

Analysis of Effecting Survival Factors in Patients with Gastric Carcinoma*

Chuanding Yu, Shenhua Xu, Xinghao Ni, Xinming Zhou, Yutian Ling, Gu Zhang, Chihong Zhu, Xianglin Liu

Zhejiang Cancer Hospital, Hangzhou 310022, China

Abstract Objective To understand the clinical significance of effecting factors on the survival of the patients with gastric carcinoma. **Methods** The expression of P-glycoprotein (P-gp), CD44 and CD25 were detected with FCM assay in 98 cases with gastric carcinoma, the DNA index (DI), cellular percentage of S phase (SPF) and cell proliferation index (PI) were tested in the 95 cases of gastric carcinoma, the cancer cell's C-erbB-2, P16, P53 expression levels were detected with immunohistochemistry assay in 98 cases. And to combine the data of clinical pathology and followed up to do the relativity analysis. **Results** The following up results of 98 cases with gastric cancer: 60 cases died (of 11 cases were missed, as a dead in statistics), a total of 38 cases (38.8%) were survival over three years. Compared the dead group I-II stage/III-IV stage (5/55 cases) to the survival group I-II stage/III-IV stage (21/27 cases) there was obviously significance ($P=0.0000003$); Compared the dead group to the survival group, both in clinical stages, lymph nodes metastasis, tumor embolus with involving nerves, serous membrane infiltration, differentiation, the size of the tumor, the number of sites involved with gastric cancer, and the blood type A/O, there were obviously different with significance in statistics ($P<0.01$), all these were an important effecting factors on survival of patients. And the expression of the P-gp, CD44, CD25, C-erbB-2, P16, P53 and the cancer cellular DI, SPF, PI and patient's sex, age, BMI, with or without tumor family history, with or without in operation blood transfusion were not related to three years survival ratio of the patients with gastric carcinoma. **Conclusion** The clinical stages, lymph node metastasis, tumor embolus involved with nerves, serous membrane infiltration, differentiated degree of tumor cell, the size of tumor and the their cancer cell be involved with ranges of the gastric cancer, all these may be as prognosis effectors for the patients with gastric carcinoma.

Key words Gastric carcinoma; Rate of survival; Effecting factor; Flow cytometry; Immunohistochemistry

It was known that there were a number of factors for effecting survival of the patients with gastric carcinoma, which of them is the important effecting factor? In the present study, the DNA index (DI), cellular percentage of S phase (SPF), cell proliferation index (PI), and the expression levels of P-glycoprotein (P-gp), adhesion molecule CD44 and interleukin-2 receptor of lymphocyte membrane (CD25) were detected with FCM assay and the expressive levels of C-erbB-2, P16,

P53 in the cancer cells were detected with immunohistochemistry, and combining the data of clinical pathology and followed up to do the relativity analysis.

MATERIALS AND METHODS

Clinic materials

Ninety-eight specimens of gastric carcinoma come from the operated patients in thoracic surgery of Zhejiang cancer hospital from December 1998 to September 2000. they were not treated with chemotherapy before operation. The expressed levels of P-gp, CD44 and CD25 were detected by flow cytometry in the cancer cells and the normal cutting margin of gastric mucosa cells, DI, SPF, PI were detected in 95 cases of them. And also to detect the expressed levels of C-erbB-2,

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Correspondence to: Chuanding Yu.

Email: yuchuanding@126.com,

Tel: 0571-88122588(O)

P16 and P53 with immunohistochemistry, and they all were reexamined by pathologic diagnosis and case data. Among them, 67 cases were males, 31 were females. Age ranged from 30~80 years, the median age was 58 years old. All the cases were followed up regular. Of them, 60 cases died (of 11 cases were missing, as a died cases), 38 cases (38.8%) were survivals over three years.

Reagents

The antibodies applied in FCM are a mouse-anti-human monoantibody of P-gp-PE, CD44-PE, CD25-PE and IgG2a-PE (CD44-PE and CD25-PE is BD company product, other is Immunotech company product). The nuclear DNA was stained by iodination diiodide one step assay of inserted fluorescence.

The immunohistochemistry monoantibody C-erbB-2 (CB11), P16 and P53 are Zymed company product (purchased from Mai Xin Bio-tech company, Fujian China), work concentration 1:200. Envision kit (immediately type) is DAKO company product (purchased from Shanghai gene company).

Sample preparation

To take a mucosa from the tissue cell of gastric carcinoma and normal mucosa from gastric operating incision. After separating by machine, and single cells were obtained by passing a 200 whole nylon net. 20ul antibody was added into 12×75mm plastic tube, five tubes for each case. Tube A is P-gp-PE, tube B is CD44-PE, tube C is CD25-PE, tube D is IgG2a-PE as a negative control, tube E is cellular DNA staining, tube F is normal human peripheral blood monocytes DNA as a straining control. In each tube 1×10^6 single cells suspension was added, after enough stirring, put them in the dark-room 30min, then 3ml PBS (containing 0.1% sodium azide) was added, shaking and stirring the centrifuging (200g) 5min, and poured out of the supernatant, and added 0.5ml 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 positive cell percentage of P-gp, CD44 and CD25 by B.D Celquest software and scatter-graph. DNA ploidy detected will be collected to 10000 cells in acquisition window,

and then analyzed Modfit LT 2.0 software for analyzing the DNA of scatter-graph and each phase of cellular ratio, as well as to copy the results.

The judging standard of DI: The diploid of DI between 0.9-1.1, the other is an aneuploid.

The percentage of S phase (SPF): The cell of S phase accounts for the percentage of total cell number.

The cell proliferation index (PI): The cells of S, G2 and M phase accounts for the percentage of total cell number.

The judgment standard of P-gp expression: According to average expression rate of P-gp in normal gastric mucosa was 24.7%, therefore, when the positive cell < 25% was defined as a negative, which were 40 cases (40.8%), between 25%~40% as the low expression, which were 14 cases (14.2%), between 41%~60% as the moderate expression, which were 17 cases (17.3%), and >60% as high expression, which were 27 cases (27.5%).

The judgment standard of CD44 expression: According to the average expression rate of was 9.56% in a total of 98 cases with a normal mucosa of gastric incision, therefore, where the positive cell < 10% were negative, there were 55 cases (56.1%); 10~20% (low expression) were 20 cases (20.4%); 21~50% (moderate expression) were 13 cases (13.2%); >50% (high expression) were 10 cases (10.2%).

The judgment standard of CD25 expression: According to the the average expression rate of CD25 was 7.9% in a total of 98 cases with a normal mucosa of gastric incision, therefore, where the positive cell < 8% were negative, there were 61 cases (62.2%) > 8% as positive expression, which were 37 cases.

Envision immunohistochemistry was used of the C-erbB-2 (CB11), P16, P53 kit (DAKO). The staining of step as following: (1) The paraffin sections were de-waxed to the water. (2) Hot restoration: put the section 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, with TBS buffer washing again, then respective with the first antibody C-erbB-2, P16, P53 incubated for 60 min at room temperature. (5) After washing by TBS buffer, with the polymer second antibody against Envision 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, restaining with hematoxylin, dehydration, clearing and mounting. With TBS substitution the first antibody, as a negative control, as well as using a known positive tissue as a positive control.

Assessment criteria of C-erbB-2: The positively expression of c-erbB-2 was mainly located at cell membrane. According to the 035E61 monoantibody assessment criteria of DAKO company: The negative (-), no staining cells or staining cells was <10% under the high power microscope; positive (+), weak or incompletely staining cells was >10% under the high power microscope; strong positive (++ or +++), the whole membrane of tumor cells were completely strong stained >10% under the high power microscope.

Assessment criteria of P16: The positive expression of P16 was mainly located at 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 16%~50% ,strong positive (+++), the tumor cells were completely strong stained >50% under the high power microscope.

Assessment criteria of P53: The positive expression of P53 was mainly located at 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%; moderately expression (++) , positive cells was 41%~50% , strong positive (+++), the tumor cells were completely strong stained >50% under the high power microscope.

Statistical analyses

Statistical analysis was done with SPSS11.0 statistics software and the χ^2 test for quantitative data. $P < 0.05$ was considered as statistical significance.

RESULTS

The important effecting factors for survival of the patient with gastric carcinoma (Table 1)

The effection of operation fashion on survival of gastric patients

According as the medical history, the operation fashion to be divided into a subtotal gastrectomy radical operation, total gastrectomy radical operation and palliation resection. The survival of the patients with subtotal gastrectomy radical operation was compared to that of all of other group, there was no statistics significance (Table 2).

Some effecting factor of without statistics significance

The expression of P-gp in the dead group (negative/positive, 25/35 cases) was compared to that in the survival group (negative/positive, 15/23 cases), there was no significance ($P = 0.830$, $\chi^2 = 0.046$).

The expression of CD44 in the dead group (negative/positive, 32/28 cases) was compared to that in the survival group (negative/positive, 23/15 cases) was no significance ($P = 0.484$, $\chi^2 = 0.489$).

The expression of C-erbB-2 in the dead group (negative/positive, 40/20 cases) was compared to that in the survival group (negative/positive, 25/13 cases) was no significance ($P = 0.929$, $\chi^2 = 0.008$).

The expression of P16 in the dead group (negative/positive, 15/45 cases) was compared to that in the survival group (negative/positive, 10/28 cases), there was no significance ($P = 0.884$, $\chi^2 = 0.021$).

The expression of P53 in the dead group (negative/positive, 27/33 cases) was compared to that in the survival group (negative/positive, 20/18 cases), there was no significance ($P = 0.461$, $\chi^2 = 0.543$).

The diploid/aneuploid (28/30 cases) in the dead group was compared that in survival group (19/18 cases), there was no significance ($P = 0.770$, $\chi^2 = 0.085$).

The PI < 12% / PI > 12% in the dead group (15/43 cases) was compared to that in the survival group (PI < 12% / PI > 12%, 11/26 cases) there was no significance ($P = 0.680$, $\chi^2 = 0.170$).

In addition, the SPE, the expression of CD25, and the sex, ages, the body weight of the patients in dead group were compared that in survival group, there were no significance ($P > 0.05$).

The results of this experiment demonstrated that

patients with/without family history of tumor, blood transfusion and with/without chemotherapy after operation were not related to the survival of the patients ($P > 0.05$).

DISCUSSION

The gastric carcinoma is one of the common malignant tumor, and both morbidity and mortality were at first place of the digestive tract. The factors of effecting survival are complex, which not only include biological behaviour of gastric carcinoma, such as the type of pathology, degree of cellular differentiation, tumor cell DNA ploidy, clinical stage, serosa was inversed, lymph node metastasis, situation of oncogene expression and so on, but also be related to the state of organism, namely, antitumor ability of host, their ages, sex, response of immune surveillance and blood type and so on. And to apply a many kinds of treatment, including operation fashion, blood transfusion, chemotherapy and so on. In general, the more advanced stage of patient with gastric carcinoma, the more poor prognosis, but sometimes the advanced patients could survival for a long time. Opposite, although the case belong to early pathological

changes, but there will be soon die from relapsed by tant factors in the effecting survival of patient. This results of this study was consistent with the data of abroad and at home [1-7].

Up to now, without a report on the relation between the type of blood and survival of patient with gastric carcinoma. The results of this study demonstrated that the survival rate of "A" type blood patient was more than that of "O" type blood patients, which had a obviously significance.

From the data, we also found that the survial of the patients with subtotal gastrectomy radical operation was compared to patients with total gastrectomy radical operation, there was no a significance between them ($P = 0.1113$). The result as same as Ling^[8], they reported that the subtotal gastrectomy had the same sarvival rate as the total gastrectomy for treating the gastric carcinoma. Because of the patients with subtotal gastrectomy may have a better nutrient state and life quality, a more conservative resection operation of stomach will be select ed for the focus located in the distal part of the stomach.

Palliation resection operation does not only to relief of pyloric obstruction, bloody, pain, but also to extend

Table 1 The important effecting factors for survival in the patient with gastric carcinoma

Group (cases)	dead group (60)	Survival group(38)	P	χ^2
I-II /III-IV stage	5/55	21/17	0.0000003	26.288
With or without Lymph node metastasis	3/57	17/21	0.000002	22.616
With or without tumor embolus or be Involved with nerves	21/39	26/12	0.00125	10.412
With or without serous membrane infiltration	6/54	12/26	0.0072	7.225
Moderate differentiation / low differentiation	9/51	14/24	0.0129	6.180
$\leq 5\text{cm} / > 5\text{cm}$	18/42	21/17	0.0128	6.198
1 site /2-3 site	16/44	18/20	0.0359	4.401
Blood type: A/ O	10/31	14/13	0.0204	5.376

Note:

1. low differentiated cancer includes moderate-low differentiated adenocarcinoma, low differentiated adenocarcinoma, signet-ring cell carcinoma and low- adenocarcinoma anaplastic.
2. According to clinical record situs, A: antrum pylori; M: body of stomach; C: gastric fundus pars cardiaca; involving 2 situs record as AM or MC or CM and involving 3 situs record as AMC
3. Blood type of AB were 9 cases, B type were 21 cases compared the other groups was no statistical significance..

the life of the patients. So, if the cancer could not to be resected by radical, do not blindly to give up the resection operation, for a number of gastric carcinoma patients with palliation resection could survival for five years. In the present group, there were 7 cases undergone the palliation resection. One of them did this kind of operation (March, 2000), the mass size was 10 cm×8 cm of tumor, and adhesion with the transverse colon which was to be resected partially. Under the microscope, the low differentiated adenocarcinoma cell could be observed to invade into serous membrane, some of tumor embolus and 3/31 lymphonode metastasis. The patient was followed up by phone in 5th June 2006, he said his health was in a good condition. Therefore, we should operate the resection operation with the actively and optimistic manner, even the patients had a metastases, which overstepped the range of resection operation, but if the patient's health was in a good state, the palliation resection operation should be taken into account.

At present time, each report had a different result on the DNA ploidy of gastric carcinoma. In this study, we found 48 cases (50.5%) were aneuploid tumor in a total of 95 cases with gastric carcinoma, which was the same as Chistyakova OV report^[9], they detected aneuploid tumor that was account for 52.7% (49/93). In present data showed the aneuploid tumor's PI, SPF was high than the diploid, the difference was a obviously significance ($P < 0.01$). But the SPF, PI and ploidy in each group with different clinical stages, with or without lymph node metastasis, different site of the cancer, different tumor size, and different three year survival rate, there were no significant difference. Our results is the same as Jiao YF^[10] and Lee JH *et al*^[11]'s reports, which found the DNA ploidy in the gastric carcinoma with the intestine

type was common, and the other clinical pathology was not related to the ploidy.

The significance of over-expression of C-erbB-2 gene in breast cancer was a hot-point in anti-cancer studying, one kind of anti-C-erbB-2 monoclonal antibody medicine (Herceptin), is applied to treat breast cancer with stage III, which can expend patient survival rate and also to improve their life quality. Now the study of C-erbB-2 have been extended to others tumor, but the study on gastric carcinoma was still not many^[12, 13]. In generally, when C-erbB-2 was positive expressed, the patient's prognosis was poor, so which could be taken as a marker of judging prognosis in the gastric carcinoma. Then in present data of death group of the C-erbB-2 expressed negative/positive (40/20 cases) was compared to the survival group of the C-erbB-2 expressed negative/ positive (25/13 cases) that was not a statistics significance ($P = 0.929$). However, of those positive patients whether or not could be selected to do the Herceptin treatment, which will be valuable to further study.

The data of the present literature demonstrated that the expression of P16 decreased in gastric carcinoma^[14, 15], but there was difference about the relationship between the expression of P16 protein and clinical pathologic factors. Xiao *et al*^[14] reported that expression of P16 protein was related with the lymph node metastasis. But Han *et al*^[15] reported that expression of P16 protein were not related to the types of gastric carcinoma, clinical staging, lymph node metastasis. The result of this study indicated that the difference between the expression of P16 protein and the tumor's size, differentiation degree, clinical staging, lymph node metastasis, ages and so on, was not significant. In the death group the negative and positive expression of P16 were 15 cases and

Table 2 The effecton of operation fashion on survival of gastric patients

Group (cases)	dead group (60)	survival group (38)	<i>P</i>	χ^2
Subtotal gastrectomy radical operation	43	34		
Total gastrectomy radical operation	11	3	0.1113	2.536
Palliation resection	6	1	0.1248	2.355

45 cases, and in the survival group they were 10 cases and 28 cases respectively, the difference was not statistically significance ($P=0.884$).

P53 gene is an important suppressor, the expression rate of P53 protein in the gastric carcinoma was about 40%~60%. The present result was 52.02% that was in agreement with the majority of study. Up to now the relationship of P53 protein expression of gastric carcinoma tissues with the tumor clinical pathologic factors was not a definite conclusion. Xiao *et al* [14] reported that the P53 expression was significant in the early stage and in the advance of the cancer ($P<0.05$), and the P53 protein expression was closely related to the differentiation degree and the situation of the advance ($P<0.05$). Han and Duan *et al* [15,16] reported that the expression of P53 were related to the lymph node metastasis ($P<0.05$), but not related to the survival prognosis ($P>0.05$) [16]. The present study indicated that expression of P53 were not related with the tumor size, differentiation degree, clinical staging, with or without lymph node metastasis, ages, sex and so on ($P>0.05$).

In the present time, there is some different opinion about the expression of P-gp in the gastric carcinoma. Wang *et al* [17] using the immunohistochemistry assay detected the P-gp expression, which was 65.21% (30/46) in gastric carcinoma, and was related with the clinical stages closely ($P<0.05$), the more late clinical stage, the higher degree of P-gp expression, so it could assess the prognosis as a marker. Liu *et al* [18] with the immunohistochemistry assay to detect the expression of P-gp in the 67 cases with gastric carcinoma, found that the expression intensity of P-gp was evidently related to their histological types and lymph node metastasis. The present study found that the expression rate of P-gp in a total of 98 cases was 59.2%, and it was not related with the tumor's size, differentiation degree, clinical staging, lymph node metastasis, sex and three years survival rate ($P>0.05$), which was the same as Chen [19] and Cai *et al*'s [20] results. That indicated that detecting the expression of P-gp was not clinically significant in prognosis of patient with gastric carcinoma, but it will benefit to evaluate the sensitiveness of chemotherapy. It may be taken as a guide in rational selection of the anti-cancer drug in order to avoid the blindness in using of

medicine. Many studies indicated that the MDR chemotherapy medicine mediated by P-gp are vincristine, vinorelbine and adriamycin, especially vincristine. Therefore, for the patient with gastric carcinoma with the positive expression of P-gp, we should as far as possible avoid using the lipid and alkaline medicine, and to choose the alkylating agent and anti-metabolism medicine that was not related to the P-gp.

CD44 is a cell surface transmembrane glycoprotein distributed broadly. It takes part in the specific adhering process of cell-cell and cell-matrix interaction, and may be related to the cell migration and tumor's metastasis. Of the literature data on the relationship between CD44 expression of gastric carcinoma and the prognosis of the patients was without agreement. The present study found that the expression of CD44 in the death group and the survival group there was not significant difference ($P=0.484$).

Many literature data indicated that the index of body mass index was related to the carcinogenesis of gastric, and obesity is one of the risk factors in the carcinogenesis of gastric, but a few reports on the index of body mass index was related to survival of patient with gastric carcinoma. Alici *et al* [6] reported that the index of body mass index was <20 , which will be a poor prognosis factor of survival. The results of the present study indicated that the data of index of body mass index from 59 cases, there was not a prognosis significance.

Whether or not effecting on the patient's survival for the blood transfusion during the operation was not in agreement up to now. Han *et al* [3] reported that blood transfusion during the operation would be an important factor effecting on patient's survival. We found that the death group of with/without (33/27 cases) as compared with the survival group of with/without (19/19 cases) that difference was not statistically significant ($P=0.629$).

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