737E5, 770E9; BAC 516O23, 573G21
AA504989	AA504989		Unidentified	YAC 715C10, 729E12, 737E5, 770E9; BAC 271O23, 367I24, 269E3
H16027	H16027	WI-8953	Cathepsin B precursor, Hs. 84898	YAC 809H8

(Table 5). Eight of these 11 ESTs appeared to be unique since they did not identify homologous sequences in the publicly available databases. ESTs N29512 and AA018590 identified by the marker SGC30677 in Unigene were developed from cDNA clones with sequence homology to human farnesyl diphosphate farnesyltransferase. EST H16027, which is one of the ESTs detected by the marker WI-8953, is derived from a cDNA with homology to human cathepsin B precursor.

In addition to the previously reported ESTs from the region, both end sequences generated from BAC clones were analyzed with BLASTN. Only the Sp6-end sequence from clone 269E3 identified a homologous sequence, Stratagene's fetal retina *Homo sapiens* cDNA clone (AA504989). About 1.5 kb of this cDNA clone has been sequenced. Another cDNA clone (W68256, 0.6 kb) identified by the marker WI-6800 was isolated, and the insert was fully sequenced. A total of nearly 25 kb of sequence was generated from BAC insert ends and four cDNA inserts.

DISCUSSION

DCIS is a preinvasive form of breast cancer that is being detected at an increasing rate due to screening mammography. In some cases it represents a step in the pathway to invasive breast cancer, although it is not always an obligate precursor (Rogers, 1987). In our study of 65 cases of DCIS, using 18 markers on 8p and 1 on 8q, LOH was seen on 8p in 29% of informative cases. Our data identify the smallest common region of LOH to be located at 8p22–p23 in an approximately 1.4-cM region between D8S265 and D8S520. The pattern of LOH for tumor 79 suggests the presence of at least one other tumor suppressor gene proximal to the 8p22–p23 locus.

Other studies have also reported evidence of LOH on 8p in DCIS (Aldaz *et al.,* 1995; Seitz *et al.,* 1997b; O'Connell *et al.,* 1998; Anbazhagan *et al.,* 1998). Anbazhagan *et al.* (1998) found the most common region of loss in 60 invasive ductal breast tumors to be at 8p21.3–p23.3 between D8S560 and D8S518. The 1.4-cM region of loss we have identified lies within this region. Several studies have attempted to correlate 8p LOH and the ability of a tumor to metastasize (Aldaz *et al.,* 1995; Yaremko *et al.,* 1996; Anbazhagan *et al.,* 1998; Dahiya *et al.,* 1998). Yokota *et al.* (1999) observed a minimal region of LOH on 8p, in a region flanked by markers D8S511 and D8S1991, that was associated with advanced tumor stage and aggressive histologic subtype. This region does not overlap with our minimal region. However, the two studies analyze different tumor types. In our data we observed no correlation between LOH on 8p and tumor grade or the presence of an invasive component. Both observations are thus consistent with the presence of more than one putative tumor suppressor gene on 8p, one associated with early events in breast oncogenesis and one associated with tumor invasion.

Chromosome 8p22–p23 has also been associated with potential tumor suppressor genes involved in other types of human cancers. Wright *et al.* (1998) defined three LOH regions of overlap in ovarian cancer, one at 8p22, near marker LPL, and two at 8p23, near D8S549 and the region distal to D8S1759, with the highest LOH rates at D8S264, D8S550, and D8S1827. Frequent allelic losses at D8S550 have also been observed in hepatocellular carcinoma (Nagai *et al.,* 1997). Thus it is likely that a putative tumor suppressor gene in the region of D8S550 may be involved not only in preinvasive breast cancer but also in other human cancers.

To clone and characterize efficiently the putative tumor suppressor gene in this region of 8p22–p23, we constructed a meiotic linkage map to resolve the ambiguity of marker order within this region. A radiation

hybrid map for the 1.4-cM LOH deletion interval in the region was also constructed. The recently published GeneMap'98 (Deloukas *et al.,* 1998; http://www.ncbi.nlm.nih.gov/genemap98) has several positional discrepancies between GB4 and G3 RH maps in the deletion interval. For example, marker WI-6800 was placed distal to D8S520 on the GB4 map while the G3 map placed WI-6800 proximal to D8S550. A direct comparison of the two RH maps reveals a limited number of shared STSs; thus precise marker orders cannot be determined from these data. When comparing markers D8S520, D8S550, WI-6800 (SHGC-24302), and SGC33822 (T96924), our RH data agreed more closely with the locus order on the G3 map than that on the GB4 map. Although our RH map position for the marker D8S1759 is different from that on the G3 map, the position was verified with our BAC clone-based contig.

Previously, physical maps that include part of 8p22–p23 have been constructed based primarily on assembly of existing YAC contigs or RH mapping (Bookstein *et al.,* 1994; Bova *et al.,* 1996; Farrington *et al.,* 1996). However, the calculated order and the distances between loci can be affected by the rearrangements, internal deletions, and chimerisms that can occur in YACs. We have confirmed marker orders with BAC clones, which are known to have low frequencies of these events. Our integrated YAC/BAC contig, extending from the distal marker 65L19-Sp6 (~200 kb distal to D8S520) to the proximal marker WI-8953 (~100 kb centromeric to D8S1759), has enabled a more precise measurement of the physical distance and marker order of the 8p22–p23 region. The contig is also a valuable framework to integrate additional cDNAs, STSs, and ESTs reported in the region. Eleven ESTs (Fig. 3, Table 5) were placed onto the clone contig, and they will be used for preliminary candidate gene analysis.

Additional genes of interest from this region may be found in the literature. Hughes *et al.* (1998) found a novel amplicon at 8p22–p23 resulting in cathepsin B (CTSB), a cancer-related gene that is overexpressed in esophageal adenocarcinoma. The EST H16027, which is similar to the CTSB gene, maps to the proximal boundary of the contig at the marker WI-8953 (Fig. 3, Table 5) and may represent this gene. Harder *et al.* (1997) isolated the gene for human β-defensin-2 (human BD-2, also known as DEFB2), and mapped it to YAC clone 773G4, within the preexisting 8p22–p23 YAC contig WC-1195, which is anchored to our contig by the marker D8S1695.

The STS information and the overlapping BAC clones presented in this report provide the starting material for large-scale sequencing of this genomic region. The contig will also be a useful resource contributing to gene identification and studies of gene expression patterns in the region.

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