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NEU (C-erbB-2) ONCOGENE IN BREAST CANCER AND ITS POSSIBLE ASSOCIATION WITH THE RISK OF DISTANT METASTASES

A retrospective study and review of literature

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Abstract

By immunohistochemical staining C-erbB-2 (neu) oncogene was found on the cell membrane in 19 out of 44 primary breast cancers from a pathology archive. No obvious relation was found between neu oncogene, age, and lymph node status and tumor size. There was a tendency towards smaller primary tumors and more estrogen receptor-negative tumors in the oncogene-positive group. No case of distant metastasis during follow-up was found among the oncogene-negative patients, while 6 oncogene-positive patients developed such metastases. This suggests that the neu oncongene is an independant prognostic factor, which might predict the development of distant metastasis. Further studies including more patients and long-term survival analysis are, however, needed in order to evaluate the prognostic significance of the neu oncogene.

Key words: Breast cancer, neu oncogene, metastasis.

Protooncogenes present a group of genes identified by their genetic sequences with known tumorigenic or transforming potential (1–3). Activated by amplification, mutation, translocation and deletion they can promote tumor formation (3, 4).

In 1981 a novel transforming gene was identified in chemically induced rat retinoblastomas (3, 5, 6). This gene was called neu. It is related with but distinct from the C-erbB protooncogene (5, 7). The neu protooncogene differs from the neu oncogene in its point of mutation, an adenine-thymidine transversion in the transmembrane portion of the molecule (8–10). Amplification of C-erbB-2 has only been found in adenocarcinomas (8, 11, 12). Zhou et al. (11) reported that amplification was about 3 times more frequent in breast carcinomas than in other adenocarcinomas, except for gastric cancer (1, 11, 13, 14) and ovarian cancer (3). In contrast to C-erbB-2, its protooncogene C-erbB-1 is not amplified in adenocarcinoma (8, 11, 12, 15) but highly expressed on the membranes of some squamous cell carcinomas (12, 15) and brain tumors (16). The human homology is located on chromosome 17 q 21 (7, 17). It specifies a transmembrane receptor like phosphoglycoprotein that is closely related to epidermal growth factor (C-erbB-1). It has a cell external ligand binding domain and a cell internal domain with tyrosine kinase activities involved in signal transduction. When induced by the neu oncogene the malignant phenotype is associated with increased tyrosine kinase activity (3, 18).

During the last years different groups have studied the relation between C-erbB-2 and primary adenocarcinoma of the breast (1, 3, 5, 11, 13, 19–27) but the results are controversial. Most authors have tried to correlate neu oncogene with other prognostic parameters like age, lymph node status, histological grade and receptor status. In the present study we are reporting findings from 44 archived primary breast adenocarcinomas in which the presence of neu oncogene was examined by immunohistochemical method.

Material and Methods

Forty-four paraffinized specimens were collected from the pathology archive in St Camillus and St Augustinus Hospitals. Immunohistochemical staining was done in the Department of Pathology in the University of Ghent.

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Hospital charts were reviewed concerning age, menopausal state, size of primary tumor, hormonal receptor status, lymph node involvement, histological grade, disease-free interval and development of metastases or local recurrences. None of the patients had received radiation therapy or chemotherapy prior to surgery. The immunohistochemical staining was done and evaluated without knowledge of the clinical findings and the outcome of the patients.

The 44 specimens derived from 43 different patients (one patient had bilateral breast cancer) with an average age of 59.1 years and operated on in 1987–1988. None of the patients had distant metastases at the time of surgery. Follow-up periods varied between 9–24 months, average 12 months.

The biopsy specimen for light microscopic examination was fixed in 10% formalin during 12 h and embedded in paraffin at 58°C following routine procedures.

The peroxidase-anti-peroxidase technique was applicated as follows:

1. Deparaffinized sections were immersed into methanol-containing 0.03% hydrogen peroxide for 20 min to block the endogenous peroxidase activities and were incubated with 5% bovine serum albumin for 30 min.

2. Incubation with mouse monoclonal 3B5 and 9G6 anti-neu-antibody (50 to 100 μl per slide) for 30 min at room temperature in a moist chamber; dilution of the antibody in TBS + 1% BSA was 1/100 (supernatant) and 1/6 000 (ascites) for 3B5 and 1/10 (ascites) for 9G6.

3. Addition of rabbit anti-mouse immunoglobulin (Dakopatts, Glostrup, Denmark) diluted at 1/40 in TBS + 1% BSA for 45 min.

4. Addition of mouse peroxidase–anti-peroxidase complex (Dakopatts, Glostrup, Denmark) diluted at 1/200 in TBS + 1% BSA for 30 min.

5. Addition of 3-3'diaminobenzide (Sigma, St. Louis, USA) with 0.01% hydrogen peroxide in TBS for 10 min, followed by washing in tap water.

All sections were dehydrated and mounted on Merckoglas (F. Merck, Darmstadt, West Germany). Control sections were prepared by omission of the primary antibody. The specificity of the 3B5 antibody was tested by incubating the primary antibody with the antigen.

Estrogen and progesterone receptors were determined with dextran coated charcoal technique. They were expressed as fmol/mg protein. Values of more than 10 fmol/mg protein were considered positive.

Results

Immunohistochemical staining of neu oncogene was positive in 19/44 tumors (18/43). The average age of the oncogene-positive cases was 59 years. Of the 19 oncogene-positive tumors, 16 were poorly differentiated ductal carcinomas, 2 comedocarcinomas and 1 lobular carcinoma.

Of the 25 oncogene-negative tumors one was a well differentiated ductal carcinoma, 13 were poorly differentiated ductal carcinomas, 2 comedocarcinomas and 9 lobular carcinomas.

There were no obvious differences between the oncogene-positive and oncogene-negative cases concerning menopausal state, axillary lymph node state and progesterone receptor positivity (Table). There was a tendency towards more estrogen receptor-positive tumors and larger tumors in the oncogene-negative group. The most striking difference, however, concerned distant metastases during follow-up. Such metastases occurred in 6 out of 17 patients with oncogene-positive tumors (one patient was lost to follow-up) but in none of the 25 patients with oncogene-negative tumors.

Discussion

Different groups of investigators have found that C-erbB-2 is overexpressed in some primary adenocarcinoma of the breast (1, 3, 5, 11, 13, 19–27). The degree of amplification varied from 8% in some reports (19) to 47% in other reports (5). Slamon et al. (1) were the first to report C-erbB-2 amplification in breast carcinoma, which they found in about 30% of the tumors. They found a positive correlation between gene amplification and number of positive lymph nodes and negative estrogen receptor and inverse relation between gene amplification and survival rate.

Varley et al. (20) found that gene amplification was limited to cases with poor short-term prognosis. Cline et

Table

Relation between neu oncogene findings in 43 breast cancer patients (44 breast carcinomas as one patient had bilateral tumors) and some tumor parameters

	Oncogene-positive	Oncogene-negative	χ^2-test
Premenopausal	4/18 (22%)	9/25 (36%)	$0.3 < p < 0.5$
Positive axillary lymph nodes	9/19 (47%)	12/25 (48%)	$p = 0.7$
Positive estrogen receptors	11/19 (58%)	21/25 (84%)	$p = 0.05$
Positive progesterone receptors	8/19 (42%)	15/25 (60%)	$0.2 < p < 0.3$
Tumor size >2 cm	7/18 (39%)	18/26 (69%)	$p = 0.05$
Distant metastases during follow-up	6/17* (35%)	0/25 (0%)	$p < 0.005$

* One patient lost to follow-up.

al. (14) found a positive correlation between gene amplification and number of positive lymph nodes. Zhou et al. (11) reported that gene amplification was frequently associated with advanced stage and regional lymph node metastases. Fontaine et al. (5) reported correlation between neu oncogene amplification and tumor grade. They suggested that neu copy number and activity might be altered when the tumor metastasizes. Venter et al. (21) reported that amplification of the product C-erbB-2 gene correlated with increased gene expression. Guerin et al. (22) found that overexpression of C-erbB-2 neu proto-oncogene was correlated with the number of positive lymph nodes and with estrogen and progesterone receptor negativity. Van den Vijver et al. (23, 24) and Ali et al. (25), however, reported that amplification was independant of receptor status, stage of the disease, lymph node involvement, and tumor grade and survival rate.

There has thus been considerable disagreement regarding the prognostic significance of C-erbB-2 amplification. The development of antibodies to the C-erbB-2 gene product using synthetic peptides which can react with paraffin-embedded tissue has facilitated retrospective studies of archival pathology specimens from large groups of patients with known clinical outcome (8, 13, 26).

Immunostaining for neu oncogene can be easily performed in a routine pathology laboratory. Heterogenicity within the tumor can obscure the result when gene amplification is studied, but even in such cases the neu oncogene can be detected by immunostaining (13).

Using antibody staining of C-erbB-2 protein, Gusterson et al. (27) could not find an association between neu staining, lymph node metastases or estrogen receptor level. Berger et al. (28) reported that positive C-erbB-2 staining correlated with poor nuclear grade and number of positive lymph nodes. They also reported that immunostaining could occur without gene amplification. Van den Vijver et al. (29) found that immunostaining of C-erbB-2 correlated with tumor size and survival but not with lymph node involvement, disease-free interval or histological grade.

In the present small pilot study there was a striking difference concerning the development of distant metastases during follow-up between patients with oncogene-positive and oncogene-negative tumors. Interestingly 4 of our 6 oncogene-positive patients with distant metastases had no axillary lymph node metastases and 4 of the patients had estrogen receptor-positive tumors. This might suggest that the neu oncogene is an independant marker that predicts hematogenous spread. The study is, however, preliminary and must be evaluated with caution. Further studies on larger materials and analysis of long-term survival (which is clearly related to generalized metastases) are needed in order to evaluate the possible prognostic significance of the neu oncogene in breast cancer and if this oncogene could be used as an indicator for selection of adjuvant therapy (30).

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