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Advances in Brief

Allelic Loss and the Progression of Breast Cancer[1]

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Abstract

To study genetic changes and the evolution of breast cancer, we assayed for loss of heterozygosity (LOH) in 12 sets of synchronous carcinoma *in situ* (CIS) and invasive cancer, compared to normal control DNA. Microsatellite markers were used, which map to each nonacrocentric autosomal arm. Eight tumor sets demonstrated LOH of the same allele in both concurrent invasive cancer and ductal CIS, for a total of 18 chromosomal loci. Three of nine tumor sets showed LOH on 11p. In two of these sets, LOH was seen on 11p only in the invasive tumor, not the corresponding CIS. One of these tumors also exhibited allelic loss in the invasive tumor for 4 loci, all of which were retained in the noninvasive tumor. For two tumor sets, LOH was mirrored in matched ductal CIS, invasive tumor, and lymph node metastasis. The maintenance of LOH for certain loci throughout the stages of breast cancer suggests clonality of the cancer cells.

Introduction

Human solid tumors are believed to arise due to a multistep process involving the activation of oncogenes and the inactivation of tumor suppressor genes. This cascade of genetic events releases the cell from normal regulatory controls, and allows the formation of the malignant phenotype, followed by the development of invasion and metastasis. In certain cancers, a chronology for these events has been determined, such as in the transition from benign polyp to invasive cancer of the colorectum (1). Little is known about the events that are involved in the transition of CIS[3] of the breast to invasive cancer. DCIS is a noninvasive carcinoma and is a precursor to invasion in some cases, although it is not an obligate precursor (2). The subtypes of DCIS (such as comedo, cribriform, solid, papillary, and micropapillary) differ in biological behavior. We have studied the allelic loss of chromosomal loci in DCIS (3–5) and have established that the chromosomal arms that show the most frequent allelic losses in DCIS are 8p, 13q, 16q, 17p, and 17q. Other authors have reported LOH in DCIS on chromosomes 11q, 2p, and 4q (6, 7). To determine which chromosomal loci are involved in breast cancer progression, we continued our studies on examples of synchronous CIS and adjacent invasive breast cancer and, when available, lymph node metastases.

Materials and Methods

Twelve samples of tumor and control were obtained; 10 from the archives of the Department of Pathology, St. Louis University, 1 from Jewish Hospital (St. Louis, MO) and 1 from Barnes Hospital (St. Louis, MO). Paraffin-embedded, formalin/alcohol-fixed material was archived between 1988 and 1993. In two of these cases, lymph nodes involved with metastatic tumor were also obtained for LOH assay. The pathologists (N. J. P. and J. H. R.) determined the subtype of the DCIS component and its nuclear grade (high, intermediate, or low). A microdissection technique was used to separate invasive tumor from CIS and from adjacent normal stroma (3). Uninvolved lymph node DNA from the same patient was used as normal control. DNA was extracted as described previously (3). LOH was assayed using PCR of microsatellite markers. The markers used and their PCR conditions have been described (5). PCR products were separated on 3M urea denaturing polyacrylamide sequencing gels and were dried before exposure to Kodak XAR film. LOH was determined by a combination of visual inspection and scanning densitometry of the autoradiographs. The technique used for scanning densitometry is fully described in Ref. 5. A 3-fold difference in the relative allele intensity ratios between tumor DNA and normal DNA in an informative tumor normal pair was scored as LOH (allele 1/allele 2 in tumor compared to allele 1/allele 2 in normal).

Results

Of the 12 samples of CIS available for study, 7 were comedo, high nuclear grade DCIS (tumors 46, 47, 49, 50, 57, 58, and 69). Tumor 48 was of the cribriform subtype of DCIS, intermediate nuclear grade; tumor 55: cribriform, low nuclear grade; tumor 56: micropapillary, low nuclear grade; tumor 70 terminal duct CIS (a variety of CIS that has histological features that resemble both lobular and DCIS), high nuclear grade; and tumor 72: mixed variety of DCIS, intermediate nuclear grade. In all cases the invasive component was of the invasive ductal variety.

We studied chromosomal deletions by assaying for LOH using 48 microsatellite markers that map to 39 nonacrocentric autosomal arms. The data obtained are summarized graphically in Fig. 1. For a total of 18 chromosomal loci, LOH could be demonstrated in both the CIS and invasive component of the tumor. LOH was observed in both DCIS and invasive tumor for loci on 1p (1 of 8 informative tumor sets), 1q (1 of 7), 7q (2 of 4), 8p (2 of 5), 11p (1 of 9), 13q (2 of 5), 16q (3 of 7), 17p (2 of 5), 17q (2 of 8), 18p (1 of 4), 18q (2 of 8), and 22q (1 of 6). In tumor 47, LOH at loci on 7q and 17p was seen in DCIS and LNM; the invasive tumor could not be assayed due to insufficient sample. Similarly, for tumor 48, LOH was seen at locus *CD3D* (11q) for both DCIS and LNM. At several loci the LOH pattern in DCIS and invasive component was also mirrored in the LNM [*e.g.*, loci *D1S165*, *APOA2*, and *NM23* (tumor 47) and loci *D16S266* and *D16S402* (tumor 48)].

The DNA from two cases (cases 46 and 49) showed LOH on 11p in the invasive tumor but not the DCIS component. Fig. 2 shows examples of LOH for these tumors. Examination of Fig. 2, *A–C* shows virtually complete loss of an allele in both the DCIS and the invasive component of tumor 46 for loci on 13q, 17p, and 22q. This indicates that the sample was carefully dissected and that stromal contamination is not obscuring LOH in the DCIS component. Fig. 2 *D* shows the pattern of allelic loss for the same tumor sample using a marker on 11p. Two alleles are seen in the DCIS; however, there is a great reduction of the signal from one allele in the invasive tumor. These data were confirmed by densitometry. The fold difference in integrated allele ratios between DCIS and normal for the marker *D11S861* was 1.42 for tumor 46 and 1.74 for tumor 49. These figures do not

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[3] The abbreviations used are: CIS, carcinoma *in situ*; DCIS, ductal CIS; LOH, loss of heterozygosity; LNM, lymph node metastases.

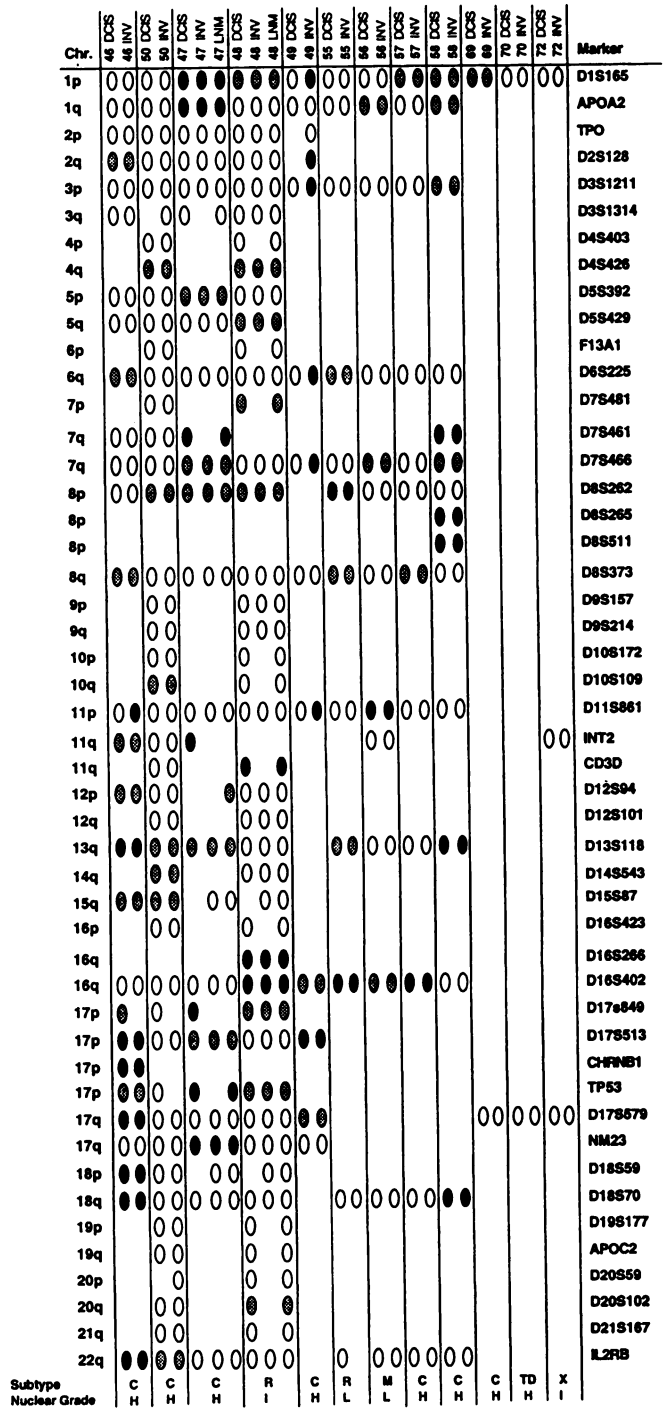

Fig. 1. Summary of LOH data for 10 sets of synchronous CIS and adjacent invasive cancer and for two sets of DCIS, invasive cancer, and lymph node metastasis. The chromosomal arm is listed on the *left*, the marker used is on the *right*, and tumor number is on the *top*. ●, no LOH; ○, LOH; ◍, noninformative; *INV*, invasive carcinoma; *LNM*, lymph node metastasis. *C*, comedo subtype; *R*, cribriform; *M*, micropapillary, *TD*, terminal ductal; *X*, mixed; *H*, high nuclear grade; *L*, low nuclear grade, *I*, intermediate.

meet our criteria for scoring LOH. However, the fold differences for the corresponding invasive components were 5.0 and 19.7, respectively. Densitometry at the other loci showing LOH for tumor 46 confirmed a difference >3-fold in each case for both DCIS and invasive tumor, compared with normal control.

For tumor 49, the invasive cancer also exhibited allelic loss for loci on 1p, 3p, 6q, and 7q. In the matched DCIS sample, both alleles were retained. This tumor also showed LOH in the invasive component using a 2q marker, but insufficient DNA was available to assay the DCIS. The fractional allelic loss (number of chromosomal arms showing LOH/total number of informative chromosomal arms) for the invasive component of tumor 49 was 0.63 (7 of 11). In every case where LOH of loci was observed in concurrent DCIS and invasion (and LNM), the same allele was lost. Tumors 50, 69, 70, and 72 did not show allelic loss for any locus examined, although for 3 of these tumors, 4 loci or less were studied. All LOH results were verified with repeat assays at least once.

Discussion

Inactivation of tumor suppressor genes plays a central role in the development of human cancers and their progression. In invasive breast carcinoma, LOH of loci on virtually every chromosome has been observed (8, 9), making it difficult to ascertain which genetic events are the most crucial in oncogenesis. Our allelotyping study has revealed that loci on 8p, 13q, 16q, 17p, and 17q are lost most frequently (>14%) in DCIS, implying that tumor suppressor genes near these loci are important in the early stages of breast cancer (5).

Circumstantial evidence that DCIS is a precursor lesion to invasive ductal carcinoma is based on three observations: (*a*) the frequent coexistence of DCIS and invasive cancer in the same breast (10); (*b*) the greatly increased risk of subsequent invasive breast cancer in women with biopsy-proven DCIS (11); and (*c*) 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 (12). 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 noninvasive stage.

Studies of the molecular changes in DCIS and invasive breast cancer are few. Davidoff *et al.* (13) studied 6 examples of synchronous DCIS and invasive cancer for expression of p53 and found the same levels of protein expression in each tissue type. Expression of the oncogenes c-erbB-2 and c-myc is also consistent between coexisting preinvasive and invasive breast cancer (14, 15). Zhuang *et al.* (6) studied allelic loss for two loci on 11q13 (*INT2* and *PYGM*). They found that for every case of DCIS that showed LOH (n = 15), loss of the same allele was seen in the corresponding invasive tumor (6). O'Connell *et al.* (7) studied four loci [*TPO* (2pter), *D4S192* (4q25–34), *D16S265* (16q21), and *D17S579* (17q21)] and found that 8 of 10 cases of DCIS shared LOH patterns with more advanced lesions for at least 1 of the 4 loci.

Our study provides information on 48 loci representing the 39 nonacrocentric autosomal arms. The data show that if allelic loss at a locus is found in the DCIS component, it persists in the synchronous invasive cancer and LNM. This indicates that, in those tumors where DCIS and invasive cancer are adjacent, the invasive component has arisen in all likelihood from the DCIS. Our data are in accord with the clonal expansion theory of Nowell (16), which states that tumors progress through the sequential acquisition of genetic and biological features. It should also be noted that this clonal expansion is seen for examples of comedo, micropapillary, and cribriform varieties of DCIS.

In two invasive cancers (tumors 46 and 49), LOH occurs on 11p but is absent in DCIS. Allelic loss on 11p has been reported to occur in 10–41% of invasive breast cancer (5, 8), although we have found it to be a very infrequent event in DCIS (1 of 32 tumors). This suggests that inactivation of tumor suppressor loci on 11p may be one of the features of the invasive phenotype. The difference in the LOH pattern for tumor 46 for the locus *D11S861*, when compared with the other loci shown in Fig. 2, A–C, suggests that there is a clone of cells in the

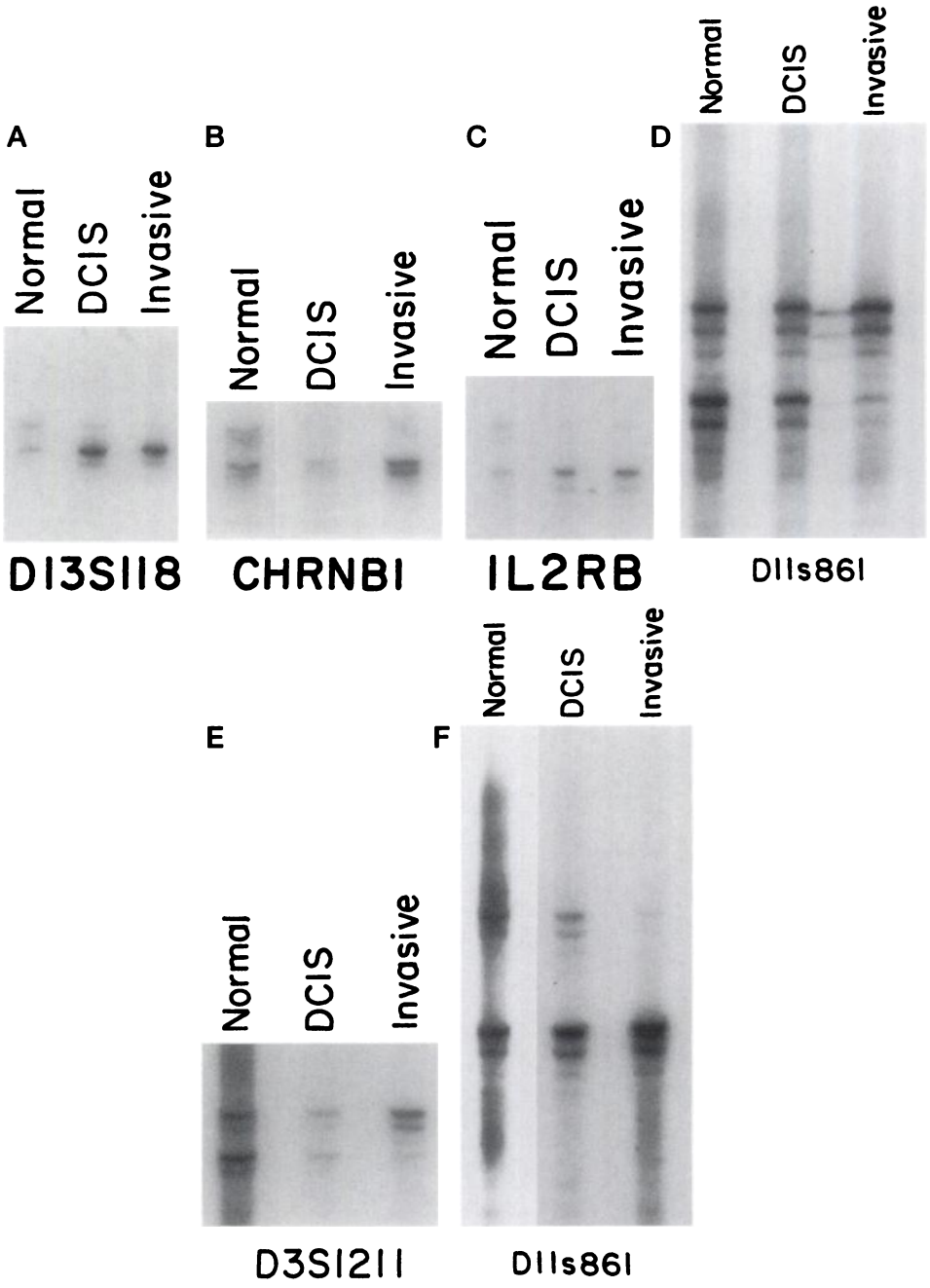

Fig. 2. A, B, and C, tumor 46. Loss of the same allele in both DCIS and invasive component for loci *D13S118* (13q), *CHRNB1* (17p), and *IL2RB* (22q). The fold difference in integrated allele ratios between tumor and normal are: *A*, DCIS 4.12, invasive 7.25; *B*, DCIS 7.78, invasive 9.2; and *C*, DCIS 6.7, invasive 10.5. *D*, tumor 46. Loss of one allele in the invasive component but not DCIS for the locus *D11S861* (11p). The fold differences between tumor and normal are DCIS, 1.42 (insufficient to call LOH) and invasive, 5.0 (LOH). E and F, tumor 49. Loss of an allele in the invasive component but not DCIS for loci *D3S1211* (3p) and *D11s861* (11p). The fold difference in integrated allele ratios between tumor and normal are E, DCIS 1.95, invasive 9.7; *F*, DCIS 1.74, invasive 19.7.

invasive tumor that retains two 11p alleles, although the majority of these cells have lost one allele. In contrast, the loss of one allele is virtually complete in both DCIS and invasive component for this tumor at the other loci shown. According to the theory of Nowell, this suggests that LOH on 11p may be a later event chronologically in the pathway to invasion.

LOH on 11p has been correlated with low estrogen receptor protein and tumor size in invasive breast cancer, both of which are indicators of poorer prognosis (17). Concordant loss of 11p and 17p in invasive breast cancer is more frequently associated with the development of lymph node metastases (18). One invasive cancer (tumor 49) also showed loss of loci on 1p, 2q, 3p, 6q, 7q, and 17p, whereas the corresponding DCIS displayed loss only at the locus on 17p. Loss at all these sites has been reported for invasive breast cancer (8, 9) and may indicate a more aggressive phenotype. For example, LOH on 1p has been correlated with the presence of lymph node metastases, larger tumor size, and nondiploidy (19), and LOH of loci on 7q with impaired survival (20). The high fractional allelic loss of the invasive component of tumor 49 suggests that multiple genetic events have taken place in this tumor.

In summary, these data suggest that the subtypes of DCIS represent precursor lesions of invasive ductal carcinoma of the breast. LOH of regions most commonly involved in DCIS (8p, 13q, 16q, 17p, and 17q) is maintained in the concurrent invasive component. Loss of alleles at other loci such as 11p may be later events which are a feature of the invasive phenotype.

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