Clinical Significance of Lung Resistance Protein (LRP) Expression and MTT Assay in Patients with Malignant Tumor in Hematology

Qing Wang, Xiaojing zeng, Jiayi yan, Rugang Shen, Li Ma, Jie Yang, Lin He

Department of Hematology, the First Affiliated Hospital, Guiyang Medical College, Guizhou, 550004, China

Abstract **Objective** To investigate the relationship between the expression of lung resistance protein (LRP) and drug resistance in patients with malignant tumor in hematology. **Methods** Flow cytometry was used to examine the expression of LRP in 43 patients with malignant tumor in hematology and 38 patients with non-malignant tumor in hematology. Meanwhile, the chemosensitivity of malignant tumor cells to 9 anti-tumor drugs were estimated in 20 patients with malignant tumor in hematology by MTT assay. **Results** The positive rate of LRP in newly diagnosed group was 65.52%; The first complete1 remission rate was 38.46% and 85.71%, in LRP-positive and LRP-negative patients respectively. The difference between them was significant (P<0.01). The positive rate of LRP in relapsed group and in newly diagnosed group were significantly higher than that in non-malignant tumor group in hematology, respectively (P<0.01 and P<0.05). And the difference of the total positive rate of LRP between the relapsed group, newly diagnosed group and control group was significant (P<0.01). The results of the study reveals that the chemosensitivity assay correlated well with the outcome of the treatment with overall coincidence rate of 75%, a positive coincidence rate of 100% and a negative coincidence rate of 66.67 %, respectively. **Conclusion** LRP expression was found to be a useful parameter to predict the outcome of the treatment. High expression of LRP leads to clinical drug resistance.

Key Words Lung resistance protein(LRP); MTT assay; Hematology; Malignant tumor

Multidrug resistance (MDR) is one of the main reasons that cause chemical treatment failure in malignant tumor of hematology. The reason that produces the MDR is affected by many factors and the mechanism is different in different drugs, creature species and different source of tissue cells. The phenomenon of drug resistance was caused by P-glycoprotein (PGP), lung resistance protein (LRP), multidrug resistance-associated protein (MRP) and the increased activity of the glutathione (GSH). Previous studies of MDR is more relevant to the PGP in china. LRP is still not much, only minor studies applied the method of RT-PCR or immunohistochemistry. The RT-PCR method made more false positivity and immunohistochemistry made more mistakes. The foreign assays indicated that the method of flow cytometry is more accurate than the above two methods. To investigate the relationship between the expression of lung resistance protein (LRP) and drug resistance in patients with malignant tumor in hematology, we applied the flow cytometry to measure the expression of LRP in 43 patients and 38 normal patients with non−malignant tumor in hematology. Meanwhile, the chemosentivity of malignant tumor cells to 9 antitumor drugs were estimated in 20 patients with malignant tumor in hematology by MTT assay.

MATERIALS AND METHODS

Patient samples

The 43 patients with malignant tumor in hematology were all from admission patients in the hospital during the June to December in 2002. The age of patients ranged from 13 to 81 years old (averaging 44 years old). Among them, male 33, female 10. 17 cases (M2, 4; M3, 8; M4, M5, 1)are acute non-lymphocytic leukemia (ANLL); The others are acute lymphocytic leukemia (ALL) 9 cases, hybrid acute leukemia (HAL) 2 cases; the chronic leukemia(CML) 4 cases, myelodysplastic syndrome (MDS-RAEB) 5 cases, multiple myeloma (MM) 6 cases, respectively. The newly patients are 29 cases, relapsed 6 cases, complete remission 8 cases. All

cases were diagnosed according to the Criterion of Diagnosis and Treatment Effect. Control group were 38 common patients with blood disease (iron deficiency anemic, (IDA) 6, megaloblastic anemia (MA) 2, idiopathic thrombocytopenic purpura (ITP) 5; hemolytic anemia (HA) 3, hyperplasia anemic 10) and 12 normal persons.

Methods

Assay of FCM The expression of LRP in bone marrow of all samples was detected and analyzed by flow cytometry (FCM) with the direct stainning method. The specific LRP monoclonal antibody LRP-56 was purchased from Becton Dickinson Company. All samples were operated according to the illustrations of the reagent. The results were analyzed by the FCM and described in the content of LRP (percentage).

We applied FACSCalibur (company of BD, USA) to analyze all samples: the FACSCalibur argonion laser emitting at 488nm, the power was 300 MW, and the fluorescence of PE was collected by the computer through a 585/42 BPs(bandpass). The fluorescence signal passes through 4000% logarithms class enlargers, the light signal spread through the linear enlarger. To set a gate according to the dot plot of FSC/SSC and establish the flow type analysis of FL2 (PE)-the COUNTS, we collected 20,000 cells for every sample and deposited them in the disk with the form of List Mode and analyzed with the software of Cell Quest (company of BD).
The 38 negative examples were examined with the same method. The average LRP value as the normal reference value, which exceeding this value is regarded as high expression.

The MTT assay in vitro We withdraw mononuleus from the samples to set up a cellar liquid (density is 2×10^9/L) in a microbiological safety cabinet at appropriate containment level. We joined 9 kinds of chemotherapy medicine 10ul (the work liquid density was installed previously) liquid in cell culture plate (company of BD) with 96 well respectively. This process was repeatedly 4 times. The concentration of the chemotherapy medicine installed with the NS is as follows:

DNR1.6 × 10^{-2}/L; THP1.2 × 10^{-2}/L; ADR1.6 × 10^{-2}/L;
MIT5.0 × 10^{-2}/L; HAT1.2 × 10^{-3}/L; Ara–C6.0 × 10^{-2}/ L;
VCR0.8 × 10^{-3}/L; MTX8.0 × 10^{-3}/L; VP16. 164.0 ×10^{-2}/L

100μl cell liquid was added in each well, the blank bore and the bore added cell liquid without drugs as the control. This process was repeated 4 times , respectively. Placing it in a 5% CO_2 incubator for 24 hours at 37℃, then 12.07 mmol/L MTT 20ul was added in each well, cultured for 4 hours. Centrifuge the plate and discard the suspension and add 100μl of the 0.26 mmol/L SDS 0.01mol/L-HCL in each well, placing it in the incubator overnight. The light density (the optical density, A) value of each bore was measureed by the Bio-Rad automatic enzyme mark instrument with 570 nm as the absorb the wave-length and the 630 nm to be the reference wave-length. Take the average value of 4 groups as the final value. Compute the cell toxicity index (CI)[2]

CI=(1−experiment group A value/the control group A value) ×100%

The judgment criterion of clinical effects

CI≤30%: resistance in vitro; >30%: sensitive in vitro; CR in 2 therapy courses: sensitive in vivo; (NR) MBDI ≥60%: Sensitive in vivo; MBDI<60%: resistance in vivo[3].

The judgment of the relationship between the result of drug sensitive in vitro and vivo: true positive (vitro/vivo S/S) (S: Sensitive) and false positive: (vitro/vivo S/R) (R: resistance) true negative: (R/R); false negative (R/S)[4].

Statistical Analyses

Date analysis was performed using the SPSS 11.0 statistical software package. All results were expressed with $\bar{x}\pm s$. We adopted t-test, chi-square test and Cox-regression analysis data. The level of significance was set up at $P<0.05$ for all analyses.

RESULTS

The expressions of LRP in monocleaus in bone marrow.

LRP was expressed in 62.79 %(27/43) of bone marrow samples of patients with malignant tumor in hematology. The positive percentage of LRP expression in newly diagnosed group was 65.52%, in relapsed group was 83.33% and in complete remission (CR) was 37.50% group, respectively. The positive expression of LRP was 68.57% in newly diagnosed and relapsed group while it was only 31.58% in control group. The difference was significant ($P<0.01$). The expression of LRP and the positive rate of LRP in relapsed group and in newly diagnosed group were significantly higher than that in non-malignant tumor in hematology, respectively

(P<0.01 and P<0.05).

The relation of the expression of LRP and the types of malignant tumor in hematology

The expression of LRP and the positive rate of LRP in ANLL group(64.71%) and in MM (83.33%) group were significantly higher than that in control group (31.58%), respectively (P<0.05 and P<0.01). There was no significant difference between the ALL, CML, MDS, HAL groups and control group, and neither was in the subtypes groups of ANLL groups and between the ALL group and ANLL group.

The relation of the positive expression of LRP and CR1 in patients with M3

The positive rate of LRP expression in newly diagnosed group was 80% (4/5), CR rate was 25% (1/4). 2 patients with DIC were dead. 2 patients gave up treatment. 1 patient attained CR. The expression of LRP was negative in 1 patient who attained CR. The expression of LRP was negative in the 3 patients of CR group. There was a significant difference between the newly diagnosed group and CR group in the expression of LRP (P<0.05).

The relation of the expression of LRP clinical outcome

Five out of 13 patients with the positive expression of LRP attained good outcome, and 6 cases of 7 patients with the negative expression of LRP attained good curative effect. The high expression of LRP is a poor predictive parameter for patients.

The relation of the expression of LRP and drug sensitivity in vitro in patients with malignant tumor in hematology

The results of this study indicate that the expression of LRP in MTX resistance group was significantly higher than that in MTX sensitive group after compared the expression of LRP with the sensitivity to chemotherapy drugs in vitro and in 20 patients with malignant tumor in hematology.

The MTT assay and clinical outcome

We studied the relation of the clinical outcome and the sensitivity of 20 patients with malignant tumor in hematology to 9 chenmotherapy drugs. 2 patients were sensitive and 4 were resistant to the drugs in vitro in 8 patients. Results was as follows: S/S: 2; R/R:2; R/S:2; S/R 0.The results of the study reveals that the outcome of the treatment with overall coincidence rate of 75%, a positive coincidence rate of 100%, and a negative coincidence rate of 66.67%, respectively. The predicative sensitivity is 50% and the specialty is 100%.

DISCUSSION

The LRP is a kind of new protein related to MDR. LRP was discovered firstly in SW-1573/2 R120 of PGP negative MDR in the non-small cell lung cancer. Its gene was located in 16 p13.1-16p11.2, and the whole length of LRP cDNA is 2.8 kb, and it codes an non-sugar protein which the quantity of molecule is about 1.1×10^5. The LRP distributes extensively in normal and tumor tissues, it distributes with a certain organization specificity in normal tissues [5]. We found that the average value of LRP is 9.9305% in 38 control samples, so take the LRP≥9.9305 (percentage) be the criterion of positive expression of LRP. The expression of LRP is different in different tumor and it is higher in tumor tissues than that in normal tissue.[5,7]. It may cause the MDR through two kinds of mechanisms[5]: first, it can block drugs to be transmitted into the nucleus through nucleus pore and redistributing the drug from the nucleus (drug target) to the cytoplasm. Second, it could make the drug in cytoplasm into transmit vesicle and distributing in atria and ventricle and finally was expelled from the cell. The LRP can lead to drug resistance which can´t be mediated by PGP and MRP such as carboplatin, cisplatin and cyclophosphamide and so on. The same character of these medicines is taking the DNA as the target, which implicated that LRP could lead to drug resistance mainly through the mechanism of block of nucleus-target.

In present study, the LRP has the lower expression in marrow mononuleu cells and the average rate is 9.9305%. The expression of LRP in the newly diagnosed group (65.52%) is higher than that in control group (31.58%), the difference is significant (P<0.01). The results was same as Filipits[8]. Damiani [9]. found that LRP was over-expressed in relapsed ANLL(33/54) (FCM method). In our study, we found that the expression of LRP is 83.33% in relapse group and it is higher than that in control group (P<0.05); The percentage of LRP is 17.81% higher than that in newly diagnosed group but there is not significant difference between the two

groups. The result is more close to the result of Chi et al. Whose study showed that the expression rate of LRP was 68.0%(17/25) in newly diagnosed group and 80.0% (8/10) in relapse and refractory group (RT-PCR method)[10]. Zhang et al. Found that the positive rate of LRP was 28.95(11/38), the CR rate was significantly lower in LRP-positive childhood acute leukemia patients than that in LRP-negative patients (27.3% vs. 85.2%, $P<0.05$) (RT-PCR method)[11]. These results indicated that LRP is related to clinical drug resistance. Schwarzenbach[12] discovered that the LRP was expressed in 12.5%(9/72) MM patients with the RT-PCR method. We discovered the positive expression rate of LRP was 83.3% (5/6) in MM, and was significantly different from the control group ($P<0.05$).

Renjinhai.[13] found that the expression of LRP gene was related to types of AL in FAB, and it is higher in ALL group than that in ANLL group. In our study, there was no significant difference between control group and ALL, CML, MDS, HAL groups or among the subtypes of ANLL. Filipits [8] found that the expression of LRP in M3 is low and well reacted to Tretinoin. We studied 8 patients with M3, 5 cases of them was newly diagnosed, the expression rate of LRP is 80% (4/5), CR is 20%. The expression of LRP was all negative in the CR group with 3 patients. There was a significant difference between the newly diagnosed group and CR group ($P<0.05$).

We studied 8 patients with malignant tumor in hematology with the MTT method. Two cases of them were sensitive and 4 cases were resistance to drugs in vitro. The relation of the sensitivity in vitro and the sensitivity in vivo is: S/S, 2; R/R, 2; R/S, 2; S/R, 0. The results of the study indicated that the rate of outcome of the treatment was 75%, a positive coincidece rate was 100% and a negative coincidece rate 66.67 %, respectively. Compared to the result of Luomeihua[14] whose overall coincidece rate is 93.3%, positive predictive rate is 94.9%, the negative predictive rate is 83.3%, our positive coincidece rate and negative coincidece rate is lower, which maybe due to the number of sample limited. In our study, 5 out of 13 with LRP expression positive had good outcome, the rate of CR was 38.46%; 6 out of 7 with LRP expression negative had good effect, the rate of CR was 85.71%. Low expression of LRP predicts a good prognosis, vice versa. The results was same to Damiani [9] whose conclusion indicated that expression of LRP is related to clinical outcome.

In present study, the results indicated that LRP is a valid parameter of drug resistance in chemotherapy. The high expression of LRP can cause the clinical drug resistance and is a poor-prognostic factor for the patients with malignant tumor in hematology. It has been reported that LRP was closely related to the daunorubicin resistance[15, 16]. We found that there was a significant difference of the expression of LRP between the MTX sensitive group and MTX resistance group in vitro ($P<0.05$). Therefore, LRP may lower the drug concentration inside the cell, causing cell resistance to the chemotherapy drugs such as MTX through two mechanisms (as former) when high level expression of LRP occur. If we look both the expression of LRP and the assay of the sensitivity of chemotherapy drugs in vitro as the index of drug resistance, it could be helpful to elevate the accuracy of judgment in clinical chemotherapy drug resistance and good to the design of clinical chemotherapy scheme.

REFERENCES

1. Zhang zhinan. The criterion of diagnosis and treatment effect 2nd edition Beijing: Science publisher, 1998. 373–379.
2. Zhang ti, Zhang chunqing, Ji chunyan, et al. Chemosesitivity assay in vitro and expression of multidrug resistance gene mdr-1 in acute myeloid leukemia patients and Their Clinical Significance. Journal of Experimental Hematology, 1997, 5(3): 259–262.
3. Bian gengshou, Shao zonghong, Wang jinghua, et al. The study of the clinical significance of comprehensive drug sensitivity assay in acute leukemia. Chinese Journal of Hematology, 1990, 11(3): 113–116.
4. Hwang W S, Chen L M, Huang S H, et al. Prediction of chemotherapy response in human leukemia using in vitro chemosensity test. Leuk Res, 1993, 17(8):685–688.
5. Scheper R J, Broxterman H J, Scheffer G L, et al. Overexpression of a M(r) 110,000 vesicular in non-p-glycoprotein-mediated multidrug resistance. The Cancer Res, 1993, 53(7): 1475–1479.
6. Scheffer G L, Wijnggaard P LG, Flens M J, et al. The drug resistance-related-protein LRP is the human major vault protein. Nature Med, 1995, 1(6): 578–582.
7. Slovak M L, Ho J P, Cole S P, et al. The LRP gene encoding a major vault protein with drug associated maps proximal to MRP on chromosome 16: evidence that

Qing wang M.D.0851-8048084;(0)13984802958
E–mail: wq4066718@163.com

chromosome breakage plays a key role in MRP or LRP gene amplification. Cancer Res, 1995, 55(19): 4214–4219.
8. Filipits M, Pohi G, Stranzl T, et al. Expression of the lung resistance protein predicts poor outcome in de novo acute myeloid leukemia. Blood, 1998, 91(5):1508–1513.
9. Damiani D, Michieli M, Ermacora A, et al. P-glycoprotein (PGP), and not lung resistance–related protein (LRP), is a negative prognostic factor in secondary leukemias. Haematologica, 1998, 83(4): 290–297.
10. Chi zuohua, Zhao hongguo, Wu shaolin, et al.The expression and clinical significance of lung resistance protein gene in acute leukemia. Chinese Journal of laboratory Diagnosis, 2002, 6(6): 366–368.
11. Zhang jianbai,Sun yuan, Dong juan et al. Expression of lung resistance protein and multidrug resistance-associated protein in na?ve childhood acute leukemia and their clinical significance. Chinese Journal of Cancer, 2005, 24(8): 1015–1017.
12. Schwarzenbach H. The Expression of MDR1/ P-glycoprotein, the multidrug resistance protein MRP, and the lung-resistance protein LRP in multiple myeloma. Med Oncol, 2002, 19(2): 87–104.
13. Renjinhai, dongzuoren, guoxiaonan et al. The clinical significance of lung resistance protein (LRP) gene expression in patients with acute leukemia. Chinese Journal of Hematology, 2000, 21(1): 10–13.
14. Luomeihua, biangengshou, fengmin, et al. The predictive value of drug sensitivity experiment MTT method to the effects of chemotherapy in acute leukemia. Journal of Experimental Hematology, 1995, 3(1): 57–61.
15. Den Boer ML, Pieters R, Kazemier KM, et al. Relationship between major vault protein/lung resistance protein, multidrug resistance-associated protein, P-glycoprotein expression, and drug resistance in childhood leukemia. Blood, 1998, 91(6): 2092–2098.
16. Valera ET, Scrideli CA, Queiroz RG, et al. Multiple drug resistance protein (MDR-1), multidrug resistance-related protein(MRP) and lung resistance protein (LRP) gene expression in childhood acute lymphoblastic leukemia. Sao Paulo Med J, 2004, 122(4):166–171.