Relationship Between Expression of P-glycoprotein and cell Proliferation of Esophageal Carcinoma

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Abstract **Aim** To understand the relationship between the expression of P-glycoprotein(P-gp)and the cell proliferation of esophageal carcinoma. **Methods** To examine the expression level of P-gp, DNA index(DI), the percentage of cell in S-phase fraction (SPF)and cell proliferation index (PI) in the operating samples which were from 64 cases with esophageal carcinoma by flow cytometry and to analyze its relationship. **Results** The negative expression(positive cells<25%) were 25 cases(39.1%)and positive expression were 39 cases(60.9%) of P-gp in a total of 64 patients with esophageal carcinoma. Among these, the light expression(positive cells, 25%~40%)were 11 cases (17.2%), the middle expression(positive cells, 41%~60%)were 14 cases (21.8%), and the high expression(positive cells>60%)were 14 cases(21.8%). The cell proliferation of esophageal carcinoma were obviously negative correlated with the overexpression of P-gp. And the tumor cells with lower expression of P-gp was high percentage of SPF and PI, it might show those cancer cells of proliferation phase were sensitive to medicine. compared the PI and SPF of tumor cells of the patients with aneuploid esophageal carcinoma to that of diploid esophageal carcinoma, the difference was significant (P<0.05 and P<0.01 respectively), but the difference was no significant between the expression of P-gp% in the patients with the aneuploid esophageal carcinoma and with diploid esophageal carcinoma. **Conclusion** To detect the P–gp expression level, DI, SPF and PI in the patient with esophageal carcinoma by using FCM have more quick, convenient, and accurate detecting character. It provides a choice proposal of chemistry treatment for patient with esophageal carcinoma.

Key Words Esophageal carcinoma; cell proliferation; P-gp; Flow cytometre

It is well known that the one of direct reason for the fail of chemotherapy may related with the multidrug resistance gene (MDR) and its overexpression product of P-gp protein (P-gp). It has not been reported on the relativity between the proliferation of cancer cells and with its clinical significance of the esophageal carcinoma. In order to understand the significance of DNA index (DI), percentage of cell S-phase fraction (SPF) and cell proliferation index (PI) and the expression of glycoprotein (P-gp), this study is to detecting the expression level of DI, SPF, PI and P-gp by means of flow cytometre (FCM) and explorate the ralation–ship between them. So as to provide scientific base of the esophageal carcinoma in the synthesis therapy and for selecting the proposal of chemotherapy.

MATERIAL AND METHODS

Study subject To take the operating sample for detecting the expression level of DI, SPF, PI and P-gp in a total of 64 cases with esophageal carcinoma which are hospitalized patients in department of chest-surgery of Zhejiang Cancer Hospital from Dec 13, 1998 to Sept 7, 2000, including 51 male, 13 female, ages ranged 39~72 years old, and the mean age is 54.8 years old.

Agent The applied antibodies are a mouse-anti-human monoantibody (P-gp-PE) with PE (phycoerythrin) marker, the controls are IgG2a-PE (immunotech product), the nucleic DNA were stained by means of a propidium iodide single step with a inserting assay of fluorescence.

Methods To take a mucous membrane, which was from the tissue of esophageal carcinoma and the normal esophagus at the operating section. After separating by machine, a single cells were obtained by passing a 200 whole nylon net. To take a 20 ul antibody added into 12 mm×75 mm plastic tube, four tubes for each case. A tube is P-gp-PE; B tube is IgG2a-PE as a negative control; C tube is stained DNA from esophageal carcinoma; D tube is stained DNA from peripheral blood of normal subject as a control. In each tube 1×10^6 cell suspension was added, after enough stirring, put them in the darkroom for 30 minutes, then 3 ml PBS (containing 0.1% sodium azide) was added, shaking and

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stirring, then centrifuging (200g) for 5 minutes, and poured out of the supernatant, and added 0.5 ml 1% paraformalin for fixation, and then to detecting by TACS caliber FCM (BD company) on the same day, For each tube the FCM collected more then 2000 cells within the cell gate. The percentage of positive labeled cell was analyzed by using Cell Guest software and histogram. collecting about 10000 cells within the cell gate for detecting the DNA polyploid, and by means of the Modfit LT2.0 software to analyzing the DNA histogram and its cellular proportion in each phase of cell cycle. And coping the result.

Result judgment

The DNA index: Range 0.9~1.1 is a diploid, and the other is an aneuploid.

The cell proliferation index (PI): The cell number of the S and G2+M phase in the percentage of a total cell number.

The P-gp expression: negative (positive cell< 25%), the light expression(positive cells 25%~40%), the middle expression (positive cells 41% ~60%), and the high expression (positive cells>60%).

The results are analysed with the student´s t test.

RESULTS

The negative expression of P-gp in the total of 64 cases with esophageal carcinoma were 25 cases (39.1%); The positive expression of P-gp were 39 cases (60.1%). Among those, low expression were 12 cases (18.8%), middle expression 13 cases (20.3%), and high expression 14 cases(21.9%).

The cell proliferation index of esophageal carcinoma and its relationship to SPF and P-gp expression see table 1.

The percentage of a S-phase cell in esophageal carcinoma (SPF) in relation to the expression of PI, P-gp see table 2.

Table 1 and 2 showed that the cell proliferation of esophageal carcinoma and SPF were obvious negative relation with P-gp expression. The cancer cell with lower expression of P-gp was high percentage of SPF and PI. The result indicated that the propagation cell was sensitivity to the drug.

The DNA index (DI) of esophageal carcinoma cell in relation to PI, SPF, P-gp expression to see table 3.

It showed the PI, SPF of aneuploid tumors were higher than that of the diploid tumors and the difference was significant in statistics (P<0.05 and P<

Table 1. Cell proliferation index (PI) of esophageal carcinoma and the expression of SPF, P-gp

	PI ($\bar{X}$±S)	SPF ($\bar{X}$±S)	P-gp ($\bar{X}$±S)
>12%(n=36)	25.09 ± 1.89	20.21 ± 1.91	29.79 ± 3.76
<12%(n=28)	7.56 ± 0.53	6.00 ± 0.55	47.34 ± 5.82
P value	4E−11	3E−08	0.0106
t value	5E+06	5E+06	2.6324

4E−11=0.00000000004 3E−08 =0.00000003 5E+06=5000000.0

Table 2. SPF of Esophageal carcinoma in relation to the expression of PI, P-gp

	SPF ($\bar{X}$±S)	PI ($\bar{X}$±S)	P-gp ($\bar{X}$±S)
>10%(n=32)	22.04 ± 1.92	26.01± 2.04	30.66 ± 4.02
<10%(n=32)	5.94 ± 0.45	8.83 ± 0.82	44.28 ± 5.43
P value	2E−11	9E−11	0.048
t value	5E+06	5E+06	2.156

2E−11=0.00000000002 9E−11=0.00000000009

Table 3. DNA index (DI) of esophageal carcinoma cell in relation to PI, SPF and P-gp expression

DI	SPF ($\bar{X}$±S)	PI ($\bar{X}$±S)	P-gp ($\bar{X}$±S)
aneuploid(n=30)	21.23 ± 2.83	18.70 ± 2.57	35.10 ± 4.31
diploid(n=34)	14.06 ± 1.25	9.84 ± 0.95	39.56 ± 5.32
P value	0.019	0.001	0.524
t value	2.412	3.384	0.640

0.01 respectively) but the difference of the P-gp expression level betwen the aneuploid tumor and diploid tumor was no significant.

DISCUSSION

The mechanism of multidrug resistance gene (MDR) is a very complex problem, in there, the over-expression of P-glycoprotein (P-gp), which is the product of the amplification by the MDR gene, may be a principal reason. The molecular weight of P-gp is a 170KD, and belonge 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 the hydrophobic-lipophilic medicine Such as VCR, alkaloid, ADM and so on from the cells, and to result in the drug concentration decrease of the cells, which makes the action of cytotoxicity reduce or a complete loss, at the same time, 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]

The studies over a ten years on the mechanism of MDR have showed that the MDR1 gene amplification and the over expression of product of P-gp, may be one of the directly reasons of the clinical chemotherapy failure, which make the patient producing resistance to this medicine, result in metastasis and recurrence of cancer. At the present study we found the proliferation of esophageal carcinoma cells were negative related to the P-gp expression. The tumors with the lower expression of P-gp, was often high percentage of cell S-phase fraction (SPF) and high cell proliferation index (PI), that was to say the cancer cell during its proliferation stage will possess more higher sensitive for the chemotherapy. That also was consistent with the clinical chemotherapy being well efficacy for proliferating tumor cell. The positive expression of P-gp of the esophageal carcinoma without chemotherapy patient occupied 61%, it suggested that those tissues of esophageal carcinoma all existed the primary MDR gene, which was one of an important reason why it always had a poor efficacy using the chemotherapy to treat the patient with esophageal carcinoma. Now for treating the esophageal carcinoma will still emphasize the position of both the operation and radiotherapy in the synthesis treatment. At the same time, we must emphasize the screening of patients suitable to for chemotherapy. Especially when the patient possess a high percentage of cell S-phase fraction and high cell proliferation index as well as when the tumor with a negative expression of the P-gp. We can choose these kinds of drug such as an alkaloids, cyclo-anthracences and podophylum medicine. And we must have reasonably to propose a chemotherapy for the patient with P-gp positive expression and to eschew a blindness in chemotherapy, especialy forbidding application of medicine which produce the tolerance easily such as alkaloids, VCR and so on. We could choose the alkylating and anti-metabolism agent such as 5-Fu and folinic acid etc. We also must actively develop a study for reversing the MDR of tumor. It will benefit to enhance the dose of chemotherapy and promote the efficacy of medicine.

At present time, there are a many reports about the methods of the detection of tumor resistance. Now there have two kinds of methods for the detecting MDR1 and P-gp. One is at a cell level, including Immunohistochemistry[2-3], immunoblotting, FCM and so on; the other is at an RNA level, including: Northern Blot, Slot blot, RNA in situ hybridization and RT-PCR in esophageal carcinoma research. In a lot of literatures RT-PCR method[4-10] were commonly applied to detecting a situation of the MDR1 gene expression. Chen et al.[4-5] applied RT-PCR to detecting MDR1 gene in 46 esophageal carcinoma with chemotherapy and 30 cases with before and after chemotherapy to finding the positive ratio of MDR1 gene was 37%, and its expression without relation to the tumor stages, classification, and the metastasis of lymphnode. Chen et al.[6] reported that a esophageal carcinoma biopsy specimen of 58 cases, MDR1 gene positive ratio was 82%, and the expression was also without relation to the tumor stages, classification, and the metastasis of lymph node. Zhang et al.[7] reported a positive ratio of the MDR1 gene was 35.71% in the tissues of 42 cases esophagus squamous carcinomas. and also to prove the MDR1 gene expression ratio without relation to the tumor stage, classification and the metastasis of lymphnode, and Wang et al[8] reported that the positive expression rate of MDR1 gene was 75%, which might be suggestion the tumor possessed a poor differentiation, and also reflected a deepen infiltration. Tu et al.[9] detected the tissues of 30 cases esophageal carcinoma to finding the positive expression of the

MDR1 gene was 33.3%, and to prove its expression without lelation to patient´s clinical data such as: sex, age, tumor size and classification as well as the metastasis of lymph node, TNM phrase. they reported the positive ratio was 33.3%~82.8%, the reasons for the bigger difference is the RT-PCR to be quantitative determination only and can´t do qualitative determination.

To detect the expression level of P-gp in the tumor cell by using FCM, have more quick, convenient and to possess the accurate detecting characters. But a very few literatures detecting P-gp of tumor tissues with FCM reported[11-20]. Fujii et al.[11] using FCM to analysis the P-gp expression in 24 cases of stomach carcinoma, 8 cases were positive expression of P-gp. The positive incidence was higher in the progressive stage. Up to present, we have not seen the report on detecting a P-gp expression of the esophageal carcinoma cell by using FCM. In the study, we applied the FCM to detecting the P-gp expression level of esophageal carcinoma and to make further study of the DNA index, the percentage of cell S-phase fraction and cell proliferation index. It might be provide a scientific basis for choosing an appropriate proposal of chemotherapy.

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