The clinical significance of detecting expression of P-gp in the cell of gastric carcinoma by flow cytometry

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Abstract **Objective** To understand the clinical significance of the expression level of MDR1 gene product P-glycoprotein (P-gp) in the cell of gastric carcinoma. **Methods** The expression level of P-gp in the operating samples from 98 patients with gastric carcinoma and the normal gastric mucasa near the operating section were detected by flow cytometry. **Results** In the expressing positive cell of P-gp in 98 patients with gastric carcinoma, <25% were 40 cases (40.8%); 25-40% were 14 cases (14.2%); 41-60% were 17 cases (17.3%); >60% were 27 cases (27.5%). The expression rate in gastric carcinoma involved in two site (AM/MC/CM) or three site (AMC) was higher than the normal gastric mucosa. compared with normal gastric mucosa, the expression rate of P-gp was higher in ulcer type, infiltrating ulcer type carcinoma in infiltrating type, diffused infiltrating type carcinoma; It were higher in gastric carcinoma with larger size and with lymph node metastasis, The differences were statistical significance (P<0.01). The expression rate of P-gp were related to the differentiation of the cancer and with clinical stages. **Conclusion** FCM in detecting the P-gp expression level in gastric carcinoma may well be more quick, convenient, and to possess an accurate detecting character. It could provide a reference for an electing proposal of clinical chemotherapy and to judge the prognosis.

Key Words Gastric carcinoma; P-glycoprotein; Flow cytometry

It is well known that one of the direct reason for the fail to chemotherapy may be related to multidrug resistance gene (MDR1) and its over expression product P-glycoprotein (P-gp). But only a few reports about the expression of MDR1 in gastric cancer up to now. It has not been reported about using the flow cytometry (FCM) to detecting the P-gp in the patients with gastric carcinoma. In order to understand the clinical significance of the P-gp expression level in the cell of gastric carcinoma, the present study examined the expression level of P-gp in 98 cases patients with gastric carcinoma without chemotherapy before operating and normal gastric mucosa by FCM, It may be to provide a scientific basis for combining treatment and choosing the drug of chemotherapy in gastric carcinoma.

MATERIALS AND METHODS

Clinical materials A total 98 cases with gastric carcinoma, which were hospitalized patients in department of chest-surgery of Zhejiang Cancer Hospital were collected from December, 1998 to Sept ember 2000. Among them, the male were 67 cases, female 31 cases. The mean age is 58 ±12 gears old (ranging 30-80).

Agents The antibody is a mouse-anti-human monoantibody (P-gp-PE) marked with PE (phycoerythrin), The control is an IgG2a-PE (Immunotech product).

Methods We took a mucosa from gastric carcinoma and gastric operating section site after separating by machine, and obtained a single cell by passing a 200 whole nylon billetingnet. Taking a 20μl antibody added into 12×75mm plastic tube, two tubes for each case, A tube is P-gp-PE, B tube is IgG2a-PE as a negative control. In each tube added 1×10^6 cells suspension, after enough stirring, put it in the dark-room for 30 minutes, then 3ml PBS (containing 0.1% sodium azide) was added, shaking and stirring, then centrifuging (200g) for 5 minutes, and poured out of the supernatant, and added 0.5ml 1% para-formalin for fixation. On the day, it was detected by the TACS Caliber FCM (BD Company).

Judgement of results From each tube more then 2000 cells was collected within the cell gate. The percentage of positive labeled cell was analyzed by using cell guest software and histogram.

Grades of positive cells were classified as negative (25%), low (25-40%), middle (41-60%) and high (<60%), the results are presented by using the student´s t test for statistical analyses.

RESULT

The expression of P-gp in the total of 98 patients with gastric carcinoma was as follows: positive cell <25%(negative expression) were 40 cases (40.8%); 25-40% (low expression) were 14 cases (14.2%); 41-60% (middle expression) were 17 cases (17.3%); >60% (high expression) were 27 cases (27.5%).

Site of tumor growth The expression level of P-gp with the site of tumor growth was showed in table 1.

Macropathology types The relation between expression of P-gp of gastric carcinoma and the macropathology types was showed in table 2.

Size of tumor The relation of expression of P-gp of gastric carcinoma to the size of tumor was showed table 3.

Differentiation degree of tumor The relation of the expression P-gp to their differentiation degree was showed in table 4.

The relation between the expression of P-gp of gastric carcinoma and lymph node metastasis The expression positive rate of P-gp were (40.78± 3.15)% in 57 cases gastric carcinoma with lymph node metastasis. There was the most significant difference when compared to the expression in normal gastric mucosa (P=0.00002, t= 4.387). The pos-

Table 1. The relation between expression of P-gp in gastric carcinoma and the site of tumor

Site of tumor growth	Cases(n)	P-gp %($\bar{X}\pm S\bar{x}$)	P	t
One (A/M/C).	34	32.39 ± 4.07	0.076	1.787
Two(AM/MC/CM)	57	43.37 ± 3.88★	0.00001	4.619
Three(AMC)	7	52.21 ± 7.37★	0.0009	3.408
Normal mucosa	98	24.74 ± 2.09		

★ P<0.001 vs normal mucosa.

Note: clinical records A: indicate gastric pyloric antrum; M: indicate body of gastric; C: indicate cardiac gastric.

Table 2. The relation between expression of P-gp of gastric carcinoma and the macropathology types

Macropathology type	Cases(n)	P-gp %($\bar{X}\pm S\bar{x}$)	P	t
Raised, depressed	8	38.11 ± 10.4	0.092	1.701
Ulcer	40	37.96 ± 4.59★	0.003	3.015
Infiltrating ulcer	31	36.59 ± 4.16★	0.008	2.701
Infiltration, diffused infiltrating	19	51.63 ± 6.48★	0.000004	4.880
Normal mucosa	98	24.74 ± 2.09		

★ P<0.001 VS normal mucosa.

Table 3. The relation between expression of P-gp of gastric carcinoma and the size of tumor

Size of tumor	Cases(n)	P-gp % ($\bar{X}\pm S\bar{x}$)	P	t
≤4cm	27	37.44 ± 4.79★	0.008	2.702
5-8cm	57	41.46 ± 3.93★	0.0001	4.123
>8cm	14	40.32 ± 6.15△	0.011	2.601
Normal mucosa	98	24.74 ± 2.09		

★ P<0.01 vs normal mucosa. △ P<0.05 vs normal mucosa.

Table 4. The relation between the expression of P-gp and the differentiation degrees of gastric cancers

Differentiation degree	Cases(n)	P-gp % ($\bar{X}\pm S\bar{x}$)	P	t
Middle differentiation adeno-ca.	23	36.00±5.72△	0.030	2.202
Middle-poor differentiation adeno-ca.	28	37.28±5.34△	0.011	2.581
Poor differentiation adeno-ca.	35	41.75±4.09★	0.0001	3.991
Signet ring cell carcinoma	12	50.45±9.41★	0.0002	3.791
Normal mucosa	98	24.74±2.09		

★ P<0.01 vs normal mucosa. △ P<0.05 vs normal mucosa.

itive rate of P-pg was (37.88±5.82)% in 41 cases without lymph node metastasis, which is also higher than the expression of it in normal gastric mucosa (P= 0.015, t= 2.475).

The relation between the expression of P-gp of gastric carcinoma and the clinic stages of tumors The positive rate of P-gp was (38.93 ± 5.28)% in 26 cases with I-II stage of gastric carcinoma, and was (40.65±3.26)% in 72 cases with III-IV stage of gastric carcinoma, compared to their expression in normal gastric mucosa, the expression of P-gp in various stages of gastric cancer was higher, the difference was statistical significant (P= 0.004, t =2.912; P=0.0003, t =4.293).

DISCUSSION

The pathologic mechanism of the MDR is a very complex problem, in which, the overexpression of P-gp may be a principal cause. The molecular weight of P-gp is 170KD, it belong to a translocation ATP binding protein family. The structure of P-gp possesses a "drug-pump" function, which is dependent on its energy, and it can pump out the hydrophobic-lipophilic medicine, such as VCR, alkaloids, ADM and so on. Then it able to flow out of the cell and result in the drug concentration decrease of the cells, which makes away the cell-toxin action of the medicine or a complete loss. At the same time, it makes the cells produce the drug tolerance. So the P-gp is a molecular basis for producing the drug tolerance. If the tumor cell possesses a MDR gene, the P-gp will express in proportion to its tolerance degree[1].

Over a ten years´ study on the mechanism of MDR has showed that the MDR1 gene amplification and over expression of P-gp may be one of reasons failure to clinical chemotherapy. It makes the patient producing drug resistance and result in metastasis and recurrence. Therefore, to detect the MDR of tumor is of an important significance for choosing and conducting the clinical practice of using the anticancer drug.

Now, It has been used the gene chip technique to study the gene amplification[2] in home and in abroad. Someone[3] to apply the comparative genomic hybridization (CGH) technique to study the abnormal MDR1 gene. But a great number of studies were on the expression of P-gp, the product of MDR1 gene, by using immunohistochemistry [4-5]. Therefore, only a few of paper[6-11] for detecting the expression of P-gp and MDR1 gene by FCM in the gastric carcinoma cell was reported. Fujii et al[6] detected the expression of P-gp in 40 cases of solid carcinoma (including 15 cases of gastric carcinoma) by FCM and the positive rate was 37.5%. Their results showed that the MDR1 was one of an useful marker for the prognosis of this kind of solid carcinoma (P<0.01) with the multi-variable analysis. So the solid carcinoma with a positive MDR1 or P-gp, may possess a high malignancy; It could have reference significance for the clinical appraisal.

Tanigawa et al[7] detected the MDR1 gene with RT-PCR method in the cell of gastric carcinoma, and also using the FCM and immunohistochemistry to detect the P-gp, they found that the date from the FCM was closely in relation to its resistance degree of thymine by combinatorial way, and thinking that the FCM method is a suitable for the clinical chemotherapy to calculate the relative resistance.

Hotta et al[8] examined the P-gp expression in 29 cases with fresh gastric-intestine carcinoma (16 cases of gastric carcinoma) by the FCM. The result showed the P-gp expression was obviously related to the inhibition rate of the Adriamycin.

In home, some reports[9-10] applied the FCM to detecting the P-gp expression in various kinds of malignant tumor samples.

The present study detected the P-gp expression level with FCM in 98 cases gastric carcinoma and normal operating section cell of mucosa. The result showed that there were 40 cases (40.8%) with a negative expression of the P-gp in 98 cases of gastric carcinoma. A low expression were 14 cases (14.2%), A middle expression were 17 cases (17.3%), A high expression were 27 cases (27.5%). That is to say about 40% cases with gastric carcinoma could be given consideration to treat with auxiliary chemotherapy before or after operation. It still was 14.2 % of patients may choose the operation and to add reasonable plan of the chemotherapy, and try to avoid application of medicine such as alkaloid, VCR and so on, which are easily to produce the tolerance with a high degree. For the other 45% gastric carcinoma patients with a middle and high P-gp expression should be given the study for reversing the MDR1 gene of tumor. It may beneficial to increase the proportion of chemotherapy and to promote an efficacy of the synthetic therapy.

The results of this study suggested that the P-gp expression of gastric carcinoma was related involved with the locality of tumor, the bigger the involved range, the higher the P-gp expression is. The P-gp expression of gastric carcinoma was related to their type of tumor mass. But, it was poorly related to the tumor biological behavior of clinical infiltrating type and diffused infiltrating type; The P-gp expression of gastric carcinoma was positively related to their size of tumor. In addition, the P-gp expression of gastric carcinoma was related to the degree of differentiation, the state of the lymphnode metastasis, and the clinical stage. Therefore, to detecting the P-gp expression of gastric carcinoma may provide a choice for the chemotherapy and as a reference marker for the prognosis.

In addition, Huang et al[11] detected the MDR1 gene expression from a peripheral blood lymphocyte (PBL). in 39 cases of solid carcinoma, to their thinking, detecting the MDR1 gene expression level may benefit for judging the prognosis and to make a choice of chemotherapy. However, we have been detected the P-gp expression of PBL in 200 cases with solid carcinoma and the result suggested that there was not difference compared with normal person group. In spite of the patient was at the last stage, and was obviously tolerance from a clinical observation, but their P-gp expression was still the same as normal people. The reason may be a few cell number of solid tumor in the PBL and a great number of normal lymphocyte in there. So there was not a real reflecting the tolerance of the tumor cell. Therefore, the clinical significance of using the FCM for detecting the P-gp expression in the PBL with a solid carcinoma was still be verified. However, It was more significant by means of FCM to detect the P-gp expression of PBL in the patient with blood diseases[12-14].

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