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Expression of Her2/neu, Steroid Receptors (ER and PR), Ki67 and p53 in Invasive Mammary Ductal Carcinoma Associated with Ductal Carcinoma *In Situ* (DCIS) *Versus* Invasive Breast Cancer Alone

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Abstract. *Aims: (a) To assess the expression patterns of HER2/neu, steroid receptors (ER and PR), Ki67 and p53 in invasive ductal cancer (IDC) and IDC associated with carcinoma in situ (IDC/DCIS) and (b) to determine if there is a differential expression of these molecular markers between IDC and IDC/DCIS. Materials and Methods: Paraffin-fixed breast cancer samples, diagnosed with only one histological invasive tumor (IDC (n=130), and IDC/DCIS (n=36) were analyzed by immunohistochemical means. The non-parametric Mann-Whitney and χ^2 tests were used to evaluate any statistical differences between different groups. Significance was assumed at p<0.05. Results: A significant increase of the tumor grading was observed between IDC and IDC/DCIS (p<0.05). Her2/neu amplification was demonstrated in 49.6% of IDC compared to 31% of IDC/DCIS (p<0.05). ER expression showed no statistical differences between IDC and IDC/DCIS. The PR expression was demonstrated in 71% of IDC with significantly lower intensity than IDC/DCIS (p<0.05). The Ki67 expression was significantly higher (p<0.05) in IDC cases (64%) versus IDC/DCIS (49.7%). No differences were observed between IDC and IDC/DCIS for p53 expression. Conclusion: We demonstrated significantly different expression patterns of Her2/neu, PR and Ki67 in IDC versus IDC/DCIS. Since these molecular markers play important roles in carcinogenesis and tumor progression, IDC/DCIS could be an important subtype of mammary invasive ductal cancer. Differences in expression of the evaluated markers might suggest a higher malignant potential of invasive carcinomas alone. The lower expression of Her2/neu and Ki67 in IDC/DCIS could implicate a less malignant behavior compared to a differentiated IDC. Additionally, these results might suggest that DCIS might be a malignant preform and the interaction with neoplastic tissue could result in an aggressive type of invasive tumor.*

Breast carcinoma has become the most common malignancy in the female population, affecting one in eight women and is one of the leading causes of mortality among women in Western countries (1, 2). The most important prognostic factors are the tumor size, histological grade and lymph node stage. Several studies have shown that patients who have involved axillary lymph nodes have much poorer prognosis than those without nodal metastasis (3). Breast cancer is thought to derive from aberrant non-invasive breast lesions, such as atypical ductal hyperplasia (ADH) and ductal carcinoma *in situ* (DCIS) (4). Over 14% of breast cancers diagnosed in the United States annually are DCIS and approximately 50% of untreated DCIS developed into an ipsilateral invasive breast cancer within 24 years after the original biopsy (5). However, it is quite unclear how invasive breast cancer develops through these lesions. There is increasing evidence that there are several progression routes leading to invasive breast cancer, depending on histology and differentiation grade (6, 7). The importance of several molecular markers in breast cancer has been of considerable interest during recent years, not only as prognostic markers, but also as predictors of response to therapy. Especially the steroid receptors (estrogen receptor (ER), progesterone receptor (PR)) (8, 9), Her2/neu (10, 11), Ki67 (12, 13) and p53 (14, 15) have gained increasing interest. However, the

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Table I. *Analyzed cases with IDC and IDC associated with DCIS.*

	IDC	IDC/DCIS
ER	123	30
PR	120	29
Her2/neu	121	28
Ki67	122	26
p53	107	20
Total examined specimens	130	36

expression of these molecular markers has not been assessed in invasive ductal carcinomas (IDC) *versus* IDC with DCIS (IDC/DCIS) yet.

Therefore, the aims of this study were: (a) to assess the expression patterns of HER2/neu, steroid receptors (ER and PR), Ki67 and p53 in IDC and IDC/DCIS and (b) to determine if there is a differential expression of these molecular markers between IDC and IDC/DCIS.

Materials and Methods

Breast cancer tissue was obtained from 130 patients diagnosed with just one histological tumor type by classical pathological evaluation, between October 1999 and October 2002 at the Department of Obstetrics and Gynecology of the University of Rostock, Germany. Paraffin-fixed breast cancer samples, diagnosed with only one histological invasive tumor form and invasive ductal cancer (IDC) associated with ductal carcinoma *in situ* (DCIS), were analyzed by immunohistochemical means with antibodies against Her2/neu, ER, PR, Ki67 and p53 using the immunostaining system from Ventana® (Ventana Medical System, Tuscon, AZ, USA). Tissue was classified as IDC alone (n=130) or IDC with DCIS (n=36). Due to lack of additional breast tissue in several cases, the actually analyzed cases are listed in Table I.

Immunohistochemical staining with antibodies (Table II) was performed with the Nexus©Autostainer and a DAB staining protocol with the use of a microwave (Ventana Medical System), as previously described (16, 17). Briefly, the slides were air-dried, incubated with inhibitor serum and protease solution. This protease solution (protease reagent 1; Ventana Medical System) contains an alkaline protease, an endopeptidase of the serine protease family. Incubations with biotinylated antibodies and avidin-peroxidase were subsequently performed, followed by several washing steps with PBS after each incubation step, as described by the manufacturer. Visualization of peroxidase activity was performed with DAB and H_2O_2 and counterstaining was performed with hematoxylin/blueing reagent. Positive cells showed a brownish color and negative controls as well as unstained cells were blue.

The intensity and distribution patterns of the specific Her2/neu immunohistochemical staining reaction were evaluated using the semi-quantitative score (graded as 0 = no, 1 = weak, 2 = moderate and 3 = strong staining). Her2/neu expression was evaluated as amplified (n=3) or not amplified (n=0 to 2). Steroid receptors were analyzed using the immunoreactive score (1 to 12), as previously described for human endometrium (18) and breast cancer tissue (19). A cut-off value of IRS=2 was used to determine the expression of steroid receptors in breast tissue (19). Ki67 and p53 expression were evaluated as positive or negative with a cut-off value of 10% of positively stained cells. Sections were examined using an Olympus (Tokyo, Japan) photomicroscope. Digital images were obtained with a digital camera system (Olympus) and were saved on computer. The results were evaluated using the χ^2 test (SPSS, Chicago, IL, USA). Significance was assumed at $p<0.05$.

Table II. *Antibodies used for immunohistochemical characterization of DCIS and invasive breast cancer tissue.*

Antibody	Clone	Isotype	Dilution	Source
ER	R1	mouse IgG_{2a}	undiluted	Dako, Glostrup, Denmark
PR		rabbit IgG	undiluted	Dako, Glostrup, Denmark
Her2/neu		rabbit IgG	1:400	Dako, Glostrup, Denmark
Ki67		goat anti-mouse IgM	1:10	Dianova, Hamburg, Germany
p53	1801	mouse IgG1	undiluted	Biogenex, San Ramon, CA, U.S.A.

Results

A significant increase of the tumor grading was observed between IDC and IDC/DCIS ($p<0.05$). Her2/neu amplification was demonstrated in 49.6% of IDC cases compared to 31% of IDC/DCIS ($p<0.05$) (Table III). Immunohistochemically, a typical membrane-staining pattern was observed (Figure 1). Steroid receptors were also demonstrated in malignant breast cancer tissue (Figure 2). ER showed an expression in 54.1% *versus* 48.4% of IDC and IDC/DCIS examined cases, with no statistical differences (Table III). PR immunohistochemical expression was demonstrated in 71% of IDC. Interestingly, the PR expression in IDC/DCIS was significantly lower ($p<0.05$) than IDC (Table III). Ki67 expression could also be observed with a variable staining pattern in malignant mammary tissue (Figure 3). The Ki67 expression was significantly higher ($p<0.05$) in IDC cases (64%) *versus* IDC/DCIS (49.7%). No differences were observed between IDC and IDC/DCIS for p53 expression (Table III).

Discussion

We demonstrated a significantly lower amplification of Her2/neu in IDC/DCIS compared to DCIS. Additionally, the

Table III. *Frequency of the immunohistochemical expression of steroid receptors, Her2/neu, Ki67 and p53 in IDC and IDC/DCIS.*

	Invasive ductal carcinomas				Invasive ductal carcinomas with DCIS				Significance
	negative/ not amplified		positive/ amplified		negative/ not amplified		positive/ amplified		
	n	%	n	%	n	%	n	%	
ER	50	45.9%	59	54.1%	16	51.6%	14	48.4%	n.s.
PR	31	29%	76	71%	16	53.3%	13	46.7%	$p<0.05$
Her2/neu	57	50.4%	56	49.6%	20	69%	9	31%	$p<0.05$
Ki67	40	36%	71	64%	16	59.3%	11	49.7%	$p<0.05$
p53	71	70.3%	30	29.7%	14	70%	6	30%	n.s.

HER2/neu status was similar in both the *in situ* and invasive components of the single tumor, confirming previous results (20, 21). This growth factor receptor HER2/neu generally is not overexpressed in normal or benign breast lesions (22). However, a significantly lower HER-2/neu expression in invasive carcinoma than in DCIS has been reported previously (23-25). Interestingly, due to these results, it has been suggested that the HER2-positive phenotype develops during the progression from ADH to DCIS, but may frequently be lost during progression to IDC, perhaps as a result of HER2-directed immune response (7). An alternative hypothesis is that HER2-negative ductal carcinomas do not derive from DCIS, but develop from ADH *via* an alternative pathway (7). Since the Her2/neu expression is significantly lower in IDC/DCIS compared to IDC, we suggest that IDC/DCIS may be a precursor for the development of a more aggressive and malignant IDC.

These speculations seem to be confirmed with a significantly lower Ki67 expression in IDC/DCIS than IDC alone. Ki67 has been suggested as a prognostic marker in breast cancer patients (26, 27). The interest in Ki67 has increased because it may even be considered as a predictive marker for chemotherapy response (12). Interestingly, no significant Ki67 differences were observed between DCIS and IDC/DCIS (28). However, it seems that an increasing proliferation rate occurs during progression of IDC/DCIS to IDC, suggesting a significant role in tumorigenesis.

In addition to the above risk factors, ER and PR status have also been shown to have prognostic value in breast cancer, although the importance of hormone-receptor status lies rather as a predictor of response to endocrine therapy (8, 9). We could demonstrate a significantly higher PR expression in IDC/DCIS compared to IDC, while ER did not show any statistical differences. These results demonstrate that IDC/DCIS may better respond to endocrine therapy. Additionally, p53 mutations have been suggested to be predictive of risk for subsequent breast carcinogenesis, since p53 mutations are more likely to be found in highly-invasive, poorly-differentiated, high-grade breast tumors (29, 30). Therefore, mutant p53 has been suggested to be a biomarker predicting risk for subsequent breast carcinogenesis (14, 15). Recently, a coexistence of HER2 over-expression and p53 protein accumulation has been suggested to be a strong prognostic molecular marker in breast cancer (31). However, we could not observe differences in the p53 expression between IDC/DCIS and IDC. Probably, the immunohistochemical detection of the p53 protein alone is insufficient to assess the functional state of the p53 gene and the subsequent gene product (30).

Overall, we demonstrated significantly different expression patterns of Her2/neu, PR and Ki67 in IDC *versus* IDC/DCIS. Differences in expression of the evaluated markers may suggest a malignant potential of invasive carcinomas. The lower expression of Her2/neu and Ki67 in IDC/DCIS could implicate a low degree of malignant behavior compared to a differentiated IDC.

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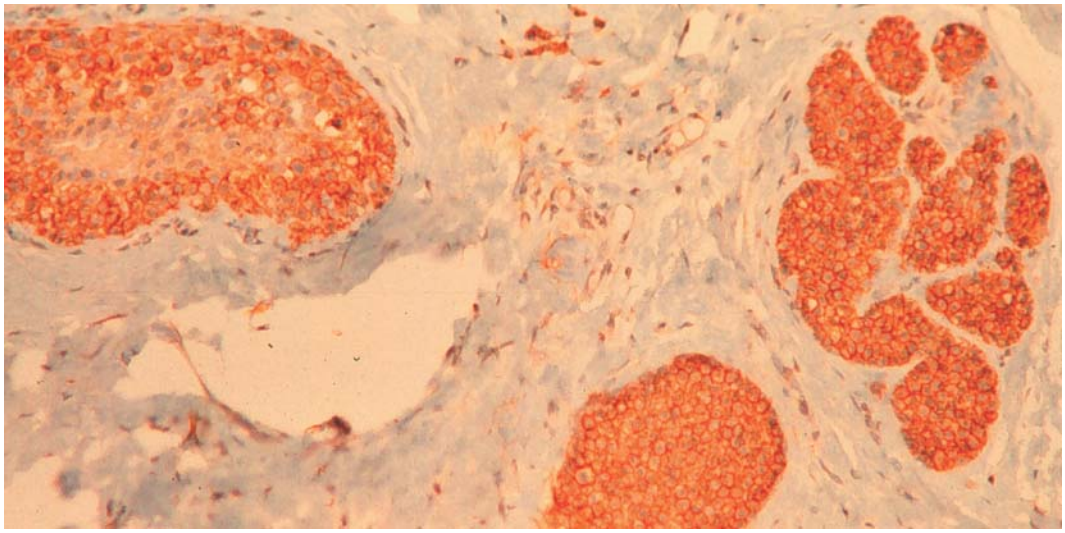

Figure 1. *Characteristic Her2/neu expression in invasive ductal mammary carcinoma (x100).*

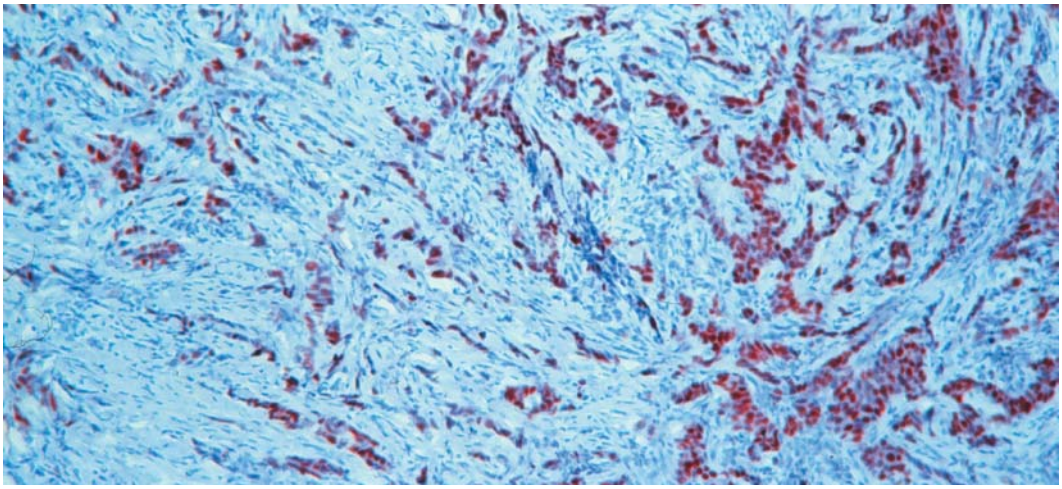

Figure 2. *Progesterone receptor expression in invasive ductal carcinoma (x100).*

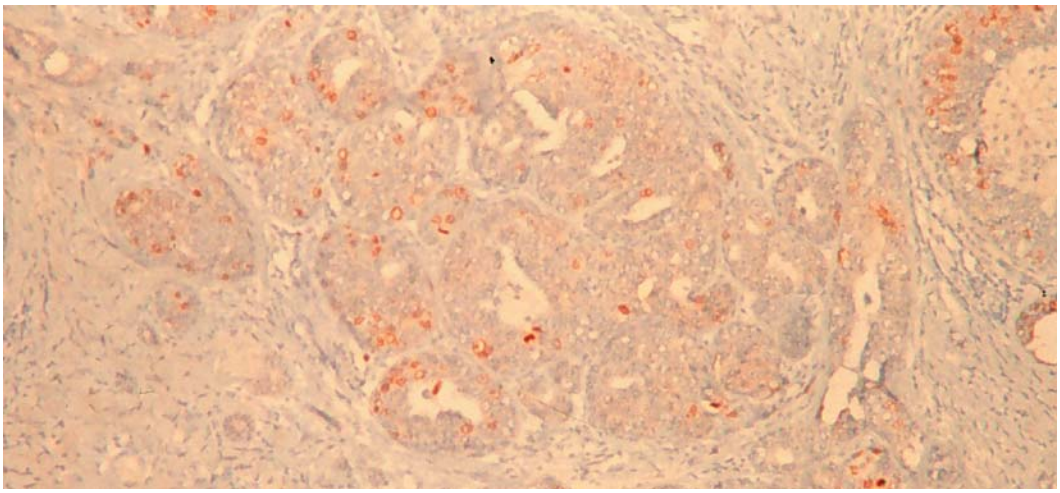

Figure 3. *Ki67 with immunostaining lower than 10% of the cells (x100).*

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