Pattern of Chromosome 16q Loss Differs between an Atypical Proliferative Lesion and an Intraductal or Invasive Ductal Carcinoma Occurring Subsequently in the Same Area of the Breast

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Atypical proliferative lesions of the breast, such as atypical ductal hyperplasia and atypical papilloma, are considered to be precursors of breast carcinomas and have frequently been shown to have loss of heterozygosity (LOH) on chromosome 16q at the DNA level. We evaluated whether an atypical proliferative lesion and a carcinoma that subsequently occurred in the same area of the ipsilateral breast were of identical clonal origin in seven patients. Using DNA isolated from microdissected archival tissue of epithelial components of both the biopsy specimen of the atypical proliferative lesion and the mastectomy specimen of the carcinoma, the pattern of LOH on 16q was compared between these two lesions using polymerase chain reaction–microsatellite LOH analysis. As a control, LOH on 16q was examined in 13 cases of usual ductal hyperplasia, 10 usual papillomas, and 6 atypical ductal hyperplasias. In the seven cases, LOH on 16q was detected in three of the six atypical proliferative lesions and in five of the seven carcinomas, but the allele with LOH or a deleted region always differed between the two. LOH was detected in both atypical proliferative lesions and carcinomas in one case, only in the atypical proliferative lesion in two cases, and only in carcinomas in three cases. In the controls, LOH on 16q was absent in usual ductal hyperplasias or usual papillomas but was detected in two of six atypical

ductal hyperplasias. Although atypical proliferative lesions were frequently confirmed to be of clonal nature with LOH on 16q, these lesions and carcinomas were considered to be clones, probably originated from a field with these clones.

KEY WORDS: Breast cancer, Chromosome 16, Loss of heterozygosity, Natural history, Precancer lesions.

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Intraductal carcinomas, or ductal carcinomas *in situ* (DCIS), are considered to be the early, preinvasive stage of breast cancer, and, if followed up, are often found to progress eventually to invasive cancers (1, 2). DCIS are subclassified into low-grade noncomedo type (*i.e.*, cribriform, papillary, and low-papillary types) and high-grade comedo type. Atypical ductal hyperplasia (ADH) is defined as a small lesion of atypical epithelial cells with structural atypia but where the degree of atypia is not sufficient to classify DCIS. ADH is a risk factor for breast cancer occurrence, which becomes four times as likely as in women without the lesion (3, 4). Therefore, ADH stands in an intermediate position both histologically and as a cancer risk factor between the hyperplasia without atypia and DCIS. Atypical papillomas, or papillomas with an atypical or borderline part, which are frequently the feature of multiple papillomas, are also shown often to be accompanied by cancer and/or to be a risk factor of breast cancer (5–7).

Loss of heterozygosity (LOH) on chromosome 16q frequently occurs in human breast cancers, including low-grade DCIS (8–11). ADH is also reported to show LOH frequently on chromosomes 16q and 17p (12). Therefore, ADH is demonstrated to be a monoclonal proliferation, although it is undetermined whether the ADH is a hyperplastic lesion or a lesion at the earliest stage of low-grade

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DCIS. On the other hand, these chromosomal alterations are rare or absent in usual ductal hyperplasias and usual intraductal papillomas (10, 13–15). Thus, LOH on 16q is assumed to be involved in the acquisition of clonal proliferative properties by mammary glandular epithelial cells at the very early developmental stage.

A comparison of the pattern of LOH among different lesions in an ipsilateral breast will reveal their clonal origin (16). Several studies revealed clonal identity between the components of DCIS and invasive carcinoma in identical tumors (17, 18). We often observe the metachronous occurrence of an atypical proliferative lesion, for example, ADH or atypical papilloma, and a carcinoma in the same breast in a patient. However, it is still unclear whether these atypical proliferative lesions are the direct precursors of carcinomas occurring later in the same area of the breast. In the present study, to clarify this subject, we examined LOH on 16q among the breast lesions metachronously resected from the same patients. Initially, these patients underwent excision of a breast tumor that was disclosed to be an ADH/atypical papilloma; 2.7 to 16.1 years later, the patients underwent a mastectomy because of primary breast cancers that occurred at the area of previous excisional biopsy. We isolated DNA from archival tissue blocks of both the previous atypical lesions and the later carcinomas by means of microdissection and compared the pattern of LOH by polymerase chain reaction (PCR)-mediated microsatellite analyses between these lesions.

MATERIALS AND METHODS

Diagnostic Criteria

The criteria of usual ductal hyperplasia, or hyperplasia of usual type, and usual intraductal papilloma have been given in previous studies (7, 13–15). The diagnosis of ADH was made according to the criteria of Page *et al.* (3, 19). The diagnosis of atypical papilloma was made if there were parts of borderline lesion or ADH within the papilloma (3, 6, 7). In atypical papillomas, the part of borderline or ADH could be recognized solely on the basis of the relative homogeneity and hyperchromasia of the cellular mass. The histological type and grade of carcinomas were determined according to the criteria described by Page and Anderson (20, 21).

Patients

We selected 7 women who had undergone excision of a breast tumor diagnosed as an atypical proliferative lesion of duct epithelial cells (e.g., ADH, atypical papilloma) and a subsequent mas-

tectomy because of a primary breast carcinoma that had appeared at the site of previous biopsy. By reviewing hematoxylin and eosin (HE)-stained tissue sections, the histological diagnosis was confirmed.

Case 1

A left breast tumor was biopsied and diagnosed as atypical hyperplasia. This lesion was confirmed as compatible with ADH (Fig. 1, A-B). After 16 years, the patient noticed a 2.8 × 2.5-cm tumor at the site of the previous biopsy. The tumor was diagnosed as invasive ductal carcinoma (IDC), solid-tubular type, Grade 3 (Fig. 1C), and a modified radical mastectomy was performed.

FIGURE 1. Histological presentation of an atypical hyperplastic lesion and a carcinoma resected metachronously from Patient 1. (A, B) Earlier atypical ductal hyperplasia (A, ×100; B, ×200) and (C) later invasive ductal carcinoma (×200). Hematoxylin and eosin staining.

Case 2

A 56-year-old woman underwent excisional biopsy of a right breast tumor, which was diagnosed as atypical hyperplasia. The lesion was slightly atypical duct papillomatosis and was included in the category of ADH. After 5.5 years, she noticed a right-breast tumor (1.8 × 2.0 cm) at the biopsy site during a follow-up exam. She underwent a mastectomy and was diagnosed to have an IDC with a DCIS component and another incidental lobular carcinoma *in situ*.

Case 3

A 33-year-old woman underwent excisional biopsy of a right breast tumor measuring 2.5 × 2.0 cm. The tumor was diagnosed as atypical intraductal papilloma and confirmed as such by review (Fig.

FIGURE 2. Histological presentation of an atypical hyperplastic lesion and a carcinoma resected metachronously from Patient 3. (A, B) Earlier atypical papilloma (A, ×100; B, ×200) and (C) later invasive ductal carcinoma (×200). Hematoxylin and eosin staining.

2, A-B). Sixteen years later, she visited the hospital complaining of a very large (10-cm) tumor occupying the right breast, including the biopsy site, and after diagnosis of IDC, scirrhous type, Grade 3, she underwent a mastectomy and adjuvant chemotherapy (Fig. 2C).

Case 4

A 42-year-old woman underwent excisional biopsy of a right breast tumor after complaining of bloody nipple discharge; this tumor was diagnosed as atypical papilloma (borderline case). A review showed the lesion to be equivocal between atypical papilloma and DCIS of low-papillary type. After 5.8 years, the patient noticed a tumor at the biopsy site and underwent a simple mastectomy after diagnosis of IDC, papillotubular type, Grade 2.

Case 5

A 45-year-old woman underwent excision of a left breast tumor, which was diagnosed as atypical hyperplasia. The lesion was confirmed to be ADH by review. After 11.2 years, she noticed another tumor at the biopsy site and underwent a mastectomy after diagnosis of DCIS with early invasion.

Case 6

A 53-year-old woman underwent a biopsy of a left breast tumor. After 5.3 years, a 3.2 × 3.1-cm tumor recurred at the same site. The previous lesion was diagnosed as atypical papilloma and the latter as IDC, papillotubular, Grade 2 (Fig. 3, A-C).

Case 7

A 58-year-old woman underwent a biopsy because of a left breast tumor, and after 2.7 years, she underwent a mastectomy because another tumor had occurred at the same site. The former was diagnosed as duct papillomatosis with atypia, which was categorized as mild ADH by review, whereas the latter was identified as IDC of scirrhous type with a DCIS component.

Control Cases

Among women who underwent excision of breast tissue because of tumor or abnormal nipple discharge at the National Cancer Center Hospital between 1986 and 1998, we randomly selected 13 cases of usual ductal hyperplasia, 10 cases of usual papilloma, and 6 cases of ADH. None of these lesions were accompanied by an ipsilateral, synchronous carcinoma, and all of them differed in location.

Microdissection and DNA Extraction

From the formalin-fixed paraffin-embedded tissue blocks of these lesions and tumors stored at the

FIGURE 3. Histological presentation of an atypical hyperplastic lesion and a carcinoma resected metachronously from Patient 6. (A, B) Earlier atypical papilloma (A, $\times 100$; B, $\times 200$) and (C) later invasive ductal carcinoma ($\times 200$). Hematoxylin and eosin staining.

hospital, tissue sections were cut in 10- μ m thicknesses for the selective microdissection of the epithelial component of atypical lesions and carcinomas. The sections were mounted on noncoated glass slides and stained with HE.

Using LM200 laser capture microdissection (Arc-turus Engineering, Santa Clara, CA), the epithelial component of the atypical lesions was collected from the HE-stained tissue sections (22). For normal DNA, adipose tissue or lymphocytes were dissected. From the collected tissue, DNA was isolated using a QiaAmp DNA mini kit (Qiagen, Basel, Switzerland) according to the supplier's recommendation. The DNA was dried up and dissolved in 100 μ L of 10 mM Tris-HCl (pH 8.0)–1 mM EDTA.

PCR-LOH Analysis

For PCR, oligonucleotide primers were synthesized by Sawady Technology (Tokyo, Japan). The sense primer was labeled with 6-carboxyfluorescein. The primer sets used were *D16S409* (q12.1), *D16S304* (q12.1), *D16S408* (q13), *D16S514* (q21), *D16S512* (q22), *D16S515* (q22), *D16S303* (q22), *D16S518* (q23.3–q24.1), *D16S305* (q23–q24), and *D16S393* (q23–q24) (23, 24). With 10 μ L of DNA, the final concentration of a 20- μ L PCR reaction mixture comprised a 1 $\times$ Gold PCR buffer; 2.5 mM $MgCl_2$; 112.5 μ M each dATP, dGTP, dCTP, and TTP; 4 μ M each primer; and 0.5 U AmpliTaq Gold (Perkin-Elmer, Foster City, CA). This was subjected to PCR composed of 1 cycle at 95°C for 10 minutes, 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by 1 cycle at 75°C for 5 minutes, using the GeneAmp PCR system 9600 (Perkin-Elmer). DNA products amplified by PCR were analyzed directly by an ABI Prism 310 genetic analyzer and a GeneScan Analysis software program (Perkin-Elmer). If the peak representing an allele in the lesion DNA was a third or less of the peak of the same allele in normal-cell DNA, the lesion was judged to have LOH. The PCR analysis was repeated twice, and when results were uncertain, the experiment was further repeated until the reliability of the results became convincing.

RESULTS

The results of the study of seven follow-up cases are summarized in Figure 4 and Table 1. In Patient 1, LOH was detected in both the ADH and the IDC. LOH was commonly detected at the *D16S514* and *D16S393* loci but only in the IDC at *D16S512*. In Patient 2, LOH was detected in the DCIS that was

Case	Lesions		Interval	Chromosome markers									
				\$409	\$304	\$408	\$514	\$512	\$515	\$303	\$518	\$305	\$393
1	1st	ADH	16 yr	NI	NI	NI	●	○	NI	NI	ND	ND	●
	2nd	IDC		NI	NI	NI	●	●	NI	NI	ND	ND	●
2	1st	ADH	5.5 yr	NI	NI	NI	NI	○	NI	NI	ND	NI	NI
	2nd	DCIS		NI	NI	NI	NI	○	NI	NI	NI	NI	NI
	IDC	NI		NI	NI	NI	ND	NI	NI	NI	NI	NI	NI
	LCIS	NI		NI	NI	NI	○	NI	NI	ND	ND	NI	NI
3	1st	Atyp. papilloma	16.1 yr	●	NI	ND	●	○	●	ND	NI	ND	●
	2nd	IDC		○	NI	○	○	○	○	ND	NI	ND	ND
4	1st	Atyp. papilloma ~DCIS	5.8 yr	ND	NI	○	●	ND	ND	NI	NI	ND	●
	2nd	IDC		ND	NI	○	○	ND	ND	NI	NI	ND	○
5	1st	ADH	11.2 yr	NI	NI	ND	○	ND	ND	ND	ND	ND	ND
	2nd	DCIS		NI	NI	ND	●	ND	ND	ND	ND	ND	ND
6	1st	Atyp. papilloma	5.3 yr	NI	NI	ND	○	○	ND	ND	ND	○	ND
	2nd	IDC		NI	NI	NI	●	●	ND	ND	●	●	ND
7	1st	ADH	2.7 yr	NI	NI	NI	NI	ND	ND	ND	ND	NI	ND
	2nd	DCIS		NI	NI	NI	NI	ND	ND	ND	ND	NI	○
	IDC	NI		NI	NI	NI	ND	ND	●	ND	NI	○	○

FIGURE 4. A summary of microsatellite loss of heterozygosity (LOH) analysis in an atypical hyperplastic lesion and a carcinoma resected metachronously from seven patients. ADH, atypical ductal hyperplasia; Atyp. Papilloma, atypical papilloma; DCIS, ductal carcinoma *in situ*; IDC, invasive ductal carcinoma; LCIS, lobular carcinoma *in situ*. S409, S304, and so forth indicate loci of chromosome markers: *D16S409*, *D16S304*, and so forth; NI, not informative; ND, not done; ●, LOH; ○, no LOH.

resected later by mastectomy but not in previous biopsy specimens of the ADH and in the later lobular carcinoma *in situ*. In Patient 3, LOH was detected in the previously resected atypical papilloma but not in the IDC resected later (Fig. 5). The same results were observed for Patient 4, in which the earlier low-papillary DCIS/atypical papilloma had LOH, but the later IDC did not. In Cases 5 and 6, LOH was only detected in the carcinomas resected later but not in the previous ADH or atypical papilloma (Fig. 6). In Patient 7, data on LOH was not available for the ADH.

LOH was absent at any loci on chromosome 16q examined in epithelial cells in the 13 usual ductal hyperplasias and 9 usual papillomas (Fig. 7). Only one usual papilloma was judged to have microsatellite instability at only one (*D16S512*) of the two informative loci. In the six ADH cases, two (33%)

FIGURE 5. Loss of heterozygosity (LOH) on chromosome 16q in an atypical hyperplastic lesion and a carcinoma resected metachronously from Case 3. (A) Earlier atypical papilloma and (B) later ductal carcinoma. Abscissa, size of polymerase chain reaction (PCR) product; ordinates, intensity of fluorescence; darker curves, PCR products from normal cells; lighter curves, PCR products from cells of the atypical papilloma or carcinoma. LOH is detected only in the atypical papilloma (A, arrow).

FIGURE 6. Loss of heterozygosity (LOH) on chromosome 16q in an atypical hyperplastic lesion and a carcinoma resected metachronously from Case 6. (A) Earlier atypical papilloma and (B) later ductal carcinoma. Abscissa, size of polymerase chain reaction (PCR) product; ordinates, intensity of fluorescence; darker curves, PCR products from normal cells; lighter curves, PCR products from cells of the atypical papilloma or carcinoma. LOH is detected only in the carcinoma (B, arrow).

revealed LOH on 16q: one (Case 25) had LOH at distal loci (*D16S305* and *D16S393*) but not at a proximal site (*D16S304*). The other (Case 26) showed LOH at *D16S512* and *D16S393* but not at other loci.

DISCUSSION

LOH on chromosome 16q is frequently detected in both DCIS and invasive carcinomas of the breast, irrespective of histological grade or type (8–11). However, recent studies have disclosed that the statuses of chromosome 16 markedly differ between the group comprising noncomedo DCIS and low- to intermediate-grade invasive carcinomas and another group comprising comedo DCIS and high-grade invasive carcinomas. In the former

Case/Age	16q chromosome marker									
	S409	S304	S408	S514	S512	S515	S303	S518	S305	S393
Hyperplasia										
1/50	ND	○	ND	NI	ND	ND	NI	ND	ND	NI
2/44	ND	NI	ND	○	NI	ND	ND	ND	NI	NI
3/39	○	ND	ND	○	ND	NI	ND	ND	ND	NI
4/49	○	NI	ND	○	NI	NI	NI	ND	○	○
5/42	○	NI	NI	○	○	○	NI	○	○	○
6/47	NI	○	NI	NI	ND	NI	NI	ND	NI	○
7/44	ND	ND	ND	○	○	○	NI	NI	○	○
8/42	ND	NI	ND	NI	NI	NI	NI	○	NI	NI
9/31	ND	NI	NI	○	NI	○	NI	○	○	NI
10/43	○	NI	NI	NI	NI	○	NI	○	NI	○
11/51	NI	NI	○	○	NI	○	NI	ND	NI	NI
12/49	NI	NI	○	○	○	NI	NI	○	○	NI
13/49	ND	NI	○	NI	NI	○	NI	○	○	○
Papilloma										
14/50	ND	NI	ND	NI	NI	ND	ND	ND	NI	ND
15/43	○	○	ND	○	○	○	ND	ND	NI	NI
16/34	ND	NI	ND	○	MI	ND	ND	NI	NI	ND
17/45	○	○	ND	○	ND	○	NI	○	NI	○
18/47	NI	NI	NI	○	ND	NI	ND	ND	NI	○
19/42	NI	NI	NI	○	NI	○	ND	ND	ND	○
20/29	ND	○	NI	○	ND	ND	ND	ND	ND	NI
21/38	○	○	○	○	NI	NI	ND	NI	NI	NI
22/50	NI	○	○	NI	NI	NI	ND	○	○	○
23/61	○	○	NI	○	NI	○	ND	○	NI	○
ADH										
24/39	NI	NI	ND	NI	ND	ND	ND	ND	ND	○
25/47	ND	○	ND	NI	NI	NI	ND	ND	●	●
26/50	ND	○	○	NI	●	○	ND	ND	○	●
27/50	NI	○	○	NI	ND	NI	NI	ND	○	○
28/51	○	NI	ND	NI	ND	ND	ND	ND	ND	○
29/61	○	○	NI	○	NI	○	NI	○	NI	○

FIGURE 7. A summary of microsatellite loss-of-heterozygosity analysis in usual ductal hyperplasias, usual papillomas, and atypical ductal hyperplasias. MI, microsatellite instability; S409, S304, and so forth indicate loci of chromosome markers: *D16S409*, *D16S304*, and so forth; NI, not informative; ND, not done; ●, LOH; ○, no LOH.

TABLE 1. Cases

Case	Age, y (1st/2nd)	Interval between 1st and 2nd Surgery (y)	Histology of Tumor	
			1st Biopsy	2nd Surgery
1	39/55	16	ADH	IDC, G3
2	56/62	5.5	Duct papillomatosis with slight atypia	DCIS, LCIS
3	33/49	16.1	Intraductal papilloma with atypia	IDC, G3
4	42/48	5.8	Low-papillary DCIS/atypical papilloma	IDC, G2
5	45/57	11.2	ADH/duct papillomatosis	DCIS
6	53/59	5.3	Intraductal papilloma with atypia	IDC, G2
7	58/60	2.7	Duct papillomatosis with atypia	DCIS, IDC

ADH, atypical ductal hyperplasia; IDC, invasive ductal carcinoma; G3, Grade 3; DCIS, ductal carcinomas in situ; LCIS, lobular; carcinoma in situ; G2, Grade 2.

group, LOH on 16q is mostly accompanied by the surplus 1q arm(s) and clonal fusion(s) between the 1q and the residual fragment of chromosome 16, which generates a translocation *der(16)t(1;16)(25–27)*.

The incidence of LOH on 16q was similar between carcinomas and atypical proliferative lesions of the breast but was very low or absent in usual ductal hyperplasias or usual papillomas. It was confirmed that there was a continuous spectrum between ADH/atypical papilloma and low-grade DCIS in terms of high incidence of LOH on 16q, as well as

histological similarity. These atypical lesions would be situated as the earliest developmental stage of the group of low-grade DCIS/low- to intermediate-grade IDC. The incidence of *der(16)t(1;16)* formation in these atypical lesions remains to be elucidated.

The pattern of LOH on 16q always differed between metachronous atypical proliferative lesions and carcinomas. In some cases, LOH on 16q was only detected in the later carcinoma, and the carcinoma might have occurred directly from residual ADH/atypical papilloma by the accumulation of other chromosome alterations, such as LOH on 16q, and subsequent inactivation of certain tumor-suppressor genes. However, in two patients, the later carcinoma was not considered to develop simply by the addition of chromosomal changes because LOH was only detected in the earlier atypical proliferative lesion. Examining the data from the seven cases, we could instead conclude that the atypical proliferative lesion and the carcinoma were usually clones, probably originated from a field with these clones, and that the former was not the direct precursor of the latter.

The ADH and atypical papilloma have been shown to be indicators of high risk of breast cancer (3–5, 7, 28). However, these cancers occurred after the excision of those atypical lesions. Therefore, there might be a field of macroscopic size for cancer predisposition in the breast, or the whole breast might be the field for the predisposition. Even after the ADH or atypical papilloma was removed, the cell in the field could transform later to another ADH/atypical papilloma or to a malignancy. In such a field, certain predisposing factors might induce LOH on 16q and carcinogenesis in a relatively random manner, as well as ubiquitous proliferation of mammary gland cells.

LOH also has been shown to occur frequently in unremarkable mammary glands adjacent to cancer tissue (29). That result also suggested that there is a “localized predisposed region” from which the cancer arises, and in that study, genomic instability appeared to occur throughout the patch area of the predisposed region. Otherwise, the whole breast may be the predisposed region. The present result supports the idea that such a predisposed region existed in the present seven cases. The detection of LOH on specific chromosomal regions in a noncancerous epithelial component of mammary glands might be reasonable as the tool for monitoring the levels of genomic instability and cancer predisposition. Larger studies with analysis of a broader spectrum and other markers will be necessary to further our understanding of these elements of carcinogenesis in the breast.

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