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EXPRESSION OF mdr-1 GENE IN CANCER TISSUE AND ITS ASSOCIATION WITH MORPHOLOGICAL INDEXES OF ESOPHAGEAL CARCINOMA IN ANYANG

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Objective: Overexpression of mdr-1 gene is associated with multidrug resistance (MDR) and aggressive characteristics of malignance. Our purpose was to detect the levels of P-gp expression in fresh untreated esophageal carcinomas, and to correlate these levels to current prognostic indicators of morphology.

Methods: Reverse transcription polymerase chain reaction (RT-PCR) was used to investigate mdr-1 gene expression of 46 samples from untreated esophageal carcinoma, and compared the positive incidences among differentiated grades, TNM stages and macroscopic types.

Results: All 46 samples were pathologically squamous cell carcinoma. The positive incidences of mdr-1 gene expression were 37% (17/46) in whole group, 35% (6/17), 40% (8/20), 33% (3/9), for I, II and III differentiated grades, respectively. The expression rates of 33% (6/18), 40% (5/12), and 37% (6/16), were found in IIa, IIb, and III stage of TNM, respectively. In macroscopic type view, the positive incidence was 37% (3/8) in constrictive, 33% (5/15) in fungating, 40% (6/14) in marrowlike, 33% (3/9) in ulcerative type. There were no statistically significant differences among each category system of morphology.

Conclusion: The result, high level expression of mdr-1 gene in untreated esophageal carcinoma, suggested the poor efficacy of chemotherapy for some esophageal carcinoma patients. And we should cautiously choose cases who will receive chemotherapy. Surgery is still the best treatment for carcinoma of esophagus. Besides, the data also revealed that the expression of mdr-1 gene in untreated esophageal cancer was independent of morphologic prognostic indexes, and that there were no correlation between mdr-1 gene expression and morphological indexes.

Key words: MDR, Esophageal tumor, mdr-1 gene, Gene expression, Surgery.

Multiple drug resistance (MDR) of cancer cells is one of the most interesting areas in the current cancer researches. It has been shown that many tumors have MDR. One of the molecular base of MDR is the amplification of mdr-1 gene and overexpression of its product, p170, which were thought as the direct cause of chemotherapy failure by many investigators.[1-9] Moreover, others[10] believe that mdr-1 gene expression in cancer tissue was a malignant biological indicator for neoplasms. Up to date, few research reports were found in the literature on mdr-1 gene expression in esophagus cancer tissues, and no investigations were done on the interrelations between mdr-1 gene expression and the morphological parameters of esophagus cancer. In order to study these problems, we made the following phase I investigation.

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MATERIALS AND METHODS

Main Instrument and Reagents

Bio-RAD Gene Cycler™ (Gene Amp lifier) (Japan), LG15-w high speed centrifuge (Beijing Medical Centrifuge Factory), SA- U94- 11 ultraviolet transilluminator (Shanghai Zhongya Biological Institute), Reverse Transcription Polymerase Chain Reaction (RT-PCR) Reagent Kit (Beijing Jinghai Biological Engineering Company).

The Sequences of mdr-1 Gene Primers

5'ACCATCATTGCAATAGCAG3'
5'TGTTCAAACTTCTGCTCCTG3'

The Sequences of Inner Control β_2-microglobulin Gene Primers

5'ATGGCTCGCTCGGTGACCCTAC3'
5'TCATGATGCTTGATCACATGTCTCG3'

Specimens

Forty-six esophagus cancer patients were randomly chosen from the First Department of Thoracic Surgery, Anyang City Tumor Hospital, Henan Province, from September 1994 to September 1995. The samples were taken immediately after surgical resection. All of the patients had no treatment prior to operation. And they were all permanent residents of Anyang citizenship. 26 were male and 20 were female. The age distribution ranged from 72 years to 21 years, with mean age of 54.3 years. 8 were in the upper thoracic section, 30 in the middle, and 8 in the lower, according to 1987 UICC criteria. TNM classification was 18 in stage IIa, 12 in stage IIb, and 16 in stage III. All of the 46 cancers were squamous cell carcinomas, and 17 were in grade I, 20 in grade II, and 9 in grade III, acceding to Sun Shaoqian's (孙绍谦) grading system for squamous cell carcinoma. 9 were ulcerative type, 6 constrictive type, 15 fungating type, and 16 medullary type according to Wu Yingkai's (吴英恺) gross pathological typing method.

Methods

Methods for determining mdr-1 gene expression developed by Charpin, et al.[1] were used with minor modifications. Major steps included extraction of total tumor RNA by guanidine isothiocyanate method; Synthesis and amplification of complementary DNA (cDNA) to mdr-1 gene by reverse transcription polymerase chain reaction (RT-PCR). The products were separated by electrophoresis on agarose gel containing EB. DNA bands were made visible by transillumination with ultraviolet and photographed.

Assessment Criteria

In the negative results, there was only one band, a 300 bp band. The positive results had two bans, inner control band and mdr-1 gene 170 bp band. Gene expression was calculated on a concentration scanner by the relative yield of the mdr-1 gene to the β_2 inner control gene as the following formula.

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

The ratio <0.1 means negative expression; >0.4 means high expression; 0.1–0.4 means moderate expression.

The parameters observed included mdr-1 gene expression positively in macro- and microscopic morphology 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 statistical significance.

RESULTS

In the 46 cases, 37% (17/46) had mdr-1 gene expression. The expression was not relevant to morphological parameters of the tumor. It was an independent molecular biological characteristic of the tumor (Tables 1–3).

Table 1. mdr-1 gene expression in TNM stages of esophagus cancer

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

P>0.05, no statistical differences among the three stages

Table 2. mdr-1 gene expression in Sun Shaoqian's (孙绍谦) grading system of esophagus squamous cell carcinoma

Grade	No. of positive cases	Positive rate (%)
I (n=17)	6	35
II (n=17)	8	40
III (n=17)	3	33

$P>0.05$, no statistical differences among the three grades

Table 3. mdr-1 gene expression in Wu Yingkai's (吴英恺) macroscopical typing system of esophagus carcinoma

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

$P>0.05$, no statistical differences among the four types

DISCUSSION

The treatment for esophagus carcinoma is mainly surgical, the efficacy of which depends on many morphological factors including TNM stages, tissue differentiation grades, histological types, and gross showing of tumor. Generally speaking, early stage and well differentiated squamous cell carcinoma can expect better treatment results than poorly differentiated adenocarcinoma or undifferentiated cancer. However, in clinical practices, many exceptions do occur. This indicates that like many other malignant tumors, esophagus cancer has its own unique biological characteristics that mere morphological parameters cannot reveal. These problems warrant further research and exploration, especially researches at the level of molecular biology.

Since the finding of mdr-1 gene and its product P-gp (P-glycoprotein, p170), lots researches have been done 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. The congenital expression of mdr-1 gene in the esophagus cancer tissues, however, received little attention. In this study, 37% of the total 46 esophagus cancer patients who had no chemotherapy before operation were found to have congenital mdr-1 gene expression in their cancer tissues. The rate was much higher than reported in leukemia[2-4,9] melanoma,[6] and breast cancer.[1,5,7] This fact may account for the unsatisfactory chemotherapy for esophagus cancer, and demands greater attention for selecting more effective drugs and better regimens in order to enhance the efficacy of chemotherapy. This study also indirectly stresses the importance of surgical treatment for esophagus cancer. Many researches have demonstrated that cancer is a genetic disease. The prognostic factors for esophagus cancer, besides traditional morphological indicators, should also include antioncogene, oncogene and their abnormal expression products, and other factors at the level of genetic molecules. And this new insight is gradually become a guide to clinical practice. Some researchers[10] maintain that mdr-1 gene expression is a negative prognostic factor indicting more vicious biological behavior as well as drug resistance property. In 1979, Goldie and Codman[11] put forward a drug resistance theory of tumor based on genetic changes. They believed that the drug resistance property was due to genetic mutations of tumor cells, that tumor cell genes mutated constantly at a set frequency, and that the larger the tumor, the more proliferation frequency, and the stronger drug resistance. This theory may indicates that the drug resistance property of tumor cells is directly linked to clinical stage of neoplasm. In this research, however, we studied the expression of mdr-1 gene in different TNM stages, gross morphological types, differentiated grades of esophagus cancer, and the relations each of them with mdr-1 gene expression, and found no statistical differences within the various groups. This indicates that mdr-1 gene expression is an independent molecular parameter irrelevant to pathological morphology of the cancer. The results also show that whether the cancer is in early or late stage, there is always a chance of mdr-1 gene expression, and all esophagus cancers may have some degree of MDR property. Nowadays, surgery is better than chemotherapy and still remains the first choice in the treatment for esophagus cancer. Nevertheless, there are many mechanisms to account for MDR property of cancer cells.[12, 13] This study just dealt one of them and cannot overtake others. Further studies are needed.

Some researchers[14] hold that mdr-1 gene also expressed in normal cells, besides cancer cells, and it is a protective mechanism of cells. Our present study results that mdr-1 expressions were found in any stage

and any grade with no statistical differences may agree with this opinion. In this phase I study, we didn't set normal tissues control, and there was no comparison between the results pre- and post-chemotherapy. So a clear judgment cannot be made just now. As to the question whether mdr-1 gene overexpression is another parameter of malignant biological behavior of esophagus cancer, long follow-up is needed. At present, it cannot be ascertained.

Although surgical resection of esophagus cancer is destructive and compromises the quality of life of the patients, it remains the treatment of choice in the comprehensive therapy regimen, because so far there is no major break through in chemotherapy. At the sometime, more researches are required to reverse the MDR so that better chemotherapy can be achieved, thereby enhance the efficacy of comprehensive therapy.

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