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PRINCIPAL INVESTIGATOR: Carol L. Rosenberg, M.D.

CONTRACTING ORGANIZATION: Boston University
Boston, Massachusetts 02215

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Breast cancer probably develops as multiple genetic abnormalities accumulate, but the timing and nature of the earliest steps in this progression remain unknown Proliferative breasat lesions, considered benign but associated with increasaed breas t cancer risk, were recently shown to contain clona l genetic abnormalities. Therefore, we hypothesized that clonal genetic abnormalities might be detectable before any phenotypic abnormalities develop: i.e., in microscopically normal brea st tissue. We examined DNA from 95 normal-appearing ducts or terminalductal-lobular units (TDLU) from 20 individuals with varying breast cancer risk: those undergoing reduction mammoplasty, those with proliferative lesions, and those alreaady with breast cancer. Using 9 microsatellite markers, we looked for genetic instability, or allele imbalance, most likely loss of heterozygosity (LOH). We found genetically abnormal clones in 21/95 (22%) of seemingly normal samples from 10/20 (50%) women from all 3 risk groups. In women under age 50, trends towards increased rates of abnormalites were noted with increased cancer risk. Abnormalities were more likely to be at sites of known or postulated tumor suppressor genes than at random or neutral loci. Our data indicate that genetic abnormalities potentia lly critical to breas t cancer genesis accumulate before detection of high risk lesions.

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Carol Rosenberg 1/5/99

PI - Signature Date

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Introduction:

Little is known about the earliest genetic events in the process of breast carcinogenesis. Carcinoma in situ (CIS) is generally accepted as the first truly malignant breast lesion, but as CIS are characterized by multiple genetic abnormalities, other, earlier lesions must occur. Previous work by ourselves and others has demonstrated that some high risk proliferative breast lesions (atypical hyperplasia, or AH), which are considered to be "benign" are, in fact, monoclonal cell populations. This implies that these histologically "benign" lesions may actually represent early steps in the process of neoplastic transformation. However, no information is available regarding either the time at which clonal genetic abnormalities first appear, or the genetic relationship between monoclonal "benign" lesions and any breast cancers developing in the same subject.

We proposed to use a novel and powerful system we had developed to investigate these issues, which could lead to a greater understanding of the earliest events in breast carcinogenesis. The specific goals of this research study are 1) to identify whether genetic abnormalities are detectable in the earliest postulated stage of breast tumorigenesis, while breast tissue remains not only histologically benign, but even normal; and 2) to explore the clonal relationships between these genetically aberrant but microscopically normal lesions, "benign" but high risk, proliferative lesions, and breast cancers.

Body:

As outlined in the Statement of Work, during the first year of the award work was performed on Tasks 1 (selection of microsatellite primers), 2 (identification of specimens), 3 (section acquisition), 4 (microdissection and DNA extraction), 5 (PCR), 6 (analysis of data) and 7 (statistical analysis).

Methods:

Microsatellites (Task 1): 9 microsatellite markers at 9 genomic loci have been combined into several robust multiplex combinations. The nine microsatellite sequences examined were: MYCL1 (1p), D1S549 (1q), D2S123 (2p), D7S486 (7q), THO1 (11p), TP53 (17p), D17S579 (17q), D18S34 (18q), AR (Xq). Primers were purchased from Research Genetics (Huntsville, Alabama), or synthesized commercially. Although this task was supposed to be completed by this time, we will be continuing to optimize the primer selection.

Selection of samples (Task 2). All samples were reviewed by a single breast pathologist to identify histologically normal tissue. In total, ninety-five samples of histologically normal single ducts or TDLUs were obtained from 20 subjects; an average of 5 ducts or TDLUs were examined per subject. In addition, lymph node and stromal tissues were examined when available. Six cases of reduction mammoplasties were identified at random from the pathology department archives; candidates are screened prior to surgery to eliminate those in whom there is a personal or familial history of breast cancer. Nine cases of AH had been identified previously(1). Five cases of breast cancers diagnosed in pre- or perimenopausal women were selected at random from the pathology department archive. Breast cancer cases were selected in an attempt to match the uniformly young age of the reduction mammoplasty subjects, and the relatively young age of those with AH. Most specimens dated from 1994 or 1995; a few were older. Initial diagnostic or therapeutic specimens were used, therefore, no subjects had received prior chemotherapy or radiation. Tissue from both right and left sides was identified in all reduction mammoplasty and in breast cancer subject #34, who had bilateral disease. Except in this case, no subject with breast cancer was aware of a positive family history. Tissue from only the affected side was available in the remaining cases. Although Task 2 was supposed to be completed by the end of this year, we will continue to obtain additional specimens.

Preparation of DNA (Tasks 3 and 4): Histologically normal single ducts or TDLUs, stromal tissue and lymph nodes, were identified by hematoxalyn and eosin staining of the top and bottom of seven consecutive sections cut from a tissue block; the five intervening unstained sections were then microdissected as previously described(1). DNA was extracted using standard techniques(2, 3). To make sure abnormalities were not artifacts due to small amounts of template DNA(4), we performed serial dilution experiments. Using normal lymphocyte DNA, we determined the concentration at which the pattern of the products amplified by the AR primers was no longer reproducible. In each case, the amplified products were identical in size and intensity when 100pg or more of template DNA was used, but with 10pg the results were inconsistent (data not

shown). To approximate the minimum DNA concentration in each reaction using DNA from ducts or TDLUs, we determined that the number of cells microdissected from a normal duct varied between 250-1000; a TDLU contains far more cells. Assuming 6.5 pg DNA per cell and 50% loss of DNA during extraction, and given that 1/10 of the final volume of DNA solution was used per reaction, we estimate that a minimum of between 80-325 pg of template DNA was available per reaction.

PCR and data analysis (Tasks 5 and 6): We performed multiplex PCRs as described elsewhere(1) . Briefly, 1/10 of the DNA solution served as a template in a 50 ul reaction volume. After 40 cycles of amplification incorporating [α^{32}P]dCTP, with annealling temperatures of 55^{o}, 58^{o} or 60^{o} C, one-fifth of the amplified products were electrophoresed through 7% denaturing gels. Microsatellite changes were scored by visual inspection as instability (when a novel-sized amplified product was present), or as allelic imbalance suggestive of LOH when unequivocal loss of intensity of one allele was seen at heterozygous loci. In order to be scored as abnormal, demonstration of instability or LOH needed to be reproduced at least twice with identical results. Because of the limited quantities of DNA available, unequal amplification in early PCR cycles could lead to inaccurate relative allele intensities, therefore ratios of relative allele intensities at heterozygous loci based on densitometry were not calculated, and relative allele imbalance was scored as no loss. After scoring, the total number of abnormal samples and of abnormal alleles, and the nature of the abnormalities, were determined.

Statistical analysis (Task 7): We compared the three groups on the following measures: 1) the proportion of subjects with at least one abnormality, 2) the mean percent of abnormal loci, and 3) the mean percent of abnormal ducts. The data were examined for all subjects, and, separately, for women under age 50, by analysis of variance (weighting by the number of ducts or TDLUs).

Results:

Histologically normal breast ducts and TDLUs (samples) were microdissected carefully (see Figure 1). We detected genetically abnormal clones of cells in these normal-appearing samples in 10/20 (50%) subjects studied; 21/95 (22%) ducts or TDLUs were involved. Table 1 presents the data. Alterations were present in subjects from all three groups, i.e., in 2/6 (33%) women without apparent increased risk of breast cancer (reduction mammoplasty), in 4/9 (44%) women with an increased risk of breast cancer (AH), and in 4/5 (80%) women who already had developed the disease. More than one independent abnormal clone was detected in 1/6 (17%) subjects who had under gone reduction mammoplasty (#32), in 1/9 (11%) subjects with AH (#19) and in 4/5 (80%) subjects (#34, #35, #36, #37) with breast cancer.

Samples characterized by microsatellite changes were histologically indistinguishable from those without (see Figure 2); and no preference for abnormalities in single ducts compared with TDLUs was seen. Somewhat more microsatellite instability (22 examples) than LOH (13 examples) was evident, but this may have been skewed by one subject (#12) with 9 instances of instability and only 1 of LOH. In addition, because relative allele imbalance was scored as no loss (due to the small quantity of template

DNA: see Methods), it is possible that we underestimated the number of cases characterized by LOH. However, since each duct or TDLU likely represents the progeny of a single ductal stem cell(5), allelic alterations should usually affect at least a substantial fraction of the cells comprising the sample, and therefore should, generally, be detectable.

From 6 subjects undergoing reduction mammoplasties, who had no increased risk of breast cancer, 32 histologically normal samples were examined. Five ducts, from 2 individuals, contained clonal genetic changes. Subject #24 had a single abnormality identified in 1 of 5 samples (data not shown). In subject #32 (see Figure 3*a*), 4 of 10 samples, all from the right breast, had evidence of 5 microsatellite abnormalities, 4 of which involved a single microsatellite locus. In this subject, more than one abnormal clone was present. To determine with certainty this subject's germline configuration at these loci, three geographically distinct samples of stromal tissue were microdissected from the same blocks. All three demonstrate a single pattern, identical to that seen in the majority of the ducts (see Figure 3*a*).

From 9 subjects diagnosed with high-risk AH lesions (some of which have been shown to contain clonal abnormalities(1)), a total of 26 histologically normal samples were examined. Because the diagnosis of AH is usually made from a biopsy, the amount of tissue available for investigation is much smaller than from reduction mammoplasty or cancer specimens, and it is always unilateral. Despite the smaller number of samples examined per subject in this group than in either of the others, we identified evidence of microsatellite alterations in 5 of 26 histologically normal breast samples from 4/9 subjects in this high risk group (#12, #19, #20, #21). Subject #12 demonstrated 10 microsatellite alterations, all present in 1 of 6 samples (see Figure 3*b*). (This subject had colon cancer diagnosed at age 60, 3 years prior to breast biopsy, and 9 years later remains free of disease. It is possible that she represents a case of HNPCC). Subject #19 demonstrated microsatellite instability in each of the 2 samples examined. A different locus was altered in each sample, thus this subject had more than 1 abnormal clone. In subjects #20 and #21, 1 of 3 samples were abnormal, each with evidence of LOH (data not shown). Interestingly, the AH lesions from these 4 subjects were not found to have allelic alterations(1). Similar dissonance between genetic abnormalities in AH and in simple hyperplastic lesions from the same subject has been reported recently(6).

The third group studied consisted of 5 subjects with breast cancer, from whom a total of 37 histologically normal ducts and TDLUs were microdissected. Seemingly normal tissues from 4 of 5 subjects contained genetic abnormalities. In all 4 subjects multiple abnormal clones were found. Three of 7 samples from subject #34 contained genetic alterations at 3 different loci (see Figure 3*c*). (Samples from both left and right breast were abnormal). As a control to confirm the subject's germline configuration at the apparently altered loci, DNAs from 3 lymph nodes pathologically free of tumor were examined. Their microsatellite patterns were the same as the predominant pattern seen in the ducts or TDLUs (see Figure 3*c*). Two of 11 samples from subject #35 contained 4 genetic alterations involving 2 microsatellite loci (see Figure 3*d*). As a control, lymphoid tissue from 3 separate nodes was examined: no microsatellite alterations were detected. Three of 8 samples from subject #36 demonstrated 3 genetic alterations at three separate

loci (see Figure 3*e*). Finally, 3 of 6 samples from subject #37 revealled 4 abnormalities at 3 microsatellite loci (data not shown). The remaining subject (#38), with 5 samples examined, demonstrated no abnormalities.

Overall, a total of 35 clonal alterations were detected among 1348 alleles examined, yielding a mutation rate of 2.6%. There were suggestions that as the risk of breast cancer increased so did the number of alterations, particularly for women less than 50. (For example, the percent of all subjects with any abnormality increased from 33.3% to 44.4% to 80.0% across the three groups, and the mean percent of abnormal alleles rose from 1.2% in women with reduction mammoplasties, to 3.9% in women with AH, to 3.0% in women with breast cancer. Similarly, the mean percents of abnormal ducts increased with risk of breast cancer, from 15.6% in the reduction mammoplasty group, to 19.2% in the AH group and 29.7% in the breast cancer group). However, perhaps due to the relatively small sample size of this pilot study, these observations did not achieve statistical significance. When women under age 50 were examined, the trends were more pronounced and reached significance when comparing mean percent of abnormalities betwen the subjects with reduction mammoplasty (1.2%) vs those with breast cancer (3.4%) (P=.049) (see Table 2).

Certain microsatellite loci were altered much more commonly than others. Four of the 9 loci examined accounted for 28/35 (80%) abnormalities. In contrast, the remaining 5 loci accounted for only one-fourth as many: 7/35 (20%) abnormalities. When LOH alone was considered, the results were similarly skewed: 11/13 examples of LOH were at these 4 loci, whereas only 2/13 examples of LOH were at the other 5 loci. The 4 frequently altered loci are all situated near sites of known or putative tumor suppressor genes postulated to be relevant to breast tumorigenesis: 7q31(7), 11p15(8, 9), 17p13(10), and 17q21(11, 12). In contrast, the 5 less frequently altered loci are situated at genes or sites less commonly associated with breast cancer. When microsatellite instability was considered, we found that 12/22 abnormalities were seen at the 5 dinucleotide repeat markers, and 10/22 at the 4 tri and tetra nucleotide repeat markers. This pattern reflects that reported in breast cancer(13, 14, 15).

Discussion:

We have found multiple genetically abnormal clones existing in breast tissue while that tissue looks not only benign but histologically completely normal. These data indicate that genetic abnormalities which may be critical to breast tumorigenesis start accumulating far before pathological detection even of high risk lesions. The eventual fate of a given clone is unknown, as is the risk to a woman whose breast contains these occult lesions. It is noteworthy that we find mutant clones both in women at low and at high risk of developing breast cancer, because the majority of women who develop breast cancer have no identifiable risk factors. Clinical follow-up of the individuals in this study is not currently available.

It is possible that the mutant clones we detect are relevant to the earliest stages of breast tumorigenesis. Several observations support the speculation that these clones may indicate tissue at increased risk of cancer development. First, 4 of 5 women with breast

cancer had multiple abnormal clones, whereas only 2 of 15 women without the disease had more than a single abnormal clone. Second, 80% of the microsatellite abnormalities were at 4 loci believed to play a role in breast tumorigenesis. LOH, suggesting the presence of a tumor suppressor gene, has been found at 7q31(7) in a subset of breast cancers; the recently identified TSG101 putative tumor suppressor gene, located at 11p15(8, 9) is mutated in a fraction of human breast tumors; mutations of the P53 tumor suppressor gene, located at 17p13(10) are the most frequently identified genetic abnormalities in breast cancer; and the breast cancer susceptibility gene, BRCA1, and possibly other relevant tumor suppressor genes, are found at 17q21(11, 12). (Although *BRCA1* itself has not been found to be mutated in a significant percentage of sporadic human breast cancers(16), LOH in the region of the gene is detectable(11, 12). Thus, another mechanism of *BRCA1* inactivation or another gene may be playing a role).

Although these microsatellite loci were selected because of their chromosomal location, the overrepresentation of abnormalities at these sites indicates that they may predispose to the formation of genetically aberrant clonal populations. In contrast, mutation at arbitrary or more neutral sites may not confer a growth advantage and a detectable mutant clone may never arise. This would suggest that the genetic alterations we have detected are less likely to be random changes, and more likely to be relevant to the earliest stages of breast cancer development. Finally, it is noteworthy that the pattern of microsatellite instability seen in normal-appearing tissues is similar to the type of instability reported in breast cancers: i.e., overall, a low-level of microsatellite alterations, with a substantial proportion of changes seen in tri and tetra nucleotide repeat markers(13). Microsatellite instability has been detected in all stages of breast cancer, and consequently it has been postulated that this abnormality occurs early in the course of disease development(14, 15, 17, 18, 19, 20).

Our findings in subjects with breast cancer are consistent with the limited data available from studies in other tissues indicating that histologically normal tissue at increased risk for the development of cancer can contain specific clonal genetic abnormalities. For example, clones of p53 mutated ketatinocytes occur in sun-exposed normal-appearing human skin(21, 22), and in normal-appearing mucosa from patients with cancers of the upper aerodigestive tract(23). Microsatellite alterations have been seen in normal-appearing colonic mucosal epithelium of patients with chronic ulcerative colitis, who are at increased risk of developing colon cancer(24). Finally, LOH at chromosome 3p has been reported recently in breast cancers and in directly adjacent, but not more distant, histologically normal breast tissue(25). In contrast, cytogenetic studies examining macroscopically normal breast tissue surrounding breast cancers(26), and investigations of LOH and/or microsatellite instability in breast cancers have not reported abnormalities in normal-appearing tissues(13, 14, 15, 17, 18, 19, 20, 27, 28). This may be due, in part, to the relatively large amount of "normal" tissue generally used as a control, making detection of small abnormal clones difficult.

Even if only a rare abnormal clone expands by acquiring additional mutations, and the others represent "dead ends" (i.e., they would involute or remain stable), these data could help explain the genetic heterogeneity noted in many breast cancers. Multifocal

breast cancers can represent independent, not metastatic, malignancies(29), single breast malignancies can contain karyotypically unrelated clones(30, 31), and heterogeneous patterns of allelic loss have been reported in DCIS tumors(32). It is unclear how all the distinct clones could represent outgrowths from a single original population. The presence of multiple genetically distinct abnormal clones, several of which could progress independently and simultaneously, could represent one explanation.

Finally, it is notable that a trend may exist in the rate of abnormalities among the three groups of women studied, particularly when in women <50 years. Several factors may explain the absence of statistical significance associated with most of these associations. First, the baseline rate for somatic mutation in normal breast tissue may be relatively high, even in women at no identifiable increased risk of breast cancer. We obtained a rate of 1.2% in women with reduction mammoplasties, and an overall rate of 2.6%, both of which are higher than the baseline rate of somatic mutation estimated to be <0.5% in clones derived from normal T cells(33). The rate is suspected to be low in other normal tissue, but, as far as we are aware, has not been measured. In addition, the data from the reduction mammoplasty group could have been skewed by the presence of subject #32; this individual had quite a few clonal abnormalities. One could speculate about whether women with abnormalities similar to subject #32's could be at higher risk for the eventual development of breast cancer. Second, although the number of alleles examined was large, the number of individuals in each of the three groups may have been too small to detect small but significant differences. Examination of additional specimens from more subjects could clarify this important point. Third, the rate of abnormalities seen in each group may not be related to risk, but may reflect the effects of aging. The average age was lowest in the reduction mammoplasty group (31 yrs) with the lowest rate of abnormalities, intermediate in the breast cancer group (41 yrs) with the highest rate of abnormalities, and highest in the AH group (53 yrs), with an intermediate rate of abnormalities, but in this last group more abnormalities were seen in specimens from older women. Examination of specimens from a larger group of women of varied ages may answer this question. Finally, it is possible that the critical event is mutation of a postulated breast tissue-specific "gatekeeper" gene, without which progression of any nascent clone does not occur(34). Thus, the observed mutation rate might not be the key factor.

Recommendations:

The work on each task is progressing. Although it has taken longer to accumulate samples and to optimize the primer combinations than anticipated, other tasks have taken less time than anticipated, and are already yielding statistically significant results. These results have just been published (35). Therefore, we plan to continue work on the tasks as outlined in the original application.

Conclusions:

Breast cancer is believed to develop as multiple genetic abnormalities accumulate, each conferring some growth advantage, but the timing and nature of the earliest steps in this progression are not yet elucidated. Proliferative breast lesions, associated with an increased risk of breast cancer although considered benign, recently were shown to contain clonal genetic abnormalities. Therefore, we hypothesized that clonal genetic abnormalities might be detectable before any phenotypic abnormalities are evident: i.e., in histologically normal breast tissue. We examined DNA extracted from 95 normal-appearing breast ducts or terminal ductal-lobular units (TDLU)s from 20 individuals at varying degrees of risk (those undergoing reduction mammoplasties, those with atypical hyperplastic (AH) proliferative lesions, and those already diagnosed with breast cancer). Using 9 microsatellite markers, we sought evidence of genetic instability, or of allelic imbalance (most likely representing loss of heterozygosity (LOH)). We found genetically abnormal clones in 21/95 (22%) seemingly normal samples from 10/20 (50%) women, from all three risk groups. In women under age 50, trends towards increased rates of abnormalities were noted with increased cancer risk. The abnormalities identified were more likely to be at sites of known or postulated tumor suppressor genes, rather than at random or neutral loci. Our data indicate that genetic abnormalities potentially critical to breast tumorigenesis accumulate before pathological detection even of high risk lesions, and are detectable in tissue that is not only histologically benign, but completely normal.

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Appendices:

Tables 1 and 2

Figures 1, 2, and 3 with Figure Legends

Table 1: Microsatellite Alterations in Histologically Normal Breast Tissue Samples

Subject	Age	# micro-satellite loci	# altered samples/total samples	# altered alleles/ total alleles examined*	Type of alteration
Histologically normal ducts or TDLUs from reduction mammoplasty specimens:					
#24	34	9	1/5	1/90	1 instability
#25	25	9	0/4	0/72	
#26	24	9	0/3	0/54	
#29	42	9	0/3	0/48	
#31	23	7	0/7	0/92	
#32	36	8	4/10	5/138	1 instability, 4 LOH
Subtotal:6		*9*	*5/32 (15.6%)*	*6/494 (1.2%)*	*2 instability, 4 LOH*
Histologically normal ducts or TDLUs from AH biopsies:					
#10	58	9	0/3	0/50	
#12	63	8	1/6	10/92	9 instability, 1 LOH**
#13	37	8	0/2	0/32	
#17	51	8	0/3	0/48	
#18	31	8	0/2	0/32	
#19	41	8	2/2	2/32	2 instability
#20	74	7	1/3	2/40	2 LOH
#21	59	5	1/3	1/26	1 LOH
#23	63	8	0/2	0/32	
Subotal: 9		*8*	*5/26 (19.2%)*	*15/384 (3.9%)*	*11 instability, 4 LOH*
Histologically normal ducts or TDLUs from subjects with breast cancer:					
#34	39	7	3/7	3/84	1 instability, 2 LOH
#35	38	7	2/11	4/154	4 instability**
#36	39	7	3/8	3/98	2 insability, 1 LOH
#37	38	8	3/6	4/74	2 instability, 2 LOH**
#38	53	6	0/5	0/60	
Subtotal:5		*7*	*11/37 (29.7%)*	*14/470 (3.0%)*	*9 instability, 5 LOH*
Total: 20		**~8**	**21/95 (22.1%)**	**35/1348 (2.6%)**	**22 instability, 13 LOH**

*Occasional amplifications either were not successful or did not yield reproducible results; in these cases the alleles were not scored, hence the actual number of alleles examined is in some instances slightly smaller than the maximum possible number would be. The maximum possible number of evaluable alleles equals: (no. primers) x (no. ducts) x (2 alleles per locus)

**one or more biallelic changes noted

Table 2: Rates of genetic abnormalities in normal-appearing breast tissue from subjects < 50 yrs in three breast cancer risk groups

	Group			
	Mammo-plasty	AH	Breast cancer	
% subjects with abnormality:	33.3	33.3	100.0	$p = 0.107$
mean % abnormal alleles:	**1.2**	**2.1**	**3.4**	$\mathbf{p = 0.049}$
mean % abnormal ducts:	15.6	33.3.	34.4	$p = 0.384$

* mammoplasty vs. breast cancer

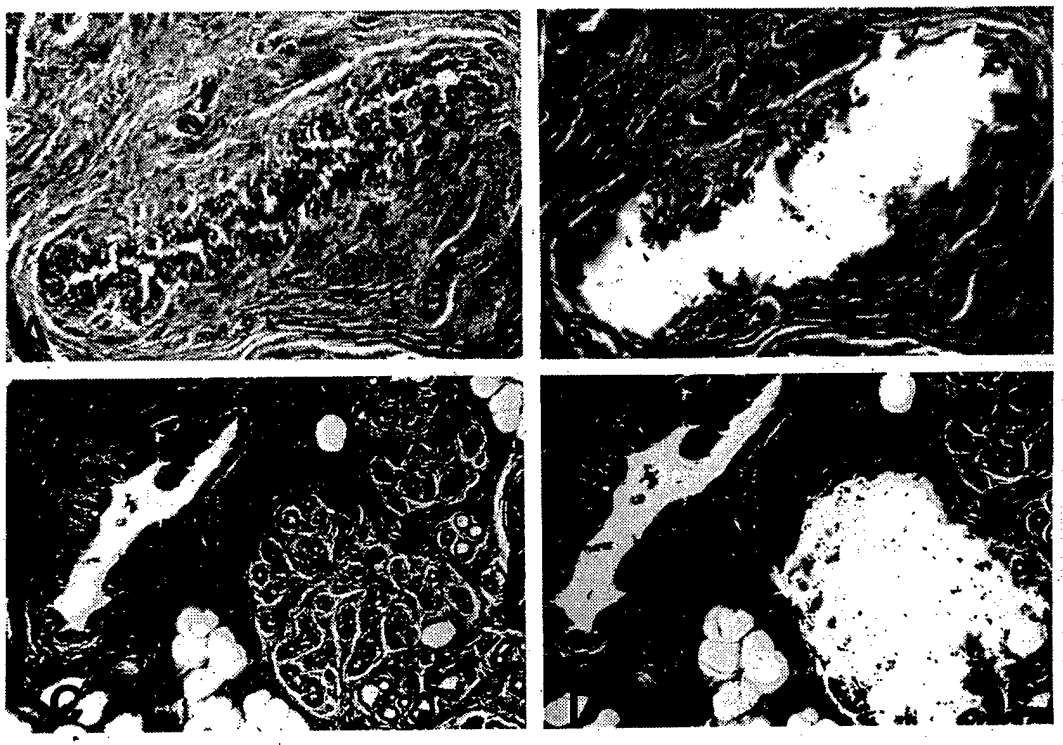

Figure 1

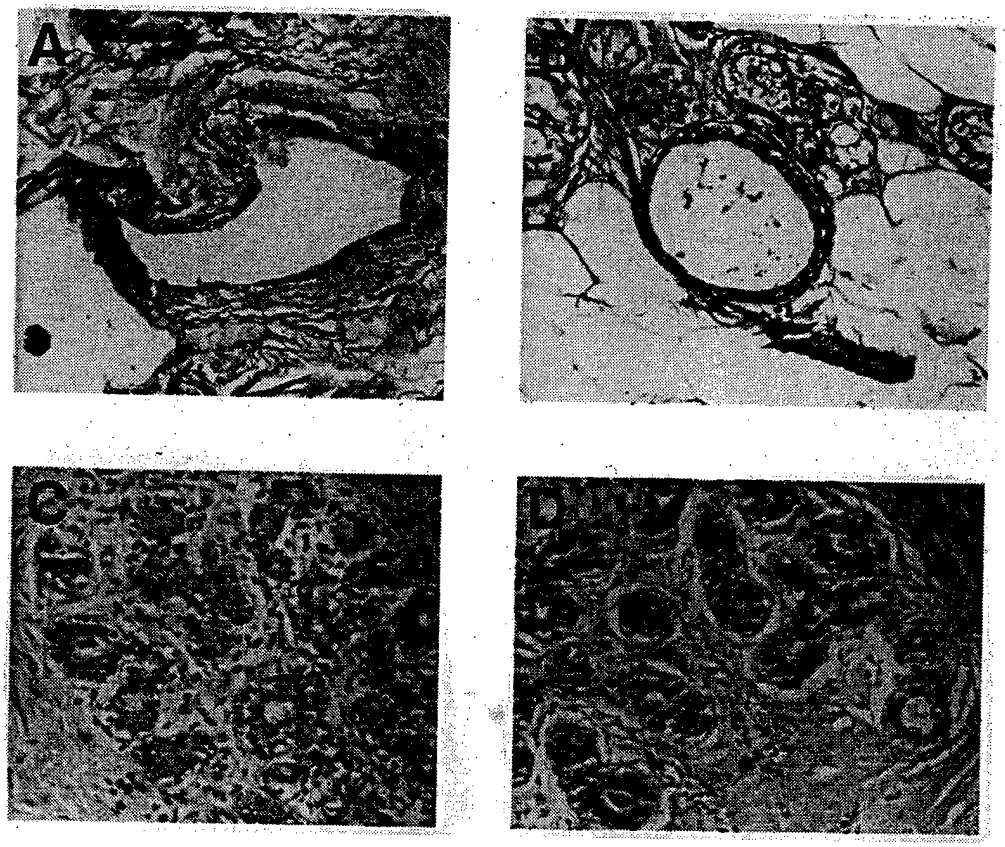

Figure 2

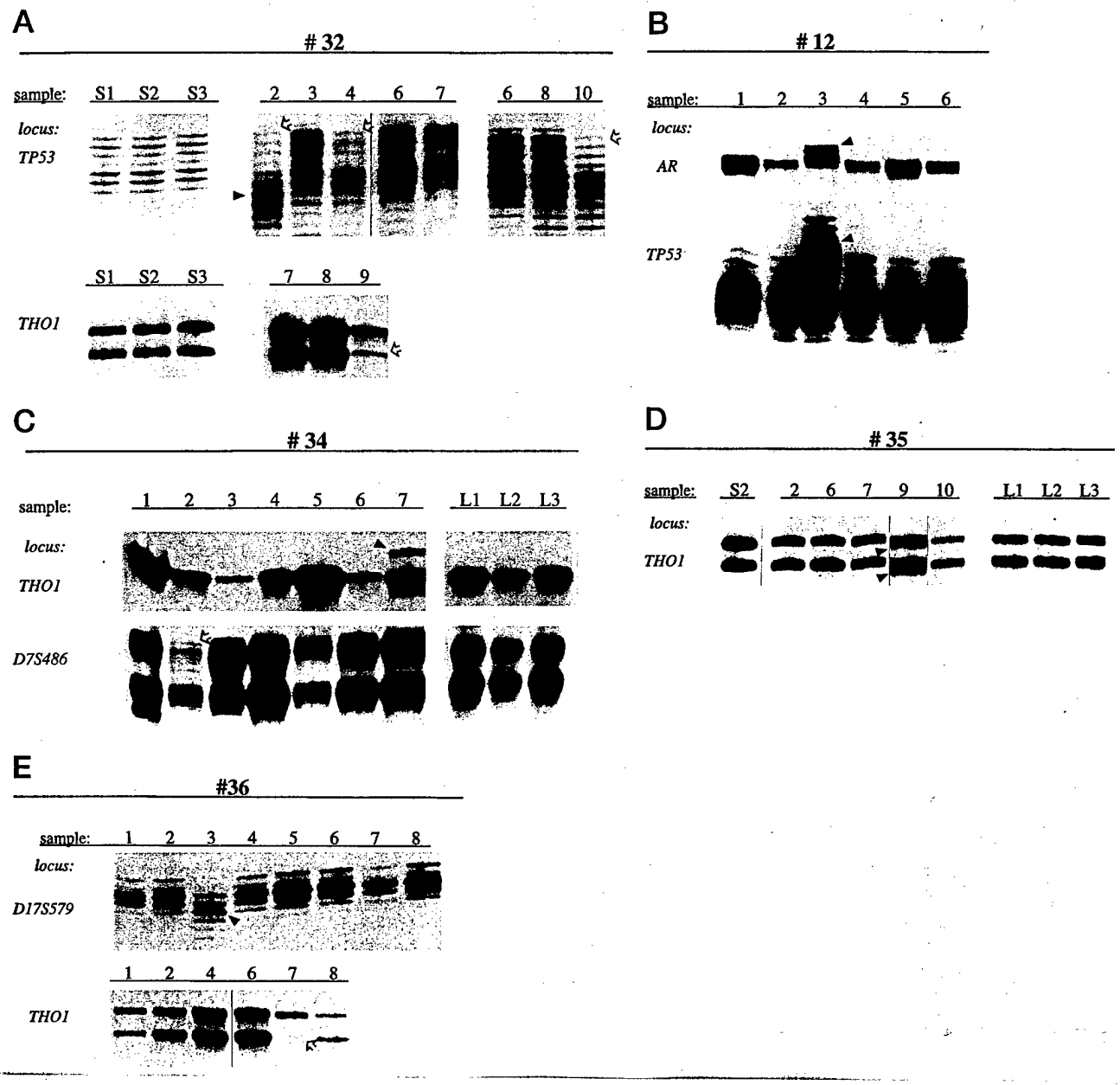
A
32
sample:
S1 S2 S3
2 3 4 6 7
6 8 10
locus:
TP53
S1 S2 S3
7 8 9
THO1
B
12
sample:
1 2 3 4 5 6
locus:
AR
TP53
C
34
sample:
1 2 3 4 5 6 7
L1 L2 L3
locus:
THO1
D7S486
D
35
sample:
S2 2 6 7 9 10
L1 L2 L3
locus:
THO1
E
#36
sample:
1 2 3 4 5 6 7 8
locus:
D17S579
1 2 4 6 7 8
THO1

Figure 3

Figure Legends

Figure 1. Examples of tissue before and after microdissection. Slides were stained with hematoxylin and eosin for the purpose of illustration. Subject #35's sample 6 (duct) is shown before (panel A) and after (panel B) microdissection. Magnification 200x. Subject #35's sample 2 (TDLU) is shown before (panel C) and after (panel D) microdissection. Magnification 100x. Amplification of the DNA extracted from these two samples is shown in Figure 3*d*.

Figure 2. Normal-appearing ducts and TDLUs: those with, or without, genetic alterations are histologically indistinguishable. From subject #12: panel A is sample 6, a genetically normal duct, and B is sample 3, which is genetically aberrant. From subject #32: panel C is sample 3, a genetically normal TDLU, and panel D is sample 2, which is genetically aberrant. Magnifications are 100X.

Figure 3. Representative examples of genetic abnormalities seen in histologically normal ducts or TDLUs from 5 subjects.

a demonstrates LOH and microsatellite alteration at two loci in subject #32, who had a reduction mammoplasty. LOH at the upper allele of the TP53 microsatellite is seen in samples 2, 4 and 10 (open arrows). In addition, in duct 2, the lower TP53 allele is smaller than normal (closed arrow). The bottom part of the panel demonstrates LOH at the lower THO1 allele in duct 9 (open arrow). S1, S2, S3 are three separate stromal specimens, each demonstrating the same unaltered pattern at both loci.

b demonstrates microsatellite alterations at two loci in subject #12, diagnosed with AH. A larger band, representing a novel allele, is seen at both the AR and TP53 microsatellites in sample 3 (closed arrows), but in none of the other 5 ducts.

c depicts LOH and microsatellite alteration at two loci in subject #34, with breast cancer. A larger band, representing a novel allele, is seen in sample 7 at the THO1 microsatellite (closed arrow) and LOH of the upper allele at the D7S486 microsatellite is seen in sample 2 (open arrow). L1, L2, L3 are three separate lymph nodes each demonstrating the same pattern of microsatellite bands, representing the germline pattern.

d depicts biallelic alterations (closed arrows) at the THO1 locus in sample 9 from subject #35. One sample of stromal tissue (S2) and three lymph nodes (L1, L2, L3) were also examined, and demonstrated no alterations. (Photographs of samples 2 and 6 before and after microdissection are shown in Figure 1). All ductal samples were amplified and electrophoresed simultaneously but different exposures have been placed adjacently.

e depicts LOH and microsatellite alteration at two loci in subject #36, with breast cancer. At the D17S579 microsatellite, a shortened allele replacing the upper allele is seen in duct 3 (closed arrow). At the THO1 microsatellite, LOH of the lower allelle is seen in duct 7.