CLINICAL SIGNIFICANCE OF P-GLYCOPROTEIN EXPRESSION IN BREAST CANCER

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ABSTRACT

Objective: to study the clinical significance of P-glycoprotein (P-gp) in breast cancer. Methods: Expression of P-gp in 60 cases of breast cancer was examined by immunohistochemistry. P-gp expression and response to chemotherapy were comparatively investigated in 19 patients with metastatic breast cancer. Results: The P-gp was positive in 48.3% of the 60 cases of breast cancer. P-gp expression was not related to patients' age, menstruation status, number of axillary lymph nodes involved, clinical stage, histological type, and hormonal receptor status ($P>0.05$). The frequency of metastasis (62.1%) and mortality (51.7%) were higher in P-gp positive cases than in negative cases (16.1% vs 12.9%, $P<0.005$). The 5-year survival rate of P-gp positive cases (48.3%) was significantly lower than that of negative cases (87.1%) ($P<0.05$). In patients who received adjuvant chemotherapy distant metastasis occurred more frequently in the P-gp positive cases (94.7%) than in the P-gp negative cases (57.1%) (P=0.0468). More P-gp negative patients (7/9) than positive patients (1/10) were responsive to chemotherapy (P=0.0055). Conclusion: Immunohistochemical examination of P-gp expression is useful in predicting response to chemotherapy and prognosis in breast cancer patients. P-gp positivity is associated with poor prognosis.

Key words: Breast cancer, P-glycoprotein, Immunohistochemistry, Prognosis.

Multidrug resistance (MDR) is one of the important factors which limits the chemotherapeutic effects. MDR phenotype is associated with overexpression of MDR_1 gene and P-glycoprotein (P-gp) (encoding product of MDR_1), which can limit chemotherapeutic effects by pumping high lipophilic and hydrophobic drugs out of the cells. In this test, we tried to detect P-gp expression in normal tissue, benign tumors and carcinoma of breast; analyse its benign tumors and carcinoma of breast; analyse its relation to clinical pathologic factors, chemotherapeutic effects and prognosis; and to evaluate its clinical significance.

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MATERIALS AND METHODS

Selection of Cases

Sixty cases of breast cancer diagnosed pathologically in our hospital from 1989 to 1992, plus 15 cases of normal breast, 16 cases of breast hyperplasia and 15 cases of fibroadenoma of breast; this also included 19 cases with distant metastasis postoperation of breast cancer in our hospital during Jan, 1996–Feb, 1997 were used for this study.

Patients' Characteristics

Of 60 patients with breast cancer, the median age was 50 (from 28–80), <50 32 patients, ≥50 28 patients; 27 were premenopausal and 33 postmenopausal; 5 were at stage I, 40 at stage II and 15 at stage III; 27 were without axillary lymphatic node metastasis (No), 17 with metastasis 1–3 (N1–3), and 16 with metastasis more than 4 (N≥4); 30 were with carcinoma simplex, 14 infiltrating ductal carcinoma, 5 infiltrating lobular carcinoma, 5 medullary carcinoma, 3 mucinous adenocarcinoma and 3 scirrhoma. Average follow-up time was 52.8 months. Postoperative adjuvant chemotherapy with CAF regimen for 6 cycles was performed on 26 patients with high risk, of which, 22 had distant metastasis (7 at stage II b and 15 at stage III). Of 19 postoperation patients with distant metastasis, 17 were female and 2 were male, with a median age of 48.5 (28–67). Chemotherapy regimen: CTX+ADR+5-Fu, PTX+ADR or EPI. The patients had been treated for 2 cycles at least, 3–4 weeks as a cycle. According to the standard evaluation criteria by WHO (1981), the chemotherapeutic response was classified as CR PR, S and PD.

P-gp Determination by SABC

Paraffin sections of the tumor were subjected to deparaffization and then down to alcohol; Antigen was repaired by microwave after 0.3% H_2O_2 incubation of the sections in methanol solution. After routine serum blocking, slices were incubated overnight at 4°C in a 1:10 dilution of P-gp monoclonal antibody (BM, Germany); incubating with biotinated antimouse Ig G and a dilution of 1:100 Streptavidin Biotin–Peroxide Complex (SABC) for 60 minutes; routine mounting was performed for microscopy after DAB coloration and hematoxylin re-staining. Estrogen receptor (ER) and progesterone receptor (PR) tests were done according to the directions of an affinity histochemistry kit of enzyme-linked estrogen and progesterone derivatives (from Oncology Institute of Shanghai Medical University). P-gp stainings were classified into 4 grades:[1] negative (-), positive (+), mid-range positive (++) and high positive (+++).

Statistical Analysis

x^2 test and Fisher's test were performed by using SAS statistical software and the survival rate was calculated by using the Kaplan-Meier method and was analyzed by the Log-rank test.

Results

P-gp Expression in Breast Cancer, Benign Breast Disease and Normal Breast Tissue

P-gp expression in 60 breast cancers and other breast tissues is as shown in the Table 1. Of 60 breast cancers, 29 were positive (48.3%). Brownish yellow granular staining in cancers could be seen scattering or in areatus statue in cytoplasm and the cell membrane; Intensity of P-gp expression varied for different tissues or different locations in the same tissue and their distribution was heterogeneous (Figures 1, 2). Positive expression at various intensities with heterogeneous distribution could be seen in breast lobular hyperplasia around the cancer; no positive expression was seen in normal tissue around the cancer. Staining of a few cells was scattering within normal breast tissue. ER (+) was in 46 cases, ER (-) in 14 cases; PR (+) in 16 cases and PR (-) in 44 cases. P-gp expression in breast cancer had no relation to patients' age, menstrual status, clinical stage, number of axillary lymphatic node metastasis, histological type of cancer and hormone receptor ($P>0.05$), the extent of P-gp expression also had no relationship with menstrual status and hormonal level ($P>0.05$). Distant metastatic frequency and mortality in the positive P-gp group were significantly higher than those in the negative P-gp group (62.1% and 16.1%, 51.7% and 12.9%, respectively) ($P<0.005$). Five-year survival rate in the P-gp positive group was 48.3%, which was significantly lower than that in the P-gp negative group (87.1%) ($P<0.05$). When distant metastasis occurred, the difference was quite significant (P=0.00005). After adjuvant chemotherapy by CAF regimen the distant metastatic frequency in the positive group (94.7%) was higher than that in the negative P-gp group (57.1%, P=0.0468).

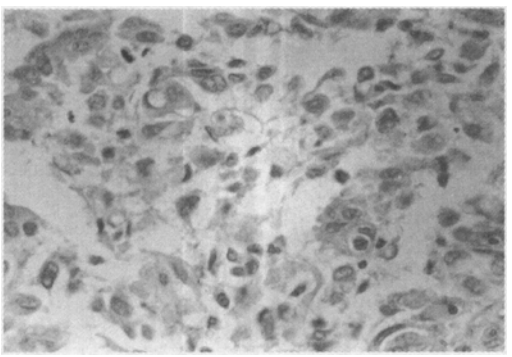

Fig. 1. Infiltrating ductal carcinoma P-gp (+) SABC, ×400.

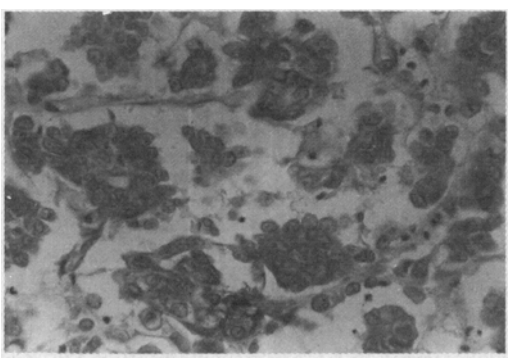

Fig. 2. Carcinoma simplex P-gp (+++) SABC, ×400.

P-gp Expression in Breast Cancer Metastasis

P-gp positive rate in 19 breast cancer cases with metastasis was 52.6% (10/19); total response rate of chemotherapy was 42.1% (8/19). Of 10 cases with positive P-gp, there was 1 case with PR, 4 with S and 5 with PD; of 9 cases with negative P-gp, there was 1 case with CR, 6 with PR, 1 with S and 1 with PD. Response rates for cases with positive P-gp and cases with negative P-gp were 1/10 and 7/9, respectively. The difference between the two rates was significant (P=0.0055).

DISCUSSION

The study indicated that there was no P-gp expression in normal breast tissue, low P-gp expression in breast hyperplasia and fibroadenoma of breast, various low P-gp expressions in hyperplasia around breast cancer, and high P-gp expression in breast cancer. Whether the activated MDR_1 gene is the cause for the increase of P-gp

expression in the development of normal breast tissue into cancer, there is no reporte yet. P-gp positive rate in the breast cancer in our study is close to that reported by other investigators.[2,3] We observed that in different pathological tissue types, or in same tissue types, or different focuses in one slice, there was heterogeneous distribution of the cells and with various staining intensities. This may be related to the heterogeneity of breast cancer cells and one of the reasons for individual variation and heterogeneous difference in tumor shrinkage and intensity during chemotherapy for breast cancer.

Table 1. P-gp expression in breast cancer, benign tumor and normal tissues

Source of Tissues	No. of cases	P-gp expression			
		+++	++	+	−
Breast cancer	60	7	12	10	31
Breast hyperplasia	16	0	0	6	10
Fibroadenoma of breast	15	0	0	4	11
Normal breast	15	0	0	0	15

P-gp expression is not related to patients' age, menstruation status, clinical stage of the tumor, number of axillary lymphatic node metastasis, histological type and hormonal receptor status etc; the intensity of P-gp also has no relation with ER and PR level. It indicated that P-gp in breast cancer is an independent prognostic indicator and not depending on the cellular reproduction rate.[2–4] Compared with the negative P-gp, the five-year survival rate of positive P-gp was lower and the frequency of distant metastasis was higher. This indicated that the patients with positive P-gp in breast cancer had higher frequency of distant metastasis and relapse, lower survival rate and a shorter survival time.[5] Distant metastasis frequency of the postoperation patients with positive P-gp was higher than those with negative P-gp after the CAF adjuvant chemotherapy. This indicated that adjuvant chemotherapy with MDR drugs didn't reach the goal of controling the subclinical micrometastasis.

Our study has shown that the total response rate of adjuvant chemotherapy by combined ADR and PTX regimen for the patients with positive P-gp was obviously lower than that for those with negative P-gp. This is consistent with the result of adjuvant chemotherapy and that found in literature.[6–8] These findings suggest that the P-gp drug pump increases the efflux of drugs and decreases the killing power of drugs and then decreases the response to chemotherapy. Our results suggest that MDR drugs should be avoided when using chemotherapy for patient with positive P-gp expression. In conclution an immunohistochemistry measurement for P-gp expression is helpful in evaluating MDR, chemotherapy effect and prognosis, and guiding the design of a chemotherapy regimen.

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