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Congenital expression of *mdr*-1 gene in tissues of carcinoma and its relation with pathomorphology and prognosis

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Abstract

AIM To detect the congenital expression patterns of *mdr*-1 gene in commonly encountered malignant tumors in clinic, and the relationship between the expression of *mdr*-1 gene and the prognostic morphology in esophageal carcinomas.

METHODS A total of 151 resected samples of malignant tumors without preoperative treatment were taken from Anyang City Tumor Hospital. The congenital expression of their *mdr*-1 gene was detected with reverse transcripti on polymerase chain reaction (RT-PCR) and was compared with each other. The positive incidence of *mdr*-1 gene in 46 samples of esophageal carcinoma was compared with their differentiated grades, TNM stages and macroscopic types, and the precautions and advantages of RT-PCR were evaluated.

RESULTS All the 151 samples were confirmed to be malignant histopathologically, including cancers of stomach and gastric cardia (n = 51), esophagus (n = 46), colorectum (n = 16), breast (n = 15), thyroid (n = 10), lung (n = 9) and uterine cervix (n = 24). The positive expression rate of their *mdr*-1 gene was 33.3%, 37%, 31.3%, 13.2%, 40%, 55%, and 0% respectively. All the 46 samples of esophageal carcinoma were pathologically confirmed to be squamous cell carcinoma. The total expression rate of their *mdr*-1 gene was 37% (17/46), 35% (6/17), 40% (8/20), and 33% (3/9) for differentiation grade I, II and III respectively. The expression rate of TNM classification was 33% (6/18), 40% (5/12) and 37% (6/16) in stage IIa, IIb and III. The expression rate was 33% (3/9) in ulcerous type, 37% (3/8) in constrictive types, 33% (5/15) in fungoid types, and 40% (6/14) in medullary types. No statistically significant difference was found.

CONCLUSION Compared with other methods, RT-PCR is more simple, reliable and accurate in detecting *mdr*-1 gene expression in tissues of tumor. The overexpression of *mdr*-1 gene in these neoplasms suggested that cases should be handled differently for chemotherapy with rational use of drugs. Excision is the chief treatment for carcinoma of esophagus. The expression of *mdr*-1 gene in tissues of esophageal cancer is correlated with the parameters of tumor molecular biology which are independent of histopathological mor phology.

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INTRODUCTION

Multidrug resistance (MDR) of malignant tumor cell has aroused widespread interest. It has been shown that MDR is present in many malignant tumors. One of its molecular bases is *mdr*-1 gene amplification and its expression product. Failure of chemotherapy was chiefly due to drug resistance of tumor cells[1-9]. It is very important to detect-MDR *in choosing reasonable* treatment, espec ially in using effective chemotherapeutic drugs for a specific patient. Others[10] believe that *mdr*-1 gene expression in cancer tissue is a malignant biological indicator for neoplasms. Few research reports have been found in the literature on *mdr*-1 gene expression in tissues of esophageal carcinoma, and on its relation with the morphological parameters of esophageal carcinoma. For these reasons, a primary study was made.

MATERIALS AND METHODS

Specimens and clinical data

One hundred and fifty-one specimens were taken

from the Surgical Department of Anyang City Tumor Hospital, Henan Province soon after they were excised. Of the 151 cases, 78 were male and 73 female. They were aged from 21 to 80 years, averaging 52.1. All the cases were confirmed to be malignant tumors without preoperative treatment. Of them, 51 were cancers of stomach and gastric cardia, 46 cancers of esophagus, 16 cancers of colorectum, 15 cancers of breast, 10 cancers of thyroid, 9 cancers of lung and 4 cancers of uterine cervix. The 46 patients of esophageal carcinoma were all permanent residents of Anyang citizenship, 26 male and 20 female. The site of cancer was found in the upper, middle and lower thor acic segment in 8, 30, and 8 cases respectively according to 1987 UICC criteria. Eighteen cases were in stage IIa, 12 in stage IIb, and 16 in stage III according to TNM classification. All the cases were squamous cell carcinomas. Of them, 17 were in grade I, 20 in grade II, and 9 in grade III according to SUN Shao-Qian's grading system for squamous cell carcinoma. Nine were found to be ulcerous type, 6 constrictive type, 15 fungoid type, and 16 medullary type according to WU Ying-Kai's gross pathological typing method.

Main instrument and reagents

Reverse Transcription Polymerase Chain Reaction (TT-PCR) Reagent Kit was supplied by Beijing Jinghai Biological Engineering Company. Bio-RAD Gene CyclerTM (Gene Amplifier) was made in Japan. LG15-w high-speed centrifuge was made by Beijing Medical Centrifuge Factory. SA-U94.11 ultraviolet transilluminator was made by Shanghai Zhongya Biological Institute.

The sequences of *mdr*-1 gene primers

5'ACCCATCATTGCAATAGCAG3'
5'TGTTCAAACTTCTGCTCCTG3'

The sequences of inner control β2-microglobulin gene primers

5'ATGGCTCGCTCGGTGACCCTAC3'
5'TCATGATGCTTGATCACATGTCTCG3'

METHODS

Methods for determining mdr-1 gene expression

Methods for determining *mdr*-1 gene expression[1] was used with minor modifications. Major steps were extraction of total tumor RNA by guanidine isothiocyanate, synthesis and amplification of complementary DNA (cDNA) to *mdr*-1 gene by RT-PCR. The products were separated by electrophoresis on agarose gel containing EB. DNA bands were made visible by transillumination with ultraviolet.

Assessment criteria

Only one 300bp band was found in the negative results. Inner control band and *mdr*-1 gene 170 bp band were found in the positive results. Gene expression was calculated on a concentration scanner by the relative yield of the *mdr*-1 gene to the β_2 inner control gene. Its formula is expressed as:

$$\text{Mdr-1 expression ratio} = \frac{\text{mdr-1 band absorption}}{\text{Inner control band absorption}}$$

The ratio < 0.1 means negative expression, > 0.4 high expression, 0.1 - 0.4 moderate expression. The observed parameters included *mdr*-1 gene expression positively in macro- and micro-scopicmorphology of the specimens, and comparison *mdr*-1 expression in various groups.

Statistical analysis

Chi-square test was used, and *P* value less than 0.05 stands for statistica l significance.

RESULTS

The expression of mdr-1 gene in the studied samples was over 30%, except that in cancer of uterine cervix and breast (Table 1).

The total expression rate of *mdr*-1 gene was 37% (17/46) in the 46 cases of esophageal carcinoma with no relation to the morphological parameters of the tumor. It was an independent molecular biological characteristic of the tumor (Tables 2-4).

Table 1 *mdr*-1 gene expression in 151 specimens (%)

Site of tumors	*n*	H	M	L/N	H&M
Esophagus	46	10.9	26.1	63.0	37.0
Gastric cardia	35	17.1	11.9	71.0	29.0
Stomach	16	25.0	12.5	72.5	27.5
Colorectum	16	18.8	12.5	68.7	31.3
Lung	9	22.0	33.0	45.0	55.0
Breast	15	6.6	6.6	86.8	13.2
Thyroid	10	20.0	20.0	60.0	40.0
Uterine cervix	4	0.0	0.0	100.0	0.0
Total	151	15.2	18.6	66.2	33.8

H: high expression, M: middle expression, L(N): lower / no expression

Table 2 *mdr*-1 gene expression in TNM stages of esophageal carcinoma

TNM stage	*n*	No. of positive cases	Positive rate (%)
IIa	18	6	33
IIb	12	5	40
III	16	6	37

P>0.05, among the three stages.

Table 3 *mdr*-1 gene expression in SUN Shao-Qian's grading system of squamous cell carcinoma of esophagus (*n* = 17)

Grade	No. of positive cases	Positive rate (%)
II	6	35
II	8	40
III	3	33

P>0.05, among the three grades.

Table 4 *mdr*-1 gene expression in WU Ying-Kai's macroscopical typing system of esophageal carcinoma

Types	*n*	No. of positive cases	Positive rate (%)
Ulcerative	9	3	33
Constrictive	8	3	37
Fungoid	15	5	33
Medullary	14	6	40

P>0.05, among the four types.

DISCUSSION

It is believed that mdr-1 gene is one of the normal sequences of human genome. Nevertheless, its expression and expressive level are decided by different cell type and environmental factors. The *mdr*-1 gene expression can be investigated with several molecular methods including evaluation of protein expression and mRNA. Protein may be detected by Western blot analysis and immunohistochemical techniques. Immunohistochemical staining is commonly used, but it is not suitable for quantitative determination of protein due to its complicacy and influence of experimental conditions. Since all organisms store their genetic information in nucleic acid, methods of direct detection of *mdr*-1 at mRNA level have advantages of high efficiency, sensitivity, and specificity. Traditional methods for mRNA such as S_1 nuclease test, RNA slot blots, RNA protection assays, *in situ* hybridization and Northern blot analysis are greatly limited due to their overlaborate procedure and poor sensitivity. RT-PCR was used in present study. After cDNA of *mdr*-1 gene was synthesized according to the transcribed mRNA of *mdr*-1, it was detected by PCR *in vitro*. Compared with other gene measure ments, RT-PCR is one of the most sensitive, specific, reproducible, effective, simple, and time-saving methods[2]. We believe that the prospects of its application in clinic are quite broad.

One of the mechanisms of drug resistance to cancer cells is called MDR which is known as the resistance to lipophilic drugs such as daunorubicin, adriamycin, vincristin, and colchicin. It is very unfavourable to chemotherapy, because once tumor cells develop resistance to one of the these drugs, they will develop resistance to all lipophilic drugs[5-7]. How to evaluate multidrug- resistant tumor cells is a problem demanding prompt solution. Recently, there were several papers on the mechanism, evaluation and reversion of MDR[5-8]. Most of them focused on *mdr*-1 and its products, p-170[2-6]. p-170 (P-gp) was considered as an ATP-dependent drug molecular pump, which would lead to failure of chemotherapy as a result of drugs being pumped out from cells. This fact has been proved in many researches of diseases such as leukocythemia, breast can cer and melanoma. These recearches were of no significance in clinical practice because they were usually focused on a certain kind of tumors and their results were obtained from malignant cell line with different methods. In the present study, the expression of *mdr*-1 gene in commonly encountered malignant tumors was synchronically studied by the same technicians with same instruments, experi mental methods and reagents. The results showed that all the detected neoplasms except cervical carcinoma, expressed *mdr*-1 gene in different degrees (Table 1). Breast cancer also had a low expression of *mdr*-1. The positive number of *mdr*-1 gene in the other tumor tissues was more than 1/3 except that in breast and cervical cancers. This kind of expression was congenital because these tumors had not received chemotherapy when they were detected. It indicated tumor carried *mdr*-1 gene and its product-Pgp from the development of tumor. Clinicians should pay great attention to the mechanisms of its drug resistance if they are tenable. They should differentiate the subgroups of malignant tumors from molecular level in addition to taking other clinical indexes such as tissue differentiation, TNM staging system, and tissue type into consideration, in order to avoid unsuitable chemotherapy and use of MDR-drugs especially lipophilics. Combined treatment with reverser of *mdr*-1 gene and supressor of P-gp should be used to improve curative effect if it has been proved to be effective. Some researchers held that MDR of tumors could not be completely explained by *mdr*-1 gene and its P-gp system[10], and further research should be made. In our phase II study, a control study will be made on the difference of *mdr*-1 gene expression in cancer and normal tissues as well as in those before and after chemotherapy. We believe that the theory of MDR will be an important reference index for chemotherapy of tumors as a result of our better understanding of it.

Surgical treatment is the commonly used treatment for esophageal carcinoma. Its curative effect is chiefly decided by TNM stages, histological differentiation and types. Generally speaking, it is better for the early and well differentiated squamous cell carcinoma than the late and poorly differentiated adenocarcinoma or the undifferentiated one. However, exceptions in clinic suggest that further research at the molecular level of gene is needed in esophageal carcinoma which has its unique biological characteristics independent of its morphological parameters as other malignant tumors.

Since the finding of *mdr*-1 gene and its product P-gp (P-glycoprotein, p17 0), they have been ap-

plied to researches on their relations to cytotoxic chemotherapeutic drugs, especially to the lipophilic drugs. Many of these researches were in the field of hematic malignancies, and new ways were explored to reverse MDR. However, attention was seldom paid to the congenital expression of *mdr*-1 gene in tissues of esophageal carcinoma and its relation with morphological parameters. It was found in this study that the congenital expression of *mdr*-1 gene was 37% in tissues of the 46 cases of esophageal carcinoma without chemoth erapy before operation. It was much higher than that reported in leukemia[4,9-13], melanoma[6], and breast cancer[1,3,5,7]. This is one of the possible reasons why no progress has been achieved in the curative effect of chemotherapy for esophageal carcinoma in the past years. It indicates that only by attaching importance to the selection of chemotherapeutic drugs and suitable chemotherapy for esophageal carcinoma, can its curative effect be achieved. It also suggests that surgical treatment for it at present should be stressed. Many researches have demonstrated that cancer is a genetic disease. Besides traditional morphological indexes, the following factors were found to be related with the prognosis of esophageal carcinoma such as antioncogene, oncogene and their abnormal products as well as others at the level of gene molecules, and have been taken into account in clinic. Overexpression of *mdr*-1 gene was believed to be an index of drug resistance and further malignization of the histogical behavior of cancer cells[10]. The theory of drug resistance of tumors was advanced by Goldie and Codman[8] in 1979 in the light of gene change. It held that drug resistance was resulted from gene mutation of tumor cells produced in frequency of the tumor, and that the larger the tumor, the more the frequency of proliferation and the stronger the drug resistance. This theory also indicates that drug resistance of tumors has a positive correlation with the stage of tumors. No significant difference was found in the expression of *mdr*-1 gene in tissues of esophageal carcinoma on the basis of its morphology, differentiati on and TNM. It was suggested that expression of *mdr*-1 gene is a molecular parameter independent of surgical pathomorphological indexes. This results show that surgical treatment is the first choice for esophageal carcinoma at present due to its drug resistance.

It was held that expression of *mdr*-1 gene was a protective mechanism of cells, which was found in some normal tissue cells in addition to cancer cells[10]. The results in our study seemed to support it. Long follow-up study is needed to decide whether overexpression of *mdr*-1 gene in esophageal carcinoma is an index of its further malignization, because no comparison was made for its difference in cancer and normal tissues as well as before and after its chemotherapy.

Although surgical treatment of esophageal carcinoma is destructive and will exert some influence on the quality of life of the patients, it remains the first choice before a breakthrough is made in chemotherapy. In order to improve the curative effect of chemotherapy, the reverse mechanism of MDR should be further studied while the drug-resistant mechanism is comprehensively researched.

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