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Expression of c-*myc*, erbB-2, p53 and nm23-H1 gene product in benign and malignant breast lesions: Coexpression and correlation with clinicopathologic parameters

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Abstract

The aims of this study were to assess the expression of protein products of c-*myc*, erbB-2, p53 and nm23-H1 gene in benign and malignant breast lesions, to estimate their possible coexpression and to correlate the results of immunohistochemical analysis with various clinicopathologic parameters. The method used was the immunohistochemical detection of the corresponding protein. Expression of c-*myc* protein was high in both malignant and benign lesions (95% and 100%). Expression of erbB-2 and mutated p53 proteins in malignant lesions was 27% and 34%. These proteins were present in benign lesions as well: 7.8% of benign lesions were positive for erbB-2 protein and 19.6% for p53 protein. The expression of nm23-H1 protein was similar in benign and malignant lesions: 47% and 54%. The coexpression of nm23-H1 and mutated p53 protein was found in 14 carcinomas (16.5%). We found a tendency of negative correlation between the expression of these two proteins. We also found a negative correlation between the size of breast carcinomas and the expression of nm23-H1, a higher proportion of nm23-H1-positive carcinomas in the group of erbB-2-negative, p53-negative carcinomas and a higher proportion of nm23-H1-positive carcinomas in the group of malignant lesions with negative axillary lymph nodes. Our results support the hypothesis that in women with breast cancer the expression of nm23-H1 gene may contribute to more favorable phenotype. We also showed that some changes found in malignant breast tumors such as the presence of mutated p53 protein and the expression of erbB-2 protein may be found in benign lesions as well.

Keywords: Breast lesions; c-*myc*; erbB-2; p53; nm23-H1; Gene product expression

Introduction

The alterations of oncogenes and tumor suppressor genes play an important role in the pathogenesis of cancer, including breast cancer. The detection of such alterations might be useful for exploring the process of carcinogenesis and for predicting the phenotype that is more prone to metastasis (Keen and Davidson, 2003). Breast cancer remains the most common non-cutaneous malignancy in women (Richie and Swanson, 2003; Sasco et al., 2003). Several studies have described the correlation between amplification of c-*myc* oncogene and the course of breast cancer disease (Borg et al., 1992; Schlotter et al., 2003). It has been shown that the erbB-2 gene plays a role in the neoplastic transformation (Keen and Davidson, 2003) and in some studies an overexpression of erbB-2 gene was correlated with poor prognosis of patients with breast cancer (Emi et al., 2002; Slamon et al., 1989). Breast tumors with alterations in the p53 tumor suppressor gene have an aggressive phenotype

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(Greenblatt et al., 1994) and are more prone to develop metastases (Bergh et al., 1995). Data regarding nm23 gene in breast cancer suggest its potential role in the suppression of metastasis (Hennessy et al., 1991; Leone et al., 1993).

The aim of this study was to assess the expression of protein products of two oncogenes (c-*myc* and erbB-2) and two tumor suppressor genes (p53 and nm23-H1) in benign and malignant breast lesions, using the immunohistochemical method. We were interested in the comparison of the expression of these genes between benign and malignant breast tissue and the estimation of their possible coexpression. We were also interested in possible correlations between the results of immunohistochemical analysis and various clinical, pathological and biochemical parameters.

Patients, materials and methods

Patients and tissue specimens

We analyzed 85 malignant breast tumors-carcinomas and 51 benign breast lesions. All specimens were obtained through routine surgery performed at the University Hospital Center Zagreb, Croatia. Some clinical, pathohistological and biochemical parameters of patients and their tumors are shown in Tables 1 and 2. Macroscopic tumor size was defined as the largest tumor diameter measured by the pathologist. The histological grading was performed according to Scarff, Bloom and Richardson (Bloom and Richardson, 1957). Fresh samples of breast lesions, immediately adjacent to the segment of tissue that was fixed in formalin, were snap-frozen in liquid nitrogen and stored in the Human Tumor Bank (Spaventi et al., 1994b) at −80° until further use. Four-micrometer frozen sections were cut, mounted on glass slides and fixed in 100% acetone for 10 min. The frozen sections were analyzed by the immunohistochemical method. Before inclusion in the study, each specimen was verified by a histopathologist.

Table 1
Data on 85 patients with breast carcinoma

Patients with breast carcinoma	*N* = 85	
	Number	%
Age (years)		
≤50	17	20.00
>50	68	80.00
Tumor size[a]		
≤2 cm	18	21.18
>2 cm	59	69.41
>5 cm	7	8.24
Histological grade[b]		
Well differentiated (I)	21	24.71
Moderately differentiated (II)	20	23.53
Poorly differentiated (III)	16	18.82
Axillary lymph nodes		
Negative	38	44.05
Positive	41	55.95
1–3 nodes	25	29.76
4–9 nodes	13	15.48
>10 nodes	3	3.57
Not known	6	7.14
Estrogen receptors (ER)		
≤10 fmol/mg proteins	38	44.71
>10 fmol/mg proteins	47	55.29
Progesterone receptors (PgR)		
≤20 fmol/mg proteins	47	55.29
>20 fmol/mg proteins	38	44.71
Cathepsin D		
High value (>45 pmol/mg proteins)	26	30.59
Low value (≤45 pmol/mg proteins)	19	22.35
Histology—PHD		
D-invasive ductal carcinoma	55	64.71
L-invasive lobular carcinoma	18	21.18
DL-invasive ductal and lobular carcinoma	2	2.35
O-other	10	11.76

prot. = proteins.
[a] The tumor size was not available in one case.
[b] Histological grading was done for invasive ductal and invasive ductal and lobular carcinomas.

Cell lines

Human breast carcinoma cell line SK-BR-3 was tested for the presence of all four proteins and was used as a positive control in all experiments. Human breast carcinoma cell line MCF-7 was also used as a positive control in the analysis of nm23-H1 protein.

The cells were cultured in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% fetal calf serum (Gibco). For immunocytochemical studies the cells were grown in chamber slides (Nunc, Roskilde, Denmark) overnight in a humidified atmosphere with 5% CO_2 at 37°C.

Antibodies

Mouse monoclonal antibodies were used as follows: antibody c-*myc* (Evan et al., 1985), antibody (MAb) c-neu/erbB-2 (Ab 3; Oncogene Science), antibody p53 (Ab 3-PAb240; Oncogene Science) for the detection of mutated p53 and antibody nm23-H1 (Molecular Oncology).

Immunohistochemistry

The slides were fixed and the endogenous peroxidase activity was quenched by 15 min incubation in methanol with 3% hydrogen peroxide. After fixation, the slides were washed with phosphate-buffered saline (PBS). Nonspecific binding was blocked by normal rabbit serum (dilution 1:10) in the humidity chamber for 30 min. The slides were blotted and primary antibodies applied. For monoclonal antibody (MAb) c-neu/erbB-2 the incubation was 45 min at room temperature (concentration of 5 μg/ml). For c-*myc*, p53 and nm23-H1 incubation with primary antibodies was carried overnight at 4°C (dilution 1:250 for myc, concentration of 10 μg/ml for p53 and 5 μg/ml for nm23-H1). The slides were washed three times in PBS containing 3%, 2% and 1% normal human serum. Secondary antibody (rabbit to mouse