Association of Multiple Copies of the c-erbB-2 Oncogene with Spread of Breast Cancer

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ABSTRACT

Amplification of c-erbB-2 was at least three times more frequent in breast cancer than in most other types of carcinoma, and was not found in sarcomas or hematological malignancies. Amplification of c-erbB-2 was found in 15 of 86 primary breast cancers and in 3 of 12 secondary breast cancers. Amplification was more common in breast tumors of advanced stage, and in tumors which had metastasized to regional lymph nodes sites. Gene amplification was observed in 21% (4 of 19) of primary tumors which recurred within 3 years of mastectomy and in 6% (2 of 32) of nonrecurrent tumors.

INTRODUCTION

Altered protooncogenes have been implicated in the pathogenesis of certain human and animal malignant tumors. Three of the known protooncogenes encode molecules related to cellular growth factors or their receptors. c-erbB-1 encodes a receptor for EGF (1). c-sis encodes the B chain of platelet-derived growth factor (2). c-fms encodes a molecule which is related to or identical with the receptor for macrophage colony-stimulating factor (3).

c-erbB-1 is a normal gene, which is homologous to v-erbB, the transforming gene of avian erythroblastosis virus (1, 4). Recently, another protooncogene has been identified in the human genome, c-erbB-2, which also has structural similarity with v-erbB (5). Genes similar to c-erbB-2 have also been identified in the human genome by other investigators and called HER-2 (6) and the -erbB-related gene (7). Moreover, the neu oncogene, originally identified in a series of ethylnitrosourea-induced rat neuroblastomas, has been found to be related to v-erbB (8). For the sake of simplicity, we shall continue to use the designation c-erbB-2, since comparison of the nucleotide sequences of these v-erbB-related genes revealed that they are the same gene. Both HER-2 and the human counterpart of neu have been mapped to the same chromosomal locus (17q21) as c-erbB-2 (6, 9, 10).

The protein product of the c-erbB-2 gene resembles the EGF receptor (5, 6, 8). Polyclonal antibodies to this receptor cross-react with the M, 185,000 rat c-erbB-2 gene product (8), and comparison of the human c-erbB-2 gene product with the EGF receptor reveals about 50% homology in amino acid sequence (6, 11). Based on the deduced amino acid sequence of the c-erbB-2 protein, it has been postulated that c-erbB-2 encodes a growth factor receptor similar to the EGF receptor. However, the growth factor ligand which binds to this putative receptor has not yet been identified.

Because many tissues express c-erbB-2 transcripts, it was not possible to deduce the role of c-erbB-2 either in normal tissues or in oncogenesis (6). However, when we surveyed a number of human cancers, we observed amplification of c-erbB-2 restricted to carcinomas of glandular epithelial origin (adenocarcinomas). Amplification was not found in squamous cell carcinomas, sarcomas, or hematological malignancies (12). This correlated with other observations that c-erbB-2 was amplified in an adenocarcinoma cell line, and suggested that the gene encodes a growth factor receptor associated with glandular epithelium. Because we observed two instances of amplification of c-erbB-2 in a small series of cases of carcinoma of the breast (12), and because amplification of this gene has previously been observed in another case (7), we undertook a larger survey of breast cancers for alterations of the c-erbB-2 gene.

PATIENTS AND METHODS

Breast cancer tissues were obtained from 98 patients undergoing modified radical mastectomy between 1978 and 1984. In 11 cases, normal breast tissues were available from the same surgical procedure. In 86 cases, the primary tumors were analyzed and in 12, tumors metastatic to a regional lymph node were available for analysis. Other solid tumors were obtained at surgery and leukemic cells were obtained from the peripheral blood. All tissues were stored at −70°C until analyzed. High molecular weight DNA was extracted and 10 µg were digested with restriction endonucleases HindIII, EcoRI or BamHI as previously described (12, 13). The digests were electrophoresed on 0.8% agarose gels, and the fractionated DNAs were denatured and transferred to Bio-Rad nylon membranes (ICN). The 3.0-kilobase HindIII-KpnI fragment of human c-erbB-2 complementary DNA clone pCER204 (12) was prepared as a c-erbB-2-specific probe. The 2.4-kilobase CiaI-CiaI fragment of human EGF receptor complementary DNA clone pE7 was prepared as a c-erbB-1/EGF receptor-specific probe (12). The filters were hybridized with 32P-labeled (nick translated) c-erbB-2 or c-erbB-1/EGF receptor probes, washed at a final concentration of 0.1 × saline sodium citrate with 0.1% sodium dodecyl sulfate at 65°C, dried, and exposed to Kodak XAR-5 film. The intensity of the hybridization signal was quantitated with a Bio-Rad Model VD620 optical densitometer. The filters were then hybridized with a human β-globin probe and the intensity of this signal was used as an internal control for the amount of DNA in the filters as previously described (13). Amplification of c-erbB-2 was defined as at least a 3-fold increase in the intensity of the hybridization signal relative to control normal DNA. In order to determine whether the increased c-erbB-2 hybridization signal was the result of gene amplification or chromosomal duplication, another chromosome 17 probe was also used on the same filters (14).

Hospital charts were reviewed to determine size of primary tumors, lymph node involvement, grade of histological differentiation, interval to recurrent disease, hormone receptor status, sites of metastases, and interval to death. Recurrence or progression of disease at 36 months after initial surgery was determined by chart review. Stage of disease was defined by the criteria of the American Joint Committee on Cancer after histological analysis of axillary lymph nodes. Histological grade of tumor was determined by several different pathologists at the time of tumor resection, and was reviewed by one of us (H. B.) without knowledge of the oncogene analysis.

The method of χ2 analysis was used to ascertain the statistical significance of differences between groups of breast cancers with differing anatomic or behavior patterns. Reported levels of statistical significance were not adjusted for numbers of comparisons.
RESULTS

Two hundred forty-eight malignant tumors were examined for alterations of the c-erbB-2 oncogene. Tumors examined included adenocarcinomas, squamous carcinomas, sarcomas, leukemias, lymphomas, neuroendocrine, germ cell, childhood, and brain tumors (Table 1). As previously observed, alterations of the c-erbB-2 oncogene were confined to the subgroup of adenocarcinomas (12). Amplification of this gene was detected in 22 of 186 adenocarcinomas (12%). It was particularly common in breast cancer and was observed in 15 of 86 primary tumors (17%) and in 3 of 12 metastatic breast cancers (25%). The only other adenocarcinoma with frequent amplification of c-erb-B-2 was gastric carcinoma, and amplification was observed in 2 of 8 of these tumors (Table 1). It was rare in most other adenocarcinomas; amplification was observed in only 1 of 45 colon cancers and in none of 12 hepatomas or 7 lung adenocarcinomas.

Amplification of c-erb-B-2 in breast cancers varied between 3-fold (Fig. 1) and 30-fold (Fig. 2). In each case hybridizations to a β-globin probe was used as an internal control of the gene copy number. Amplification of c-erbB-2 was an acquired abnormality in breast tumors. Two cases, previously reported, had c-erbB-2 amplification in the primary tumor or in a metastasis, but not in normal breast tissue (12).

Amplification of c-erbB-2 was more common in breast cancers of stages III and IV than in stage I and II tumors (N = 58; P < 0.01; Table 2), and amplification of c-erbB-2 was somewhat more common in primary tumors with metastasis to regional lymph nodes (8 of 37) than in primary tumors which were determined to be confined to the breast by standard morphological analysis (1 of 21). However, the difference between lymph node-positive and -negative tumors was of borderline statistical significance (P = 0.08; Table 2). Only primary tumors were included in this analysis.

Amplification of c-erbB-2 was also more common in those breast cancers which recurred. Amplification of c-erbB-2 was more frequent in primary breast cancers of stages I to III which recurred within 3 years of mastectomy (4 of 19 = 21%) than in tumors which did not recur (2 of 32 = 6%) (P = 0.06; Table 2).

The relationship between stage of disease, c-erbB-2 amplification and tumor recurrence or progression is also shown in Table 3. Several points in Table 3 are worth commenting upon since

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**Table 1** Frequency of c-erbB-2 amplification in breast and other cancers

<table>
<thead>
<tr>
<th>Tumor</th>
<th>No. of cases</th>
<th>Number with c-erbB-2 amplification</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>86</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Secondary</td>
<td>12</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Adenocarcinomas*</td>
<td>88</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Sarcomas</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hematological malignancies</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other tumors</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Colon (45), ovary (11), gastric (8), renal (5), hepatoma (12), and lung (7).

* Includes 2 of 8 gastric carcinomas.

* Squamous, neuroendocrine, Wilms', hepatoblastoma, brain, and germ cell.

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**Table 2** Correlation between amplification of c-erbB-2 protooncogene and characteristics of primary breast cancer

<table>
<thead>
<tr>
<th>Tumor characteristic</th>
<th>No. studied</th>
<th>No. with c-erbB-2 amplification</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I and II</td>
<td>34</td>
<td>2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stage III and IV</td>
<td>24</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Axillary nodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>21</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>No recurrence, stages I-III</td>
<td>32</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>Recurrence, stages I-III</td>
<td>19</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>No recurrence, progression at all stages</td>
<td>34</td>
<td>3</td>
<td>0.08</td>
</tr>
<tr>
<td>Recurrence, or progression at all stages</td>
<td>23</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

* Studies in which only metastases were examined were excluded from this analysis.

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**Table 3** Correlation between stage, progression, or recurrence and c-erbB-2 amplification in primary breast cancers

<table>
<thead>
<tr>
<th>Primary cancers</th>
<th>Stage I c-erbB-2</th>
<th>Stage II c-erbB-2</th>
<th>Stage III c-erbB-2</th>
<th>Stage IV c-erbB-2</th>
<th>Uncertain c-erbB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurred or progressed</td>
<td>13</td>
<td>0</td>
<td>19</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Did not recur or progress</td>
<td>10</td>
<td>0</td>
<td>15</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

* Neg., negative; Amp., amplification.
they indicate that the c-erbB-2 oncogene is an imperfect guide to tumor recurrence. Seven stage I and II breast cancers recurred within 36 months. None of these had detectably amplified c-erbB-2. Furthermore, two stage II tumors with amplified c-erbB-2 did not recur in this time period. However, the 4 stage III tumors with c-erbB-2 amplification, all recurred.

There was no apparent correlation of the frequency of c-erbB-2 amplification with any of the following parameters, the estrogen receptor status of the tumor, histological type, histological grade, size of the tumor, and age of the patient.

**DISCUSSION**

King et al. (7) originally identified a 5- to 10-fold amplification of c-erbB-2 in a human mammary carcinoma. Our observations in 248 human malignancies, including 86 primary breast cancers, suggest that c-erbB-2 amplification is about three times more frequent in breast cancers than in other adenocarcinomas except perhaps gastric cancer, and is many times more common in breast cancer than in squamous carcinomas, sarcomas, neuroendocrine tumors, and hematological malignancies (Table 1). In these latter tumors the gene is rarely if ever found to be amplified. There were increased copies of the c-erbB-2 gene in 17% of our series of 86 primary breast tumors and in 18% of another recent series (15).

EGF receptors are the product of the c-erbB-1 protooncogene, and are highly expressed on the membranes of some squamous carcinomas (16) and brain tumors (17). The c-erbB-1 gene is amplified, rearranged, or translocated in some of these malignancies but not in other types of human tumors. These observations suggest that alterations in the c-erbB-1/EGF receptor gene are associated with induction or progression of malignancy of squamous epithelium. In contrast, alterations of the c-erbB-2 gene in humans have thus far been limited to tumors of glandular epithelium (12). We report here that amplification of this gene is particularly common in breast cancer. Certain correlations between increased copies of the c-erbB-2 gene and the clinical characteristics of primary breast cancers were noted. c-erbB-2 amplification was more common in advanced primary cancers than in early stage primary cancers. Increased copies of this gene were found in 29% of primary tumors of stages III and IV, in none of 13 stage I cancers, and in only 2 of 21 stage II cancers. Stage reflects both size of the tumor and the presence of metastases. Therefore the data were also analyzed in regard to the presence or absence of morphologically identifiable metastases in lymph nodes and distant sites. Amplification of c-erbB-2 was found in only 1 of 21 tumors confined to the breast, but was found in 22% of cases with regional lymph node metastases. An intriguing observation is that the recurrence of tumors stages I through III within 3 years of mastectomy was about three times as frequent in those cancers which had increased numbers of c-erbB-2 genes than cancers lacking this alteration. Such a correlation has been observed by other investigators (15). However, it should be noted that the correlation between breast cancer recurrence and c-erbB-2 amplification was not statistically significant in our series. Only when additional protooncogenes, including c-myc, c-ras-Ha, c-mycb and c-ras-ki are examined does the difference become statistically significant (18).

The high incidence of c-erbB-2 amplification in breast cancers, and the frequent association of c-erbB-2 amplification with primary breast cancers which have metastasized at the time of initial diagnosis and surgical treatment, suggest that activation of c-erbB-2 often plays a role in the progression and spread of breast cancers. A recent study provides evidence that breast cancers with c-erbB-2 amplification overexpress the protein (19). One can speculate that an increase in numbers of a growth factor receptor may provide these adenocarcinoma cells with a proliferative advantage. The possible prognostic significance of this observation suggests that alterations of this oncogene should be examined prospectively in a larger number of cases of breast cancer.

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**REFERENCES**


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