BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Sep; 17 (5): 471-473

Correlation between expression of *mdr*-1 gene and oncogenes in human promyelocytic leukemic HL60 cell line and sublines[1]

ZHOU Wei-Dong, ZHANG Hong-Qing, FANG Min, XUE Shao-Bai
(*Department of Biology, Beijing Normal University, Beijing 100875, China*)

KEY WORDS oncogenes; multiple drug resistance; flow cytometry; acute promyelocytic leukemia

AIM: To study the relationship between expression of oncogenes and multiple drug resistant (MDR) phenotype. **METHODS:** The drug resistant level of HL60 cell line and its sublines were determined with flow cytometry. RNA Dot blot hybridization was used to identify the expression of oncogenes and *mdr*-1 gene. **RESULTS:** The expression of *mdr*-1 gene was in the opposite relation with c-*myc* expression, but in the positive relation with c-H-*ras* gene expression in the multiple drug resistant cell lines. In non-MDR cell line HL60/RA, the expression levels of *mdr*-1, c-*myc*, and c-H-*ras* were the same as HL60 parental cells. **CONCLUSION:** Multiple drug resistance is related to not only *mdr*-1 expression, but also some oncogenes expression level.

Amplification and overexpression of c-*myc* and c-H-*ras* is closely correlated with malignant transformation and poor prognosis. Still, chemotherapy failure due to cellular drug resistance remains a major problem in most patients. The multiple drug resistance (MDR) *mdr*-1 gene expression was found to be inversely correlated with N-*myc* expression[1]. Transformation of rat liver epithelial cells with v-H-*ras* or v-*raf* causes increased expression of *mdr*-1 and MDR phenotype[2]. So the relationship between MDR and oncogenes activation is deserved to be investigated in this study.

MATERIALS AND METHODS

Drugs and reagents Rhodamine 123 (Rho-123), diethyl pyrocarbonate (DEPC), verapamil (Ver) were from Sigma Co. Guanidine isothiocyanate was from GIBCO. Harringtonine (Har) was from Institute of Materia Medica, Chinese Academy of Medical Sciences. [α-^{32}P]dCTP (370 GBq·L^{-1}) was from Beijing Furei Co.

Cell lines Anti-differentiation cell line HL60/RA was a kindly gift from Prof HAN Rui (Institute of Materia Medica, Chinese Academy of Medical Sciences). HL60, and HL60/Har were preserved in our Lab. HL60/Har + Ver and HL60/Ver were obtained from HL60/Har and HL60 cells cultured in RPMI 1640 medium containing Ver at increasing concentrations for over 6 months, respectively.

Flow cytometry The detection of intracellular fluorescent intensity with flow cytometry is a useful mark of resistant level[3,4]. Cells were labeled with Rho-123 by incubation in supplemented RPMI 1640 containing Rho-123 10 mg·L^{-1} at 37 ℃ for 30 min, then were resuspended in fresh culture medium after rinsed twice with cold phosphate buffered saline (PBS) by centrifugation. The fluorescent intensity of cells was analyzed with FACS 420 flow cytometer, 1×10^4 cells were examined in each sample.

Dot blot hybridization Total RNA was prepared from cell cultures[5]. Denaturing gel electrophoresis of RNA was done in order to confirm the purity of extracted RNA. The concentration of extracted RNA was determined by uv excitation with DU-50 uv spectrophotometer. RNA samples were blotted onto nitrocellulose filter in the amount of 20, 10, 5, 2.5 μg using a Dot blot apparatus. Filters were baked in vacuum oven at 80 ℃ for 2 h. Baked filters were prehybridized, hybridized and washed, autoradiographed at −70 ℃ with a cronex fluorescence enhance screen.

RESULTS

Multiple drug resistance of each cell line In contrast to parental HL60 cells, the fluorescent intensity of Rho-123 was decreased in HL60/Har, HL60/Har + Ver, and HL60/Ver. The retention of Rho-123 of HL60/Har was much less than that of HL60/Har + Ver, and HL60/Ver. The fluorescent intensity of Rho-123 in HL60/RA was approximately the same as parental cells (Fig 1). The difference of intracellular Rho-123 retention among these cell lines indicated that the resistance of

[1] Project supported by the National Natural Science Foundation of China, № 39270785 and The Doctoral Programme Foundation for Institution of Higher Education, № 9202702.
Received 1995-06-12 Accepted 1996-05-22

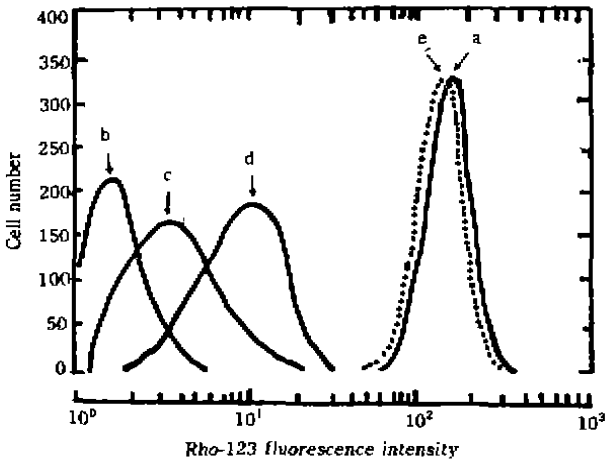

Fig 1. Flow cytometry distribution of Rho-123 fluorescence of HL-60 (a), HL-60/Har (b), HL60/Har + Ver (c), HL60/Ver (d), and HL60/RA (e) incubated with Rho-123 10 mg·L^{-1} in RPMI 1640 medium for 30 min.

HL60/Har, HL60/Har + Ver and HL60/Ver was due to expression of P-glycoprotein, however; the resistance of anti-differentiation cell line HL60/RA was subject to non-Pgp resistance.

Expression of intracellular *mdr*-1 gene Compared with HL60 parental cells, the expression level of *mdr*-1 in HL60/Har, HL60/Har + Ver, and HL60/Ver cells was increased evidently. HL60/Har cells exhibited higher degree of *mdr*-1 expression than HL60/Har + Ver and HL60/Ver cells. But the level of *mdr*-1 mRNA of HL60/RA cells was almost the same as parental cells (Fig 2).

Expression of c-*myc* and c-H-*ras* In contrast to parental HL60 cells, the c-*myc* mRNA level of HL60/Har, HL60/Har + Ver, HL60/Ver cells was decreased and that of c-H-*ras* was increased abviously. HL60/Har, HL60/Har + Ver, HL60/Ver cells almost containd the same amount of c-*myc* and c-H-*ras* mRNA, respectively. No alteration about the c-H-*ras* and c-*myc* mRNA level was detected in HL60/RA and drug-sensitive parental cells (Fig 2).

DISCUSSION

In the present study, we found that two cell lines resistant to Har and Har + Ver exhibited higher degree of *mdr*-1 expression. The levels of *mdr*-1 expression identified by Northern hybridization was in accordance with the retention of Rho-123 detected by flow cytometry. Compared with drug-sensitive parental cells, the two cell lines exhibited higher expression of c-H-*ras* and lower c-*myc*. All the results suggest that *mdr*-1 expression be inversely correlated with c-*myc*, but positively with c-H-*ras*. The results coincide with previous reports[1,2]. No alteration of *mdr*-1, c-*myc*, c-H-*ras* expression in anti-differentiation HL60/RA cells suggests that multiple drug resistance and differantiation resistance of tumor cells be modulated by different mechanism.

The relationship between *mdr*-1 and oncogenes expression probably varied in different cell lines[6-8]. Stimulation of c-*raf* kinase increased the activity of *mdr*-1 promoter in NIH3T3 cells, The c-*raf* responsive sequence is localized between −69 and −41 in *mdr*-1 promoter. Cotransfection with dominant negative raf mutant c-*raf*-c4 blocked

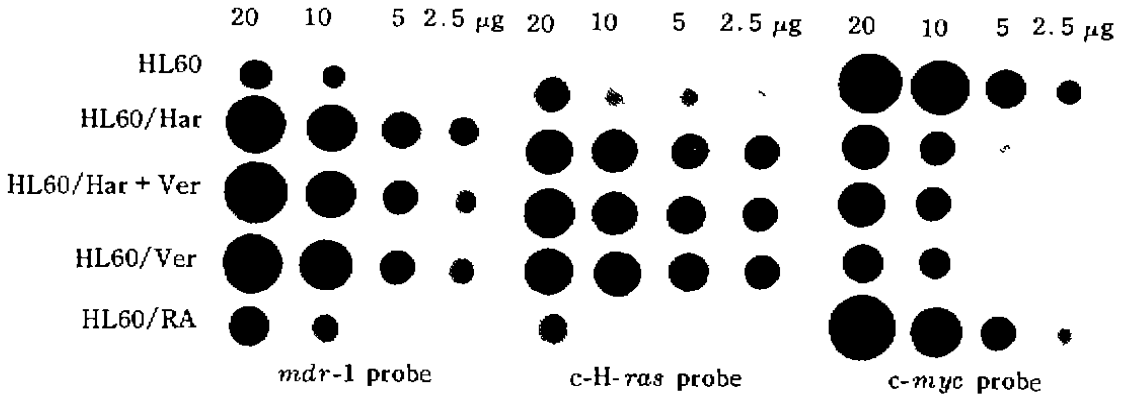

Fig 2. Cell total RNA Dot hybridization.

serum stimulation of the *mdr*-1 promoter and resulted 20-fold decreasc in *mdr*-1 promoter activity[9]. All this suggested that increased activity or expression of growth regulatory proto-oncogenes may influence *mdr*-1 expression in human tumor cells. The results of this report, that MDR cell lines exhibited increased c-H-*ras* and decreased c-*myc* expression, implicated that *mdr*-1 probably execute some influence on c-*myc* and c-H-*ras* expression or that expression of *mdr*-1, c-*myc* and c-H-*ras* be modulated by same transcriptive factors.

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471 - 473

人早幼粒白血病 HL60 细胞及其亚系中 *mdr*-1 基因和癌基因表达的关系

R733.
R 318

周卫东，张鸿卿，方 敏，薛绍白
(北京师范大学生物系，北京 100875，中国)

关键词 癌基因；多种抗药性；流式细胞计量术；急性早幼粒白血病

HL60，表达

目的：研究人早幼粒白血病 HL60 细胞系及其分别抗 Har，Har+Ver，RA 的抗药性细胞亚系中癌基因与多药抗性表型的相关性 方法：流式细胞计量术测定各细胞系的多药抗性程度，点杂交检测各细胞系 *mdr*-1 及 c-*myc*、c-H-*ras* 的表达. 结果：多药抗性细胞模型中 *mdr*-1 基因和 c-*myc* 基因表达存在逆相关性，和 c-H-*ras* 基因表达存在正相关性，非多药抗性细胞模型(HL60/RA)中 *mdr*-1，c-*myc* 和 c-H-*ras* 表达水平与亲本 HL60 细胞相同. 结论：多药抗性不仅与 *mdr*-1 表达有关，也与一些癌基因表达状态的改变有关.