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Genomic instability demonstrates similarity between DCIS and invasive carcinomas

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Abstract

Purpose—To assess telomere DNA content (TC) and the number of sites of allelic imbalance (AI) as a function of breast cancer progression.

Experimental design—TC and AI were determined in 54 histologically normal tissues, 10 atypical ductal hyperplasias (ADH), 122 in situ ductal carcinomas (DCIS) and 535 invasive carcinomas (Stage I–IIIA).

Results—TC was altered in ADH lesions (20%), DCIS specimens (53%) and invasive carcinomas (51%). The mean number of sites of AI was 0.26 in histologically normal group tissue, increased to 1.00 in ADH, 2.94 in DCIS, and 3.07 in invasive carcinomas. All groups were statistically different from the histologically normal group ($P < 0.001$ for each); however, there was no difference between DCIS and the invasive groups.

Conclusions—Genomic instability increases in ADH and plateaus in DCIS without further increase in the invasive carcinomas, supporting the notion that invasive carcinomas evolve from or in parallel with DCIS.

Keywords

Allelic imbalance; Breast cancer; Ductal carcinoma in situ; Genomic instability; Telomere DNA content

Introduction

It is widely accepted that genomic instability is a pre-requisite for the initiation and progression of virtually all cancers [1]. Accordingly, the progression of breast cancer can be characterized by the accumulation of genetic mutations in critical genes accompanied by histological progression from normal epithelium to atypical ductal hyperplasia (ADH), to ductal carcinoma in situ (DCIS) to the development of an invasive breast carcinoma [2,3].

A significant cause of genomic instability is telomere dysfunction [4–7]. Telomeres are nucleoprotein complexes that are comprised of 1,000–2,000 tandemly repeated copies of the hexanucleotide DNA sequence (TTAGGG) [8]. These repeat regions are associated with numerous telomere binding proteins, such as Telomeric Repeat-binding Factor 1 (TRF1), Telomeric Repeat-binding Factor 2 (TRF2) and Protection of Telomeres 1 (POT1), which play important roles in telomere maintenance [9,10]. Telomeres are located at and stabilize the ends of eukaryotic chromosomes, thus preventing degradation and recombination [11–13]. However, telomeres can be critically shortened, and thereby become dysfunctional, by several mechanisms, including incomplete replication of the lagging strand during DNA synthesis [14], loss or alterations of the telomere-binding proteins involved in telomere maintenance [15], and DNA damage induced by oxidative stress [16]. Telomere loss may be compensated by the reactivation of the enzyme telomerase, as seen in 85–90% of human cancers [17].

Abnormalities in telomere length are early and frequent events in the malignant transformation of numerous types of carcinomas [18,19]. In breast, telomere shortening has been observed in invasive carcinomas, in situ lesions, and histologically normal tissue proximal to breast tumors [20,21]. Additionally, our laboratory has recently demonstrated that telomere DNA content (TC), a proxy for telomere length, in breast tumor tissues is a prognostic marker for clinical outcome [22,23].

Genomic instability can also be manifested by the presence of allelic imbalance (AI), which is a deviation from the normal 1:1 ratio of maternal and paternal alleles. Numerous studies have shown that the presence of AI is characteristic of invasive breast carcinomas [24,25] and is also present at the in situ stage of the disease [26,27]. Additional studies have demonstrated that AI occurs within atypical breast hyperplasias [28,29], histologically normal tissue proximal to breast tumors [21,30–32], and, in some instances, breast tissue from women with benign breast disease [33]. AI has also been found in the stromal compartment of cancer-associated breast tissues [34].

Numerous groups have investigated AI in the development of breast cancer. Notably, Ellsworth et al. [35] developed a panel of microsatellite markers specific for loci commonly lost in breast cancer. This group examined the evolution of genomic instability by characterizing AI in tissue samples representing a continuum of breast cancer development and concluded that DCIS lesions contain AI levels characteristic of advanced invasive tumors [36].

To evaluate the link between telomere dysfunction and the generation of allelic imbalance in the progression of breast cancer, we assessed alterations in TC and the extent of AI in a continuum of breast tissues ranging from histologically normal tissue derived from reduction mammoplasty, to ADH, DCIS and invasive carcinomas ranging from Stage I to IIIA. Here, we demonstrate that genomic instability (i.e. changes in TC or AI that exceed values typically observed in normal tissues) increases along the continuum of breast disease; however, it plateaus in DCIS without further increase in the invasive carcinomas.

Materials and methods

Tissue samples

A total of 721 human breast tissues were used in this study. Fifty-four normal, disease-free breast tissue samples from women undergoing reduction mammoplasty (mean age = 35.6 years; range: 17–68) were obtained from the National Cancer Institute Cooperative Human Tissue Network (Nashville, TN). Ten atypical ductal hyperplasia lesions (mean age = 56.3 years; range: 41–70) were obtained from the Department of Pathology at University of New Mexico Hospital (UNMH). Two independent cohorts of breast tumors were analyzed. The first cohort (test set) was obtained through the New Mexico Tumor Registry (NMTR) and Department of Pathology at UNMH and consisted of 163 specimens including DCIS ($N = 27$), and Stage I ($N = 104$) and IIA ($N = 32$) invasive breast carcinomas (mean age = 47.5 years; range: 25–77). The second cohort (validation set) was obtained through the Health, Eating, Activity and Lifestyle (HEAL) Study, an ongoing population-based, multi-center prospective cohort study [37], and consisted of 494 cases including DCIS ($N = 95$), and Stage I ($N = 244$), IIA ($N = 112$), Stage IIB ($N = 39$) and IIIA ($N = 4$) invasive breast carcinomas (mean age = 59.3 years; range: 29–89). Clinical data for the two tumor cohorts are shown in Table 1. Experiments were performed in accordance with all federal guidelines as approved by the University of New Mexico Health Science Center Human Research Review Committee.

Histological review

All tissue sections were examined microscopically to confirm diagnosis. Tissue sections were not microdissected, but typically contained from 75 to 100% tumor cells. A single pathologist reviewed the histological slides for the 10 ADH lesions and cohort two (validation set); whereas, the reduction mammoplasty specimens and cohort one (test set) were reviewed by numerous pathologists. The criteria used for the ADH specimens were based on morphological characteristics of a proliferative lesion that fulfills some but not all the criteria for DCIS.

DNA isolation and quantification

DNA was isolated from fresh, frozen or formalin-fixed, paraffin-embedded (FFPE) tissue samples using the DNeasy® silica-based spin column extraction kit (Qiagen; Valencia, CA) and the manufacturer's suggested animal tissue protocol. FFPE samples were treated with xylene and washed with ethanol prior to DNA extraction. DNA concentrations were measured using the Picogreen® dsDNA quantitation assay (Molecular Probes, Eugene, OR) using a λ phage DNA as the standard as directed by the manufacturer's protocol.

Telomere DNA content (TC) assay

TC was measured in known DNA masses, typically 5–10 ng, by slot blot titration assay, as previously described [21–23]. TC is expressed as a percentage of the TC in a placental DNA standard measured in parallel, which is defined as 100%. Each measurement was repeated independently three times and the coefficient of variation for each sample was $\leq 10\%$. The content of telomere DNA sequences can easily be measured in genomic DNA obtained from fresh, frozen and paraffin-embedded tissues [22,38]. We have previously shown that TC is (i)

directly proportional to telomere length determined by Southern blotting, (ii) not affected by TTAGGG sequences outside the telomere, and (iii) not affected by DNA fragmentation less than 1 KB in length [22,38].

Determination of allelic imbalance

The extent of AI was determined using a straight-forward, economical, and high-throughput method recently developed by our laboratory [39]. This method evaluates AI in a panel of 16 randomly selected microsatellite markers (i.e. markers with no known relationship to breast cancer) thereby preventing measurement bias by selection of genes whose products are involved in tumorigenesis [39]. Briefly, DNA (~1 ng) was amplified using the AmpFISTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA) using the manufacturer's protocol. Each multiplex PCR reaction amplifies 16 short tandem repeat (STR) microsatellite loci from independent locations in the genome (Amelogenin, CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX and vWA). Each of the PCR primers is labeled with one of four fluorescent dyes (6-FAM, PET, VIC and NED), each with a unique emission profile, allowing the simultaneous resolution of 16 amplicons of similar size. PCR products were resolved by capillary gel electrophoresis and detected using an ABI Prism 377 DNA Sequencer (Perkin Elmer, Foster City, CA). The height of each fluorescence peak in the electropherograms was quantitated using the ABI Prism GeneScan and Genotype Analysis software (Applied Biosystems, Foster City, CA) and a ratio of the peak heights of each pair of heterozygous allelic amplicons was calculated. By convention, the allele with the greater fluorescence intensity was designated the numerator. Thus, the ratio was always ≥ 1.0 , with 1.0 representing the theoretical ratio for normal alleles. We previously defined an operational threshold of AI (i.e. ≥ 2 sites of AI) that could differentiate between a variety of normal and cancerous tissues independent of storage conditions (i.e. fresh, frozen or paraffin-embedded, formalin-fixed) [39]. Of the 118 normal specimens, only 1 (0.8%) specimen demonstrated ≥ 2 sites of AI. In contrast, of the 239 tumor specimens, 161 (67.4%) demonstrated ≥ 2 sites of AI.

Statistical methods

The mean number of sites of AI and TC distributions for histologically normal, ADH, DCIS and invasive carcinoma specimens were analyzed by non-parametric Rank Sums tests. Chi-square tests were used to determine differences for individual allelic frequencies between the DCIS and invasive groups. JMP® statistical package (SAS Institute, Cary, NC) was used for all analyses and P -values < 0.05 were considered to be significant.

Results

TC in histologically normal tissue and ADH lesions

TC was determined in 54 histologically normal breast tissues obtained from women who underwent reduction mammoplasty. TC was tightly regulated within these histologically normal breast tissues; 95% of these normal specimens fell within the range of 75–154% (Fig. 1), nearly identical to the 75–143% range previously reported in a diverse set of 70 specimens of normal tissue from multiple organ sites, including breast [22]. Next, TC was determined in a set of 10 ADH lesions. TC values in two specimens (20%) fell outside the 95% range found in the histologically normal specimens.

Telomere DNA content in a test cohort of breast tumors

TC next was determined in a cohort of 27 DCIS, 104 Stage I and 32 Stage IIA breast tumors. In contrast to the histologically normal group, there was a wide range of TC distribution in the tumor specimens within the test cohort (Fig. 1). Of the 27 DCIS cases, 10 (37%) fell outside

the normal range. Similarly, 44 of the 104 Stage I tumors (42%) and 14 of the 32 Stage IIA tumors (44%) fell outside the normal range. However, the DCIS specimens as a group had longer telomeres than the Stage I ($P = 0.0152$) and Stage IIA ($P = 0.0338$) tumors.

Telomere DNA content in a validation cohort of breast tumors

The results were validated in an independent population-based breast tumor cohort comprised of 494 specimens. TC was determined in 95 DCIS, 244 Stage I, 112 Stage IIA, 39 Stage IIB and 4 Stage IIIA breast tumors. Fifty-five of the 95 DCIS cases (58%), 127 of the 244 Stage I (52%), 65 of the 112 Stage IIA (58%), 20 of the 39 Stage IIB (51%) and 3 of the 4 Stage IIIA (75%) tumors fell outside of the normal range defined by the histologically normal breast tissues. Again, the DCIS group had longer telomeres than the Stage I ($P < 0.0001$), Stage IIA ($P = 0.0005$) and Stage IIB ($P = 0.0048$) tumors (Fig. 1). In both the test and validation cohorts, TC did not correlate with ethnicity, nodal status, or ER and PR status.

Extent of AI in histologically normal tissue and ADH lesions

To extend and confirm these findings, AI, another independent marker of genomic instability, was measured and compared in the same tissue cohorts. The mean number of sites of AI was 0.26 in the histologically normal and 1.00 in the ADH groups (Fig. 2). As compared to the histologically normal group, the ADH group showed a significant increase in the extent of AI ($P = 0.0002$), although the small number of ADH specimens must be noted.

Extent of AI in a test cohort of breast tumors

Next, the extent of AI was analyzed in the test cohort. The mean number of sites of AI was 2.63 in DCIS, 3.24 in Stage I tumors and 2.84 in Stage IIA tumors (Fig. 2). All groups were statistically different when compared to the histologically normal group ($P < 0.0001$ for each). As observed for TC, there was no difference in the extent of AI in the DCIS group compared to any of the invasive groups. Additionally, there was no difference between Stage I and Stage IIA tumors.

Extent of AI in a validation cohort of breast tumors

These findings were replicated in the validation cohort. The mean number of sites of AI was 3.03 in DCIS, 3.08 in Stage I, 2.98 in Stage IIA, 2.92 in Stage IIB and 3.50 in Stage IIIA (Fig. 2). All categories were statistically different from the histologically normal group ($P < 0.001$ for each). There was no statistically significant difference between the DCIS group and the groups of invasive carcinoma or between any of the invasive groups. Additionally, there was no statistical difference in the mean number of sites of AI between paired groups by stage between the test and validation cohorts of breast tumors. Next, we tested our previously operationally-defined threshold for AI (i.e. ≥ 2 sites of AI) in these tissue cohorts [39]. Using this threshold, 0 of the 54 (0%) histologically normal breast specimens contained ≥ 2 sites of AI (Table 2). In contrast, 131 of the 163 tumors in the test cohort (80.4%) and 402 of the 494 tumors in validation cohort (81.4%) contained ≥ 2 sites of AI (Table 2). AI did not correlate with ethnicity, nodal status, or ER and PR status in both the test and validation cohorts.

Allelic frequency in DCIS and invasive tumors

Since the mean number of sites of AI in specimens of DCIS was nearly identical to the invasive tumors in both study cohorts, we next determined whether there was a difference in the allelic frequencies at each locus as a function of stage of progression. Since the individual loci have no known involvement in the development of breast cancer, there should be no selection pressure and the frequency of AI at a particular locus should not differ as a function of progression. For this analysis, the DCIS and invasive tumors were combined from the two tumor cohorts. As shown in Table 3, there were no statistically significant differences in the

allelic frequencies of 14 of the 15 markers between the DCIS and invasive groups, except at the TH01 locus which showed an increase in AI in the DCIS samples compared to the invasive tumors ($P = 0.014$).

Discussion

The accumulation of genomic instability is characteristic of all carcinomas, including breast [1]. It has been proposed that breast cancer progression can be modeled as a sequence of events progressing from normal epithelium to ADH, to DCIS, to finally the development of an invasive breast carcinoma [2,3]. However, the genetic changes that underpin these histological changes still remain to be fully understood.

In this investigation we used TC and AI, two independent quantitative markers of genomic instability, to demonstrate that genomic instability increases as a function of the extent of breast disease (i.e. histologically normal tissue to ADH to DCIS). Alterations in TC and the extent of AI plateau in DCIS and do not increase further with increasing stage in invasive carcinomas. However, TC measurements show further telomere shortening between DCIS lesions and invasive carcinomas. The later finding is consistent with our previous studies demonstrating low TC compared to high TC confers an adjusted relative hazard of 4.43 (95% CI 1.4–13.6, $P = 0.009$) [22] in a cohort of 77 women. Additionally, in a population-based study of 530 women, low TC conferred an adjusted relative hazard of 2.88 (95% CI = 1.16–7.15; $P = 0.022$) [23].

Our TC findings are consistent with our previous reports that TC correlates with Stage in invasive carcinomas [22]. Here, we show that 95% of the histologically normal breast tissues analyzed in this study fall within a range of 75–154% of the placental DNA control, nearly identical to the range previously reported [21], demonstrating that TC is tightly regulated regardless of inherent tissue properties that may affect TC, such as organ site or patients' age. However, evidence of telomere dysregulation (i.e. attrition or elongation) was present in all the tumor cohorts. Speculatively, the finding of telomere elongation in tumors reflects the reactivation of telomerase, which is reactivated in 85–90% of tumors [40]. However, the extent of reactivation varies amongst tumors as demonstrated by Hines and colleagues who showed an approximate 800-fold difference in telomerase expression among a panel of 36 breast tumors [41]. Additionally, it has been postulated that early telomerase activation results in longer telomeres as compared to late activation, thus providing an opportunity for continued telomere shortening and accumulation of genomic instability.

Our observations confirm and extend the results of Ellsworth et al. [35] which demonstrated that levels of genomic instability are equivalent in DCIS lesions and advanced invasive tumors. However, that particular study utilized a panel of markers that were previously identified as important genes in the development of breast cancer. This confounds the ability to clearly interpret AI across these markers as genomic instability since these markers may be linked to oncogenes or tumor suppressor genes involved in the development of breast cancer. In contrast, the assay used in this study is based on AI at 16 random microsatellite regions that have no known involvement in the development of breast cancer, and thus reflect genomic instability independent of their linkage to genes involved with breast tumorigenesis. The differences in the extent of imbalance among the particular loci may reflect the proximity of the microsatellite region to the telomere ends. Chromosomal differences in telomere length may also contribute to the individual heterogeneity.

In conclusion, the level of genomic instability assessed by (i) dysregulation in TC (i.e. outside the 95% range found in normal breast tissue) and (ii) extent of AI assessed at 16 microsatellite loci located throughout the genome, increases along the continuum of breast disease from

histologically normal, to ADH lesions to DCIS and the level of genomic instability did not differ between DCIS and invasive carcinomas. In all, these findings suggest that DCIS lesions have the same extent of genomic instability (i.e. TC alterations and increased AI) as invasive carcinomas; thus supporting the notion that invasive carcinomas evolve from or in parallel with DCIS.

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References

1. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature* 1998;396:643–649. [PubMed: 9872311]
2. Simpson PT, Reis-Filho JS, Gale T, Lakhani SR. Molecular evolution of breast cancer. *J Pathol* 2005;205:248–254. [PubMed: 15641021]
3. Allred DC, Mohsin SK, Fuqua SA. Histological and biological evolution of human premalignant breast disease. *Endocr Relat Cancer* 2001;8:47–61. [PubMed: 11350726]
4. Lo AW, Sabatier L, Fouladi B, Pottier G, Ricoul M, Murnane JP. DNA amplification by breakage/fusion/bridge cycles initiated by spontaneous telomere loss in a human cancer cell line. *Neoplasia* 2002;4:531–538. [PubMed: 12407447]
5. O'Hagan RC, Chang S, Maser RS, Mohan R, Artandi SE, Chin L, et al. Telomere dysfunction provokes regional amplification and deletion in cancer genomes. *Cancer Cell* 2002;2:149–155. [PubMed: 12204535]
6. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, Harley CB, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J* 1992;11:1921–1929. [PubMed: 1582420]
7. Chin K, de Solorzano CO, Knowles D, Jones A, Chou W, Rodriguez EG, et al. In situ analyses of genome instability in breast cancer. *Nat Genet* 2004;36:984–988. [PubMed: 15300252]
8. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, et al. A highly conserved repetitive DNA sequence, (TTAGGG)_n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* 1988;85:6622–6626. [PubMed: 3413114]
9. de Lange T. Protection of mammalian telomeres. *Oncogene* 2002;21:532–540. [PubMed: 11850778]
10. Smogorzewska A, de Lange T. Regulation of telomerase by telomeric proteins. *Annu Rev Biochem* 2004;73:177–208. [PubMed: 15189140]
11. de Lange T, Shiue L, Myers RM, Cox DR, Naylor SL, Killery AM, et al. Structure and variability of human chromosome ends. *Mol Cell Biol* 1990;10:518–527. [PubMed: 2300052]
12. Saltman D, Morgan R, Cleary ML, de Lange T. Telomeric structure in cells with chromosome end associations. *Chromosoma* 1993;102:121–128. [PubMed: 8432193]
13. Hande MP, Samper E, Lansdorp P, Blasco MA. Telomere length dynamics and chromosomal instability in cells derived from telomerase null mice. *J Cell Biol* 1999;144:589–601. [PubMed: 10037783]
14. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol* 1973;41:181–190. [PubMed: 4754905]
15. Smogorzewska A, van Steensel B, Bianchi A, Oelmann S, Schaefer MR, Schnapp G, et al. Control of human telomere length by TRF1 and TRF2. *Mol Cell Biol* 2000;20:1659–1668. [PubMed: 10669743]
16. Bohr VA, Anson RM. DNA damage, mutation and fine structure DNA repair in aging. *Mutat Res* 1995;338:25–34. [PubMed: 7565878]

17. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;266:2011–2015. [PubMed: 7605428]
18. Meeker AK, Argani P. Telomere shortening occurs early during breast tumorigenesis: a cause of chromosome destabilization underlying malignant transformation? *J Mammary Gland Biol Neoplasia* 2004;9:285–296. [PubMed: 15557801]
19. Meeker AK, Hicks JL, Iacobuzio-Donahue CA, Montgomery EA, Westra WH, Chan TY, et al. Telomere length abnormalities occur early in the initiation of epithelial carcinogenesis. *Clin Cancer Res* 2004;10:3317–3326. [PubMed: 15161685]
20. Meeker AK, Hicks JL, Gabrielson E, Strauss WM, De Marzo AM, Argani P. Telomere shortening occurs in subsets of normal breast epithelium as well as in situ and invasive carcinoma. *Am J Pathol* 2004;164:925–935. [PubMed: 14982846]
21. Heaphy CM, Bisoffi M, Fordyce CA, Haaland CM, Hines WC, Joste NE, et al. Telomere DNA content and allelic imbalance demonstrate field cancerization in histologically normal tissue adjacent to breast tumors. *Int J Cancer* 2006;119:108–116. [PubMed: 16450377]
22. Fordyce CA, Heaphy CM, Bisoffi M, Wyaco JL, Joste NE, Mangalik A, et al. Telomere content correlates with stage and prognosis in breast cancer. *Breast Cancer Res Treat* 2006;99:193–202. [PubMed: 16752076]
23. Heaphy CM, Baumgartner KB, Bisoffi M, Baumgartner RN, Griffith JK. Telomere DNA content predicts breast cancer free survival interval. *Clin Cancer Res* 2007;13:7037–7043. [PubMed: 18056180]
24. Balmain A, Gray J, Ponder B. The genetics and genomics of cancer. *Nat Genet* 2003;33:238–244. [PubMed: 12610533]
25. Miyakis S, Spandidos DA. Allelic loss in breast cancer. *Cancer Detect Prev* 2002;26:426–434. [PubMed: 12507227]
26. Farabegoli F, Champeme MH, Bieche I, Santini D, Ceccarelli C, Derenzini M, et al. Genetic pathways in the evolution of breast ductal carcinoma in situ. *J Pathol* 2002;196:280–286. [PubMed: 11857490]
27. Ellsworth RE, Ellsworth DL, Love B, Patney HL, Hoffman LR, Kane J, et al. Correlation of levels and patterns of genomic instability with histological grading of DCIS. *Ann Surg Oncol* 2007;14:3070–3077. [PubMed: 17549568]
28. O'Connell P, Pekkel V, Fuqua SA, Osborne CK, Clark GM, Allred DC. Analysis of loss of heterozygosity in 399 premalignant breast lesions at 15 genetic loci. *J Natl Cancer Inst* 1998;90:697–703. [PubMed: 9586667]
29. Aubele MM, Cummings MC, Mattis AE, Zitzelsberger HF, Walch AK, Kremer M, et al. Accumulation of chromosomal imbalances from intraductal proliferative lesions to adjacent in situ and invasive ductal breast cancer. *Diagn Mol Pathol* 2000;9:14–19. [PubMed: 10718208]
30. Ellsworth DL, Ellsworth RE, Love B, Deyarmin B, Lubert SM, Mittal V, et al. Genomic patterns of allelic imbalance in disease free tissue adjacent to primary breast carcinomas. *Breast Cancer Res Treat* 2004;88:131–139. [PubMed: 15564796]
31. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* 1996;274:2057–2059. [PubMed: 8953032]
32. Forsti A, Louhelainen J, Soderberg M, Wijkstrom H, Hemminki K. Loss of heterozygosity in tumour-adjacent normal tissue of breast and bladder cancer. *Eur J Cancer* 2001;37:1372–1380. [PubMed: 11435067]
33. Euhus DM, Cler L, Shivapurkar N, Milchgrub S, Peters GN, Leitch AM, et al. Loss of heterozygosity in benign breast epithelium in relation to breast cancer risk. *J Natl Cancer Inst* 2002;94:858–860. [PubMed: 12048275]
34. Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res* 2000;60:2562–2566. [PubMed: 10811140]
35. Ellsworth RE, Ellsworth DL, Lubert SM, Hooke J, Somiari RI, Shriver CD. High-throughput loss of heterozygosity mapping in 26 commonly deleted regions in breast cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:915–919. [PubMed: 14504204]

36. Ellsworth RE, Ellsworth DL, Deyarmin B, Hoffman LR, Love B, Hooke JA, et al. Timing of critical genetic changes in human breast disease. *Ann Surg Oncol* 2005;12:1054–1060. [PubMed: 16228814]
37. Baumgartner KB, Hunt WC, Baumgartner RN, Crumley DD, Gilliland FD, McTiernan A, et al. Association of body composition and weight history with breast cancer prognostic markers: divergent pattern for Hispanic and non-Hispanic White women. *AmJ Epidemiol* 2004;160:1087–1097. [PubMed: 15561988]
38. Bryant JE, Hutchings KG, Moyzis RK, Griffith JK. Measurement of telomeric DNA content in human tissues. *Bio-techniques* 1997;23:476–478.480, 482
39. Heaphy CM, Hines WC, Butler KS, Haaland CM, Heywood G, Fischer EG, et al. Assessment of the frequency of allelic imbalance in human tissue using a multiplex polymerase chain reaction system. *J Mol Diagn* 2007;9:266–271. [PubMed: 17384220]
40. Hiyama E, Hiyama K. Clinical utility of telomerase in cancer. *Oncogene* 2002;21:643–649. [PubMed: 11850791]
41. Hines WC, Fajardo AM, Joste NE, Bisoffi M, Griffith JK. Quantitative and spatial measurements of telomerase reverse transcriptase expression within normal and malignant human breast tissues. *Mol Cancer Res* 2005;3:503–509. [PubMed: 16179497]

Fig. 1.

TC distributions in histologically normal breast tissues derived from reduction mammoplasties, ADH lesions, and two independent cohorts of DCIS lesions and invasive breast carcinomas (Stage I–III). The numbers of tissues analyzed are indicated (*N*). TC is expressed as a ratio of TC in a placental DNA control. The boxes represent group medians (line across middle) and quartiles (25th and 75th percentiles) at its ends. Lines above and below boxes indicate 10th and 90th percentiles, respectively. The gray shaded area indicates 95% of TC measurements in the histologically normal group (75–154%). *Note:* Although the individual data points are horizontally shifted, some are still overlapping and therefore may not be visible

Fig. 2.

Extent of allelic imbalance in histologically normal breast tissues derived from reduction mammoplasties, ADH lesions, and two independent cohorts of DCIS lesions and invasive breast carcinomas (Stage I–III). The numbers of tissues analyzed are indicated (*N*). The bars indicate the mean number of unbalanced loci (shown for each group) $\pm$ standard errors.

Abbreviations: ADH, atypical ductal hyperplasia; DCIS, ductal carcinoma in situ

Table 1

Clinical and pathological characteristics of the analyzed breast tumors

Characteristic	Cohort #1 (N = 163)		Cohort #2 (N = 494)	
	N	%	N	%
<i>Ethnicity</i>				
NHW	106	65	380	77
Hispanic	27	17	114	23
Unknown	30	18	0	0
<i>TNM stage</i>				
0 (in situ)	27	16	95	19
I	104	64	244	49
IIA	32	20	112	23
IIB	0	0	39	23
IIIA	0	0	4	1
<i>Node status</i>				
Negative	163	100	261	53
Positive	0	0	107	22
Unknown	0	0	126	25
<i>ER status</i>				
Positive	80	49	418	85
Negative	46	28	72	14
Unknown	37	23	4	1
<i>PR status</i>				
Positive	72	44	340	69
Negative	53	33	151	30
Unknown	38	23	3	1
<i>Age</i>				
Mean	47.5		59.3	
Rang	25–77		29–89	

TNM stage was assigned using the 2002 AJCC revised criteria. Ethnicity was self-reported. *N*, Number of specimens; ER, estrogen receptor; PR, progesterone receptor; NHW, non-Hispanic White. For additional details, see Materials and Methods Section

Table 2

Extent of AI in cohorts of breast tissue

Group	N	# Samples ≥ 2 AI	%
Histologically normal	54	0	0.0
ADH	10	1	10.0
Cohort #1			
DCIS	27	20	74.1
Stage I	104	92	88.5
Stage IIA	32	19	59.4
Combined	163	131	80.4
Cohort #2			
DCIS	95	81	85.3
Stage I	244	196	80.3
Stage IIA	112	89	79.5
Stage IIB	39	32	82.1
Stage IIIA	4	4	100.0
Combined	494	402	81.4

Table 3

Frequency of AI at distinct loci in DCIS and invasive tumors

Loci designation	DCIS (<i>N</i> = 122)	Invasive (<i>N</i> = 535)	<i>P</i> value
D8S1179	0.19	0.26	0.108
D21S11	0.25	0.31	0.207
D7S820	0.03	0.08	0.066
CSF1PO	0.04	0.06	0.377
D3S1358	0.30	0.21	0.052
TH01	0.39	0.28	0.014
D13S317	0.18	0.24	0.175
D16S539	0.17	0.24	0.086
D2S1338	0.17	0.13	0.284
D19S433	0.14	0.18	0.310
vWA	0.35	0.32	0.538
TPOX	0.25	0.23	0.572
D18S51	0.10	0.10	1.000
D5S818	0.22	0.27	0.277
FGA	0.12	0.15	0.512