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CONGENITAL EXPRESSION OF mdr-1 GENE IN FRESH CANCER TISSUES FROM SEVERAL HIGH-INCIDENCE NEOPLASMS WITHOUT PREOPERATIVE CHEMOTHERAPY

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Objective: The purpose of the present study is to detect characteristics of primary expression of mdr-1 gene in several neoplasms which has high morbidity in clinic. Methods: 151 resected samples, which are pathologically malignant and clinically untreated before operation, were obtained from Anyang Cancer Hospital. All of them were investigated with RT-PCR for the expression of mdr-1 gene and correlated each other. Besides, we evaluated the advantages of RT-PCR in this study. Results: The mdr-1 gene expression rate of these 151 samples, including cancers of stomach and gastric cardia (n=51), esophagus (n=46), colorectum (n=16), breast (n=15), thyroid (n=10), lung (n=9), uterine cervix (n=4), was 33.3%, 37%, 31.3%, 13.2%, 40%, 55%, 0%, respectively. Conclusion: Compared with other methods, RT-PCR for studying mdr-1 gene expression had certain advantages in simplicity, reliability, and accuracy. Overexpression of mdr-1 gene in these neoplasms suggested that cases should be distinguished before treatment according to MDR of tumor and to choose effective drugs for individual cancer patient.

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Key words: Neoplasms, Drug resistance, Gene expression, mdr-1 gene, Surgery.

Multiple drug resistance (MDR) of cancer cells is a direct cause of chemotherapy failure. One of the molecular bases of MDR is the amplification of mdr-1 gene and overexpression of its product, p170.[1-15] How to evaluate the MDR for a selected cancer patient before treatment is very important for choosing reasonable treatment, especially for chemotherapeutic regimen. The present study detected the characteristics of primary expression of mdr-1 gene in several neoplasms which have high morbidity in clinic.

MATERIALS AND METHODS

Specimens

One hundred and fifty-one cancer specimens were chosen from surgical departments of Anyang City Tumor Hospital, Henan Province. The samples were taken immediately after surgical resection. All of the patients had no treatment prior to operation.

The age ranged from 21 to 80, averaged 52.1 years old, including 78 males and 73 females. All of 151 cases were pathologically malignant and clinically untreated before operation, including cancers of stomach and gastric cardia (n=51), esophagus (n=46), colorectum (n=16), breast (n=15), thyroid (n=10), lung (n=9), uterine cervix (n=4).

Main Instruments and Reagents

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

The Sequences of mdr-1 Gene Primers

5'ACCCATCATTGCAATAGCAG3'
5'TGTTCAAACTTCTGCTCCTG3'

The Sequences of Inner Control β_2-microglobulin Gene Primers

5'ATGGCTCGCTCGGTGACCCTAC3'
5'TCATGATGCTTGATCACATGTCTCG3'

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 bands, 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.

RESULTS

All of the detected neoplasms, except uterine cervix cancer, expressed mdr-1 gene in different degrees. Compared with other cancers, breast cancer has lower expression for mdr-1. The mdr-1 positive number in the others is more than 1/3 (Table 1).

Table 1. mdr-1 gene expression in 151 studied specimens (%)[*]

Item	H	M	L/N	H & M
Esophagus (n=46)	10.9	26.1	63.0	37.0
Gastric cardia (n=35)	17.1	11.9	71.0	29.0
Stomach (n=16)	25.0	12.5	72.5	27.5
Colorectum (n=16)	18.8	12.5	68.7	31.3
Lung (n=9)	22.0	33.0	45.0	55.0
Breast (n=15)	6.6	6.6	86.8	13.2
Thyroid (n=10)	20.0	20.0	60.0	40.0
Uterine cervix (n=4)	0	0	100.0	0
Total (n=151)	15.2	18.6	66.2	33.8

[*] H: high expression M: middle expression L (N): lower/no expression

DISCUSSION

In present view, mdr-1 gene is one of normal sequences of human gene group. Nevertheless, its expression and expressive level are decided by different cell types and its environmental factors. The expression can be investigated with several molecular methods including evaluating mRNA and protein expression. Protein may be detected by Western blot analysis and immunohistochemical techniques, with immunohistochemical staining being the most popular method. Immunohistochemical staining, a more complicated method for protein, is often influenced by experimental conditions, and can not measure protein quantitatively. Because all organisms store their genetic information in nucleic acid, methods for mdr-1 at mRNA level have advantages of high effectiveness, sensitivity, and specificity. Traditional methods for mRNA are S1 nuclease test, RNA slot blots, RNA protection assays, *in situ* hybridization and Northern blot analysis. Each method must be considered in terms of sensitivity, specificity, reproducibility, use as a quantitative assay, and its effectiveness in the detection of mdr-1 gene expression within a heterogeneous background of nonexpressing cells. Every methodology for nucleic acid therefore bears its own advantages and disadvantages. RT-PCR, chosen in present study, composed of cDNA of mdr-1 gene according to transcribed mRNA of mdr-1, and then followed by the polymerization chain reaction *in vitro*. Compared with other gene measurements, RT-PCR is one of the most sensitive, specific, reproducible, effective, simple, and time-saving methods.[2,3] We believe that it will have extensive future.

There are several mechanisms about drug resistance of cancer cells. It is so-called MDR that cancer cells resist some cytotoxic chemotherapeutic drugs, especially to the lipophilic drugs. Tumor cells that develop resistance through the MDR mechanism develop cross-resistance simultaneously to several structurally unrelated natural products due to the same mechanism, such as daunorubicin, doxorubicin, vincristine, colchicine, and so on.[5-7] This characteristic of cancer cell affects efficacy of chemotherapy. How to evaluate the MDR of neoplasm at the beginning of treatment has been one of crucial clinical problems. Recently, there were several papers on the mechanism, evaluation and reverse of MDR.[5-8] Most of them focused on mdr-1 and its products, p-170.[2-6] p-170 (P-gp), a 170 kd transmembrane glycoprotien, is an energy-dependent drug molecular pump. Pgp can transport intracellular drugs extra-cellularly in an energy-dependent manner, eventuating in decreased intracellular drug accumulation, and making chemotherapy failure for cancers. This fact has been proved by many researches in different fields such as leukocythemia, breast cancer and melanoma. But these experimental results were obtained from only one kind of malignancy or malignant cell line. The situation is different from clinic and is difficult to correlate with clinical practice. In present study, several clinical common neoplasms have been simultaneously studied by the same group of technicians with the same apparatus, reagents, and method for mdr-1 gene expression, indicating no objective and subjective effects on experiment itself. The results showed that all of the detected neoplasms, except uterine cervix, expressed mdr-1 gene in different degrees (Table 1). Compared with other cancers, breast cancer also has a lower expression for mdr-1. The mdr-1 positive number in the others is more than 1/3. This kind of expression is a congenital expression due to untreating before operation, indicating that tumor carries the characteristic of mdr-1 gene and its product-Pgp from the very beginning of tumor development. Before choosing treatment, especially for chemo-regimen, clinicians should distinguish the subgroups of malignances from molecular level beyond clinical indexes such as tissue differentiation, TNM staging system, and tissue type, avoiding unsuitable chemotherapy. The treatment regimen for patients, who have to receive chemotherapy, should base on these theories in order to avoid using those medicines which belong to MDR-drugs especially for lipophilic drugs. Besides, if it is possible, we should choose the drugs which can be used as reverser as well as inhibiter of MDR at the same time to promote the therapeutic efficacy. However, there are other mechanisms[14] about the drug resistance of neoplasm, and some researchers held that mdr-1 gene and its P-gp system can not totally explain MDR of neoplasms,[14,15] therefore, lots of research works should be done in the future. In our phase II study, we will investigate the expression under a normal tissues controlled group, as well as pre- and post-chemotherapy controlled studies. We believe that along with the depth of study, the theory about MDR will be a better reference index for choosing

reasonable chemotherapy.

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