Study on Relationship between P-glycoprotein and P53 Expression in Gastric Carcinoma

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Abstract **Objective** To evaluate the clinical significance of the expression of P-glycoprotein (P-gp) and P53 protein and their relationship in gastric carcinoma. **Methods** The expression of P-gp was examined with flow cytometry and the expression of P53 was examined by immunohistochemical staining in 98 specimens of gastric carcinoma patients who hadn´ t received chemotherapy. **Results** The positive expression rates of P-gp and p53 in a total of 98 patients with gastric carcinoma were 59.2% and 52.02% respectively, and the expressions were not related with the size of tumor, differentiation degrees, clinical stages, lymph metastasis, and the sex and survival time of the patients except the age of the patients($P<0.05$). When comparaed the expression rates in the groups of P53- and P-gp+ with that in other groups, there were no significant difference in statistics. **Conclusion** There is no clinical significance of detecting P-gp and P53 for the gastric carcinoma prognosis. But it can provide a reference for selecting of anti-carcinoma medicine.

Key words Gastric carcinoma; P-gp; P53

Up to now, there is a different report about the relationship between the expression of P-gp, P53 and their clinical pathology in the tissue of gastric carcinoma. In the present study, the expression of P-gp protein and P53 were examined with flow cytometry and Evision of immunohistochemical staining in 98 patients with gastric carcinoma, in order to study the relationship between the expression of P-gp ,P53 and the clinicpathological factors of the patients.

MATERIALS AND METHODS

Study subject

Ninety-eight patients were being operated in Zhejiang Cancer Hospital from December 1998 to September 2000, which include 67 male, 31 female, their age ranged from 30 to 80 years old (mean 58 years old). All the cases were followed-up, among them, 11 cases were lost who were considered as dead case in statistics; 38 cases (38.8%)were survive over three years long.

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Reagents

The phycoerythrin (PE) labeling mouse anti-human monoantibody was used by FCM in the experiment. P-gp-PE and control IgG2a-PE were purchased from Immanotech Co. P53 mAb was made by DAKO Company. En Vision Immunohistochemistry Reagent Kit (work concentration 1:200) was made by DAKO Company (supplied by Shanghai gene Company).

Methods

To take a mucosa membrane, which was from the tissue cell of gastric carcinoma and the normal mucosa from gastric operating incision . After separating by machine, and single cells were obtained by passing a 200 whole nylon billeting net.To take a 20 ul antibody added into 12 mm × 75 mm plastic tube, two tubes for each case. A tube is P-gp-PE and B tube is IgG2a-PE as a negative control. In each tube 1×10^6 single cells suspension was added, after enough stirring, put them in the dark-room for 30 minutes, then 3 ml PBS (containing 0.1% sodium azide) was added, shaking and stir-

ring then centrifuging (200g) for 5 minutes, and poured out of the supernatant,and added 0.5 ml 1% Polyoxymethylene for fixation. On the day, it was detected by FCM (FACS Calibur B.D Company, USA). A set number of cells to be collected to 2000 in acquisition window, then analyzed a positive cell percentage by B. D Cellquest software.

EnVision immunhistochemistry was used for the P53 kit (DAKO). The step as following: (1) The paraffin sections were dewaxed in water. (2)Hot restoration, put the sections into citrate-buffered (0.01mol/L, pH 6.0), microwave boiling for 10 min.(3)After washing by distilled water, put it into 3% H2O2 for 5 min. (4)After washing by distilled water, and washing again with TBS buffer, then with the first antibody P53 incubated for 60 min at room temperature. (5)After washing by TBS buffer, with the polymer second antibody against En Vison incubated for 30 min at room temperature. (6)After washing by TBS buffer, DAB for developing to control under the microscope. (7)After washing by distilled water, restraining with hematoxylin, dehydration, clearing and mounting. With TBS substitution the first antibody as a negative control, and the known positive tissue act as positive control.

Judgment of the P-gp expression: The P-gp expression was 24.74%, which was from the 98 cases with normal gastric incision mucosa. Therefore, positive cells < 25% was defined as a negative; weak expression: positive cells was 25%~40%; moderately expression: positive cells was 41%~60%; strong expression: positive cells was >60%.

Assessment criteria of P53: The positive expression of P53 was mainly locates in cellular nucleus. According to the assessment criteria of DAKO company: negative (-), no staining cells or equal to the staining of background; positive (+), weak or incompletely staining cells was <15% under the high power microscope; moderately expression (++), positive cells was 41%~60%, strong positive (+++), the tumor cells were completely strong stained >50% under the high power microscope. Statistical analyses t test was used, and P value less than 0.05 stands for statistical significance.

RESULTS

The expression of P-gp The negative expression of P-gp in 40 cases among 98 patients with gastric cancer (40.8%); low expression were in 14 cases (14.2%); moderate expression were 17 cases (17.3%); strong expression were 27 cases (27.5%). When they were statistics separately, there was no significant difference of P-gp expression in size of tumor, differentiation degrees, clinical stages, lymph metastasis, sex and survival except the patient´ s age. The positive expression rate in patients whose age were <60 year old was higher than that in the patients ≥60 years old, the difference had a significant in statistics (P<0.05) (table 1).

The expression of P53 The negative expression of P53 was in 47 cases (47.9%), and 51 cases (52.02%) were positive expression in a total of 98 patients with gastric carcinoma. Among them, weak expression was in 33 cases; moderate expression was in 9 cases; strong expression was in 9 cases. There was no relation between the positive expression of P53 and the tumor size, differentiation degrees of the tumor tissues, clinical stages, lymph node metastasis, ages sex and condition of survival. Of the 47 cases were negative expression of P53, Among those weak expression (33 of cases); moderate-strong expression (18 cases), there was also no a significant difference (P>0.05) in their tumor size, differentiation degrees, clinical stages with or without lymph node metastases, ages, sex and condition of survival.

There were 28 cases with the deletion of P53 suppressor and positive expression of P-gp and as compared with other group, there also were no significant difference (P>0.05) (table 2).

DISCUSSION

Table 1 The relation between expression of P-gp and age of patients with gastric carcinoma

Age(n)	P-gp+(n)	P-gp-(n)	P	X^2
≥60(42)	20	22	0.0437	4.069
<60(56)	38	18		
total(98)	58	40		

Table 2 Compared between P53−&P−gp+, P53−&P−gp−, P53+&P−gp+, P53+&P−gp− and clinical pathological factor

group(n)	P53− & P−gp+	P53− & P−gp−	P53+ & P−gp+	P53+ & P−gp−
Tumor size				
⩾6cm	17	10	18	14
<6cm	11	9	12	7
Differentiationdegree				
Moderate	7	3	7	6
Moderate−low	21	16	23	15
Lymphonode metastasis				
With	6	3	6	5
Without	22	16	24	16
Clinical stages				
II	7	5	7	5
III−IV	21	12	23	16
Sex				
Male	18	12	21	14
Female	10	5	9	7
Ages				
⩾60	10	11	11	11
<60	18	8	19	10
Survival				
<3 years	15	12	20	13
>3 years	13	7	10	8

The over expression of multidrug resistance (MDR1) gene amplification and its product of P−gp was Known to be one of a directly failure reason in the clinical chemotherapy. The molecular weigh of P−gp is a 170 KD, and belong to a translocation ATP binding protein family [1]. The transmembrane structure of P−gp possesses a "drug −pump" function, which is dependent on its energy, and it can pump the hydrophobic − lipophilic medicine, such as VCR, alkaloids, ADM and so on out of the cells and resulted in the drug concentration decrease of the cells, which makes the action of cytotoxicity decrease or a complete loss and to produce the drug tolerance. As a result the patients loss their sensitivity for the medicine therapy and produce the tumor metastasis and relapse. Therefore, detecting the MDR of tumor may be taken as a guide selecting anticancer medicine in clinical and to apply the reverse drug of P−gp was an important significance[2]. Wang *et al.*[3] reported that the expression of P−gp was 65.21%(30/46) in the 46 cases with gastric carcinoma with immunohistochemistry methods. The expression of P−gp was related to the clinical stages of the tumor,the later clinical stages the higher expression of P−gp,so it could be a marker of commenting the prognosis. Liu *et al.*[4]found that the intensity of P−gp expression was related to the histological types of the cancerous tisues and the lymph node metastasis. Zhang *et al.*[5] took the assay of immunohistochemistry to detecting the expression of P−gp in 101 cases with gastric carcinoma, 18 cases of heterotype proliferation and 20 cases of normal gastric mucosa,and found the expression of P−gp in all tissuesof the normal gastric mucosa, heterotype proliferation and the tissues of gastric carcinoma without chemotherapy. The intensity of P−gp expression was positively related to the depth of invasion, lymph node metastasis, clinical stages and the prognosis of the patients, the stronger expression of P−gp, the short the survival time. Huang *et al.*[6]used the way of immunohistochemistry to examining the positive ratio of P−gp expression which was 82.4% (89/105 cases), the positive expression rate was not related to lymph node metastasis (P>0.05). But they say the positive expression of P−gp was one of an important marker for judging the prognosis of patients. Chen *et al.*[7] reported that they used the way of immunohistochemistry to detecting expression of P−gp in the tissue of 60 cases with gastric carcinoma which expression ratio was 58.39%, and found the expression of P−gp was not related with the differentiation degrees of the tumor tissues and clinical stages (P>0.05). Cai *et al.*[8]reported that the expression rate of P−gp was 58.2%,and which were not a obvious relationship with the tissues type of gastric carcinoma,depth of invasion and lymph node metastasis.

At present study, our finding the positive P−gp of expression ratio was 59.2% in the 98 cases with gastric carcinoma, the positive expression of P−gp as compared with their tumor´ s size, differentiation degrees, clinical stages, lymph node metastasis, sex and three years survival times all was not a significant difference. But in the <60 years old patient their positive of expression ratio was obviously more than the ⩾60 years old group. Compared the difference between two groups,

there were a significant difference ($P<0.05$), which was the same with Chen[7] and Cai [8]´ s results. It suggested that detecting expression of P-gp was not clinical significance for the prognosis of the patient of gastric carcinoma, but it will be benefit to prognosticating sensitivity of chemotherapy, and so it could be taken as a guide the reasonable for selecting anticancer drug to avoid the blindness of using medicine. A number of study indicated that chemotherapeutics of MDR were vincaleukoblastine (VLB), Vincristine (VCR) and adriamycin (ADM), especially the tolerance of VLB. Therefore,selecting the chemotherapeutic for the gastric carcinoma´ s patient with a positive P-gp, to avoid using the medicine of lipid and alkaloids as possible and to selecting the alkylating and anti-metabolism agents that was not closely related to P-gp. To detecting the expression of P-gp was an important significant for the patient with positive expression of P-gp ,which could do the efficient reverse treatment. To using the P-gp reverse agent could reverse the expression P-gp. Zhou *et al.*[9] new reported that using the adenovirus vector was induced by increasing dosage of JNK (c-Jun NH2-terminal kinase) way, which can decrease the P-gp levels to control the tolerance, or even to revease the cancer cell.

p53 gene is a suppressor, and it is an important negative factor in the normal cell growth and division. It can lose their activity by the different site of mistake mutation and allele deletion. The expression rate of p53 protein in general is about 40%~60% ,our result was 52.02% which is same as the other studies[7,8].

On th relationship between the expression of p53 protein in the gastric carcinoma tissue and the parameter of the tumor clinical pathological, there were a bigger difference among the research results reported,up to now without a conclusive result. Wan *et al.*[10] reported that it was no relationship between the expression of p53 protein and the sex, ages of the patients, tumor´ s form as well as histological type, but there is a closely relationship between the invasion degree(T staging) and the lymph node metastasis (N staging), the gene mutation of p53 was considered as a critical role of the carcinogenesis and invasion metastasis. Wang *et al.*[11]reported the p53 expression of the gastric carcinoma in the early and advanced stages, which had a remarkable difference($P < 0.05$). AI-Moundhri *et al.*[12] reported to examing the positive expression of p53 in 121 arabic cases with gastric carcinoma by immunohistochemistry was 54%. There was a closely relationship between the positive expression of p53 and patient´ s ages, tumor´ s size,tumor´ s form, histological type, lymph node metastasis and clinical stages. It was an important factor for judging the prognosis of the patient. Sun *et al.*[13] reported that they applied immunohistochemistry to detecting the ratio of p53 expression in the gastric carcinoma tissue of 58 cases, it was no significant relationship to their tumor size, histological type, differentiation degree, lymph node metastasis and patient´ s ages ($P > 0.05$). Duan *et al.* [14]reported the expression of p53 was related to the lymph node metastasis, and it was not related to the prognosis of the patients. Jovanovic *et al.*[15] reported that the expression of p53 in tissues of 66 cases with gastric carcinoma hadn´ t a obviously relationship with the tumor occurring site, tumor´ s type, differentiation degree, depth of invasion, lymph node metastasis and patient´ s sex and so on. The results of this study also suggested the expression of p53 was not related to their tumor´ s size, differentiation degree, clinical stages, lymph node metastasis, ages, sex and survival condition of the patients ($P>0.05$). So we consider that there is not a clinical significance to detecting the expression of P-gp and P53 on the prognosis in the patient with gastric carcinoma. But to detecting the expression of P-gp may be taken as a guide in a reasonable selection of clinical anti-tumor medicine, to decrease the blindness of using the drug, or for selecting the revere agent of P-gp to providing a reference basis.

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