Frequency of Chromosome 7 Gain in Human Breast Cancer Cells: Correlation with the Number of Metastatic Lymph Nodes and Prognosis

KEIZO HIRATA, YUTAKA TAGAWA, KIYOTAKA KASHIMA, HIDEKO KIDOGAWA, MASAIRO DEGUCHI, TAKASHI TSUJI and HIROYOSHI AYABE

The First Department of Surgery, Nagasaki University School of Medicine and 1School of Allied Medical Sciences, Nagasaki 852-8501

HIRATA, K., TAGAWA, Y., KASHIMA, K., KIDOGAWA, H., DEGUCHI, M., TSUJI, T. and AYABE, H. Frequency of Chromosome 7 Gain in Human Breast Cancer Cells: Correlation with the Number of Metastatic Lymph Nodes and Prognosis. Tohoku J. Exp. Med., 1998, 184 (2), 85-97 —— Trisomy 7 has been reported in various malignant neoplasms, but there are no reports in breast cancer. In order to evaluate the contribution of chromosome 7 gain to breast cancer, we investigated the relationship of numerical aberration of chromosome 7 with clinicopathological variables and prognosis in seventy-nine breast cancer cases (invasive carcinomas) using the technique of fluorescence in situ hybridization (FISH) on paraffin-embedded sections. A significant correlation of the frequency of cells with extra copies of chromosome 7 (percent polysomy 7 cell score) was found with tumor size, regional lymph node status, TNM stage, histological extension, estrogen receptor (ER), and DNA ploidy. The number of metastatic lymph nodes was positively correlated with percent polysomy 7 cell score (correlation coefficient = 0.623, p < 0.01). Furthermore, cases with a high percent polysomy 7 cell score had a shorter disease-free survival and overall survival times, especially in the lymph node positive group. It was demonstrated that percent polysomy 7 cell value was closely associated with lymph node metastasis and prognosis and might be a useful prognostic predictor of breast cancer patients. —— breast cancer, trisomy 7; FISH © 1998 Tohoku University Medical Press

Trisomy 7 has been seen in various malignant neoplasms and in benign lesions, such as synovial tissue from patients with rheumatoid arthritis (Ernis et al. 1993) and colon adenoma (Longy et al. 1993). It has been reported that trisomy 7 may be the hallmark of premalignant changes in lung cancer (Lee et al. 1987; Testa and Siegfried 1992), that the copy number of chromosome 7 is strongly correlated with tumor grade in human bladder cancer (Waldman et al.

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Address for reprints: Keizo Hirata, The First Department of Surgery, Nagasaki University School of Medicine, 1–7–1 Sakamoto, Nagasaki 852-8501, Japan.
1991), that trisomy 7 could be of some importance in tumor progression in colorectal carcinoma (Bardi et al. 1991), and that trisomy 7 may be indicative of a neoplastic process in thyroid tumors (Hermann and Lalley 1992). Bandyk et al. (1994) reported that the frequency of trisomy 7 cells increased in the advanced stage prostate cancer. The fact that epidermal growth factor receptor (EGFR), platelet derived growth factor a (PDGFA), hepatocyte growth factor (HGF), and c-met/HGF receptor gene are located in chromosome 7 prompted our interest in trisomy 7 of breast cancer. Yamashita et al. (1994) found that immunoreactive HGF concentration in tumor extract of human breast cancer was the most important independent factor in predicting relapse-free and overall survival. Overexpression of c-met/HGF receptor oncogene in scirrhouis type stomach cancer (Kuniyasu et al. 1992), colorectal cancer (Liu et al. 1992; Di Renzo et al. 1995), and thyroid cancer (Di Renzo et al. 1992) has been reported.

In the present study, we analyzed the numerical abberations of chromosome 7 in breast cancer using the technique of fluorescence in situ hybridization (FISH) on paraffin-embedded sections and investigated the relationship of percent polysomy 7 cell score with clinicopathological factors and the prognosis of breast cancer patients.

**Materials and Methods**

**Patients**

The participants were seventy-nine patients with primary female breast cancer who had undergone an operation at the First Department of Surgery, Nagasaki University Hospital, Japan and at the relative hospitals between 1981 and 1994. The mean age was 55.1 years, with a range of 32 to 85 years. All cases were invasive carcinomas and the histological types were papillotubular carcinoma (15 cases), solid-tubular carcinoma (33 cases), scirrhous carcinoma (24 cases) and others (7 cases). The stages were stage I (27 cases), stage II (24 cases), stage III (18 cases) and stage IV (10 cases).

**FISH analysis**

Cancerous tissues, metastatic lymph nodes, and noncancerous tissues of the resected mammary gland as a control from breast cancer patients were used for analysis. Paraffin-embedded 10% formalin-fixed tissue sections with a thickness of 5 μm were mounted on silane-coated glass slides. Tissue pretreatment and hybridization were conducted as described before (Morinaga et al. 1993; Hirata et al. 1995). After deparaffinization, FISH was performed using a digoxigenin-labeled alpha-satellite DNA probe specific for chromosome 7 (D7Z1; Oncor, Gaithersburg, MD, USA). The probes were labeled with FITC (Anti-digoxigenin-fluorescein, Fab fragments) and nuclei were counterstained with propidium iodide (PI). FITC signals were counted (magnification, ×400) using a fluorescent microscope (Olympus, Tokyo) and regarded as the copy number of
chromosome 7. FITC signals in each nucleus between one hundred and two hundred were counted in the cancerous region and in the noncancerous breast tissue in comparison with the continuous section which was stained with hematoxylin-eosin. The frequency of the cells which had an extra copy of chromosome 7 (percent polysomy 7 cell score) was calculated in each region and the association with clinicopathological factors and prognosis was investigated.

Other parameters

Flow cytometric analysis was carried out on the fresh frozen specimen using the FACScan (Becton Dickinson, San Jose, CA, USA).

As for the prognosis, we divided the cases into two groups by the mean value of the percent polysomy 7 cell scores (25.3%) and the prognosis for 10 years was analyzed between the 7 high polysomy group (percent polysomy 7 cell score ≥ 25.3%) and the 7 low polysomy group (percent polysomy 7 cell score < 25.3%). We subdivided these groups into two subgroups by the number of metastatic lymph nodes (n = 0~3, n ≥ 4) and analyzed their differences in terms of prognosis. Clinicopathological factors and clinical stage were based on “The General Rules for Clinical and Pathological Recording of Breast Cancer” issued by the Japanese Breast Cancer Society (1996). The tumors were classified according to clinical stage using the new TNM (tnm) classification.

Statistical methods

Statistical analysis was conducted by ANOVA. Survival curves were calculated by the Kaplan-Meier method, and the significance of the difference between curves was evaluated using log-rank tests. The χ² test was used to examine the significance of the difference between groups in the frequency of various attributes. The criterion of significance was taken as a p value of 0.05 or less.

Results

A photomicrograph of fluorescence in situ hybridization on paraffin-embedded section of a primary breast cancer using a digoxigenin-labeled alpha-satellite DNA probe specific for chromosome 7 is demonstrated in Fig. 1. Some cancer cells have three signals or more.

Relationship between percent polysomy 7 cell score and clinicopathological variables

Fig. 2 shows the relationship with TNM (tnm) factors of breast cancer. Significant associations of percent polysomy 7 cell scores were found with tumor size, lymph node status, and clinical stage. Percent polysomy 7 cell score increased with larger tumor size (significant between T1 and T3, T4 [p<0.05] and between t1 and t3, t2 and t3 [p<0.01]). Significant associations were found between n0 and n1β, between n0 and n2, between n1α and n1β, and between n1α
and n2 ($p < 0.05$). As shown in Fig. 3, a positive correlation was seen between the percent polysomy 7 cell scores and the number of metastatic lymph nodes (correlation coefficient $= 0.623$, $p < 0.01$). There was no difference between the percent polysomy 7 cell scores of primary tumors and those of the metastatic lymph nodes (Table 1). As to Tnm staging and tmn staging, percent polysomy 7 cell scores increased significantly with advanced stage. Percent polysomy 7 cell score in noncancerous breast tissues was less than 5%, whereas that in cancerous tissues was more than 15% even in patients at stage I.

Fig. 4 shows the relationship of the percent polysomy 7 cell scores with the histological extension, DNA ploidy, estrogen receptor (ER), progesterone receptor (PgR), and histology. The ER-negative group had a significantly high percent polysomy 7 cell score and the PgR-negative group had a high score, but without significance. Concerning histological extension, the “s” group (extended to the skin) and the “p” group (extended to the major pectoral muscle) had higher scores of percent polysomy 7 cells compared with the “g” group (remaining in the gland) and the “f” group (extended to the fat tissue). The DNA aneuploidy group showed significantly higher percent polysomy 7 cell scores ($p < 0.01$). Percent polysomy 7 cell scores tended to increase when the stromal density was rich (data not shown). There were not significant relationships between histological types. Chromosome 7 gain was rarely seen in the noncancerous breast tissue, but was often seen in the cancerous tissue specimen from the same patient. We investigat-
Fig. 2. Correlation of percent polysomy 7 cell score with clinicopathological factors (TNM factors). Bars, s.e.; *p < 0.05; **p < 0.01.

Fig. 3. Relationship between percent polysomy 7 cell score and the number of metastatic lymph nodes. Correlation coefficient = 0.623; p < 0.01.
Table 1. **Distribution of chromosome 7 copy number in primary and metastatic breast tumors of four patients**

<table>
<thead>
<tr>
<th>Case Tnm stage</th>
<th>Tumor</th>
<th>Percent cells with number of chromosome 7 copies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Case 1</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>Case 1</td>
<td>9</td>
<td>81</td>
</tr>
<tr>
<td>Case 2</td>
<td>6</td>
<td>84</td>
</tr>
<tr>
<td>Case 2</td>
<td>10</td>
<td>81</td>
</tr>
<tr>
<td>Case 3</td>
<td>5</td>
<td>70</td>
</tr>
<tr>
<td>Case 3</td>
<td>6</td>
<td>70</td>
</tr>
<tr>
<td>Case 4</td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td>Case 4</td>
<td>8</td>
<td>59</td>
</tr>
</tbody>
</table>

T, tumor; LN, lymph node.

![Graph showing correlations](image)

Fig. 4. Correlation of percent polysomy 7 cell score with other clinicopathological factors. Bars, s.e.; *p < 0.05; **p < 0.01; n.s., not significant.

The difference in the percent polysomy 7 cell score between the central portion and the extended portion of the tumor, however, there was no difference found between them (Table 2).

**Prognostic analysis**

Disease-free survival and overall survival analyzed according to the percent polysomy 7 cell scores, lymph node status (positive/negative), and the number of metastatic lymph nodes are shown in Figs. 5, 6 and 7. Fig. 5 shows that the
TABLE 2. Distribution of chromosome 7 copy number in the central portion and extended portion of primary tumors of four breast cancer patients

<table>
<thead>
<tr>
<th>Case Tnm stage</th>
<th>Portion of tumor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 5 III</td>
<td>Central portion</td>
<td>0</td>
<td>40</td>
<td>33</td>
<td>14</td>
<td>13</td>
<td>60</td>
</tr>
<tr>
<td>Case 6 III</td>
<td>Central portion</td>
<td>0</td>
<td>84</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Case 7 III</td>
<td>Central portion</td>
<td>4</td>
<td>79</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Case 8 III</td>
<td>Central portion</td>
<td>0</td>
<td>45</td>
<td>53</td>
<td>2</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Case 8 III</td>
<td>Central portion</td>
<td>3</td>
<td>20</td>
<td>51</td>
<td>23</td>
<td>3</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>30</td>
<td>51</td>
<td>16</td>
<td>2</td>
<td>69</td>
</tr>
</tbody>
</table>

s, extended to the skin; f, extended to the fat tissue; p, extended to the major pectoral muscle.

Fig. 5. Disease-free survival and overall survival of breast cancer patients by percent polysomy 7 cell score. ○ (49 cases), 7 low polysomy (percent polysomy 7 cell score ≥ 25.3%); □ (30 cases), 7 high polysomy (percent polysomy 7 cell score < 25.3%). *p < 0.05; **p < 0.01.
Fig. 6. Disease-free survival and overall survival of breast cancer patients by lymph node metastasis (positive/negative) and percent polysomy 7 cell score. ○ (5 cases), n(−)/7 high polysomy; ■ (19 cases), n(−)/7 low polysomy; ◊ (31 cases), n(+)7 low polysomy; ▲ (24 cases), n(+)7 high polysomy; n.s., not significant.

disease-free survival times and the overall survival times of the 7 high polysomy group were significantly shorter than those of the 7 low polysomy group (p < 0.01, p < 0.05, respectively).

When the disease-free survival and the overall survival were analyzed by lymph node status (positive and negative) and percent polysomy 7 cell score, those of the 7 low polysomy subgroups were more favorable than those of the 7 high polysomy subgroups in the lymph node positive group (Fig. 6). The disease-free survival and the overall survival of four subdivided groups according to the percent polysomy 7 cell scores and lymph node status (the number of metastatic lymph node, that is, n = 0–3, n ≥ 4) were analyzed (Fig. 7). The disease-free survival times and the overall survival times of the 7 high polysomy/n ≥ 4 group were shorter than those of the other groups. Both in the n = 0–3 groups and the n ≥ 4 groups, the disease-free survival and the overall survival times of the 7 low
polytomy groups were longer than those of the 7 high polytomy groups.

**Discussion**

Our findings showed that the percent polytomy 7 cell scores of breast cancer tissues increased significantly with advance in stage and with the progression of histological extension. In particular, it was associated closely with lymph node metastasis and there was a positive correlation between the percent polytomy 7 cell scores and the number of metastatic lymph nodes. This was also seen when the cases were classified into n1α, n1β, n2, and n3 and when classified into groups in which the number of metastatic lymph nodes was 0, 1–3, and more than 4. It is interesting that there was a significant difference between n1α and n1β. This indicated that three is a significant number in lymph node metastasis of breast cancer. As lymph node status is the most important and independent prognostic
factor, the percent polysomy 7 cell score, which is closely associated with lymph node status, may also be a possible prognostic factor. Furthermore, we divided the lymph node-positive group into two subgroups by the mean value of the percent polysomy 7 cell score and it was found that the lymph node-positive/the 7 high polysomy subgroup had the poorest prognosis. When we divided the cases into two groups by the number of metastatic lymph nodes, that is, 0-3 and $\geq 4$, the 7 high polysomy subgroups had the poorer prognosis. This finding shows that it might be possible to distinguish the subgroup with the poorer prognosis from each lymph node status group by the percent polysomy 7 cell score.

It was presumed that increase in chromosome 7 was associated with the progression of breast cancer, because PDGFA, EGFR, HGF (7q21), and c-met (HGF receptor, 7q21-31) are located in chromosome 7. On the other hand, Bieche et al. (1992) reported that LOH of 7q was associated with advanced breast cancer. Atkin and Baker (1993) reported chromosome 7q deletions on 13 malignant tumors. There is no denying that chromosome 7 contains a tumor suppressor gene. In addition, Dal Cin et al. (1992) reported that trisomy 7 cells were often seen in tumor infiltrating lymphocytes in renal cell carcinomas. However, we did not find trisomy 7 cells in tumor infiltrating lymphocytes or fibroblasts in the surrounding tissues.

In breast cancers, an increase in polysomy 7 cells was seen significantly more in the DNA aneuploidy group than in the DNA diploidy group. Accordingly, it was deduced that an increase of chromosome 7 occurs frequently in breast cancer tissues with chromosomal numerical aberrations detected by flow cytometry. Bello et al. (1994) reported that molecular detection of gain of material from chromosome 7 (c-met, EGFR, and PDGFA) was obtained when trisomy 7 occurred with other chromosomal aberrations in malignant gliomas. It is probable that chromosome 7 gain has significance also in breast cancer when it accompanies other chromosomal numerical aberrations.

Breast cancer loses expression of the estrogen receptor or the progesterone receptor with progression of the tumor. Percent polysomy 7 cell score increased in ER-negative groups, suggesting that the percent polysomy 7 cell score is associated with the biological malignancy of the tumor cells. Percent polysomy 7 cell score inclined to increase when the stroma in the cancerous tissue is rich (data not shown). It is considered that the expression of c-met increased, when chromosome 7 increased, and the signal transmission of the paracrine loop (HGF/c-met) was reinforced still more when the site of production and storage of HGF was abundant. Wang et al. (1994) reported an increase in HGF mRNA in breast cancer cells. Ebert et al. (1994) reported that c-met was overexpressed in pancreatic cancer in association with increased levels of HGF mRNA. It was suggested that the breast cancer cell, which is an epithelial cell, produced HGF and an autocrine loop appeared, leading to progression of the tumor. Further investigations are required to clarify the relationships among the gain of chromosome 7,
HGF, and c-met.

Giordano et al. (1993) transfected c-met gene into the fibroblast that expressed HGF and found an increase in invasiveness rather than proliferation and Iwazawa et al. (1996) reported that an esophageal cancer cell line, which expressed HGF receptor, showed diverse invasiveness into the underlying gel containing fibroblasts, but did not invade gel without fibroblasts. Our data also showed that the percent polysomy 7 cell score increased with the advance of histological extension. However, it appeared that the extended portion of the tumor did not have a higher percent polysomy 7 cell score than the central portion. Accordingly, the percent polysomy 7 cell score is likely to increase on the whole when breast cancer extends to the surrounding tissues.

EGF receptor gene is also located in chromosome 7. It has been suggested that trisomy 7 is important in malignant disease through overexpression of the EGFR. However, Elfving et al. (1992) reported that no effect was seen when trisomy 7 cells were cultured with EGF, suggesting that it is not EGFR, but HGF or c-met that is probably responsible for progression of the breast cancer with polysomy 7 cells.

The measurement of polysomy 7 cells using FISH is simple and the procedure can be conducted within 1-2 hours. Accordingly, we consider it to be a more useful technique than the measurement of other factors such as HGF and EGFR. Paraffin-embedded sections were used in this study, however, fresh specimens such as touch smear of biopsy and needle biopsy are also available. We expect that it could be a useful diagnostic technique, including use in the prediction of prognosis.

There are some reports that chromosome 7 gain is related to malignancy of the tumor in various other organs. We reported here that chromosome 7 gain was also related to malignancy of breast cancer and the percent polysomy 7 cell score might be a useful prognostic factor in breast cancer patients.

Acknowledgment

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References


