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HER2 protein overexpression in estrogen receptor-positive ductal carcinoma *in situ* of the breast: frequency and implications for tamoxifen therapy

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Recent clinical data have suggested that the efficacy of tamoxifen in reducing the risk of local recurrence following lumpectomy and radiation therapy in patients with ductal carcinoma *in situ* (DCIS) is limited to patients with estrogen receptor (ER)-positive lesions. However, it is currently not known if HER2 protein overexpression might be associated with reduced tamoxifen benefit in patients with ER-positive DCIS, as has been suggested in patients with ER-positive invasive breast cancer and in preclinical models. Moreover, the frequency of HER2 overexpression in ER-positive ductal carcinoma *in situ* has not been previously evaluated in detail. To address this issue, we studied ER expression and HER2 overexpression in 148 cases of DCIS using a sensitive double immunostaining technique and assessed the frequency of ER expression and HER2 overexpression in relation to each other and in relation to DCIS grade. Overall, ER expression was seen in 114 cases (77%) and HER2 protein overexpression was seen in 42 cases (28%). Of 114 ER-positive ductal carcinoma *in situ*, 14 (12%) showed concurrent HER2 protein overexpression, and all 14 of these DCIS lesions were of high nuclear grade. In addition, in all 14 ER-positive DCIS cases that showed HER2 overexpression, double immunostaining demonstrated that ER and HER2 protein were coexpressed by the same neoplastic cells. We conclude that a subset of ER-positive DCIS show concomitant overexpression of HER2 protein. Whether or not HER2 overexpression is associated with a diminished response to tamoxifen in patients with ER-positive DCIS will require investigation in clinical outcome studies.
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The expression of biological markers in ductal carcinoma *in situ* (DCIS), including hormone receptors, oncogenes, tumor suppressor genes and markers of cell proliferation and angiogenesis, has been an area of active investigation for the past two decades.[1–5] Studies addressing this subject have identified important correlations between the expression of various biomarkers and certain histopathologic features of DCIS, and have served to emphasize the heterogeneous nature of these lesions with regard to their biological characteristics.

Estrogen receptor (ER) and HER2 have arguably been the most widely studied biomarkers in DCIS. Prior investigators have demonstrated that ER expression is seen in approximately 75% of DCIS cases and that the frequency of ER expression varies with the degree of differentiation, being most common in low grade and least common in high grade lesions.[1,6–26] HER2 protein overexpression has been reported in approximately 40% of DCIS, and in most studies is significantly more common in lesions of high-grade than in low-grade lesions.[1,10,12–21,23–44]

Until fairly recently, information regarding biomarker expression in DCIS was largely of academic interest, since the presence or absence of expression of any of these markers did not impact upon patient management decisions. However, in December 2002, Allred *et al*[45] presented the results of a study assessing the relationship between ER expression in DCIS and local recurrence in patients who had been treated by lumpectomy and radiation therapy, with or without tamoxifen. In that study of a subset

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of patients enrolled in the NSABP-B24 randomized clinical trial, a significant reduction of local recurrences with the use of tamoxifen was observed only in patients whose DCIS was ER-positive.[45] As a result of this observation, many clinicians have begun to take the ER status of DCIS into consideration in formulating treatment recommendations and are offering adjuvant tamoxifen only to women with ER-positive DCIS. However, whether or not all patients with ER-positive DCIS will receive an equivalent level of benefit from tamoxifen remains an unresolved issue. In particular, it is not known if the simultaneous presence of HER2 overexpression might limit or negate the beneficial effects of tamoxifen in ER-positive DCIS, as has been suggested in some clinical studies of patients with invasive breast cancer and in preclinical models.[46–54] Given this potential concern, and given the increasing use of tamoxifen in women with DCIS, an understanding of the frequency of HER2 overexpression in ER-positive DCIS assumes clinical importance.

While studies of patients with DCIS have generally shown an inverse relationship between ER expression and HER2 overexpression,[10,23,24,35] details of the relationship between ER and HER2 expression in individual examples of DCIS has previously received little attention. The purpose of this study, therefore, was to assess the frequency of ER expression and HER2 overexpression in DCIS in relation to each other and in relation to DCIS grade, and to determine the frequency and histologic correlates of HER2 overexpression in ER-positive DCIS.

Materials and methods

The study population consisted of 148 cases of DCIS accessioned at Beth Israel Deaconess Medical Center between May 2000 and December 2003 in which there was sufficient DCIS remaining for immunostaining and for which paraffin blocks were available. For each case, all available hematoxylin and eosin-stained sections were reviewed to determine the DCIS nuclear grade and to select a representative block for immunostaining. DCIS lesions were classified as low, intermediate or high nuclear grade using the criteria of Lagios.[55]

We performed double immunohistochemical staining for ER and HER2 using the Envision Double Stain System (DakoCytomation, Carpinteria, CA, USA) on 5-μm paraffin sections cut from one representative block for each case. Sections were mounted on charged glass slides and baked at 58–60°C for 2 h. Slides were allowed to cool to room temperature and were then deparaffinized in xylene and rehydrated through graded alcohols to distilled water. Subsequently, sections were subjected to heat-induced epitope retrieval (HIER) in citrate buffer, pH 6.1 (DakoCytomation Target Retrieval solution) by heating in a vegetable steamer for 40 min followed by cooling for 20 min at room temperature. Following HIER, endogenous peroxidase activity was blocked with 1% hydrogen peroxide in methanol for 10 min. The anti-ER primary monoclonal antibody (clone 1D5, DakoCytomation, 1:50 dilution) was then applied to the sections for 30 min at room temperature, followed by incubation with horseradish peroxidase-labeled polymer and then with an enzyme substrate system that employs 3,3′-diaminobenzidine as the chromogen. The tissue was then treated with Doublestain Blocking Reagent (DakoCytomation) to prevent crossreactivity between the reactions and to block endogenous alkaline phosphatase activity. The anti-HER2 primary antibody (rabbit polyclonal anti-HER2 antibody A0485, DakoCytomation, 1:800 dilution) was applied for 30 min at room temperature followed by incubation with alkaline phosphatase-labeled polymer. The reaction was then completed with a substrate system using Permanent Red (DakoCytomation) as the chromogen. Tissue sections were then lightly counterstained with Mayer's hematoxylin. Two positive controls, one consisting of an invasive breast cancer known to express ER and another consisting of an invasive breast cancer known to show HER2 protein overexpression were included in each staining run. Negative controls in which the primary anti-ER and anti-HER2 antibodies were replaced by phosphate-buffered saline were performed for each case.

Each double-immunostained slide was evaluated for the presence of ER expression and HER2 protein overexpression in the DCIS cells. Tumor cells that showed nuclear staining for ER were considered ER-positive. Of note, all ER-positive cases showed staining in at least 10% of the DCIS tumor cell nuclei, whereas all ER-negative cases showed complete absence of tumor cell staining for ER (but with staining of normal breast epithelial cell nuclei). Tumor cells were considered positive for HER2 protein overexpression when greater than 10% of the cells showed strong membrane staining (equivalent to a score of 3+ in the DakoCytomation HercepTest). ER expression and HER2 overexpression were related to DCIS nuclear grade and the frequency of expression according to nuclear grade was evaluated statistically using Fisher's exact test.

The study was approved by the Beth Israel Deaconess Medical Center Committee on Clinical Investigations.

Results

Among the 148 DCIS cases we evaluated, 18 (12%) were low nuclear grade, 56 (38%) were intermediate nuclear grade and 74 (50%) were high nuclear grade. Overall, 114 cases (77%) were ER-positive and 42 (28%) showed HER2 overexpression.

The relationships between ER expression, HER2 overexpression and DCIS grade are shown in Table 1. All 74 low and intermediate nuclear grade DCIS were ER-positive, whereas ER-positivity was seen in only 40 high-grade lesions (54%). In contrast, HER2-protein overexpression was seen exclusively in high-grade lesions. Thus, ER expression was significantly more common in non-high-grade than in high-grade lesions ($P<0.001$) and HER2 overexpression was significantly more common in high-grade than in non-high-grade lesions ($P<0.001$).

Overall, 100 cases (68%) were ER+/HER2−, 28 (19%) were ER−/HER2+, six (4%) cases were ER−/HER2−, and 14 (9%) were ER+/HER2+ (Figure 1 and Table 2). Thus, ER expression and HER2 overexpression were reciprocally related in 128 cases (86%). The patterns of ER-expression and HER2 overexpression according to DCIS grade are presented in Table 2. All 74 low and intermediate nuclear grade DCIS were ER+/HER2−. In contrast, the high-grade DCIS lesions were more heterogeneous with regard to patterns of ER and HER2 expression. Among the high-grade lesions, 26 (35%) were ER+/HER2−, 6 (8%) were ER−/HER2−, 28 (38%) were ER−/HER2+, and 14 (19%) cases were ER+/HER2+. Thus, coexpression of ER and HER2 was seen only in high-grade DCIS and such cases accounted for 19% of that population.

Of particular interest, among the 114 cases of ER-positive DCIS, 14 (12%) showed concomitant HER2 overexpression. All 14 of these lesions were of high nuclear grade. HER2 overexpression was diffuse in these cases, with strong membrane staining present in all DCIS cells. However, the extent of ER expression in these 14 cases was more variable. In two of these cases, ER expression was present in the nuclei of nearly all of the HER2-positive DCIS cells. In the remainder of these cases, the proportion of DCIS cell nuclei expressing ER was between 10 and

Table 1 ER-expression and HER2 protein overexpression according to DCIS grade

DCIS grade	*Number of cases*	*Number (%)*	
		ER-positive	*HER2-positive*
Low	18	18 (100%)	0
Intermediate	56	56 (100%)	0
High	74	40 (54%)	42 (57%)

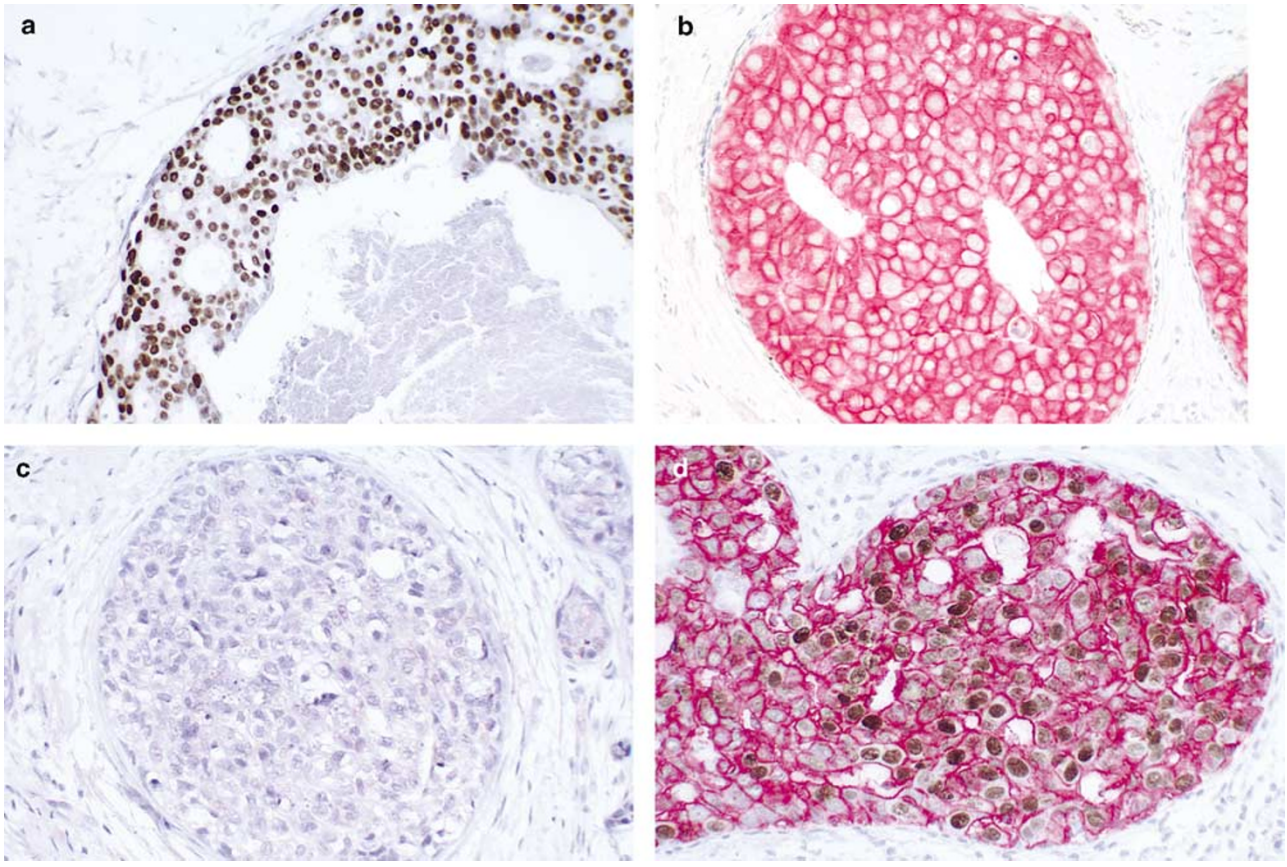

Figure 1 Examples of DCIS double immunostained for ER and HER2 protein. ER expression is denoted by brown nuclear staining and HER2 overexpression is represented by red staining of the cell membrane. (**a**) ER-positive/HER2-negative; (**b**) ER-negative/HER2-positive; (**c**) ER-negative/HER2-negative; (**d**) ER-positive/HER2-positive.

Table 2 Patterns of ER expression and HER2 overexpression according to DCIS grade

DCIS grade	*Number of cases*	*ER+/HER2−*	*ER−/HER2−*	*ER−/HER2+*	*ER+/HER2+*
Low	18	18	0	0	0
Intermediate	56	56	0	0	0
High	74	26	6	28	14
TOTAL	148	100 (67.6%)	6 (4.1%)	28 (18.9%)	14 (9.5%)

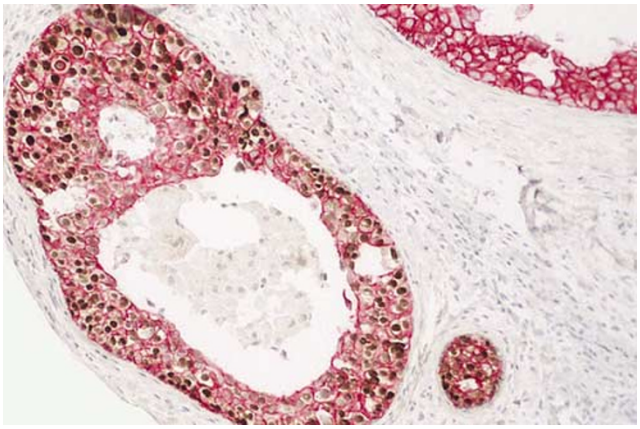

Figure 2 ER-positive/HER2-positive DCIS. In this case, some of the spaces contain DCIS cells that exhibit both ER expression and HER2 overexpression, whereas others (eg upper right) contain cells that show only HER2 overexpression.

50%. In one case, some of the ductal–lobular spaces contained HER2+/ER+ cells, whereas the DCIS cells in other spaces showed HER2 overexpression without ER expression (Figure 2). We did not identify any subpopulations of DCIS cells within any of the ER+/HER2+ cases that were positive for ER, but which lacked HER2 overexpression.

Discussion

Numerous prior studies have assessed expression of ER and/or HER2 in DCIS, and have related the expression of these markers to various histopathologic features of the lesion. In keeping with the results of many of these previous studies, we found significant relationships between ER expression and non-high-grade DCIS lesions, and between HER2 overexpression and high-grade lesions.[1,6–44,56] We also found, as have others, an inverse relationship between ER expression and HER2 overexpression in most cases of DCIS.[10,23,24,35]

However, to our knowledge, ours is the first study to utilize double immunostaining to assess ER expression and HER2 overexpression in relation to each other within individual cases of DCIS. Our results indicate that overall, approximately 10% of the DCIS cases we studied showed simultaneous ER expression and HER2 overexpression. Moreover, we found that among cases of ER-positive DCIS, 12% showed concomitant HER2 protein overexpression. This phenomenon was restricted to DCIS lesions of high nuclear grade, and in these cases coexpression of ER and HER2 was seen in the same neoplastic cells. Only one prior study has reported upon the frequency of HER2 overexpression in ER-positive DCIS. In that study of 219 cases, Claus *et al*,[23] using separate immunostains for ER and HER2, found that 19% of ER-positive DCIS also showed HER2 overexpression. Further details about these cases are not provided. It is difficult to compare the results of our study with those of Claus *et al*[23] due to methodological differences in the ER and HER2 immunohistochemical assays employed in these two studies. However, taken together, the results of these two studies suggest that approximately 10–20% of ER-positive DCIS show concomitant HER2 overexpression.

Data from several clinical studies of patients with invasive breast cancer as well as from preclinical models have suggested that HER2 protein overexpression reduces the efficacy of tamoxifen in ER-positive breast cancer,[46–54] although this remains a matter of debate.[48,57,58] Preliminary data from the NSABP B-24 trial have suggested that tamoxifen is effective in reducing the risk of ipsilateral breast tumor recurrence only in patients whose DCIS is ER-positive.[45] However, there are currently no data available from that trial or from any other clinical study to address the question of whether or not concurrent HER2 overexpression might mitigate the effects of tamoxifen in ER-positive DCIS. Nevertheless, given our observations and given the recent trend toward the use of tamoxifen in patients with DCIS, our findings are of potential clinical importance.

It could be argued that the proportion of cases of ER-positive DCIS that also show HER2 overexpression is too small to be clinically meaningful. However, it is useful to examine this issue in absolute terms to gauge the potential clinical impact of our findings. It has been estimated that there will be approximately 216 000 new female breast cancers in 2004,[59] and that approximately 20% of these (43 200) will be DCIS. If 80% of these DCIS cases are ER-positive, and if, as our data suggest, 12% of those cases show concurrent HER2 overexpression, then approximately 4100 cases of DCIS diagnosed in 2004 will show the ER+/HER2+ phenotype. Therefore, whether or not to recommend adjuvant tamoxifen in patients with ER-positive DCIS because of the

presence of simultaneous HER2 overexpression could potentially be an issue for over 4000 women each year.

In conclusion, the results of this study indicate that a subset of ER-positive DCIS cases show simultaneous HER2 protein overexpression. While this observation could have important clinical implications regarding the use of adjuvant tamoxifen in women with ER-positive DCIS, the interactions among ER expression, HER2 overexpression and response to tamoxifen will need to be evaluated in clinical outcome studies.

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