Follow-up Study of HER-2/neu Amplification in Primary Breast Cancer

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ABSTRACT

Amplification of the HER-2/neu oncogene was determined in 362 tumors from patients with primary breast cancer (185 node-negative patients and 177 node-positive patients). The overall amplification rate was 33% (30% for node-negative patients; 31% for patients with 1–3 positive nodes; 40% for patients with >3 positive nodes). Gene copy number was not associated with axillary lymph node status, steroid receptor status, or patient age but was weakly correlated with the size of the primary tumor. Amplification of the HER-2/neu gene did not correlate with either disease-free or overall survival in univariate or multivariate analyses. The results were unambiguously negative for patients with node-negative disease. Although the univariate results for node-positive patients were marginally significant (P = 0.07), the significance was not retained in multivariate analyses. Thus, while HER-2/neu amplification may be biologically important in primary breast cancer, it will only be of marginal utility as a prognostic factor for predicting clinical outcome.

INTRODUCTION

The HER-2/neu oncogene has been the subject of heated debates concerning its prognostic significance for women with breast cancer. In our initial report, we found that the HER-2/neu gene was amplified in 28% of the 189 primary breast tumors examined (1). This amplification was directly related to the number of axillary lymph nodes that were involved with tumor but was unrelated to other prognostic factors including steroid receptor status, tumor size, and patient age. The most exciting finding was that HER-2/neu amplification was an important predictor of disease recurrence and death for 86 patients with axillary node-positive disease.

Several reports have now been published by other investigators who have also studied HER-2/neu amplification in human primary breast tumors (2–26). The reported amplification rates range from 10 to 46% (Table 1), with an overall amplification rate of 20% based on 2992 patients. In general, patients with node-positive disease have greater frequency of amplification than patients with node-negative disease. However, there has been considerable controversy regarding the correlations between HER-2/neu amplification and clinical outcome. Most of these reports have described relationships between gene amplification and other prognostic factors but have not been able to correlate amplification with disease outcome because of small numbers of patients with short follow-up times. Of those studies that have examined clinical correlations, six have reported a direct relationship between HER-2/neu amplification and poor survival (1, 3, 14–16, 23), and three studies have claimed that gene amplification is not related to patient outcome (9, 12, 24).

An additional study (23) observed a significant relationship with early relapse and death in univariate analyses that disappeared in multivariate analyses. Several possible explanations for low amplification frequency and lack of clinical correlations have been proposed (10), including inadequate analytical techniques, suboptimal statistical analyses, and small numbers of patients.

In this study, we have expanded our previous study design to include 185 additional node-positive patients and 177 node-negative patients in order to examine the prognostic ability of HER-2/neu amplification for patients with primary breast cancer.

MATERIALS AND METHODS

Patients. The tumor specimens used in this study were nuclear pellets selected from our San Antonio Tumor Bank, which has been described previously (27). These tumors were originally sent to our laboratory for determination of steroid receptor content. After completion of the receptor assays, the remaining nuclear pellets were stored in freezers (−70°C) to be used in future studies. Demographic characteristics, treatment information, and follow-up status of each patient for disease recurrence and mortality are routinely collected by a specialized team of data managers.

The eligibility criteria for inclusion in this study were a tumor specimen obtained between 1973 and 1985 at the time of diagnosis from a woman with primary breast cancer, evaluable steroid receptor results, documented tumor size, and age of the patient at the time of diagnosis. The study was designed to yield approximately 400 evaluable specimens for HER-2/neu amplification analysis assuming a 60% evaluable rate.

Oncogene Amplification. HER-2/neu amplification was determined in a single laboratory by Southern analysis as previously described (1, 10, 15) in a blinded fashion without knowledge of the patient's clinical outcome or tumor characteristics. In brief, DNA from the individual tumors was digested with EcoRI and subjected to Southern blot analysis with a 32P-labeled HER-2/neu-1 probe (provided by Axel Ullrich) which is known to detect a 13-kilobase hybridizing band in human DNA. All DNA blots were stripped and reprobed with both p53 and myeloperoxidase probes to evaluate the relative loading of DNA in each lane and to exclude the possibility that amplification was caused by partial or complete duplication of chromosome 17. Blots were scanned by soft laser densitometry and the level of HER-2/neu amplification was determined by the ratio of the HER-2/neu signal relative to the single-copy p53 signal.

Statistical Analyses. The data were maintained in an AT&T 3B15 minicomputer using the Informix data base management system (Informix Software, Menlo Park, CA). Statistical analyses were performed with the BMDP statistical package (BMDP Statistical Software, Los Angeles, CA). For statistical analyses, HER-2/neu gene amplification was coded as: 1, single copy; 2, 2–5 copies; 5, 5–20 copies; 20, >20 copies. Other prognostic factors were dichotomized as steroid receptor negative versus steroid receptor positive (using 3 and 5 fmol/mg protein for ER and PgR, respectively), the number of positive axillary lymph nodes (1–3 versus >3), tumor size (<2 versus >2 cm), and age (<50 versus >50 years). Correlations between HER-2/neu amplification and other prognostic factors were evaluated using χ2 tests for trends. The primary end points in this study were disease-free survival and overall survival. Disease-free survival was defined as the interval between the diagnostic biopsy and the first recurrence of breast cancer. Patients who died without documented disease recurrence were considered censored.

Received 3/5/90; accepted 11/20/90.

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1 This work was supported in part by NIH Grant CA 30195.

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3 The abbreviations used are: ER, estrogen receptor; PgR, progesterone receptor.
sored for disease-free survival but were included as deaths when analyzing overall survival. Curves for disease-free and overall survival were calculated according to the method of Kaplan and Meier (28). The differences between curves were assessed with the log-rank test for censored survival data (29). The partially nonparametric regression model of Cox (30) was used to evaluate the predictive power of various combinations of prognostic factors in a multivariate manner.

RESULTS

A total of 662 frozen nuclear pellets obtained after performing steroid receptor assays on frozen biopsy specimens from patients with primary breast cancer were selected for inclusion in this study (323 from patients with node-negative breast cancer and 339 with node-positive disease). DNA was success-fully extracted from 362 (55%) of these samples. The primary reasons for failure to obtain DNA were (a) too few viable cells, and (b) degradation of the DNA that was extracted. There were no significant differences between patients with evaluable HER-2/neu results and patients without results with respect to number of positive axillary lymph nodes, ER status, PgR status, tumor size, and the administration of chemotherapy (Table 2). Slightly more patients with evaluable results received endocrine therapy (29%) compared to patients without results (21%) (P = 0.01), but this did not translate into significant differences between the two groups of patients with respect to either disease-free or overall survival.

The overall HER-2/neu amplification rate for the 362 evaluable patients was 33% (30% for node-negative patients; 31% for patients with 1–3 positive nodes; 40% for patients with >3 positive nodes). This compares favorably with the results in our original group (28% for patients with 1–3 positive nodes and 51% for patients with >3 positive nodes). HER-2/neu amplification was not associated with lymph node status, ER status, PgR status, age of the patient, or administration of adjuvant endocrine or chemotherapy (Table 3). There was a trend for larger tumors to have increased numbers of copies of the HER-2/neu gene (P = 0.06). Adjuvant systemic therapy (chemotherapy plus or minus endocrine therapy) was administered to 78% of the node-positive patients but to only 20% of node-negative patients. There was a tendency (P = 0.10) for node-positive patients who received adjuvant chemotherapy to have had an amplified HER-2/neu gene (39%) compared to untreated patients (31%), but administration of chemotherapy was unrelated to amplification of the gene. In contrast, only 15% of node-negative patients who received adjuvant chemotherapy had an amplified gene compared to 33% who were not treated (P = 0.055).

A total of 362 patients had evaluable HER-2/neu results. The median follow-up of these patients was 75 months (83 months for patients still alive at the time of analysis). Disease-free and overall survival are displayed in Fig. 1 and 2, respectively, by the number of copies of the HER-2/neu gene. Log-rank tests comparing the four groups of patients (single copy, 2-5 copies, 5-20 copies, >20 copies) revealed a significant trend (P = 0.01) for patients with >3 positive nodes to have decreased survival compared to patients with >5–20 copies.
Consistent with the results for the combined analyses, HER-2/neu amplification was not a significant predictor of clinical outcome for the 177 patients with node-negative disease, either as a single factor or in multivariate analyses (Fig. 3). In fact, node-negative patients with amplified HER-2/neu had slightly better clinical outcomes than patients with a single copy of the gene. Similarly, gene amplification was not predictive of patient outcome in the 185 node-positive patients (Fig. 3). When copy number was analyzed as a continuous factor, a marginally significant trend was observed between gene copy number and disease-free survival (P = 0.11), but no relationship was found for overall survival (P = 0.39). In multivariate analyses, the number of positive nodes was the most important predictor of disease-free survival (P = 0.0042), and HER-2/neu amplification remained only marginally significant (P = 0.10). ER status and the number of positive nodes were the only significant predictors of overall survival. This is in contrast to our original group of 86 node-positive patients in which HER-2/neu amplification was the most important predictor of both disease-free and overall survival.

DISCUSSION

The HER-2/neu gene was amplified in 33% of our primary breast tumors. This is consistent with our previous series of patients (1) but is slightly higher than many of the reports in

Multivariate analyses (Table 4) revealed that lymph node status, PgR status, and tumor size predicted disease recurrence, but only tumor size was statistically significant for predicting overall survival. After adjustment for other prognostic factors, HER-2/neu amplification was not a significant predictor of either disease-free or overall survival regardless of how gene amplification was represented in the models.

Since the only significant correlations that have been described in the literature between HER-2/neu amplification and clinical outcome are in patients with positive axillary lymph nodes, we next performed subgroup analyses by lymph node status.
the literature (Table 1). It might be argued that our choice of p53 as a standard of comparison might artificially inflate our amplification rate since there is now evidence that alterations of the p53 gene can occur in human breast cancer (32, 33). We have addressed this issue previously (10) by using separate probes for myeloperoxidase and p53. The p53 gene is found on the short arm of chromosome 17, while the myeloperoxidase gene is found on the long arm of chromosome 17 near the HER-2/neu gene. The use of both probes provided an assessment of the tumors for duplication of either all or part of chromosome 17 and thus addressed any increase in signal resulting from duplication of the chromosome. In no case that was called amplified did we find evidence for chromosomal duplication. Furthermore, if one allele for p53 was deleted, this would result in a maximum possibility of overestimating HER-2/neu copy number by a factor of 2. However, the presence of DNA from normal cells present in every breast tumor specimen with normal alleles for p53 would dilute this potential overestimate to a factor of always <2.

Amplification of the HER-2/neu gene was independent of the axillary lymph node status, the steroid receptor status, and the age of the patient but was weakly related to the size of the primary tumor. There is little agreement in the literature concerning the relationships, or lack of relationships, between HER-2/neu amplification and other prognostic factors. Some studies have found no association between gene amplification and lymph node status (6, 12, 14, 15, 19, 24), while others have reported a weak relationship (1, 5, 7, 21–23, 26). Several studies have reported an association with steroid receptor status (7, 13, 18, 19, 23, 24), but others have failed to confirm this (1, 6, 12, 22, 26). None of the studies have found relationships with age, and only two have reported a correlation with tumor size (23, 26).

The strong correlations between HER-2/neu amplification and disease-free and overall survival that were observed among our original cohort of 86 node-positive patients could not be confirmed in this new group of patients. The actuarial 5-year disease-free survival probabilities for the patients in this study were 70 ± 3% and 65 ± 5%, respectively, for patients with unamplified and amplified tumors. However, before concluding that HER-2/neu is not a significant prognostic factor for patients with primary breast cancer, one must be assured that the sample size is sufficient to justify such a conclusion. With 362 patients at risk for recurrence for at least 5 years, we would have approximately 80% power to detect a 15% difference in 5-year relapse rates between patients with amplified and unamplified tumors [using a two-sided test of proportions at the 5% level of significance adjusted for a 30% amplification rate (31)]. Ideally, a strong prognostic factor should separate patients into distinctive categories with different probabilities of relapse. While differences <15% may be important from a biological point of view, they would only be of marginal utility from a clinical point of view.

The results for the 177 node-negative patients were unambiguously negative. HER-2/neu amplification was not associated with either disease-free or overall survival in either univariate or multivariate analyses. This finding is in agreement with all of the published reports that have attempted to correlate HER-2/neu amplification with disease outcome in node-negative patients. Although the univariate results for the 185 node-negative patients were 70 ±3% and 65 ±5%, respectively, for patients with primary breast cancer, one must be assured that the sample size is sufficient to justify such a conclusion. With 362 patients at risk for recurrence for at least 5 years, we would have approximately 80% power to detect a 15% difference in 5-year relapse rates between patients with amplified and unamplified tumors [using a two-sided test of proportions at the 5% level of significance adjusted for a 30% amplification rate (31)]. Ideally, a strong prognostic factor should separate patients into distinctive categories with different probabilities of relapse. While differences <15% may be important from a biological point of view, they would only be of marginal utility from a clinical point of view.

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