The Significance of p53 and P-Glycoprotein Over-Expression in Gastric Cancer and Breast Cancer

Haitao Liao[1], Liyi Liu[2], Yinnong Zhao[2]

1 Nurse Training College of Guangxi Medical University, Nanning 530021;
2 Cancer Hospital of Guangxi Medical University, Nanning 530021

Objective To study the levels of p53 protein and P-glycoprotein (P-gp) and its clinical significance in gastric cancer and breast cancer. **Methods** 93 cases of gastric adenocarcinomas and 60 cases of breast cancer specimens were analyzed for p53 and P-gp by immunohistochemical staining. **Result** In breast carcinoma the positive expressions of P-gp and p53 were 38.3% and 56.7% respectively. In gastric carcinoma the positive expressions of P-gp and p53 were 44.1% and 55.9% respectively. over-expression of The P-gp and p53 were signifrecantly related to lymph node metastasis (P<0.05). Co-expression of p53 and P-gp were common (P<0.05). **Conclusion** Mutation of p53 and P-gp over-expression may play an important role in the lymph node metastasis of gastric cancer and breast cancer. Mutation of p53 gene may affect the over-expression of mdr-1 gene activity, so that p53 and P-gp gene products immunostaining assay at the early stage of gastric cancer and breast cancer may be of great clinical significance.

Key Words gastric neoplasms; breast neoplasms; P-glycoprotein; p53 protein

Malignances still are major risk diseases for human health. One of the means of treatment is improvement on chemotherapy and using new drugs. However some patients are several multi drug resistance that result in failure of chemotherapy, produce a great impact on the effect of therapy and prognosis. There may be a cooperation effect of several factors for the production of resistance to cancer therapy, including multi-drug-resistance (MDR) phenotype, which may be caused by accumulation of drug inside the body leading P-gp to increas activity, and another is the mutation of apoptosis associate gene. Three drug resistance genes, bcl-2, p53 and BCR/ABL. have been attracted more attention in order to inquire the relation between p53 protein and the expression of P-gp the present study used immunohistochemistry technique to test the p53 and P-gp, genes expression in stomach cancers and breast cancers, and analyzed the effect of P53 protein on P-gp.

MATERIALS AND METHODS

Samples and Source Paraffin-embedded tissues of 93 cases of gastric adenocarcinomas and 60 cases of breast cancer from randomly selected patients who were treated surgically or biopsy from 1996 to 2000 in cancer hospital of Guangxi Medical University. Before operation, they had not received chemotherapy. Tumors tissues were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections (4μm) were cut form each tumor specimen.

Reagents Mouse-anti-human monoclonal antibody against p53 protein, mouse-anti-human monoclonal antibody against P-gp, and SABC reagents kit (Wuhan Boster Biological Technology Company, Wuhan, China) were used in the present experiments.

Immunohistochemical Stain Immunohistochemical SABC techniques were applied. All the sections were routinely deparaffinized and rehydrated, then immersed in 3% H_2O_2 to block the endogenous enzymes, subsequently the antigen were repaired by microwave, and the rest operations were performed according to the introduction of the reagent kit, Then these sections were stained with DAB and counterstained with hematoxylin, and the results were examined under microscopy. The sections of carcinoma tissues with known protein were used as positive controls and PBS substituting the first antibody was used as negative controls.

Evaluation of results The proteins were stained brown or yellow granules or masses as positive expression. P53 protein was mainly located in the nucleus of tumor cells(Fig. 1 and 3), P-gp was mainly located in the cytoplasm and the membrane of tumor cells(Fig. 2 and 4). According to the standard set up by Leonardo[1] to evaluate the results: the protein positive cancer cells<10%(-), 10% to 30%(+), 31% to 50%(++), >50% (+++).

Correspondence: Haitao liao, professor, Nurse training college of Guangxi medical university, Nanning 530021, E-mial: ydhxlht@vip.sina.com

Statistical analysis Data was analyzed by using the χ^2 test. A P value less than 0.05 was considered significant.

RESULTS

P-gp and p53 protein expression In 93 cases of gastric carcinoma, the positive expressions of P-gp and p53

Table 1 P-gp and p53 protein expression in gastric carcinoma

Pathologic features	n	p53		P-gp	
		+	PI/%	+	PI/%
Total	93	52	55.9	41	44.1
Differentiation					
High	9	3	33.3	3	33.3
Moderate	28	16	57.1	11	39.3
Low	56	33	58.9	27	48.2
Depth of invasiveness					
Mucous or submucous layer	16	7	43.8	4	25.0
Muscular layer	14	8	57.1	6	42.9
Outer membrane	63	37	58.7	31	49.2
Lymphatic metastasis					
Negative	29	11	37.9	8	27.6
Positive	64	41	64.1*	33	51.6**

Compared with negative * x^2=5.5284, *P*<0.05 ** x^2=4.6541, *P*<0.05

Table 2 P-gp and p53 protein expression in breast carcinoma

Pathologic features	n	p53		P-gp	
		+	PI/%	+	PI/%
Total	60	34	66.7	23	38.3
Pathologic type					
Infiltrating ductal carcinoma	11	7	63.6	4	36.4
Simple carcinoma	33	20	60.6	16	48.5
Medullary carcinoma	10	5	50.0	3	30.0
Mucoid carcinoma	3	1	33.3	0	0
Scirrhous carcinoma	2	1	0	0	0
Infiltrating lobular carcinoma	1	0	56.7	0	0
Lymphatic metastasis					
Negative	21	7	33.3	4	19.0
Positiv	39	27	69.2*	19	48.7**

Compared with negative * x^2=5.7759, *P*<0.05 ** x^2=3.9057, *P*<0.05

Table 3 The relationship between P-gp and p53 protein expression in gastric carcinoma

P-gp	n	p53		
		-	+	PI/%
-	52	29	23	44.2
+	41	12	29	70.7
Total	93	41	52	55.9

x^2=5.5005, *P*=0.0189, r=0.2650

Table 4 The relationship between P-gp and p53 protein expression in breast carcinoma

P-gp	n	p53		
		-	+	PI/%
-	37	22	15	40.5
+	23	4	19	82.6
Total	60	23	34	56.7

x^2=8.5806, *P*=0.0034, r=0.4128

were 44.1% and 55.9% respectively. It was closely associated with lymphonodus metastasis (P<0.05, Table 1), but the expressions of P-gp and p53 were not related to the cell differentiation and depth of invasion in gastric carcinoma (P>0.05, Table 1). In 60 cases of breast carcinoma, the positive expressions of P-gp and p53 were 38.3% and 56.7% respectively. It was associated with lymphonodus metastasis closely (P<0.05, Table 2), but the expressions of P-gp and p53 were not related to the pathologic types of breast carcinoma (P>0.05, Table 2).

The relationship between P-gp and p53 protein expression From the Table 3 and 4, there were shown the correlation between P-gp overexpression and p53 expression in gastric carcinoma and breast carcinoma.

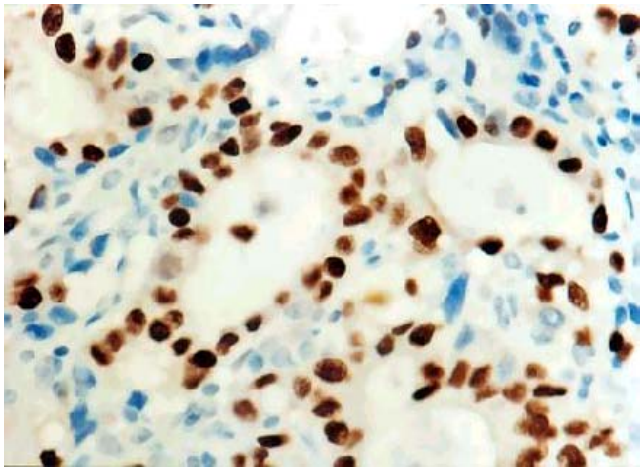

Fig.1 Immunohistochemical stain shows p53 protein in majority of nucleus in gastric glandular carcinoma cell (original magnification ×400)

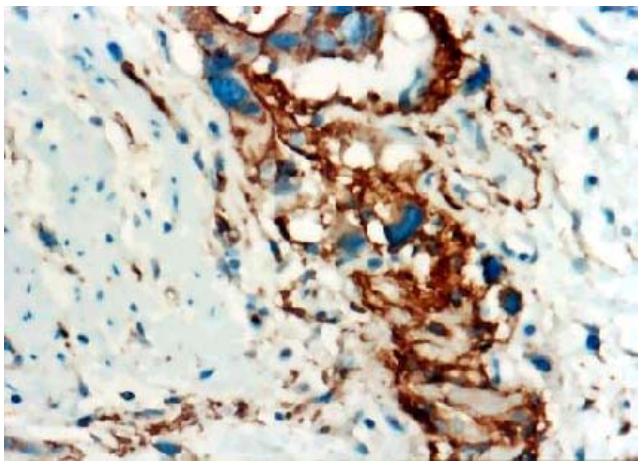

Fig. 2 Immunohistochemical stain shows P-gp in majority of gastric glandular carcinoma cell in cytoplasm and their membrane(original magnification ×400)

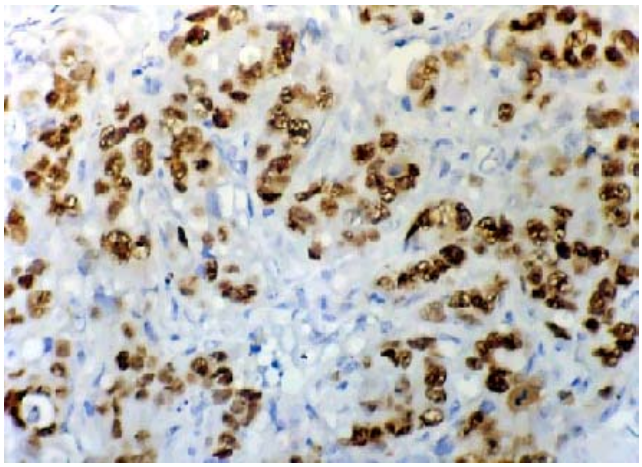

Fig. 3 Immunohistochemical stain shows p53 protein in majority nucleus of breast carcinoma cell (original magnification ×200)

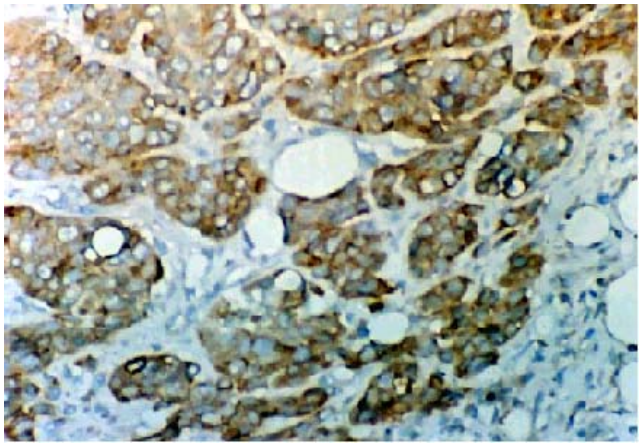

Fig. 4 Immunohistochemical stain shows P-gp in majority of breast carcinoma cell in their cytoplasm and membrane (original magnification ×200)

DISCUSSION

P-glycoproteins are encoded by multidrug resistance gene (MDR-1), its molecular wt=170kd, this membrane glycoprotein is constituted by 1280 amino acids. The level of protein expression is relative to the membrane permeability, intracellular drug concentration and the degree of drug resistance. It serve as a active transpotation pump of ATP dependence. It can maintain very low intracellular concentration of drug by pumping drug out which is the drug resistant base of the cell[2]. The level of MDR-1 expression is positive relation to drug resistance. There is a debate upon whether the level of P-gp expression can be a marker for calculation of clinical cancer chemotherapeutic effectiveness [3,4]. The present

results suggest that the positive group of lymphonodus metastasis in gastric carcinoma has a higher P-gp expression than the negative group (P<0.05). Some colonic adenoid metaplasia area where the surface of cell show P-gp positive reaction. However there are no relation to the patient's age, tumor, size, differentiation of cancer cell and depth of invasion (P>0.05). The level of P-gp expression is low in breast cancer, while P-gp positive expression in lymphonodus metastasis group is higher than non-metastasis group. It suggest that P-gp positive cancer cells have higher malignancy, more potential metastasis, and poor prognosis and may be one of the reason to reducing the effect of chemotherapy.

As far as our knowledge goes, p53 gene is the most relative gene to human neoplasm and one of the most widely investigated tumor suppressor gene. Wild-type p53 gene is suppressor gene. The function of p53 is monitoring the genomic completeness as a G1 checkpoint in the cell cycle. If there is any damage in a DNA, the cycle will be stopped at the G1 phase until the DNA has been repaired, otherwise p53 mediate apoptosis. Mutations in the p53 gene are inactive and loss its monitor function and inhibit the cancer cell apoptosis that led to tumors development[5, 6]. It is documented that mutations in the p53 gene increase in drug resistance of cancer cell[7, 8]. In the present experiment, the positive expression rate of p53 protein is 55.9% in 93 cases of gastric carcinoma group, lymphonodus metastasis group higher than non-metastasis group (P<0.05). In 60 cases of breast carcinoma, the positive expression rate of p53 protein is 56.7%, lymphonodus metastasis group and non-metastasis group are 69.2% and 33.3% respectively, the difference is significant (P<0.01). It indicates that p53 protein act an important role on metastasis and prognosis estimation both in the carcinoma stomach and breast, so that after p53 gene mutation there follows malignant cell hyperplasia and gains drug resistance.

We found that there is significant cooperation of P-gp and p53 protein expression in stomach and breast carcinomas(P<0.05 and P<0.01 respectively). It suggests that p53 gene mutation may follows a direct or indirect pathway to take part in control of the mdr-1 and influence the expression of P-gp. Therefore, early examine the above two gene products in stomach and breast carcinoma is important in calculation the patient's prognosis and clinical drug resistance.

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