Preferential Occurrence of Breast Carcinomas with Loss of Chromosome 16q and der(16)t(1;16)/der(1;16) in Middle-aged Patients with Hyperplasia of Mammary Glands

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Structural and numerical alterations of chromosome 16 are considered to be commonly involved in the genesis of breast cancer. To reveal etiological factors that predispose cells to these alterations, we examined the frequencies of chromosome 16 aneusomy, 16q loss and 1;16 fusion indicating der(16)t(1;16)/der(1;16) by multi-color fluorescence in situ hybridization in 46 tumors resected mostly from young (∥≤≤≤≤34 years old) or elderly (≥≥≥≥75 years old) women, and compared the results with those in a patient group representing a common age distribution of Japanese patients in whom chromosome 16 status in the tumor had already been studied. The correlation of these chromosome 16 alterations with age, hyperplasia in adjacent mammary glands, cancer history, and obesity indices was investigated in a total of 244 patients. In the present 46 tumors, the frequency of 16q loss and der(16)t(1;16) did not differ between 20 younger patients (30% and 15%) and 23 elderly patients (43% and 13%). However, the incidences of 16q loss and der(16)t(1;16) were low in comparison with the values of 64% and 38% in the 198 Japanese patients representing the common age distribution (P<<<<0.001). In addition, 16q loss and der(16)t(1;16) were more frequent in tumors arising in hyperplastic mammary glands (68%, 44%) than in those without (52%, 24%) (P<0.01). Such correlations were not evident for 16cen aneusomy. Other etiological factors examined were not correlated with these chromosome 16 alterations. The 16q loss and der(16)t(1;16) formation were more frequently involved in the development of breast cancer in middle-aged patients than in young and elderly patients. High-level estrogens and/or sensitivity of mammary glandular cells to estrogens might induce breast cancers with structural changes of chromosome 16.

Key words: Cancer etiology — Loss of chromosome 16 — Der(16)t(1;16)/der(1;16) — Breast neoplasms — Hyperplasia of mammary gland

Allele loss of the long arm of chromosome 16 (16q) is one of the most frequent chromosomal alterations and is suggested to be involved in the genesis of breast cancer.1—5 By fluorescence in situ hybridization (FISH), numerical and structural alterations of chromosome 16 have been detected in 87% of breast cancers and, in nearly half of cancers with 16q loss, the residual part of chromosome 16 was fused to a surplus fragment of 1q, forming der(16)t(1;16)/der(1;16).6 Most of the 1;16 fusion was found at or near α- or β-satellite repetitive sequences on both fragments of chromosomes 16 and 1.7—9 Because these repetitive sequences are a hot spot of DNA recombination, the loss of 16q with or without the 1;16 fusion is suggested to occur by heterologous chromosome pairing and subsequent DNA recombination.8, 10—11

In the previous study, der(16)t(1;16) formation was detected more frequently in the breast cancers occurring in younger patients.6 This result suggested that etiological factors can differ between der(16)t(1;16)-positive and negative breast cancers. In general, estrogens are believed to influence the occurrence of breast cancers in both premenopausal and postmenopausal women through inducing proliferation of and DNA damage in mammary glandular epithelial cells.12—14 Genetic predisposition to familial breast cancers, often found in younger patients, is in part explained by germ-line mutation of the Brca1 or Brca2 gene,15, 16 whereas aging is considered to overlap as a carcinogenic factor in elderly patients.

In vitro, the treatment of human lymphoid cells with 5-azacytidine induces undercondensation of the heterochromatin of the cell nucleus, the heterologous pairing of chromosomes 1 and 16, and structural alterations of these chromosomes, e.g., loss or gain of 1q and 1;16 fusions.16, 10, 11 Therefore, certain predisposing factors might induce structural alterations of chromosome 16 in mammary epithelial cells.

To reveal such host factors in vivo, we examined the prevalence of numerical and structural chromosome 16 alterations in 46 tumors resected from patients aged

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mostly ≤34 years or ≥75 years by multi-color FISH. We also compared the incidences with those in a group representing the common age distribution of Japanese patients, in whom chromosome 16 status in the tumors had already been studied by multi-color FISH. Furthermore, in a total of 244 primary breast cancers, we investigated the correlation of these chromosomal changes with etiological host factors, i.e., patients’ age, hyperplasia of adjacent mammary glands, menopausal status, cancer history, and Broca’s and Quetelet’s indices of overweight.

**MATERIALS AND METHODS**

**Patients** Fresh tumor tissue samples were obtained from 46 primary breast carcinomas resected from patients who received surgery to treat primary breast carcinoma between 1991 and 1996. These 46 tumors comprise 20 carcinomas from 19 young (≤34 years old) patients, 23 carcinomas from 23 elderly (≥75 years old) patients, and three carcinomas from two middle-aged patients. The tissue samples were frozen at −80°C and thawed on ice immediately before imprinting onto glass slides. The samples on the slides were fixed with Carnoy’s solution and hardened at 65°C overnight.

**FISH** The 46 breast cancers were analyzed by two-color FISH. DNA probes used were pSE16-2, an α-satellite repetitive sequence localized at D16Z2 on 16cen; pHuR195, a β-satellite repetitive sequence localized at D16Z3 on 16q11.2; cCJ52-105 localized at D16S154 on 16q24.3; and pUC1.77, a β-satellite repetitive sequence localized at D1Z1 on 1q12. Using a fluorescence microscope (BX50-34-FLA-1, Nikon, Tokyo), two observers (H.T. and T.T.) counted the fluorescent signals in 100–200 nuclei through a D-F-T triple-band-pass filter, an F-T double-band-pass filter or a B-2E filter (Chroma, Brattleboro, VT). The modal signal counts, which were regarded as representative of each tumor, were compared among the 16cen, 16q11 and 16q24 loci in each tumor. The tumor was defined to have chromosome 16 disomy if the modal count was two 16cen signals per nucleus, and the tumor was defined to have chromosome 16 aneusomy if the count was other than two per nucleus.

When there was a disparity in the signal counts between 16cen and 16q11 or between 16q11 and 16q24, the tumor was judged to have a 16q breakage. In the present study, tumors with the disparity had a larger signal number proximally than distally. Such a 16q breakage was defined as 16q loss. When the signal counts were identical at these three loci, the tumors were judged to have no 16q loss.

A 1;16 fusion, indicating der(16)t(1;16)/der(1;16) formation, was judged to exist in a tumor when the percentage of co-localized signals of D1Z1 and D16Z2/D16Z3 was greater than the mean of the control specimens plus five times the standard deviation (SD), or >24% for D16Z2-D1Z1 fusion and >30% for D16Z3-D1Z1 fusion.

The presence of chromosome 16 aneusomy at the 16cen locus, 16q loss, and der(16)t(1;16)/der(1;16) formation had been studied in 198 primary breast carcinomas by the same multi-color FISH method. In these 198, 13 tumors showed complex 16q breakages including partial gain or DNA amplification at the D16Z3 or D16S154 locus. This group was considered to represent a common age distribution in Japanese patients as the control group and comprised 184 patients aged 35–74 years, six aged ≤34 years, and eight aged ≥75 years (Table I).

**Histological examination** Mammary gland tissue surrounding each tumor was evaluated by light microscopy. Mammary glands were not observed for one patient who

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**Table I. Chromosome 16 Alterations Detected by FISH in Breast Carcinomas Examined in This Study and Previous Study**

| Age of patients | Total | 16cen aneusomy | 16q loss | der(16)t(1;16)/der(1;16) | Complex breakage |
|-----------------|-------|----------------|---------|-------------------------|-----------------|
| **A. This study (years old)** |       |                |         |                         |                 |
| ≤34             | 20    | 12             | 6       | 3                       | 0               |
| 35–74           | 3     | 2              | 2       | 0                       | 0               |
| 75≤             | 23    | 14             | 10      | 3                       | 0               |
| Subtotal        | 46    | 28             | 18      | 6                       | 0               |
| **B. Previous study (years old)** |       |                |         |                         |                 |
| ≤34             | 6     | 5              | 2       | 0                       | 0               |
| 35–74           | 185   | 109            | 124     | 74                      | 13              |
| 75≤             | 7     | 5              | 2       | 1                       | 0               |
| Subtotal        | 198   | 119            | 127     | 75                      | 13              |
| **Total (A+B)** | 244   | 147            | 145     | 81                      | 13              |
received tumor excision only. The glands were defined to have usual hyperplasia if two or more of the following were seen: adenosis or blunt duct adenosis, epitheliosis or duct papillomatosis, sclerosing adenosis, lobular hyperplasia, and fibroadenomatosis. This definition coincides very closely with those of usual ductal hyperplasia by O’Connell et al.,22) hyperplasia of usual type by Lakhani et al.,23) or proliferative breast disease by Kasami et al.24)

**Patient factors** From medical charts, patients’ age, age of the first childbirth, menopausal status, family history of breast cancer within second-degree relatives, and patients’ history of contralateral breast cancer or other cancers at the time of initial surgery were evaluated. Quetelet’s index was calculated as weight (kg) ÷ height (m)², and Broca’s index was calculated as weight (kg) ÷ [0.9 × {height (cm) − 100}].25)

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Fig. 1. Numerical and structural alterations of chromosome 16 in invasive carcinoma detected by two-color FISH analysis. A and B: Five D16Z2 signals (green) and five D1Z1 signals (red) per nucleus are seen. Two D16Z2 signals are co-localized with D1Z1 signals (arrowheads). C and D: Two D16Z3 signals (red, arrowheads) and five D1Z1 signals (green) are seen in each cell. E and F: Two signals each of D16Z3 (red) and D16S154 (green, arrowheads) per nucleus are seen. The cancer cells were judged to have 16cen pentasomy, proximal 16q breakage, and der(16)t(1;16)/der(1;16) formation. The nuclear contour is drawn in white ink. A and C: D-F-T triple-band-pass filter; B, D, and E: F-T double-band-pass filter; F: B-2E filter. Original magnification×1000.
RESULTS

We detected 16cen aneusomy in 28 of the 46 tumors (61%), 16q loss in 18 (39%), der(16)t(1;16)/der(1;16) in 6 (13%), and complex 16q breakages in 0 (Table I, Fig. 1). The chromosome 16 aneusomy, 16q loss and der(16)t(1;16)/der(1;16) were detected in 12 (60%), 6 (30%) and 3 (15%) of 20 younger patients and in 14 (61%), 10 (43%) and 3 (13%) of 23 elderly patients, respectively, and these incidences did not differ significantly between the two age groups.

In the previous studies, 16cen aneusomy, 16q loss and der(16)t(1;16)/der(1;16), and complex breakages were detected in 119 (60%), 127 (64%), 75 (38%) and 13 (7%), respectively, in the control group of 198 breast carcinomas.6,17 The incidence of 16q loss and der(16)t(1;16)/der(1;16) in the present tumors was significantly lower than in the control 198 tumors (P<0.001), but the incidence of 16cen aneusomy did not differ. In the combined 244 tumors, 147 (60%) showed 16cen aneusomy, 145 (59%) showed 16q loss, 81 (33%) showed der(16)t(1;16)/der(1;16), and 13 (5%) showed complex 16q breakages.

In the combined group, there was no difference in mean patient age between cancers with 16q loss (54.3, range 25–81, SD 12.4) and cancers without 16q loss (54.4, range 26–87, SD 17.8). In 85% (123/145) of the patients with cancers with 16q loss, the age range was 40–74 years. In contrast, the patients with cancers without 16q loss were distributed broadly in age (Fig. 2A). Breast cancer with 16q loss was detected in 69% (123/179) of patients 40–59 years old, but in only 34% (6/35) of patients 25–39 years old and 40% (12/30) of patients ≥75 years old (P<0.001).

Likewise, in the combined group, there was no difference in mean patient age between cancers with 1;16 fusion (52.4, range 32–83, SD 11.0) and those without (55.4, range 25–87, SD 16.4). Seventy-two percent (58/123) of patients with cancer with 1;16 fusion received surgery in their 40s and 50s, but only 42% (65/153) of patients with cancer without 1;16 fusion received surgery at this age (Fig. 2B). The prevalence of cancer with 1;16 fusion was 47% (58/123) in patients 40–59 years old, but only 18% (6/35) in patients 25–39 years old and 20% (17/86) in the patients ≥60 years old (P<0.001). In contrast, there was no correlation between 16cen aneusomy in tumors and age distribution of patients (Fig. 2C). The 13 patients with breast cancer with complex breakages were distributed between 35 and 74 years old.

Hyperplasia of the mammary glands adjacent to the cancer was correlated with age of the 243 informative patients (Fig. 3). The hyperplasia was seen mostly in patients of 40–54 years old (mean 48.0 years, SD 10.4), but the average age of patient without the hyperplasia was 60.5 (SD

Predisposing Factors for Breast Cancer
Table II. Association of Patient Variables with Chromosome 16 Alterations in 244 Breast Carcinomas

| Variable                                      | Clonal chromosome 16 alterations | Number of tumors (%) |
|-----------------------------------------------|----------------------------------|----------------------|
|                                               | Total                            | 16cen aneusomy       | 16q loss           | der(16)t(1;16) /der(1;16) | Complex 16q breakage |
| 1. Hyperplasia of mammary glands              |                                  |                      |                    |                           |                      |
| Positive                                      | 116                              | 64 (55)              | 79 (68)            | 51 (44)                  | 6                    |
| Negative                                      | 124                              | 80 (64)              | 65 (52)            | 30 (24)                  | 7                    |
| Lactating change                              | 2                                | 1                    | 0                  | 0                        | 0                    |
| Unknown                                       | 2                                | 2                    | 1                  | 0                        | 0                    |
| 2. Menopausal status                          |                                  |                      |                    |                           |                      |
| Premenopausal                                 | 110                              | 65 (59)              | 65 (59)            | 39 (35)                  | 5                    |
| Postmenopausal                                | 133                              | 81 (61)              | 80 (60)            | 42 (32)                  | 8                    |
| Unknown                                       | 1                                | 1                    | 0                  | 0                        | 0                    |
| 3. Family history of breast cancer            |                                  |                      |                    |                           |                      |
| Present                                       | 35                               | 21 (60)              | 21 (60)            | 11 (31)                  | 2                    |
| Absent                                        | 206                              | 123 (60)             | 122 (59)           | 69 (33)                  | 11                   |
| Unknown                                       | 3                                | 3                    | 2                  | 1                        | 0                    |
| 4. Patient history of contralateral breast cancer |                                |                      |                    |                           |                      |
| Present                                       | 34                               | 22 (65)              | 24 (71)            | 15 (44)                  | 0                    |
| Absent                                        | 207                              | 122 (59)             | 119 (57)           | 65 (31)                  | 13                   |
| Unknown                                       | 3                                | 3                    | 2                  | 1                        | 0                    |
| 5. Patient history of other cancer            |                                  |                      |                    |                           |                      |
| Present                                       | 23                               | 12 (52)              | 15 (65)            | 10 (43)                  | 2                    |
| Absent                                        | 218                              | 132 (61)             | 128 (59)           | 70 (32)                  | 11                   |
| Unknown                                       | 3                                | 3                    | 2                  | 1                        | 0                    |
| 6. Broca’s index [weight (kg) ÷ (0.9 × (height (cm) − 100))] |                  |                      |                    |                           |                      |
| ≥1.21                                         | 76                               | 47 (62)              | 46 (61)            | 22 (29)                  | 4                    |
| 1.01–1.20                                     | 101                              | 60 (59)              | 60 (59)            | 35 (35)                  | 6                    |
| ≤1.00                                         | 64                               | 37 (58)              | 37 (58)            | 23 (36)                  | 3                    |
| Not done                                      | 3                                | 3                    | 2                  | 1                        | 0                    |
| 7. Quetelet’s index [weight (kg) ÷ height (m²)] |                                |                      |                    |                           |                      |
| ≥30.1                                         | 28                               | 12 (43)              | 16 (57)            | 8 (29)                   | 1                    |
| 25.1–30.0                                     | 23                               | 16 (70)              | 13 (57)            | 7 (30)                   | 1                    |
| 20.1–25.0                                     | 145                              | 89 (61)              | 88 (61)            | 50 (34)                  | 8                    |
| ≤20.0                                         | 45                               | 27 (60)              | 26 (58)            | 15 (33)                  | 3                    |
| Not done                                      | 3                                | 3                    | 2                  | 1                        | 0                    |
| Total                                         | 244                              | 147 (60)             | 145 (59)           | 81 (33)                  | 13                   |

a) P < 0.01; b) P = 0.05.
lial cells in vitro. It is possible that exposure to high-level estrogens and/or sensitivity to estrogens might predispose the epithelial cells to structural aberrations in chromosome 16q, as well as hyperplasia.

Estrogen receptor and progesterone receptor positive statuses are known characteristics of hormone dependency of breast cancer related to the age of onset. In the previous study, the presence of der(16)(t(1;16) was shown to be correlated with a higher amount of hormone receptors in the tumor.

Der(16)(t(1;16) formation has also been reported in Ewing’s sarcoma and myelodysplastic syndrome, which are not hormone-related neoplasms. It is still possible that nonspecific etiology causes der(16)(t(1;16), or predisposing factors other than hormones might also induce der(16)(t(1;16) during the development of these cancer types.

The prevalence of 16q loss and der(16)(t(1;16)/der(1;16) was similar between young and elderly patients, but the prevalence was significantly lower than that in middle-aged patients. The prevalence of 16cen aneusomy in tumors did not differ among age groups. These data suggest that aging of the host does not play a significant role in the accumulation of numerical and structural aberrations in chromosome 16 in breast cancer.

Breast carcinomas with 16q loss and der(16)(t(1;16) tended to be associated with a history of contralateral breast cancer, although the correlation was not significant. This tendency might in part be derived from the fact that invasive ductal carcinoma of tubular subtypes and invasive lobular carcinoma, in which clonal 16q loss and der(16)(t(1;16)/der(1;16) are detected most commonly, tend to occur bilaterally. Family cancer history, past history of cancer other than breast cancer, and indices for overweight were not correlated with the status of chromosome 16 in tumors, and those factors appeared to have little relationship with accumulation of chromosome 16 alterations.

Asian women have a lower incidence of breast cancer, a better prognosis, and a prevalence at a younger age than Western women. Whether such a demographic difference derives from molecular events is not known. The incidence of 16q loss appears to be similar in European and Japanese patients. In Japanese women, der(16)(t(1;16)/der(1;16) was detected by FISH in 33% of breast carcinomas, whereas the incidence of 1;16 fusion was estimated by karyotypic analyses to be only 8–20% of presented cases in reports from Sweden, Norway, Denmark, Iceland, and the Netherlands. If the percentage of carcinomas with 1;16 fusion differs strikingly between Western and Asian patients, the etiological factors and molecular mechanisms of mammary carcinogenesis would differ substantially between the two. Accurate comparative studies using identical methods are needed to elucidate
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