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Molecular markers (c-erbB-2, p53) in breast cancer

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Abstract: The aim of our study was to evaluate the correlation between clinical characteristics, histopatologic features and c-erbB-2 as well as p53 expression in cancer tissues. Breast cancer tissue was obtained from 184 female subjects with primary breast cancer. According to hormonal status patients were divided into two groups – 64 belonged to the premenopausal group and 120 to postmenopausal group. Each patient underwent mammectomy and axillary lymphadenectomy. c-erbB-2 protooncogene was detected in 54% cases, and was correlated with infiltrating type of cancer growth, as well as larger tumor size. The presence of p53 antioncogene was observed only in 33% of cases, mainly in infiltrating duct carcinomas. The incidence of c-erbB-2 and p53 positive cases was higher among subjects, whose ultrasound and mammography revealed malignancy. There was no correlation found between of c-erbB-2 expression and axillary lymph nodes involvement It seems probable, that c-erbB-2 and p53 status of cancer tissue may prove to be useful in assessment of the level of biological aggressiveness in breast carcinomas and hence can be used as a prognostic factor.

Key words: breast cancer, molecular markers, p53, c-erbB-2

Introduction

Breast cancer is a rather common malignancy in women [1,2]. The evaluation of the biological aggressiveness of cancer cells might be used as prognostic factor. Increasing number of surgery to treat breast cancer inspired us to undertake some investigations in order to get a deeper insight into etiology and pathology of this malignancy. Well known risk factors of breast cancer are: race, age, sex, geographical environment, marital status, age at menarche, first pregnancy, age of menopause, parity, past and present breast diseases, exposure to radiation, diet and heredity. In 1999 The College of American Pathologists divided all breast cancer prognostic factors into 3 groups. First group includes factors of well-established clinical value which are obligatory in clinical practice (tumor dimensions, lymph node status, grade, histological type, mitotic index and estrogen- and progesterone receptor status). The second group consists of factors whose significance was proved by a few authors (HER-2, p53, Ki67, PCNA). Other factors of significance not confirmed in clinical studies till now (DNA ploidy, neoplastic angiogenesis, EGFR, TGF alpha, bcl-2, pS2 and cathepsin D) are included in the third group [3]. Some reports concerning the usefulness of some molecular markers as risk and as prognostic factors in breast cancer came out recently [4-7]. Aim of our study was to determine the correlation between the expression of molecular markers (p53, c-erbB-2) and clinical as well as histological picture in breast cancer in females.

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Materials and methods

Patients. Among 184 analyzed cases 148 patients (80% of all cases) were in their 4th, 5th and 6th decade of life. In accordance to TNM classification, 40 patients were T1, 140 patients were T2, and 4 patients were classified as T3 (Table 1).

All the patients underwent ultrasound breast scan as well as mammography before surgery. Only in 40 cases (21.7%) breast cancer was detected by ultrasound, in 112 patients (60.9%) the USG picture implied malignancy, and in 32 cases (17.4%) USG revealed benign tumor. Mammography detected cancer merely in 36 cases (19.6%), but in 92 (50%) patients mammography implied cancer, and in 56 cases (30.4%) benign changes was diagnosed by mammography (Table 2).

Axillary nodes were not involved (N0) in 104 patients (56.5%), and 80 (43.5%) patients histopathology revealed axillary lymph nodes metastases (Table 1).

All clinical data, including patient charts, operation notes, histopathological data and immunohistochemical study on expression of c-erbB-2 and p53 in cancer tissue were meticulously ana-

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lyzed. Patients' charts and operation notes provided us with the required information, including local clinical status, type of surgery carried out, mammography and USG findings, clinical stage of cancer according to the TNM classification and assessment concerning histopathological type and grade of breast cancer.

Tissue samples. Breast cancer tissue was obtained from 184 female subjects with primary breast cancer (clinical stage I and II), who were treated in Department of Surgery, Wrocław, from 1992 to 2001; aged from 32 to 75 years (an average of 56.5 years). According to hormonal status patients were divided into two groups – premenopausal group (n=64) and postmenopausal group (n=120). Each patient underwent mammectomy and axillary lymphadenectomy.

Immunohistochemistry. Expression of molecular markers was evaluated semi-quantitatively on paraffin slices, by the Department of Pathological Anatomy.The material was immunohistochemically stained for c-erbB-2 (polyclonal antibody Dakopatts- Dania, Code Nr. K-353) and p53 (monoclonal antibody, Dakopatts- Dania, Code Nr. K-355) as described elsewhere and analyzed under light microscopy, Olympus BX 50, with due consideration for location, range and intensity of staining.

Statistical analysis. For statistical analysis χ^2 test was used.

Results

The reults of immunohistochemistry are presented in Fig. 1 and 2. All our subjects (average age 56.5 yrs) were diagnosed with local stage of breast cancer. In accordance to the patients' age, the immunological reactivity was seen as follows: highest percentage of positive reaction against c-erbB-2 was seen in patients in 5th and 8th decade of life, whereas highest percentage of positive reaction against p53 has been detected in patients in 8th decade of life (Table 3). The highest percent expression of protein p53 was found among older patients as compared to expression of c-erb-B2 (41-70 yrs vs. 71-80 yrs). Protooncogene *c-erbB-2* was detected in 54% of cases and anti-oncogene *p53* in 33% of cases (Table 1).

As far as menopausal status of the patient is concernedd, oncoprotein c-erbB-2 was detected in similar percentages in both pre- and post- menopausal patients. Considering tumor size, highest percentage of c-erbB-2 positive cancer cells was detected in T2 and T3, whereas anti-p53 in T1 tumor (Table 3).

Protooncogene *c-erbB-2* was detected in 65% of cases with no lymph nodes involvement (N0) and in 40% of cases with lymph nodes metastases (N1). Antioncogene p53 was detected in 46.6% of (N0) cases 20% of (N1) patients (Table 3).

In 156 of 184 cases (84.8%) ductal infiltrative carcinoma or lobular infiltrative carcinoma was diagnosed. Protooncogene *c-erbB-2* was detected in 66.6% of ductal cancer cases, and in 55.5% of lobular cancer, whereas anti-oncogene *p53* was detected in 44.4% of ductal cancer and in 18.9% of lobular cancer cases (Table 3).

Table 1. Clinicopathological features reported in breast cancer patients in our study.

Characteristics	No. of cases	% of total cases
Age:		
–< 50	56	30
51-70	100	54
> 70	28	16
premenopausal	64	35
postmenopausal	120	65
Tumor size:		
T_1	40	22
T_2	140	76
T_3	4	2
Nodal involvement:		
N(-)	104	57
N(+)	80	43
Histological structure:		
Invasive ductal carcinoma	100	54
Invasive lobular carcinoma	56	30
Medullar carcinoma	16	9
Adenoid cystic carcinoma	12	7
Molecular markers:		
c-erbB-2 (+)	100	54
p53 (+)	66	33

Protooncogene *c-erbB-2* was detected in cancer tissue, in spite of the fact that USG reported a benign tumor, but the incidence of c-erbB-2 positive cells was significantly higher in group with breast cancer diagnosed by USG (p=0.035). The percentage of p53 positive cancer cells in tumors of malignancy implied by USG was similar as compared with cancer diagnosed in USG (p=0.01 vs. p=0.0003) (Table 4).

Protooncogene *c-erbB-2* was detected in cancer tissue inspite of its absence in mammography picture, but was significantly higher in tumors diagnosed by mammography as cancer. Statistically significant difference was noted in c-erbB-2 reactivity between tumors diagnosed by mammography as benign as compared to tumors of implied malignancy (p=0.02). There was no difference between mammography implied and mammography diagnosed tumors in p53 reactivity, but statistically significant difference was revealed between tumors of benign mammography appearance as compared with those of implied malignancy (p=0.007). Similarly, statistically significant difference in p53 reactivity was found between mam-

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Table 2. USG and mammography results in relation to histopathological findings in breast cancer patients.

Group	Diagnosis	USG	Mammography
		no. of cases (%)	no. of cases (%)
I	benign changes in breast	32 (17.4)	56 (30.4)
II	breast cancer suspected	112 (60.9)	92 (50)
III	breast cancer	40 (21.7)	36 (19.6)
TOTAL		184 (100)	184 (100)

Table 3. c-erbB-2 and p53 overexpression in relation to clinicopathological features.

Characteristics	No. of cases	Presence of c-erbB-2 No. of cases (%)	Presence of p53 No. of cases (%)
Age:			
=< 50	56	36 (64)	24 (43)
51-70	100	44 (44)	32 (32)
> 70	28	20 (71)	4 (14)
pre-menopausal	64	36 (56)	32 (50)
post-menopausal	120	64 (53)	28 (23)
Tumor size:			
T_1	40	16 (40)	16 (40)
T_2	140	80 (57)	40 (29)
T_3	4	4 (100)	4 (100)
Nodal involvement:			
N0	104	60 (58)	20 (19)
N1	80	40 (50)	40 (50)
Histological structure:			
Invasive ductal carcinoma	100	68 (68)	44 (44)
Invasive lobular carcinoma	56	28 (50)	12 (21)
Medullar carcinoma	16	0 (0)	4 (25)
Adenoid cystic carcinoma	12	4 (33)	0 (0)

Table 4. Immunological reactivity of antibodies with regard to USG result.

Diagnosis in USG	No. of cases	Presence of	
		c-erbB-2 No. of cases (%)	p53 No. of cases (%)
Benign changes in breast	32	4 (12.5)	0 (0)
Breast cancer suspected	112	60 (53.6)	44 (39.3)
Breast cancer	40	36 (90)	16 (40)
Total	184	100 (54)	60 (33)

Table 5. Immunological reactivity of antibodies with regard to mammography result.

Diagnosis in mammography	No. of cases	Presence of	
		c-erbB-2 No. of cases (%)	p53 No. of cases (%)
Benign changes	56	8 (14)	0 (0)
Breast cancer implied	112	64 (69)	44 (47)
Breast cancer	36	28 (77)	16 (44)
Total	184	100 (54)	60 (33)

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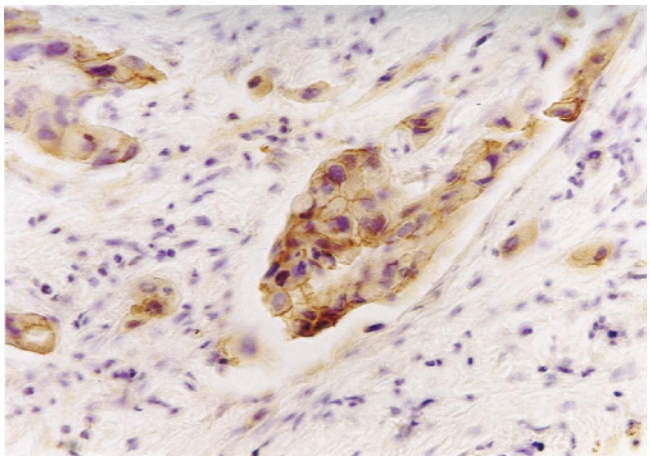

Fig. 1. Immunological staining with anti-c-erbB-2 antybody.

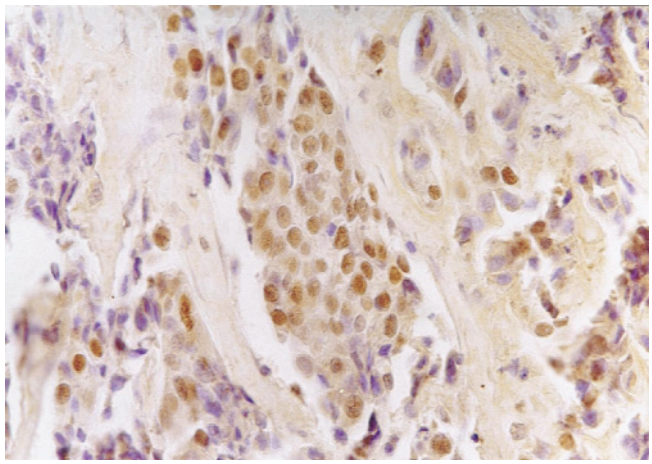

Fig. 2. Immunological staining with anti-p53 antybody.

mography benign and malignant tumors (p=0.042) (Table 5).

Among 184 analyzed cases histopathology revealed: invasive ductal carcinoma 100 cases (54%), lobular invasive cancer carcinoma 30% of cases (n=56), other types of breast cancer 16% of cases (n=28).

Discussion

Protooncogene *c-erb-B2* encodes glycoproteines which are similar to epidermal growth factor receptor (EGFR). It is considered that this product of c-erb-B2 might act as a receptor for different, still unknown growth factors. Amplification or overexpression of c-erb-B2 in female breast cancer was announced by many authors, though the percentage of c-erbB-2 positive cells found in breast cancer tissue varies among the published reports: 9% Barnes *et al.* [21], 10% Ali *et al.* [22], 11% Znou *et al.* [23], 17%, Wright *et al.* [24], 26%, Lucroix *et al.* [25], 40%, Berger *et al.* [26], 47% Guerin *et al.* [27] and 58% Wang *et al.* [28]. In our study the overexpression of c-erbB-2 was found in 54.3% cases of breast cancer. According to us, discrepancy in the results presented, by various authors may be due to the type of consolidation and the condition under which the material was consolidated .

Barnes *et al.* reported that consolidator change strongly affects estimation of c-erb-B2 expression in breast cancer [21]. Immunohistochemical staining for c-erb-B2 and p53 depended on sample preparation (paraffin embeded or snap frozen). The conformity of detection of c-erb-B2 and p53 between paraffin-embedded and snap frozen tissues cancer (breast and gastric cancer) was confirmed in 50% cases. The outcome of immnohistochemical staining may be affected by consolidater and temperature, since both factors destroy proteins structure. The same consolidater differently affected the outcome of immunohistochemical staining, depending on type of malignancy (breast cancer vs. gastric cancer), which may result from different localization of c-erb-B2 and p53 and different expression of c-erb-B2 and p53 in distinct cancers, and additionally remains under the influence of the type of immunochemistry technique employed [29,30]. Barnes *et al.* [21], Ali *et al.* [22] and Gruerin *et al.* [27] described cytoplasmic and membrane type of c-erb-B2 expression. In our study mainly the cytoplasmic type of reaction was revealed.

Protein p53 acts as a regulator of the cellular cycle and is known as a negative activator of cells growth. As DNA is destroyed the protein p53 can stop cellular cycle in G phase. After DNA reparation is initiated, p53 protein starts cellular cycle in phase S. Abnormalities of gene p53 result in dysfunction of protein p53. Beside its control function, p53 protein can also act as a transcription factor for numerous oncogenes and antioncogenes.

Ten years ago, a new gene mdm2, was localized on chromosome 12q. Three mdm2 gene products are connected to normal protein p53, whereas the one without N-terminated part of protein cannot be connect with p53 [31,32].

The correlation between alteration of gene p53 and breast cancer was observed in 1982. In 9% breast cancer patients, antibodies against the cellular protein were found. Many authors described amplification or overexpression p53 in breast cancer but their results differed. Bartek *et al.* [33] and Thor *et al.* [34] and Maru *et al.* [35] detected immunohistochemicaly p53 in paraffin skin embedded breast cancer samples in 20%, 26% and 50% cases respectively. Bartek reported higher percentage of p53 positive breast cancers in frozen tissues as compared to paraffin samples [33]. Accumulation of p53 tumor suppressor gene protein is

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generally elevated in 32% breast cancer. In our study antioncogene p53 overexpression was found in 31.4% of breast cancer cases.

Antioncogene p53 was detected more often in premenopausal period (Table 3) which is consistent with literature data [13-15]. In postmenopausal patients, antioncogene p53 was found in 50% of cases (Table 3), which was higher than it is reported in world literature (25-30%). As far as menopausal status of the patient is concerned, oncoprotein c-erbB-2 was detected in similar percentages in both pre- and post- menopausal patients, similar to the published data.

Considering tumor size, highest percentage of c-erbB-2 positive cancer cells was detected in T2 and T3 groups; whereas anti-p53 in T1 tumor group (Table 3). There is no unanimity among other authors who studied this correlation [16,17,18,19,20].

Correlation between the size of tumor and molecular findings was confirmed by some authors [36,37,38, 39]. Correlation between size of tumor and alteration of p53protein was revealed. We found accumulation of protein p53 in 37.5% T1 tumors and in 26.9% in T2 tumors, but the increase of p53 accumulation with increase of tumor size was not observed. Our results are conformable with Thompsen's results [40]. Some studies have described a correlation between expression c-erb-B2 and tumor size. In our study expression of protooncogene c-erb-B2 increased with tumor size (37.5 % of T1 tumors vs. 57.7% of T2 tumors).

Reports on correlations between USG and mammography picture of breast cancer, and molecular marker status is sparse in the literature published [41]. Among tumors diagnosed as malignant by ultrasonography, 87.5% tumors was c-erb-B2 positive and 37% was p53 positive. In cases with benign USG picture the expression of c-erb-B2 was detected in 16.6% cases and p53 was absent. Therefore our study clearly indicates that comparison of USG and mammography picture with molecular markers status may be important for early diagnosis and prognosis.

The lymph node metastases reflects systemic dissemination of cancer and plays key role in tumor host relationship. It was earlier thought that lymph nodes metastases took place earlier than systemic dissemination of cancer, but Fisher showed that cancer cells leave lymph nodes and enter the circulatory system very fast [42]. Lymph node barrier, which stops the spread of cancer cells may not be so efficient. Thus absence of metastases in lymph nodes does not exclude absence of secondary disease and distant spread. The cancer cells may also disseminate through connective tissue penetration into lymphatic vessel [43].

The necrosis of cancer tissue seemed to be related to higher histological grade of cancer and probably with increased distant spread, but in not correlated with expression of c-erb-B2 or p53 or status of axillary lymph nodes.

In our study the incidence of p53 positive cases was higher among (N+) patients as compared to (N-) ones. Our results are consistent with Rosen's observations [44]. Status of axillary lymph nodes did not affect c-erb-B2 expression in cancer tissue. Vast majority of reports described correlations between lymph node status and molecular markers in breast cancer [45-47] and merely few publications couldn't find relationship between metastases to lymph node and p53 or c-erb-B2 [48]. Carcinoma ductale infiltrans is the most common type of breast cancer. It occurs in 45-84% cases [11,12]. In our report it was found in 51.4% patients (carcinoma ductale infiltrans in 66.6% cases and multiple form in this cancer in 33.3% cases). In patients in I and II stage of carcinoma ductale infiltrans occurs more often (83.4%) than in patients in III stage (16,6%). Clinical material included: carcinoma ductale infiltrans (51.4%), carcinoma lobulare (31.4%), carcinoma medullare (8.8%), carcinoma adenoids cysticum. Only few of authors described metastases in lymphnodes. Trojani *et al.* proved that lymphnodes metastase in carcinoma ductale infiltrans occurs in 57% cases and in carcinoma lobulare in 41.4% cases [49]. Lee and Terry described that difference in occurrence of metastases in carcinoma ductale infiltrans and carcinoma lobulare is important statistically compared with carcinoma medullare and carcinoma adenoides cysticum (rare metastases) even by size of tumour more than 4 cm [50]. A correlation between molecular markers and malignant carcinoma was found.

Conclusions

In our study, expression of protooncogene c-erb-B2 was found in 66.6% cases of invasive ductal carcinoma and in 55.5% of invasive lobular carcinomas, whereas expression of protein p53 was found in 44.4% and 18.1% of cases respectively. The percentage of c-erbB-2 positive cases increased along with tumor dimensions. These findings are consistent with literature data [5,51-55]. We observed correlation, but not statistically important, between p53 expression and lymph node involvement. Marker p53 was more often detected in breast cancer tissue from premenopausal group. Both markers were detected more frequently in cases diagnosed by USG or mammography as malignant. Among all types of breast cancer, ductal carcinomas cases were more often positive for c-erbB-2 as well as p53. Above observations strongly suggest that these molecular markers may be useful in indicating the degree of biological aggressiveness of breast cancer.

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