Short Communication

HER-2/*neu* Gene Amplification in Ductal Carcinoma *In Situ* of the Breast[1]

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Abstract

This study evaluated the relative frequencies of HER-2/*neu* gene amplification in ductal carcinoma *in situ* (DCIS)-associated invasive breast cancer and DCIS alone. We examined archival tissue samples of 100 DCIS lesions with an invasive component (cases) and 100 without an invasive component (controls), with cases and controls matched by pathologic nuclear grade. HER-2/*neu* gene amplification was determined by fluorescence *in situ* hybridization. We compared HER-2/*neu* gene amplification in DCIS lesions only, irrespective of the presence or absence of an invasive component. HER-2/*neu* gene amplification occurred significantly less frequently in the cases (26%) than in the controls (40%), with an odds ratio of 0.35 (95% confidence interval, 0.17–0.72; $P < 0.004$) after adjustment for pathologic and quantitative-image-analyzed morphometric nuclear grade. The HER-2/*neu* gene also was amplified more frequently in higher- than in lower-grade DCIS alone (56% *versus* 19%, respectively; $P < 0.0001$) or in higher- than in lower-grade DCIS with invasive cancer (44% *versus* 2%, respectively; $P < 0.00001$). Future studies should examine the potential roles of HER-2/*neu* and other biomarkers (*e.g.*, p21 and Rb) as markers of the risk of DCIS patients for invasive breast cancer and as molecular targets of chemoprevention in breast intraepithelial neoplasia.

Introduction

DCIS[3] accounts for 12–14% of newly diagnosed breast cancer cases in the United States, and ~50% of untreated DCIS developed an ipsilateral invasive breast cancer within 24 years after the original biopsy (1–4). The biological mechanisms of breast carcinogenesis are not fully understood. The growth factor receptor HER-2/*neu* generally is not overexpressed in normal or benign breast lesions (5–7) and is overexpressed in DCIS lesions. However, Allred *et al.* (7) and others have reported significantly lower HER-2/*neu* expression in invasive carcinoma than in DCIS (5, 6, 8). Despite findings that HER-2/*neu* overexpression in invasive breast cancer is associated with poor prognosis (9, 10), few studies have compared HER-2/*neu* gene amplification in DCIS alone *versus* in DCIS associated with invasive cancer. In this case-control study, we evaluated the relative frequencies of HER-2/*neu* gene amplification in DCIS-associated invasive breast cancer and DCIS alone. The evaluation of HER-2/*neu* gene amplification in DCIS may help identify the highest-risk women and provide a potential molecular target for chemoprevention in this setting.

Materials and Methods

Identification of Patient Material. Paraffin-embedded archival breast tissue specimens and surgical pathology reports for 100 consecutively examined controls (DCIS alone) and 100 consecutively examined cases (DCIS with an invasive component) were identified from the pathology database of The University of Texas M. D. Anderson Cancer Center during the period 1986–1999. H&E-stained slides of each DCIS case and control were reviewed and graded by one pathologist (A. A. S.) using standard grading criteria, as described previously (11). We defined necrosis as the presence of karyorrhectic, pyknotic nuclei associated with eosinophilic debris. Only inspissated secretory material without nuclear debris was not considered to be necrosis. Necrotic material occupying less than or more than one-third of the ductal lumen was classified as focal or extensive necrosis, respectively. Cases and controls were frequency-matched according to nuclear grade. Sociodemographic characteristics and clinical variables were abstracted from patient medical records.

Analysis of HER-2/*neu* Amplification. The analysis of HER-2/*neu* gene amplification was performed by the FISH method using the Vysis Path Vysion HER-2/DNA probe kit (Downers Grove, IL). Before using HER-2/DNA probe, one pathologist (N. S.) examined the H&E-stained specimen to locate the relevant DCIS region of each of the cases and controls, and then marked the DCIS region of interest for gene amplification. Briefly, tissue sections were baked overnight at 56°C. Slides were immersed in xylene to remove paraffin, and tissue sections were dehydrated in 100% alcohol for 5 min and air-dried. Next, slides were immersed in 0.2 N HCl for 20 min to solubilize nuclear proteins. The slides were then immersed in Vysis pretreatment solution for 30 min at 80°C followed by protein digestion, in which tissues were treated with pepsin at 37°C for 10 min. After 10 μl of probe was applied to the target area of the slide, a glass coverslip was placed over the probe and sealed with rubber cement. Denaturation of slides was performed on a hot plate at 73°C for 5 min. Slides were then allowed to

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[3] The abbreviations used are: DCIS, ductal carcinoma *in situ*; FISH, fluorescence *in situ* hybridization; Rb, retinoblastoma.

Table 1 Patient characteristics

Characteristic	DCIS cases[a] (n = 100)	DCIS controls[a] (n = 100)
Demographic/reproductive		
Median age, diagnosis (range)	52 yr (27–88)	51 yr (27–77)
Race		
White	67	79
Black	12	10
Hispanic	15	6
Asian	3	4
Unknown	3	1
Median age, menarche (range)	13 yr (9–17)	13 yr (10–17)
Median age, first childbirth (range)	23 yr (15–38)	24 yr (16–39)
Pathologic/quantitative morphometric		
Nuclear grade 1	6	6
Nuclear grade 2	38	38
Nuclear grade 3	56	56
Necrosis		
Absent	48	32
Focal	20	28
Extensive	32	40
Median z score	8.42	7.67

[a] Cases, DCIS with invasive cancer; controls, DCIS alone.

hybridize overnight at 37°C in a humidified chamber. After the coverslips were removed, the slides were washed in 2× SSC/0.3% NP40 at 73°C to remove excess probes, then counterstained in 4′,6-diamidino-2-phenylindole.

The Path Vysion kit uses two directly labeled fluorescent DNA probes: locus specific identifier (LSI) HER-2/*neu*, which is specific for the *HER-2/neu* gene locus, and chromosome enumerator probe (CEP) 17, which is specific for the α satellite DNA sequence at the centromeric region of chromosome 17. The expected ratio of LSI-2/neu to CEP 17 is 2.0 for normal breast tissue. A ratio >2.0 was considered indicative of *HER-2/neu* amplification. Signals were counted for 60 tumor nuclei. Both positive and negative controls were included. We compared *HER-2/neu* amplification in DCIS lesions only, irrespective of presence or absence of invasive component.

Quantitative Morphometric Nuclear Grading (Z Score). The nuclear images were captured from Feulgen-stained tissue sections and z scores were computed according to Bacus *et al.* (12). Z score is a common scale used for objective quantitative morphometric nuclear grading system. Z scores of normal epithelia are always distributed about a mean value of zero. The individual feature z scores are weighted by a coefficient and summed to compute the quantitative morphometric nuclear grade. Four nuclear features and their weighting coefficients used include nuclear area, DNA, entropy, and valley.

Statistical Analysis. HER-*2/neu* gene amplification status was categorized as amplified or not amplified. Odds ratios and 95% confidence intervals were computed using the logistic regression model. The χ^2 test was used to compare proportions of HER-2/*neu* gene amplification status. The Mann-Whitney U test was used to compare z score values between the cases and controls. All of the statistical computations were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL) and STATA computer software programs (STATA Corporation, College Station, TX). A $P < 0.05$ was considered statistically significant.

Results

The characteristics of the women patients who provided specimens for this study are shown in Table 1. The majority of patients in the case and control groups were white. The cases and controls were similar with respect to median age at diagnosis, menarche, and at first childbirth, and with respect to the numbers of grades 1, 2, and 3 DCIS lesions. We observed no significant differences in focal and extensive necrosis between cases and controls, although controls had more (both focal and extensive) necrotic lesions than did cases. About half of the case DCIS lesions did not have necrosis. The median quantitative morphometric nuclear grades (z scores) were 8.42 and 7.67 for cases and controls, respectively.

Table 2 HER-2/*neu* gene amplification in cases and controls[a]

HER-2/*neu* amplification	DCIS cases[b]	DCIS controls[b]	OR[c]	95% CI	P
No	72 (74)	59 (60)	1.0	—	—
Yes	25 (26)	39 (40)	0.35	0.17–0.72	0.004

[a] OR, odds ratio; CI, confidence interval.
[b] Cases, DCIS with invasive cancer; controls, DCIS alone.
[c] Odds ratios were adjusted for pathologic nuclear grade and quantitative morphometric nuclear grade.

HER-2/*neu* gene amplification was less common in DCIS associated with invasive breast cancer (cases) than DCIS alone (controls). The odds ratio for HER-2/*neu* gene amplification was 0.35 (95% confidence interval, 0.17–0.72) after adjustment for pathologic and morphometric nuclear grade (Table 2). The odds ratio for pathologic nuclear grade (grades 1 and 2 *versus* grade 3) was 1.09 (95% confidence interval, 0.51–2.32) and for quantitative morphometric nuclear grade (z score ≤ 2.67 *versus* z score > 2.67) was 1.81 (95% confidence interval, 0.85–3.83). Furthermore, the odds ratio for HER-2/*neu* (0.35) did not change substantially after adjusted for necrosis (odd ratio 0.40; 95% confidence interval, 019–0.84). HER-2/*neu* gene amplification correlated with nuclear grade among both cases and controls: HER-2/*neu* gene amplification occurred in 44% of grade 3 *versus* 2% of grade 1 and 2 DCIS with invasive cancer (n = 97; $P < 0.00001$), and in 56% of grade 3 *versus* 19% of grade 1 and 2 DCIS alone (n = 98; $P < 0.0001$). As indicated by these data, HER-2/*neu* gene amplification was more pronounced in high-grade DCIS alone than in high-grade DCIS with invasive cancer. Examples of HER-2/*neu* gene amplification are shown in Fig. 1.

Discussion

We found that HER-2/*neu* gene amplification was inversely associated with the risk of invasive progression in DCIS patients, even though it correlated with high-grade lesions in both cases (DCIS-associated invasive cancer) and controls (DCIS alone). The inverse risk association was independent of pathologic and quantitative image-analyzed morphometric (z score) nuclear grade.

Several studies (5–7) have found that normal breast tissue or benign lesions (*e.g.,* hyperplasia with or without atypia) generally do not overexpress HER-2/*neu* protein but that HER-2/*neu* overexpression is more common in DCIS than in invasive cancer (5–8, 13–15). Most of these studies are case-series in which the HER-2/*neu* protein was assayed immunohistochemically. Furthermore, none of the studies used a case-control design to compare HER-2/*neu* protein expression in DCIS alone with this expression in DCIS associated with invasive cancer. We used the FISH technique instead of immunohistochemical assay to evaluate HER-2/*neu* gene amplification and a case-control study design for our comparative analysis of

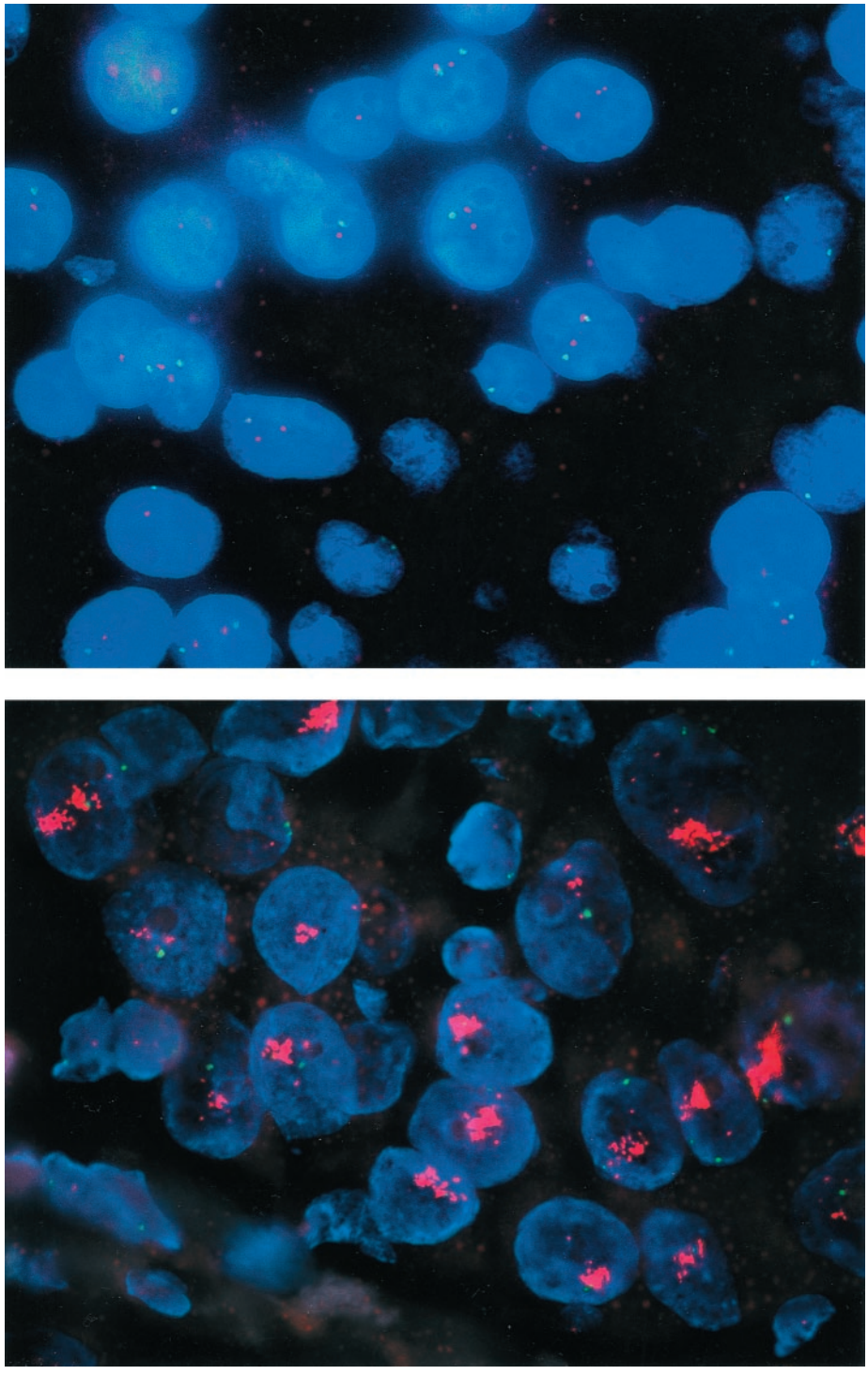

Fig. 1. An example of HER-2/*neu* gene amplification (via FISH method using the Vysis Path Vysion *HER-2*/DNA Probe kit). *Top panel*, no HER-2/*neu* gene amplification in DCIS-associated invasive cancer (×1000); *bottom panel*, HER-2/*neu* gene amplification in DCIS-alone (×1000).

HER-2/*neu* gene amplification. Although our results of HER-2/*neu* gene amplification in DCIS are consistent with earlier reports of HER-2/*neu* protein overexpression (5–8, 13–15), a recent report indicated that ~20% of breast tumors exhibited overexpression of *c-erbB-2* in the absence of *c-erbB-2* gene amplification (16). The biological basis of the higher frequency of HER-2/*neu* gene amplification in DCIS alone than in DCIS-associated invasive cancer remains unexplained.

There is the suggestion that increased cell proliferation and enhanced cell motility associated with overexpressed HER-2/*neu* may contribute to the development and progression of DCIS (17). Another speculation is that the expression of HER-2/*neu* may be down-regulated in a significant proportion of DCIS lesions as they progress to invasive cancer or that a subset of invasive cancer may develop *de novo* by mechanisms independent of HER-2/*neu* (7, 18, 19). Previous reports (20–23)

suggested that malignant tissues overexpress HER-2/*neu* while having diploid copies of the gene, indicating that mechanisms other than gene amplification contributed to the overexpression. Therefore, it is possible that HER-2/*neu* stimulates growth during the *in situ* phase only, and promotes differentiation and growth inhibition as the lesion progresses to invasive cancer. Although this seems counterintuitive, experimental overexpression of epidermal growth factor receptors may induce growth inhibition (24–27) independently of differentiation. This growth inhibition by epidermal growth factor receptors is known to result from induction of the cyclin-dependent kinase inhibitor p21 (28). Furthermore, the transfection and overexpression of HER-2/*neu* in MCF7 human breast cancer cells results in reduced proliferation, induction of p21, Rb hypophosphorylation, and differentiation (29). These regulatory proteins (*e.g.*, p21 and Rb) have not been fully evaluated with respect to their mechanistic relationship with HER-2/*neu* in DCIS patients.

Our continuing studies may help to additionally define the role of HER-2/*neu* in the biology of DCIS, including its associations with p21, Rb, and other biomarkers (30). The potential roles of HER-2/*neu* and other biomarkers, including p21 and Rb, as markers of the risk of DCIS patients for invasive breast cancer and as molecular targets of chemoprevention in breast intraepithelial neoplasia also warrant future studies (31).

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