Anti-Cancer Drugs 1997, **8**, pp. 662–665

Expression of lung resistance-related protein (LRP) in initial and relapsed childhood acute lymphoblastic leukemia

M Volm, G Stammler, F Zintl,[1] R Koomägi and A Sauerbrey[1]

Department of Oncological Diagnostics and Therapy, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany. Tel: (+49) 6221-42 3266; Fax: (+49) 6221-42 3225. [1]Department of Paediatrics, University of Jena, 07740 Jena, Germany.

Thirty-eight children with initial and 25 children with relapsed lymphoblastic leukemia (ALL) were analyzed for the expression of LRP (lung resistance protein or p110 major vault protein) using immunocytochemistry. LRP expression was found in 18 of 38 (47%) children with initial ALL and in 17 of 25 (68%) children with relapsed ALL. Children with initial ALL and without LRP expression had significantly longer relapse-free intervals than patients with LRP expression.

Key words: Childhood acute lymphoblastic leukemia, lung resistance-related protein, immunocytochemistry, prognostic value.

Introduction

There is increasing evidence that in tumor cells a wide variety of drug resistance mechanisms are present which are responsible for the outcome of patients after treatment. In earlier investigations we showed that the resistance-related factors P-glycoprotein and glutathione-S-transferase π are significant prognostic factors of children with acute lymphoblastic leukemia (ALL), whereas topoisomerase II, dihydrofolate reductase, thymidylate synthase and metallothionein showed no relationship with the relapse-free intervals.[1,2] Multivariate analyses showed, however, that P-glycoprotein and glutathione-S-transferase π solely have only limited prognostic influence.[2] Recently, a new putative membrane transporter has been isolated which is termed lung resistance-related protein (LRP) because it was first identified in a multidrug-resistant lung cancer cell line.[3] Since its identification, LRP has been found in a large number of resistant cell lines and tumors.[4–7]

In the present investigation we determined, therefore, the expression of LRP in initial and relapsed childhood ALL by immunocytochemistry, and determined whether the expression was predictive for survival.

Correspondence to M Volm

Material and methods

Patients

Thirty-eight children with initial and 25 children with relapsed ALL were analyzed for the expression of LRP. The diagnosis of leukemia was performed according to the French–American–British (FAB) classification[8] and by immunological investigation of the blast cells. The patient selection criteria for the evaluation of LRP was the availability of cell samples. Therefore, patients with high initial blast cell count ($\geq 50 \times 10^9$/l, frequently high-risk patients) are more frequent in this investigation (low n=16; high n=22).

Treatment

All patients with initial ALL were treated according to the two modified BFM protocols ALL-VII/81 and ALL-VIII/87.[9] The treatment results of the patients investigated in this study were not significantly different with respect to treatment procedures ($p > 0.1$). These treatment protocols consist of induction therapy with prednisone, vincristine, daunorubicin and L-asparaginase followed by consolidation therapy with cyclophosphamide, cytarabine, 6-mercaptopurine and methotrexate. Patients with relapsed ALL received therapy according to modified protocols for the relapse therapy of childhood ALL.[10]

Leukemic cells

Leukemic blast cells were isolated from the bone marrow. All cell samples contained at least 80% blast cells examind by May–Grünwald Giemsa staining. Cell samples were collected in heparinized flasks and mononuclear cells were isolated by Ficoll-Hypaque

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density gradient centrifugation. After separation the samples contained more than 95% blast cells. The cells were cryopreserved in liquid nitrogen with 10% dimethylsulfoxide and 5% fetal calf serum using a programmed freezer.

Immunocytochemistry

For measuring LRP, cell samples were resuspended in Hanks' balanced salt solution (Biochrom, Berlin, Germany). The cell suspensions were centrifuged with a Cytospin II (Shandon, Frankfurt, Germany), resulting in a cell monolayer. After air drying, the cells were fixed in ice-cold acetone for 10 min and stored at −20°C. The previously described biotin–streptavidin method was used to detect LRP.[11,12] Briefly, cell samples were preincubated with hydrogen peroxide (0.3%; 15 min), unlabeled streptavidin (dilution 1:50; 15 min) and non-immune normal serum. For detection of LRP, the clone LRP-56 (monoclonal anti-human P110; Dunn Labortechnik, Asbach, Germany) was used (1:20). The primary antibody was applied for 16 h at 4°C in a moist chamber. After three washes in phosphate-buffered saline (PBS) the cells were incubated for 30 min with biotinylated sheep anti-mouse IgG (1:50, 5% normal human serum; Amersham, Braunschweig, Germany). Thereafter, the streptavidin-biotinylated peroxidase complex (1:100, 30 min; Amersham) was added. Peroxidase activity was made visible with 3-amino-9-ethylcarbazole (15 min), which gives a red–brown reacting product. Counterstaining was performed with hematoxylin. Different cell lines were used as positive controls. Negative controls were obtained firstly by omitting the primary antibody and secondly by an irrelevant antibody. Three observers independently evaluated and interpreted the results of immunocytochemical staining of the cells. The immunocytochemical staining was graded either as negative or positive (>5% positive cells). The evaluations agreed in over 90% of the samples. The remaining specimens were independently re-evaluated and then categorized according to the most frequent classification. The immunostaining was scored without knowledge of the clinical parameters.

Statistic analysis

Life table analyses according to Kaplan and Meier[13] were performed for relapse-free intervals. The groups were compared by log-rank tests and based on those relative risks were calculated. The inter-relationships of clinical data and LRP expression were assessed statistically by using Fisher's exact test[14] which was used as a statistical hypothesis test for the presence or absence of an association between two factors. A p value of less than 0.05 was considered as statistically significant.

Results

Expression of LRP in childhood ALL was determined by immunocytochemistry. In Figure 1, a typical expression pattern of LRP is shown. LRP expression was found in 18 of 38 children with initial ALL (47%) and 17 of 25 (68%) children with relapsed ALL (Table 1). Statistically, this difference is not significant (p=0.1).

To analyze the prognostic value of LRP expression for relapse-free intervals, Kaplan–Meier estimates were performed. We compared groups of patients with initial ALL: patients without LRP expression and patients with LRP expression (>5% positive cells). In this analysis we found that patients without LRP expression had a higher probability of remaining in continous first remission (Figure 2, top, p=0.05). The corresponding median relapse-free intervals were >6

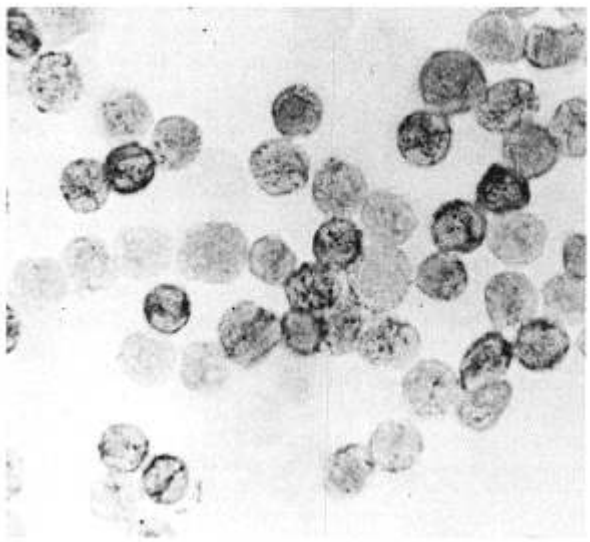

Figure 1. Typical immunohistochemical staining of LRP in ALL (about 40% of the cells are stained).

Table 1. Expression of LRP in initial and relapsed ALL of children

	Patients (n)	LRP-negative	LRP-positive
Initial ALL	38	20	18 (47%)
Relapsed ALL	25	8	17 (68%)

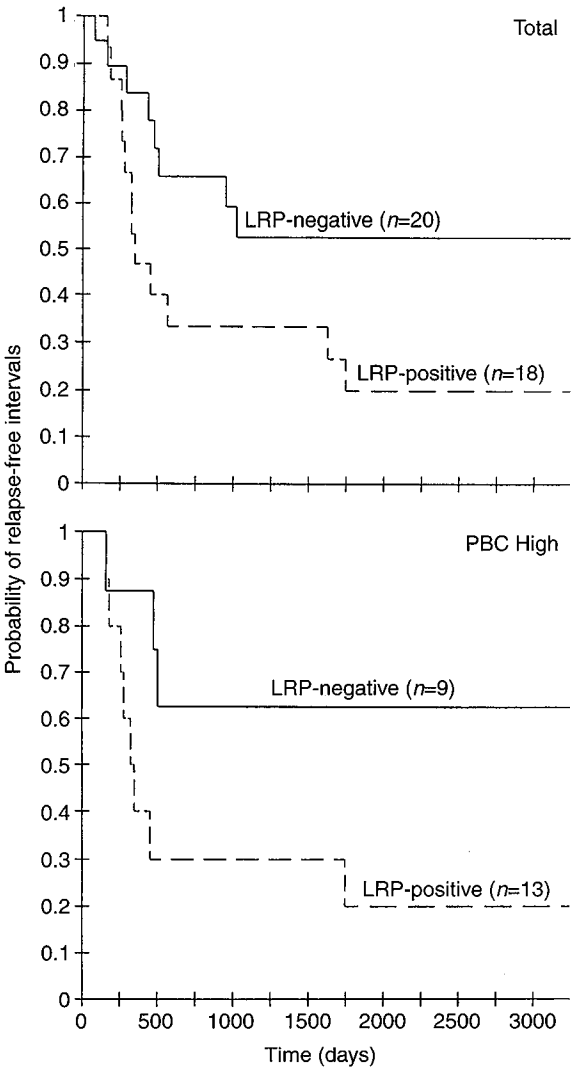

Figure 2. Relapse-free intervals: Kaplan–Meier estimates of initial ALL according to expression of LRP (negative/positive). Top: total number of investigated initial ALL. Bottom: initial ALL with high PBC ($\geq 50 \times 10^9$/l).

Table 2. Relationship between expression of LRP and peripheral blast count (cut-off: 50×10^9/l)

	LRP-negative	LRP-positive	*p* value
PBC low	11	5	0.09
PBC high	9	13	

and 1 year. The relative risk for patients with LRP expression was 2.3.

The prognosis of children with ALL is largely determined by the initial blast cell count (PBC: cut off 50×10^9/l). Therefore we grouped patients with a high blast cell count ($\geq 50 \times 10^9$/ml) according to LRP expression (Figure 2, bottom). Again, we found that patients without LRP expression had a higher probability of remaining in remission. Statistically, this difference is of borderline significance (p=0.06). However, the presence of LRP was not strongly independent of the clinical factor PBC (Table 2).

Discussion

The molecular characterization of LRP as the human major vault protein has constituted the first step to clarify its actual contribution to drug resistance.[15] Vaults are transporter units for nuclear pore complexes, thus implicating LRP vaults in nucleocytoplasmic transport processes.[16] LPR-56 staining was primary vesicular in our study. LRP has been found in different tumors. Izquerdo *et al.*[5] analyzed the expression of LRP in a panel of 61 human cancer cell lines and found expression in 78% of the cell lines. Analysis of 174 tumors comprising 27 tumor types showed LRP expression in 63% of the cases.[6] In our investigation 47% of the initial and 68% of relapsed childhood ALL showed LRP expression.

List *et al.*[7] analyzed bone marrow specimens from 87 patients with acute myeloid leukemia for expression of LRP. The overexpression of LRP was associated with an inferior response to induction therapy. Remission were achieved in 35% of LRP-positive patients as compared with 68% of LRP-negative patients. They found that only LRP had independent prognostic significance when compared with the other transport proteins. In our analysis with ALL of children we found that patients with LRP expresssion had shorter relapse-free intervals than patients without LRP expression. In agreement with the results of other authors obtained with ovarian carcinomas[15] and melanomas,[17] our data suggest that LRP appears to show prognostic value in cancers trated with chemotherapy. However, because of the small number of patients, additional studies are required to determine wether LRP can provide enhanced prognostic capabilities beyond those already available in the form of well-known clinical prognostic factors.

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(Received 12 June 1997; accepted 26 June 1997)