Advances in Brief

Pattern of Gene Alterations in Intraductal Breast Neoplasms Associated with Histological Type and Grade

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Abstract

To reveal any association between the histological type and grade of intraductal breast neoplasms and the manner of accumulation of gene alterations, eight types of gene alterations, i.e., loss of heterozygosity (LOH) on chromosomal arms 16p, 16q, 17p, 17q, and 18q, amplification of the c-erbB-2 and hst/Int-2 genes, and mutation of the p53 gene, were examined by Southern blot analysis or single-strand conformation polymorphism analysis in a total of 60 cases of intraductal breast cancer and 18 nonmalignant proliferative lesions. Among the histological types and three histological grade groups of intraductal carcinomas, the gene alterations which occurred most frequently were LOH on 16q alone in non-comedo type and Grade 1, alterations of c-erbB-2, 17p, and 16q in comedo type and Grade 2, and alterations of 17q and p53 as well as those of 16q, 17p, and c-erbB-2 in Grade 3. LOH on 16q and 18q was frequent in intraductal carcinoma of the intracytic papillary type, whereas LOH on 18q alone was detected in 27% of papillomas. Among intraductal carcinomas, the mean number of gene alterations was largest in comedo type and Grade 3, whereas it was smallest in non-comedo type and Grade 1. It was possible that LOH on 18q and 16q was involved frequently in papillary tumorigenesis and acquisition of malignant phenotype, respectively, whereas most of the other gene alterations were involved in acquisition of aggressive biological properties by intraductal carcinoma cells. It was also possible that the phenotype of breast neoplasms was determined by the combination of gene alterations at a relatively early developmental stage.

Introduction

Intraductal carcinomas, which are classified into several histological types, e.g., comedo type, non-comedo type comprising cribriform and micropapillary subtypes, and, as a special form, intracytic papillary type (1), are generally regarded as the earliest developmental stage of clinically detectable breast cancer. Intraductal carcinomas are known to be cured by mastectomy, and to take an indolent clinical course even if they are treated by local excision only (2-6). However, among the various types, there are differences not only in structural and nuclear atypia in the neoplastic cells but also in the manner of local extension (4), and the risk of local recurrence has been shown to be higher for the comedo type and/or tumors of the highest nuclear grade (5-7).

Studies of alterations in oncogenes and tumor suppressor genes, including LOH on chromosome arms, in human breast cancer have revealed a strong correlation between the type of gene alteration and both the clinical behavior and histological characteristics of the cancer cells, including prognosis and indicators of high proliferative activity, such as the histological and nuclear grade of atypia, S-phase fraction, or DNA aneuploidy (9-19). Gene alterations have also been examined in intraductal carcinoma (20-25): amplification of the c-erbB-2 proto-oncogene and overexpression of its protein were much more frequent in intraductal carcinoma of the comedo type than in invasive breast cancer (20, 22, 23). Mutation of the p53 tumor suppressor gene and accumulation of its protein in cancer cell nuclei have been also detected in intraductal carcinoma of higher histological grade and shown to consist between invasive and intraductal components (21), although the incidence of the mutation in intraductal cases is relatively lower than that in invasive cancer (23). Furthermore, LOH on chromosome 16q and 17p is frequent not only in invasive but also in intraductal cancers (15, 24, 25). Among intraductal cancer cases, LOH on 16q is shown to occur frequently, irrespective of any difference in histological grade (25). These data suggest that the manner of accumulation of the gene alterations differs among histological types and grades in breast cancers at the preinvasive stage. In the present study, to reveal any association between the accumulation of gene alterations and histological features of intraductal neoplasms, 8 gene alterations were examined in 78 surgically resected specimens comprising 60 intraductal carcinomas and 18 noncancerous proliferative lesions of the breast.

Materials and Methods

Cases, Histological Typing, and Grading. Among the patients who underwent surgical treatment for primary breast cancer at the National Cancer Center Hospital, paired samples of tumor intraductal component and nontumor tissue were obtained from a total of 78 patients: 31 with intraductal carcinomas, 29 with invasive ductal carcinomas with a predominantly intraductal component, and 18 with benign proliferative epithelial les-

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2 To whom requests for reprints should be addressed.

3 The abbreviation used is: LOH, loss of heterozygosity.
Fig. 1 Microscopic presentation of intraductal carcinoma of different histological grade. a and b, Grade 1. Cribriform structure is marked. Nuclear size and shape are uniform and mitotic figures are rare. c and d, Grade 2. e and f, Grade 3. Papillary or cribriform structure is obscure. Nuclear size and shape are variable, and mitotic figures are evident. H&E; bars, 1 mm in a, c, and e; 100 μm in b, d, and f.

Intraductal carcinomas comprised 13 intraductal papillomas, 3 atypical duct papillomatosis, 1 tubular adenoma, and 1 nipple adenoma.

The histological type of intraductal carcinoma was classified into three, i.e., comedo, non-comedo, and intracystic papillary types (1). The 29 patients who showed central coagulation necrosis in the intraductal component were classified as the comedo type regardless of histological grade. Among the patients without such comedo-type necrosis, according to the macroscopic and microscopic structure, 11 patients were classified into cribriform subtype and 2 into micropapillary subtype. These two subtypes were classified into non-comedo type. The other 18 patients were classified into intracystic papillary types. Intracystic papillary type is a unique group in which the tumor shows polypoid projection into the cystic space (8).

The intraductal carcinoma component was also classified into three histological grade groups, i.e., Grades 1, 2, and 3 (Fig. 1). The histological grade of atypia assigned to each case was the sum of the scores for (a) the degree of structural atypia, (b) the degree of nuclear atypia, and (c) the number of mitotic figures, irrespective of any difference in histological type, in the same manner as that for invasive carcinomas (Ref. 26; Table 1).

In previous studies, LOH on 16q had already been examined in 44 intraductal carcinomas (23, 25, 27), and p53 mutations and amplification of the c-erbB-2 gene were examined in
17 intraductal carcinomas. LOH on 17p was examined in two patients which carried the p53 mutation, but the result was negative for both. In addition to the previous data, LOH on 16q was examined in 16 patients, p53 mutation and c-erbB-2 amplification in 43 patients, and LOH on 17p in 58 patients. LOH on 16p, 17q, and 18p and amplification of the int-2 gene were examined in all of the 60 patients. Among the 18 nonmalignant lesions, LOH on 16q had already been examined in 11 papillomas (27). In the present study, we examined LOH on 16q in seven additional lesions and seven other gene alterations in all of the 18 lesions.

**Tissue Samples and DNA Isolation.** The intraductal carcinoma lesions were identified by careful visual observation of surgically resected specimens, and multiple portions of the lesion were sampled, embedded in OCT compound (Miles Inc., Elkhart, IN), sliced with a cryostat into 5-μm-thick sections, and stained with hematoxylin. By microscopic examination, the specimens were confirmed to contain predominantly cancer tissue, and the areas with high proportion (>50%) of cancer tissue were cut out with a razor blade and collected for DNA preparation (23, 25). In all cases of predominantly intraductal carcinoma, DNA was isolated from intraductal components (25).

**DNA Probes.** The loci of the polymorphic DNA markers used as probes for restriction fragment length polymorphism analysis and the restriction enzymes used are D16S83 (pEKMDA2.1A, Rsal) and D16S159 (Cl52.94, TaqI) for 16p (25); CETP (TaqI), D16S4 (ACH207, TaqI), HP (HP2a, BamHI), TAT (BB0.4, BamHI), D16S7 (p79-2-23, TaqI), and APRT (TaqI) for 16q (25); D17S34 (p144D6, Rsal), D17S30 (pYNZ22, TaqI), D17S31 (pmC35.1, MspI), D17S1 (pH12-2, MspI), MYH2 (p10-3, MspI), and D17S71 (p10-41, MspI) for 17p (28); D17S74 (pCMM386, TaqI) for 17q (28); and D18S8 (OLVII E10, MspI), D18S7 (OL VII A8, MspI), and D18S5 (OS4, TaqI) for 18q (28). Gene amplification was examined using the c-erbB-2, hst-1, and int-2 probes (29–31).

**Analysis of LOH, Gene Amplification, and Gene Mutation.** High-molecular-weight isolated DNA was cut with TaqI, Rsal (New England Biolabs, Beverly, MA), MspI, or BamHI (Takara, Kyoto, Japan) restriction enzyme, electrophoresed in 0.8% agarose (FMC, Rockland, ME), alkali-denatured, neutralized, and subjected to Southern blot hybridization analysis using [α-32P]dCTP-labeled DNA probes (25).

To detect mutation of the p53 gene, PCR-single-strand conformation polymorphism analyses were performed by the method described previously (32). Primers for PCR used were: 4L, 5'-TTTTTCAACCATCTACAGTCCC-3' and 4R, 5'-CTCGAGGACAACTGACCTGTC-3' for exon 4; 5L, 5'-CTCTCTTCCTCCTGAGTACTCC-3' and 5R, 5'-CTCAGAGCTGCACCTGACGCTGTCGG-3' and 6R, 5'-AGTGTGAAACCCAGACACTCA-3' for exon 5; 6L, 5'-CGATGGTACGAGCTGTCGG-3' and 7R, 5'-AAGTGCTCTGTACACTGCA-3' for exon 6; 7L, 5'-TCTAGGTGTGCTGCT-3' and 7R, 5'-CTCTAGGTGTGCTGCT-3' for exon 7; 8L, 5'-CTTCTCTTCTTAGTGGTAA-3' and 8R, 5'-CTCTAGGTGTGCTGCT-3' for exon 8; and 9R, 5'-CCCCAGCAATTAGTACCTCG-3' for exon 9. Genomic DNA (0.1 μg) was subjected to 35 cycles of PCR (94°C, 55°C, 72°C for 0.5, 0.5, 1 min, respectively) in 10 μl solution containing 0.25 pmol each of 5' end-labeled primers, 10 mm Tris-HCl (pH 8.3), 50 mm KCI, 1.5 mm MgCl2, 0.01% gelatin, 20 μM dATP, dCTP, dGTP, and TTP, and 0.5 units of Taq polymerase (Perkin Elmer/Cetus).

The PCR products were diluted 1:100 in loading solution (96% formamide, 20 mm EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue), denatured at 85°C for 5 min, and applied (1 μl/lane) to 6% polyacrylamide gel (96% formamide, 20 mM EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue) at 30 W for 2 to 5 h at room temperature with vigorous air cooling. The gel was dried on filter paper and exposed to Kodak XRP-1 film at room temperature for 2 to 24 h.

**Comparison of Number of Gene Abnormalities among Intraductal Carcinomas of Different Histology.** The number of gene alterations was calculated and compared among the groups of histological type and grade. In cases where LOH information was not available for all of the chromosome arms examined, the mean number of gene alterations per tumor was calculated by the formula,

\[
\frac{NI \times n}{N2}
\]

where \(NI\) is the number of gene alterations, including LOH, gene amplification, and gene mutation, in each tumor, and \(N2\) is the number of gene or chromosome loci that were informative for gene alterations in each tumor. In this formula, \(n\), the number of gene alterations examined, is always 8.

The incidence of each gene alteration among the groups of histological type or grade was calculated by the \(x^2\) test. Differences in the mean number of gene alterations were examined by Student's \(t\) test between two groups after confirming the equality of variance for the two groups using the F test.

**Results**

**Accumulation of Gene Alterations in Intraductal Carcinomas.** The results of previous studies (23, 25, 27) and of the present one are shown in Table 2. In total, alterations in 16q, 17p, c-erbB-2, 18q, 16p, p53, 17q, and hst-1/int-2 were detected in 43%, 28%, 22%, 16%, 12%, 7%, 7%, and 5% of the tumors, respectively (Figs. 2 and 3). The incidence of each gene alter-
Table 2  Incidence of gene alterations in intraductal carcinoma of the breast

<table>
<thead>
<tr>
<th>Gene alteration</th>
<th>Comedo type</th>
<th>Non-comedo type</th>
<th>Intraductal papillary type</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH on 16q</td>
<td>8/28 (29)</td>
<td>5/13 (38)</td>
<td>12/17 (71)</td>
</tr>
<tr>
<td>LOH on 17p</td>
<td>12/29 (41)</td>
<td>1/12 (8)</td>
<td>3/17 (18)</td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>12/29 (41)</td>
<td>1/12 (8)</td>
<td>3/17 (18)</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>3/25 (12)</td>
<td>1/12 (8)</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td>hst-1/int-2</td>
<td>3/28 (11)</td>
<td>0/13 (0)</td>
<td>1/16 (6)</td>
</tr>
<tr>
<td>Gene amplification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>12/29 (41)</td>
<td>1/13 (8)</td>
<td>0/18 (0)</td>
</tr>
<tr>
<td>hst-1/int-2</td>
<td>2/29 (7)</td>
<td>0/13 (0)</td>
<td>1/18 (6)</td>
</tr>
<tr>
<td>Gene mutation p53</td>
<td>4/29 (14)</td>
<td>0/13 (0)</td>
<td>1/18 (6)</td>
</tr>
<tr>
<td>Mean no. of gene alterations</td>
<td>1.67 ± 1.48</td>
<td>0.70 ± 0.73</td>
<td>1.41 ± 1.33</td>
</tr>
</tbody>
</table>

* a Described in Refs. 23, 25, and 27.
  b Brackets contain the data from Ref. 24.

Fig. 3 Gene amplifications in intraductal carcinoma of the breast. N, normal tissue DNA (5 μg); T, tumor DNA (5 μg). a, amplification of the c-erbB-2 gene. Five cases of the comedo type (tumors 246, 348, 357, 3358, and 3366) are presented. Signal of tumor DNA is increased in comparison to that of normal DNA. b, amplification of the int-2 gene. Two cases of the comedo type (tumors 350 and 357) are presented.

Table 3 Incidence of gene alterations in intraductal breast carcinomas of different histological types

<table>
<thead>
<tr>
<th>Gene alteration</th>
<th>Total</th>
<th>Previous studies</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH on 16q</td>
<td>25/58 (43)</td>
<td>20/44 (45)</td>
<td>5/14 (36)</td>
</tr>
<tr>
<td>LOH on 17p</td>
<td>16/58 (28)</td>
<td>0/2 (0)</td>
<td>16/56 (29)</td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>13/60 (22)</td>
<td>6/17 (35)</td>
<td>7/43 (10)</td>
</tr>
<tr>
<td>Amplification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOH on 16p</td>
<td>8/51 (16)</td>
<td>8/51 (16)</td>
<td></td>
</tr>
<tr>
<td>p53 mutation</td>
<td>3/60 (5)</td>
<td>3/60 (5)</td>
<td></td>
</tr>
<tr>
<td>LOH on 17q</td>
<td>4/57 (7)</td>
<td>4/57 (7)</td>
<td></td>
</tr>
<tr>
<td>hst-1/int-2</td>
<td>3/60 (5)</td>
<td>3/60 (5)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2 LOH on chromosome 18q, 16p, and 17q in intraductal papilloma and intraductal carcinoma. N, normal tissue DNA (5 μg); T, tumor DNA (5 μg). Arrows, LOH. a, LOH on 16q. Five cases are presented, comprising one case each of intracytis papillary (tumor 304), non-comedo (cribriform; tumor 345), and comedo (tumor 357) type, and two papillomas (tumors 311 and 340). The D18S7 and D18S8 markers are localized on 18q11.1-q11.2 and 18q21.3, respectively (28). In tumor 311, LOH was detected at D18S7 but not at D18S8. b, LOH on 16p. Cases of intracytis papillary type (tumors 243 and 248) and comedo type (tumor 229) are presented. The D16S159 and D16S83 markers are localized on 16p12.2 and 16p13.3, respectively (26). c, LOH on 17q at the D17S74 locus on 17q21 (28). Two cases of the comedo type (tumors 78 and 220) and one case of the intracytis papillary type (tumor 243) are presented.

Table 3 Incidence of gene alterations in intraductal breast carcinomas of different histological types

<table>
<thead>
<tr>
<th>Gene alteration</th>
<th>Comedo type</th>
<th>Non-comedo type</th>
<th>Intraductal papillary type</th>
</tr>
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<td>5/13 (38)</td>
<td>12/17 (71)</td>
</tr>
<tr>
<td>LOH on 17p</td>
<td>12/29 (41)</td>
<td>1/12 (8)</td>
<td>3/17 (18)</td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>12/29 (41)</td>
<td>1/12 (8)</td>
<td>3/17 (18)</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>3/25 (12)</td>
<td>1/12 (8)</td>
<td>2/14 (14)</td>
</tr>
<tr>
<td>hst-1/int-2</td>
<td>3/28 (11)</td>
<td>0/13 (0)</td>
<td>1/16 (6)</td>
</tr>
<tr>
<td>Gene amplification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-erbB-2</td>
<td>12/29 (41)</td>
<td>1/13 (8)</td>
<td>0/18 (0)</td>
</tr>
<tr>
<td>hst-1/int-2</td>
<td>2/29 (7)</td>
<td>0/13 (0)</td>
<td>1/18 (6)</td>
</tr>
<tr>
<td>Mean no. of gene alterations</td>
<td>1.67 ± 1.48</td>
<td>0.70 ± 0.73</td>
<td>1.41 ± 1.33</td>
</tr>
</tbody>
</table>

P < 0.005 between intracytis papillary type and others, calculated by χ² test.

P < 0.025 between comedo type and others, calculated by χ² test.

P < 0.05 between comedo type and others, calculated by Student's t test.

P < 0.025 between comedo and cribriform groups and between cribriform type and the others, calculated by Student's t test.
Table 4 Incidence of gene alterations in intraductal breast carcinomas differing in histological grade of atypia

<table>
<thead>
<tr>
<th>Gene alterations</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOH 16q</td>
<td>20/39 (51)</td>
<td>2/9 (22)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>17p</td>
<td>6/39 (15)</td>
<td>4/8 (50)</td>
<td>6/11 (55)</td>
</tr>
<tr>
<td>18q</td>
<td>20/39 (51)</td>
<td>2/9 (22)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>16p</td>
<td>3/32 (9)</td>
<td>1/8 (13)</td>
<td>2/11 (18)</td>
</tr>
<tr>
<td>17q</td>
<td>1/37 (3)</td>
<td>0/9 (0)</td>
<td>3/10 (30)</td>
</tr>
<tr>
<td>Gene amplification c-erbB-2</td>
<td>2/40 (5)</td>
<td>4/9 (44)</td>
<td>7/11 (64)</td>
</tr>
<tr>
<td>hst-1/int-2</td>
<td>3/40 (8)</td>
<td>0/9 (0)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>p53</td>
<td>0/40 (0)</td>
<td>1/9 (11)</td>
<td>4/11 (36)</td>
</tr>
<tr>
<td>Mean no. of gene alterations</td>
<td>1.12 ± 1.33</td>
<td>1.44 ± 1.07</td>
<td>2.33 ± 1.28</td>
</tr>
</tbody>
</table>

*P < 0.005 between Grade 1 and Grade 2 and 3 groups by χ² test.

Grade 2 and 3 groups, LOH on 17p (53%, 10/19) and c-erbB-2 amplification (50%, 11/20) as well as LOH on 16q (26%, 5/19) were frequent. In addition, in Grade 3 cases, LOH on 17q and p53 mutation were also relatively frequent, 30% (3/10) and 36% (4/11), respectively. The other gene alterations, LOH on 16p, LOH on 16q, and hst-1/int-2 amplification, were detected in only ≤20% of cases in any of the histological grade groups. The incidence of alterations of 17p and c-erbB-2 was significantly higher in Grade 2 and 3 groups than in the Grade 1 group (Table 4).

Among the total of 57 intraductal carcinomas, LOH on 17p was detected in 16 cases whereas p53 mutation was detected only in 5. Concordance of the two alterations was detected in only two cases.

The mean number of gene alterations in the comedo, intracytic papillary, and non-comedo types was 1.67 ± 1.48 (mean ± SD), 1.41 ± 1.33, and 0.70 ± 0.73, respectively, and that in the Grade 1, 2, and 3 groups was 1.12 ± 1.33, 1.44 ± 1.07, and 2.33 ± 1.28, respectively. Among cases of the comedo type, the mean number of gene alterations was 1.44 ± 1.98 in Grade 1, 1.57 ± 1.05 in Grade 2, and 2.03 ± 0.48 in Grade 3. By the F test, variances among the groups of type or grade were shown to be equal. Student's t test revealed a significant difference in the mean number between the comedo and non-comedo types (P < 0.025), between Grade 1 and Grades 2 and 3 (P < 0.025), and between Grade 1 and Grade 3 (P < 0.025).

Gene Alterations in Nonmalignant Proliferative Lesions of the Breast. In 3 (27%) of 11 papillomas, LOH on 18q was detected (Fig. 2, tumors 311 and 340). Other gene alterations were not detectable in the 18 noncancerous lesions (Table 5).

Discussion

The pattern of accumulation of gene alterations differed significantly among histological type or grade groups of intraductal carcinoma. At the same time, the number of gene alterations increased in accordance with increasing histological grade and was shown to be highest in the comedo-type and Grade 3 groups, which are known to have a high proliferation rate and aggressive clinical course in comparison to the other groups (3–7). Thus, combination of gene alterations was suggested to determine the phenotype of preinvasive breast neoplasms, and the number of gene alterations was suggested to determine the grade of their aggressiveness. In particular, a majority of the gene alterations examined, i.e., LOH on 17p, c-erbB-2 amplification, LOH on 17q and p53 mutation, were detected mostly in the Grade 3 and/or comedo-type groups and were suggested to play a role in the formation of a higher histological grade of atypia and in enhancing the speed of proliferation of intraductal carcinoma cells.

On the other hand, LOH on 16q was confirmed to occur frequently in intraductal carcinoma regardless of histological type or grade (25). Because none of the noncancerous lesions examined revealed LOH on 16q, LOH was considered to be involved in acquisition of the malignant properties of breast neoplasms. Furthermore, because LOH on 18q was detected relatively frequently in both benign and malignant intracytic papillary tumors, this gene alteration in mammary gland epithelial cells was suggested to be involved in papillary tumorigenesis or acquisition of protruding neoplastic growth.

Two manners of accumulation of gene alterations possibly occur in breast cancers at the preinvasive stage. One is stepwise accumulation of gene alterations in neoplastic cells in accordance with enhancement of malignancy grade. This would correspond to the model of colorectal tumorigenesis proposed by Fearon and Vogelstein (33). When such a tumorigenesis model is applied to breast neoplasms, the process can be explained morphologically and at the molecular level as follows: Morphologically, the benign neoplasm stage precedes that of Grade 1 or non-comedo-type carcinoma, and these precede the stage of Grade 2 or 3, or comedo-type carcinoma. Neoplasms of higher histological grade are postulated to arise always as a result of progression of lower grade ones. Lennington et al. (34) have shown that the histological pattern of intraductal carcinoma is frequently heterogeneous, and that the grade of cancer is usually higher in the central than in the peripheral portion in identical...
cases. Their data appear to provide histological evidence for stepwise progression in intraductal neoplasms. At the DNA and chromosome level, LOH on 18q occurs first at the tumorigenesis stage, LOH on 16q subsequently occurs at the stage of malignant transformation of the tumor, and then late events, e.g., LOH on 17p, amplification of the c-erbB-2 gene and other gene alterations, are added to enhance the grade of atypia and biological aggressiveness of intraductal carcinoma cells.

However, not all cases appear to confirm the multistage progression of intraductal breast neoplasms. Therefore, there is a second possibility that the histological grade and genotype of breast cancer is already determined at the earliest stage of tumor development. At the moment of tumorigenesis or soon after, some tumors arise as benign neoplasms or Grade 1 carcinomas with a smaller number of gene alterations and remain in this condition for a long period, whereas others arise primarily as Grade 2 or 3 carcinomas with a large number of gene alterations. Both of these forms of gene mutation accumulation seem to occur in vivo, and detailed examination at the morphological and molecular levels will be necessary in order to clarify whether stepwise progression is the main course of development of intraductal breast neoplasms.

LOH on 17p and p53 mutation have been shown to be linked in a large number of studies on breast and other cancers (18, 35, 36). Both of these gene alterations occur mostly in intraductal carcinomas of Grades 2 and 3 and/or the comedo type. The incidence of p53 mutation and 17p LOH has been shown to be 20–40% and 27–55%, respectively, in breast cancer (15, 18, 19, 23, 24). In this study, on the other hand, although the incidence of 17p LOH was still high (29%), p53 mutation was detected in only 7% of intraductal carcinomas. At least in the early developmental stage, the linkage between these two gene alterations did not appear to be very strong. The timing of the occurrence or the start of clonal expansion of given cancer cells appeared to differ between LOH on 17p and p53 gene mutation.

Acknowledgments

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23. Tsuda, H., Iwaya, K., Fukutomi, T., and Hirohashi, S. p53 gene mutations and c-erbB-2 amplification in intraductal and invasive breast...


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