Chinese Journal of Cancer Research 10(2):126—129, 1998

ESTABLISHMENT OF CRITERIA FOR MEASURING MDR-1 GENE EXPRESSION LEVEL IN BREAST CANCER BY RT-PCR

Liu Xiaoqing 刘晓晴 Song Santai 宋三泰 Shi Chenghua 石成华 Xu Jianming 徐建明
Tang Zhongming 汤仲明 Jiang Zefei 江泽飞

The Affiliated 307 Hospital, Academy of Military Medical Science, Beijing 100039

Objective: To formulate criteria of multidrug resistance (mdr-1) gene expression for predicting chemotherapy response and prognosis. Methods: Using reverse transcription-polymerase chain reaction (RT-PCR) assay, the expression of mdr-1 gene in 82 breast cancer samples were detected. Results: The data were treated by statistic analysis system (SAS)-singlevariate analysis. It showed that the level of mdr-1 gene expression clearly deviated from normal to right distribution ($P<0.0001$), and thus might be divided by quantiles P_{50} (mdr-1/β $_2$-MG=0.2) and P_{75} (mdr-1/β $_2$-MG=0.6), which were taken as the preliminary criteria for analyzing 56 patients' chemosensitivity to ADM、VDS and VCR *in vitro* and 32 relapsed metastatic patients' chemotherapy response *in vivo*, seperately. When mdr-1/β $_2$-MG<0.2, the ratios of resistance gradually escalated, but there were about 30%～50% of the cases who showed sensitive to the drugs *in vitro* and effective to chemotherapy *in vivo*. When mdr-1/β $_2$-MG≥0.6, the most of patients showed drug resistance both *in vitro* and *in vivo*. Conclusion: According to the above-mentioned results, criteria of evaluating mdr-1 gene expression level was formulated: the mdr-1/β $_2$-MG<0.2 (P_{50}) was considered as negative expression, the ratio≥0.2～<0.6 (P_{75}) was weakly positive expression, ≥0.6 was strongly positive expression. This indicated that different levels of mdr-1 gene expression may reflect objectively drug resistance *in vitro* and chemotherapy response *in vivo*.

Key words: Breast neoplasma, Multidrug resistance, Gene expression

Along with the extensive development of clinical research in multidrug resistance, there are more and more reports on this area. However, the conclusions are various due to the different criteria in judging mdr-1 gene expression. We hereby undertook this research in order to meet the needs of formulating a unified criteria.

MATERIALS AND METHODS

Samples

There were 35 previously untreated and 47 relapsed metastatic breast cancer samples which were confirmed pathologically.

Preparation of RNA

Total RNA of tumor cells was isolated by acid guanidine thiocyanate-phenol-chloroform extraction.[1] The quantity of RNA was tested by UV spectroscope and the quality was confirmed by agarose formaldehyde gel electrophoresis.

RT-PCR

Precedures were carried out according to

Accepted August 22, 1997

reference.[2]

MTT Drug sensitivity Testing *in vitro*

Procedures were carried out as described in reference.[3]

Chemotherapy Protocol and Evaluating Criteria of Response

32 relapsed metastatic breast cancer patients received chemotherapy protocol containing either ADM or VDS or VCR, and responses were evaluated by WHO response criteria.[4]

Statistical Analysis

Data were treated by using statistic analysis system (SAS) singlevariate analysis.

RESULTS

Character of Deviating Distribution of mdr-1 Gene Expression

Statistic analysis revealed that expression of mdr-1 gene in 82 breast cancer samples clearly deviated from normal to right distribution. Coefficient of deviation degree g_1=1.14 (P<0.01), and coefficient of peak degree g_2=0.38 (P>0.05), and deviation normality test W=0.815 (P<0.0001), and quantiles P_{75}=0.6, P_{50} (median)=0.2, and P_{25}=0.

Relationship between mdr-1 Gene Expression and Drug Sensitivity to MDR Related Drugs *in vitro*

The expression of mdr-1 gene deviated obviously from normal to right distribution and thus might be divided by quantiles P_{50} and P_{75} which were taken as boundaries for analyzing chemosensitivity of 56 samples from breast cancer patients to MDR related drugs, such as ADM、VDS and VCR *in vitro*.[5] The less ratio of mdr-1/β $_2$-MG was, the lower mdr-1 gene expression was, the more sensitive patients to drugs were. When mdr-1/β $_2$-MG<0.2, the ratios of drug resistance were merely 16.7% (5/30)、24.1% (7/29) and 50.0% (15/30) for breast cancer samples to the three drugs *in vitro*. Along with the ratio of mdr-1/β $_2$-MG elevated, the higher expression of mdr-1 gene was, the more resistant patients to drugs were. When mdr-1/β $_2$-MG≥0.2~<0.6, the ratios of drug resistance elevated slightly to 45.4% (5/11)、71.4% (10/14) and 72.7% (8/11). When mdr-1/β $_2$-MG≥0.6, the ratios of drug resistance elevated significantly to 93.3% (14/15)、81.8% (9/11) and 86.7% (13/15) (Table 1). Chi-square test showed that the difference of ratios of drug resistance to ADM、VDS and VCR *in vitro* for breast cancer samples was statistically highly significant or significant (χ^2= 18.735, P<0.01; χ^2=14.742, P<0.01; χ^2=6.672, P<0.05) among different levels of mdr-1 gene expression.

Table. 1. Relationship between mdr-1 gene expression and drug sensitivity to MDR related drugs in 56 patients with breast cancer

	ADM			VDS			VCR		
mdr-1/β$_2$-MG	All patients No.	Drug resistant patients No.	%	All patients No.	Drug resistant patients No.	%	All patients No.	Drug resistant patients No.	%
<0.2	30	5	16.7	29	7	24.1	30	15	50.0
0.2≤~<0.6	11	5	15.4	14	10	71.4	11	8	72.7
≥0.6	15	14	93.3	11	9	81.8	15	13	86.7

Relevance of mdr-1 Gene Expression and the Outcome of Chemotherapy in Patients with Relapsed Metastatic Breast Cancer

When mdr-1/β $_2$-MG<0.2, the ratio of inefficacy to chemotherapy was only 21.1% (4/19). When mdr-1/β $_2$-MG≥0.2~<0.6, the ratio of inefficacy to chemotherapy elevated to 60%(3/5). When mdr-1/β $_2$-MG≥0.6, the ratio of inefficacy to chemotherapy elevated significantly to 87.5% (7/8) (Table 2). Chi-

square test showed that the difference of ratio of inefficacy to chemotherapy for breast cancer patients was statistically highly significant (χ^2=10.624, P<0.01) among different levels of mdr-1 gene expression.

Establishment of the Criteria of mdr-1 Gene Expression

According to the above-mentioned results, the expression of mdr-1 gene was divided into three degrees by quantiles. The degrees were as follows: The ratio of mdr-1/β $_2$-MG<0.2 (P_{50}) was considered as negative expression of mdr-1 gene; ≥0.2~<0.6 (P_{75}) as weakly positive expression; ≥0.6 as strongly positive expression.

Table 2. Relevance of mdr-1 gene expression and the outcome of chemotherapy in 32 patients with breast cancer

mdr-1/β $_2$-MG	All patients No.	Inefficacy	
		Patients No.	%
< 0.2	19	4	21.1
0.2≤~<0.6	5	3	60.0
≥0.6	8	7	87.5

DISCUSSION

There have already been a lot of reports on examination of mdr-1 gene expression in clinical tumors. Whether by Slot Blot assay, or by RT-PCR, varied degrees of drug resistant cell line were always taken as the reference criteria to judge mdr-1 gene expression in tumor tissues.[6-8] This kind of criteria often affected detection results because of using varied degrees of drug resistant cell line, thus hindered the communication and comparison among different units. In addition, as for clinical researchers in our country, they rarely had varied degrees of drug resistant cell line, and that would affect the development of the research.

On the basis of detecting data from Chinese breast cancer samples, mdr-1 gene expression of 82 breast cancer samples was analyzed statistically by SAS. Data revealed that mdr-1 gene expression clearly deviated from normal to right distribution, and thus quantiles P_{50} and P_{75} were regarded as preliminary criteria for analyzing 56 patients' chemosensitivity to ADM、VDS and VCR *in vitro* and 32 patients' chemotherapy response *in vivo*, separately, in order to confirm reliability of the preliminary criteria. When mdr-1/β $_2$-MG≥0.2~<0.6, the ratios of drug resistance elevated slightly, but there were about 30%—50% of the cases who showed sensitive to the drugs *in vitro* and effective to chemotherapy *in vivo*. When mdr-1/β $_2$-MG≥0.6, the most of patients showed drug resistance both *in vitro* and *in vivo*. This indicated that P_{75} being as critical level of mdr-1 gene expression might reflect drug resistance *in vitro* more objectively and correctly.

Based on comprehensive analysis of above results, we have formulated the criteria of examining the mdr-1 gene expression in breast cancer tissues using RT-PCR assay. The significance of formulating the criteria lay in: (1) This method of formulating the criteria was not limited by the type and existence of cell lines and could be carried out routinely. (2) By these unified criteria, the detecting results of different breast cancer patients and different units in our country could be compared. (3) The method of formulating judging criteria in this research could be extended to the examination practice of other tumors, and could provide new thinking and experiences to further development of MDR research.

In respect of treatment, we suggested that patients which mdr-1 gene expression was positive in their tumor tissues might be either treated with no MDR-related drugs or admitted to clinical trial in which high dose of chemotheraputic agents will be used or chemotherapy is combined with reversing agents such as verapamil, cyclosporin or others.

REFERENCES

1. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanine thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156.
2. 刘晓晴，石成华，宋三泰，等。RT-PCR 方法检测临床乳癌组织的多药抗药基因表达。中华肿瘤杂志 1996; 18:263.
3. 徐建明，汤仲明，宋三泰，等。人乳腺癌原代培养体外药敏试验的评价。中华肿瘤杂志 1995; 17: 100.
4. Miller AB, Hoogstraten MB, Staquet M, et al. Reporting results of cancer treatment. Cancer 1981; 47: 207.
5. 杨树勤。百分位数。见：中国医学百科全书编辑委员会主编。中华医学百科全书。医学统计学分册。上海科学技术出版社 1985; 15.

6. Goldstein LJ, Galski H, Fojo A, et al. Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 1989; 81: 116.
7. Hennequin E, Delvincourt C, Pourny C, et al. Expression of mdr-1 gene in human breast primary tumors and metastases. Breast Cancer Research and Treatment 1993; 26: 267.
8. Noonan KE, Beck C, Holzmayer TA, et al. Quantitative analysis of MDR-1(multidrug resistance) gene expression in human tumors by polymerase chain reaction. Proc Natl Acad Sci USA 1990; 87: 7160.