Angiogenesis and co-expressed of ER and c-erbB-2 (HER2/neu) protein in primary breast cancer patients: an analysis of 158 needle core biopsies

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Abstract
Formation of new blood vessels from a preexisting vascular bed (angiogenesis) is a complex multistep process, which may also permit metastasis. High c-erbB-2 (HER2/neu) expression has also been shown to correlate with tamoxifen resistance in patients in some studies. To investigate how tumor angiogenesis correlates with co-expression ER/c-erbB-2 (HER2/neu) in breast carcinoma diagnosed on core biopsy, microvessels were counted (and graded the density of microvessels) within the initial invasive carcinomas of 158 patients. Using light microscopy, the number of microvessels was counted manually in a subjectively selected hot spot (in the most active areas of neovascularization per 400× field), and their values were separated as above or below median (low and high), without knowledge of the outcome in the patient or any other pertinent variable. When tumors were classified as high or low MVD, based on a cut-off value (30.70175 microvessels/mm²), cases with high MVD were significantly more numerous. When the mean values of MVD of the various groups defined by co-expression ER/c-erbB-2 (HER2/neu) were compared, significant difference was noted (P = 1.82E-05). MVD did show a relationship with groups defined by co-expression ER/c-erbB-2 (HER2/neu) (P = 0.000422). Combining endocrine treatment with these new-targeted therapies (using antiangiogenic molecules) is a promising approach to improve present treatment strategies and overcome endocrine resistance and should be investigated in future preclinical and clinical studies.

Keywords: angiogenesis, breast carcinoma, ER, c-erbB-2 (HER2/neu), needle breast core biopsy.

Introduction
Breast cancer remains the most common cancer in females. It has been estimated that one in eight women will develop breast cancer during their lifetime in the USA [1].

Breast cancer mortality rates across the entire population in the USA have remained almost unchanged since 1970 [2] and it is estimated that 43,300 women died of breast cancer in the USA in 1999 [3] as a consequence of metastatic dissemination or the treatment of metastasis. A large percentage of these women were treated with cytotoxic chemotherapy, with drugs that are demonstrated to be effective against breast carcinoma cells both in vitro and in vivo. Nevertheless, despite being treated with the optimal doses at the optimal schedule, a significant percentage of women will relapse and die. For example, recurrence-free and survival rates at 10 years for women receiving polychemotherapy, for all ages, as estimated in the Oxford Overview, were 44 and 51.3%, respectively [4].

This leads to ask several questions. First, why are these treatments unable to cure a large percentage of women? Is it the result of cells that are resistant, either kinetically or by means of clonal evolution, to the drugs? Is it a problem of inefficient delivery to the tumor cells or a problem that pertains to the tumor microenvironment? A second question, undoubtedly related to the first set of questions, is why breast cancer continues to recur up to 20 years after treatment of the primary tumor [5, 6].

The status of estrogen receptor (ER) and human epidermal growth factor receptor 2 (c-erbB-2/HER2/neu) is critical in the management of patients with invasive breast cancer [7].

Endocrine manipulation with tamoxifen, aromatase inhibitors, and ovarian ablation is a central part of adjuvant therapy for early breast cancer or therapy for advanced disease in patients with ER-positive cancers [8, 9].

Trastuzumab (Herceptin) is an important recent treatment option for advanced disease in patients with HER-2 overexpressing tumors, and is under investigation to determine if it has a role in adjuvant therapy [10].

In patients whose tumors show no expression of ER, any form of endocrine therapy is considered ineffective, [7] and trastuzumab is not considered in those whose tumors exhibit less than 2+ staining of HER-2 [10].

Estrogen receptor (ER) is a member of the family of nuclear receptors and functions as a transcriptional regulator [11].

Normal breast epithelium expresses ER to varying degrees. Benign ducts and lobules display patchy to diffuse ER expression, and ductal epithelium tends to contain higher expression levels compared with lobular epithelium [12].
ER expression levels in the normal breast are thought to remain relatively stable over time, gradually increasing with age, and increased expression is associated with increased breast cancer risk [13, 14].

The follicular phase of the menstrual cycle has been associated with increased ER expression in the breast, although the results of a recent study did not confirm this finding [15–18].

The c-erbB-2 (HER2/neu) gene is the human analog of the rat neu gene identified in rat neuroblastomas in the early 1980s [19–23]. This gene has been found to be amplified and/or overexpressed in approximately 25 to 30% of invasive breast cancers in humans, most commonly in invasive ductal carcinomas. Located on chromosome 17q21, the c-erbB-2 (HER2/neu) gene encodes a 185 kD transmembrane glycoprotein with tyrosine kinase activity that functions as a growth factor receptor. This protein is a member of the epidermal growth factor receptor family of receptor tyrosine kinases, which includes epidermal growth factor receptor, HER2, HER3, and HER4. Ligand binding to one of these receptors results in the formation of homodimers and heterodimers. Dimerization is followed by phosphorylation, which, in turn, results in a cascade of downstream signaling events that are important for cell growth and maintenance of the transformed state. Of note, although HER2 forms heterodimers with other members of the family on interaction with their ligands, it has no known ligand of its own and is therefore considered an orphan receptor. However, at very high levels of overexpression, c-erbB-2 (HER2/neu) may undergo spontaneous homodimerization, which can initiate downstream signaling, stimulating cell growth and maintaining cellular transformation. This may be clinically important in c-erbB-2 (HER2/neu)-overexpressing tumors.

Angiogenesis is the growth and proliferation of blood vessels from existing vasculature. This process is quiescent in normal tissues and becomes active in rapidly growing tissues – including solid tumors. It has been shown that, in order to overcome tissue hypoxia, tumor growth beyond 1–2 mm³ is quiescent in normal tissues and becomes active downstream signaling, stimulating cell growth and maintaining cellular transformation. This process is dependant upon the formation of new vasculature [24, 25]. Angiogenesis is, thus, an established step in solid tumor progression. This has been studied in many cancers including colorectal cancer [26] non-small cell lung cancer [27, 28], hepatocellular cancer [29], melanoma [30] prostate cancer [31], breast cancer [32–38] and bladder carcinoma [39].

Most assessments of angiogenesis in female breast carcinoma have shown it to be of significant prognostic value [32–36]. However, not all studies in this field have observed such important clinical correlations to MVD [37, 38]. The reason for this discrepancy is not known.

Steroetactic core needle biopsy (SCNB) is a faster, less invasive, and less expensive alternative to surgical biopsy for the diagnosis of breast lesions, and its results have high concordance (87–96%) with those of histopathologic findings at surgery [40–44].

Purpose

This retrospective study was to evaluate the correlations between intratumoral microvessel density (MVD) and co-expression ER/c-erbB-2 (HER2/neu), in order to identify those tumours with a prominent angiogenic phenotype. It would be an important advance if high MVD could be used to help in predicting the prognosis of patients, particularly in high-risk individuals.

5 Patients and Methods

Selections of cases

The histological slides of non-palpable, mammographically detected lesions in which percutaneous stereotactic biopsy was performed from January 2004 until December 2004 in SAPAG Hautepierre, Strasbourg, France, were retrospectively reviewed.

Lesions were defined as non-palpable when patients, surgeons, and the SCNB examiner (a radiologist) could not palpate any breast lesion during physical examination.

For all cases, mammography and ultrasonography reports and films were collected for review. In addition, medical charts were reviewed to verify that none of the patients included in the study had clinical evidence of malignancy or a history of ipsilateral breast carcinoma and also to collect clinical information, such as age, family history of breast carcinoma, parity, hormone replacement therapy received, and history of contralateral breast carcinoma.

To be eligible for this retrospective study, women had to have undergone a SNCB of a primary breast cancer. The criteria of inclusion in this study was: female sex, age older than 21 years, not pregnant, suspicious lesion of the breast (mammography), patient with node-negative breast cancer, recommendation for excisional after mammography.

Mammographic lesions were categorized according to the Breast Imaging Reporting and Data System (BI-RADS) developed by the American College of Radiology [45].

Biopsy procedure

Stereotactic localization was performed by radiologists trained in mammography using a dedicated stereotactic breast biopsy system, an automatic biopsy gun, and a 14-gauge biopsy needle with a long throw (2.3 cm excursion).

The core needle biopsy was performed by first cleansing the skin overlying the lesion with alcohol; this was followed by skin and subcutaneous infiltration with approximately 1–2 mL of 1% lidocaine.

Usually one to three biopsies were taken from different areas in each lesion utilizing the same biopsy instrument. The core needle biopsy specimens were removed from the trough in the stylet by rinsed in a container filled with sterile saline. Surgical clip was placed in patients when the entire lesion was removed by the needle core biopsy.
Tissue specimens
It was obtained a mean of 2.6 specimens (range, one to 8) per lesion. To document the presence of calcification the core specimens were radiographed. Then the core specimens were fixed in 10% formalin, paraffin embedded, sectioned, leveled ×3, and stained with Hematoxylin and Eosin. Additional levels were requested, if necessary, for histological documentation of calcification. The use of a polarizing lens assisted in the microscopic identification of microcalcification in some cases. Two pathologists retrospectively reviewed the histological slides. At the retrospective review, the pathologists knew each lesion was later excised but did not know the excisional diagnosis.

Histological review
The same senior pathologist (SAPAG) in almost all cases made the original diagnosis of invasive malignancy. For these cases, Hematoxylin and Eosin-stained slides of core biopsy samples were retrieved from the pathology archives and reviewed by a second pathologist (S.V.) to confirm the diagnosis of invasive malignancy. Diagnoses were confirmed in all cases.

Immunohistochemical evaluation and scoring
Antibodies

For the detection of estrogen receptor and c-erbB-2 (HER2/neu) protein the mouse monoclonal antibodies (Novocastra, UK) were used. All the dilutions were done in phosphate buffered saline (PBS).

The antibodies, clone, dilution, pretreatment conditions, and source for immunohistochemical studies are listed in Table 1.

### Table 1 – Antibodies used for immunohistochemistry

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Clone</th>
<th>Source</th>
<th>Dilution</th>
<th>Staining</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor</td>
<td>6F11 Novocastra</td>
<td>1:10</td>
<td>N</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>c-erbB-2 (HER2/neu)</td>
<td>CB11 Novocastra</td>
<td>1:400</td>
<td>M</td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

N – nuclear staining, M – membrane staining, H – heating, 0.01 M citrate buffer (pH 6).

Immunohistochemical staining

Immunohistochemical staining was performed on 10% formalin-fixed deparaffinized sections using the Streptavidin–Biotin method.

Immunohistochemistry for ER was performed using an automated immunostainer VENTANA (NexES) according to the manufacturer’s instructions. This system uses capillary action to draw up reagents to cover the specimens on the specially prepared slides.

Immunohistochemical staining for c-erbB-2 (HER2/neu) was performed manually using a sequenza immunostaining system (Shandon, Uncorn, United Kingdom) and rinsed with APK Wash.

Briefly, 4 µm sections were cut from the paraffin embedded blocks using a microtome. The glass slides were previously coated with poly-L-lysine. The sections were then incubated at 37°C overnight. Thereafter, the sections were deparaffinized in xylene (30 minutes, twice), sequentially dehydrated by incubating in 1:1 xylene-alcohol mixture, 100% alcohol, 90% alcohol, 70% alcohol, 50% alcohol, 30% alcohol and 1×PBS (10 minutes each). The slides were subjected to heat-induced epitope retrieval by immersing them in 0.01 M boiling citrate buffer (pH 6) in a pressure cooker for 3 minutes. They were subsequently cooled with the lid on for an additional 10 minutes. After removing the lid, the entire pressure cooker was filled with cold running tap water for 2 to 3 minutes or until the slides were cool.

**ER**

At 36°C, the stainer sequentially added an inhibitor of endogenous peroxidase, the primary antibodies (32 minutes), a biotinylated secondary antibody, an Avidin–Biotin-complex with horseradish peroxidase (30 minutes), 3,3’-diaminobenzidine (3,3’-diaminobenzidine tetrahydrochloride) (15 minutes).

The sections were counterstained with Mayer Hematoxylin, dehydrated, cleared in xylene, and mounted. The normal breast tissues adjacent to the tumor areas served as an internal control. Negative controls were obtained by staining protocols omitting the first antibody, or by using non-immune mouse sera in place of the first antibody.

c-erbB-2 (HER2/neu)

Slides were incubated overnight with the monoclonal antibody directed against HER-2 protein (diluted 1:400) at room temperature. During day 2, slides were incubated for 20 minutes at room temperature with biotinylated goat anti-mouse and goat anti-rabbit immunoglobulin (Dako), followed by another 20-minute incubation with Horseradish-peroxidase : Streptavidin–Biotin complex (Dako). Between the subsequent steps, the slides were rinsed with APK Wash for 6 minutes.

3,3’-Diaminobenzidine (Dako) was used as the chromogen. Slides were rinsed extensively in tap water and, finally, were counterstained in Harris’s Hematoxylin, dehydrated through a series of alcohols, and mounted.

For negative control, the tissue was processed in the same way, except that the primary antibody was omitted (buffer substitution).

Negative (normal breast tissue) and positive (strongly positive carcinoma) control slides were included with each essay.

Interpretation of staining results

In almost all cases immunoreactivity for ER and c-erbB-2 (HER2/neu) was evaluated semiquantitatively by the same senior pathologist (SAPAG).

Immunoreactivity was re-evaluated semiquantitatively by one pathologist (SV); the interobserver concordance was more than 95%. Both pathologists were blinded to the clinicopathologic data and patients’ outcome and status of the gene amplification.

The number of positive cells in 500 tumor cells within 4–6 non-overlapped microscopic fields at ×400 magnification was counted.
ER

Only nuclear immunostaining was interpreted as a positive result. Cytoplasmic reaction, if any, was ignored.

Immunostained slides were evaluated by light microscopy and the immunohistochemistry signal was scored using the so-called “Allred Score” [46].

Briefly, a proportion score was assigned representing the estimated proportion of positive staining tumor cells (0 = none; 1 <1/100; 2 = 1/100 to <1/10; 3 = 1/10 to <1/3; 4 = 1/3–2/3; 5 =2/3).

Average estimated intensity of staining in positive cells was assigned an intensity score (0 = none; 1 = weak; 2 = intermediate; 3 = strong). Proportion score and intensity score were added to obtain a total score that ranged from 0–8.

Based on these results, tumors were defined as ER-positive if their total IHC score was greater than 2 and ER-negative if their score was 0 or 2.

c-erbB-2 (HER2/neu)

Only the membrane staining intensity of the invasive component in hot spot areas was considered. Cytoplasmic staining was considered nonspecific staining and was not included in the assessment of membrane staining intensity.

The percentage of tumor cells showing either a complete or partial membranous pattern was scored in each case. The intensity of membrane staining was assessed as strong, moderate or weak.

The score system used for the interpretation of staining is listed in Table 2.

Table 2 – Score system used to assess c-erbB-2 (HER2/neu) status

<table>
<thead>
<tr>
<th>c-erbB-2 (HER2/neu) status</th>
<th>Staining pattern</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No staining or weak membrane staining in &lt;10% of tumor cells</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>Weak membrane staining in &gt;10% of tumor cells</td>
<td>1+</td>
</tr>
<tr>
<td>Positive</td>
<td>Moderate membrane staining in &gt;10% of tumor cells</td>
<td>2+</td>
</tr>
<tr>
<td>Positive</td>
<td>Strong membrane staining in &gt;10% of tumor cells</td>
<td>3+</td>
</tr>
</tbody>
</table>

Samples were classified as positive if they had a score of 2+ or 3+ and as negative if they had a score of 0 or 1+.

To aid in the differentiation of 1+, 2+, and 3+ staining, the Dako's Atlas for the Interpretation of HercepTest, with representative pictures of the staining intensities, was used.

Quantification of tumor vascularity

Microvessel counts and density scoring were performed manually as a single microvessel count by light microscopy in areas of invasive tumor, without any knowledge of the subjects’ previous investigations or clinical outcome, using a procedure based on a modification of the method by Weidner N et al. [33].

The slides from each tumor were at first scanned at 40× magnification, using a light microscope Olympus BX60 to select areas with the densest vascularization (hot spots). Normal mammary tissue, large areas of inflammation, granulation tissue, and tumor necrosis were excluded. Vascularity was defined by the number of microvessels (capillaries and small venules) per area counted in the fields of highest vascular density (“hot spots”) at 400× magnification.

After the individuation of the hot spots within the tumor, three adjacent, non-overlapping fields from each section were selected using a high-power magnification (40× objective and 10× ocular, 0.152 mm² per field). The count performed was the field thought to contain the highest number of microvessels found at low magnification, and each subsequent count was the field thought to be the next highest. MVD was quantified as the sum vessel count of the three fields (3 × 0.152 mm²) from each tumor.

Microvessel counts and density scoring were repeated “blind” four months later and no discrepant results were found.

All microvessel counts were standardized. The standardized microvessel score was expressed as counts per square millimeter and was obtained by dividing the actual count by the size of three-microscope field (0.456 mm²).

Statistical analysis

Descriptive statistics compared the microvessel density and co-expression ER/c-erbB-2 (HER2/neu). Results are reported as mean ± standard deviation, medians and ranges for the microvessel counts performed for each subsets. A P-value equal to or less than 5% was considered statistically significant.

Independent group t-tests were used to compare the two patient groups on both the continuous and the ordinal measures.

χ² tests of independence or Fisher’s exact test was used to compare the two groups in regard to the categorical data. One-way ANOVA was used when more than two groups of microvessel counts were compared.

If the t value that is calculated is above the threshold chosen for statistical significance (usually the 0.05 level), the null hypothesis that the two groups do not differ is rejected in favor of an alternative hypothesis, which typically states that the groups do differ.

Results

Hundred and fifty-eight women met the eligibility criteria for this report. Distribution of cases according to ER status and c-erbB-2 (HER2/neu) status and c-erbB-2 (HER2/neu) score were recorded in Tables 3 and 4.

Table 3 – Distribution of cases according to co-expression ER/c-erbB-2 (HER2/neu)

<table>
<thead>
<tr>
<th>c-erbB-2 (HER2/neu)</th>
<th>negative</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrogen receptor (ER)</td>
<td>17 (10.76)</td>
<td>8 (5.06)</td>
</tr>
<tr>
<td>negative positive</td>
<td>120 (75.95)</td>
<td>13 (8.23)</td>
</tr>
</tbody>
</table>

Note: Data are no. of patients (%).
Table 4 – Distribution of cases according to ER status and c-erbB-2 (HER2/neu) score

<table>
<thead>
<tr>
<th>Estrogen receptor status (ER)</th>
<th>c-erbB-2 (HER2/neu) score</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>17</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>0</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Data are no. of patients [%].

Among the 158 case patients, 17 (10.76%) were ER-/c-erbB-2 (HER2/neu) (Figures 1 and 2), eight (5.06%) were ER-/c-erbB-2 (HER2/neu) +, 120 (75.95%) were ER+/c-erbB-2 (HER2/neu) -, and 13 (8.23%) were ER+/c-erbB-2 (HER2/neu) + (Figures 3 and 4).

In total, there were 53 (33.54%) patients in the low-MVC group and 105 (66.46%) in the high-MVC group, one case in the low-MVC group and 16 in the high-MVC group in patients with ER-/c-erbB-2 (HER2/neu) - status, 0 cases in the low-MVC group and eight in the high-MVC group in patients with ER-/c-erbB-2 (HER2/neu) + status, 51 cases in the low-MVC group and 69 in the high-MVC group in patients with ER+/c-erbB-2 (HER2/neu) - status, one case in the low-MVC group and 12 in the high-MVC group in patients with ER+/c-erbB-2 (HER2/neu) + status (Table 5 and Figure 5).

Table 5 – Correlation of groups defined by co-expression of ER and c-erbB-2 (HER2/neu) with MVD in 158 patients with breast carcinoma

<table>
<thead>
<tr>
<th>MVD</th>
<th>ER/c-erbB-2 (HER2/neu)</th>
<th>Total [%]</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>a: ER-/c-erbB-2 (HER2/neu) -</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>b: ER-/c-erbB-2 (HER2/neu) +</td>
<td>16</td>
<td>69</td>
</tr>
<tr>
<td>High</td>
<td>c: ER+/c-erbB-2 (HER2/neu) -</td>
<td>17</td>
<td>120</td>
</tr>
</tbody>
</table>

Note: Data are no. of patients.

*The $\chi^2$ was used to evaluate the correlation between c-erbB-2 (HER2/neu) status and MVD. $P<0.05$ indicates statistical significance.

When tumors were classified as high or low MVD, based on a cut-off value (30.70175 microvessels/mm²), cases with high MVD were significantly more numerous.

When the mean values of MVD of the various groups defined by co-expression of ER and c-erbB-2 (HER2/neu) status were compared, significant difference was noted ($P = 1.82E-05$, One-way ANOVA test).

MVD did show a relationship with groups defined by co-expression of ER and c-erbB-2 (HER2/neu) status ($P = 0.000422$, Chi-square test).
Discussion

The goal was to study the relationship between angiogenesis and co-expression ER/c-erbB-2 (HER2/neu), which is in contrast to other studies that assessed angiogenesis as a prognostic factor.

Steroid hormones influence breast cancer development and progression, particularly estrogen, via their interaction with specific target cell receptors. The idea that therapeutic antagonists to estrogen action could also prevent or treat breast cancer was first suggested in the 1930s [47], long before the target either ER or antiestrogen drugs were identified.

At present, estrogen antagonist therapy is the most effective treatment for women with ER-positive breast cancer. Unfortunately, many patients present with primary (de novo) resistance to endocrine therapy, despite high tumor levels of ER, and all patients with advanced disease eventually acquire resistance to therapy.

The potential mechanisms for either intrinsic or acquired endocrine resistance are still poorly comprehended, but they clearly include ER-coregulatory proteins and cross-talk between the ER pathway and other GF and kinase networks.

Current endocrine therapies of breast cancer are based mainly on targeting the ER signaling pathway by either: (a) reducing levels of estrogen; (b) antagonizing ER function with antiestrogens such as tamoxifen; or (c) down-regulating ER levels with pure antiestrogens such as fulvestrant (Faslodex).

Tamoxifen, a non-steroidal SERM (Selective Estrogen Receptor Modulators), is the most frequently prescribed drug for the treatment of all stages of breast cancer [48], and it is now also used in prevention for women at high risk of developing breast cancer [49]. The widespread use of tamoxifen over the last 20 years is probably one of the major reasons for the observed decrease in breast cancer mortality in the past decade. Aromatase inhibitors (estrogen depletion) have recently been proven superior to tamoxifen [50].
However, a longer follow-up is required before a final benefit/risk assessment can be made to justify ousting tamoxifen in the adjuvant setting for postmenopausal women. If so, tamoxifen will remain a useful therapeutic option in advanced disease.

Tamoxifen is thought to inhibit breast cancer growth mainly through competitive blocking of the ER, thereby inhibiting estrogen-induced growth. In the adjuvant setting, tamoxifen therapy results in a 40–50% reduction in the annual odds of recurrence and leads to prolonged disease-free and overall survival [48].

In addition, tamoxifen has also been shown to induce clinical benefit in more than half of patients with metastatic disease who have ER-positive tumors [51]. However, although tamoxifen is initially effective in many patients, and in general is very well tolerated, a major obstacle to its use is tumor resistance. Almost 50% of breast cancers, despite the presence of ER, fail to respond to tamoxifen; furthermore, even patients who initially respond eventually acquire tamoxifen resistance, leading to tumor progression and death. In general, acquired resistance to tamoxifen is not attributable to loss of or alteration in the ER, and resistant tumors often respond to second-line endocrine therapy [51, 52].

Experimental and clinical evidence suggests that in many scenarios, tamoxifen resistance of breast cancers may be attributable to the ability of the tumor cells, either de novo at the beginning of the treatment or in the acquired setting after prolonged treatment, to be stimulated rather than inhibited by the drug [53–55].

The clinical implication is that tamoxifen treatment should be avoided or stopped as soon as resistance develops, because tamoxifen not only does not provide the desired protective antitumor signals, but also may instead stimulate the tumor.

c-erbB-2 (HER2/neu) can transform normal mammary epithelial cells and is overexpressed in a cohort of breast tumors, where it is associated with a more virulent behavior and poor patient prognosis [56].

Multiple lines of experimental evidence suggest that overexpression of c-erbB-2 (HER2/neu) confers antiestrogen resistance to breast tumor cells. MCF-7 human breast cancer cells transfected with either a full-length HER2 cDNA or with ectopic heregulin-ß1, the HER3/4 ligand that activates HER2, lose sensitivity to tamoxifen or estrogen dependence [57–59].

Several clinical studies have shown that tumors with high HER2 expression and/or with high circulating levels of the HER2 ectodomain exhibit a statistically lower clinical response rate and/or shorter durations of response after antiestrogen therapy [60–68], further suggesting an association between tumor levels of the proto-oncogene and resistance to endocrine therapy.

High c-erbB-2 (HER2/neu) expression has also been shown to correlate with tamoxifen resistance in patients in some studies, but this association is not strong, and other studies have failed to confirm the association [69–71].

The mechanisms by which c-erbB-2 (HER2/neu) potentially mediates tamoxifen resistance are unclear. However, HER2 overexpression results in activation of the Ras/MAPK signaling pathway in breast tumor cell lines and carcinomas [72, 73].

MAPK has been shown to phosphorylate Ser-118 in the ER, leading to ligand-independent ER activation. In the adjuvant setting, tamoxifen therapy results in activation of the Ras/MAPK signaling pathway in breast tumor cell lines and carcinomas [72, 73].

The role of angiogenesis in the development and progression of human cancers has been widely studied [78].

New blood vessels can be stimulated to grow when factors that promote angiogenesis are up-regulated or those that inhibit angiogenesis are down-regulated [24, 79]. This investigation was stimulated by the conflicting conclusions of some studies.

In this study, cases with high MVD were numerous in patients with ER+/c-erbB-2 (HER2/neu)+ profile. Therefore, the predictive value of co-expression ER/c-erbB-2 (HER2/neu) is still in question, and best current practice in adjuvant breast cancer therapy does not deny tamoxifen to patients with c-erbB-2 (HER2/neu)-positive tumors.

Conclusions

Assessment of tumor angiogenesis and co-expression ER/c-erbB-2 (HER2/neu) may prove valuable in selecting patients with early breast carcinoma for therapeutic hormonal therapy.

The correlation of angiogenesis with co-expression ER/c-erbB-2 (HER2/neu) may be a potential therapeutic target for the management of breast cancer, and open the door to the development of other novel approaches to the treatment of breast cancer using antiangiogenic molecules.

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