Multidrug resistance 1 gene expression and AgNOR in childhood acute leukemias

S. Balamurugan · D. Sugapriya · P. Shanthi · V. Thilaka · S. Venkatadesilalu · V. Pushpa · M. Madhavan

Abstract The multidrug resistance 1 (MDR1) gene product, P-glycoprotein (Pgp/p170) is a membrane protein, which acts as an ATP dependant efflux pump that expels a wide variety of organic compounds including chemotherapeutic agents from the cell. Pgp over expression has been demonstrated to be linked with poor treatment outcome and poor prognosis in a number of malignant tumors. AgNORs is a simple, reliable and inexpensive method of evaluating the proliferative activity of a tumor. We have studied MDR1 expression and AgNORs in 41 cases of acute leukemia in children. In this study, AgNOR counts in patients with acute lymphoblastic leukemia (ALL) L2 subtype (FAB classification) were significantly higher as compared to the ALL L1 subtype. Similarly, mean AgNOR count in the acute myeloid Leukemia (AML) M2 subtype was significantly higher as compared to the ALL L1 subtype. However, there was no correlation between AgNOR and treatment outcome or between AgNOR counts and MDR1 expression in any of the subtypes of acute leukemia included in this series. In AML, MDR1 gene expression was found to be related to reduced remission induction rates and hence poorer prognosis. In ALL, our study has shown no difference in remission induction between MDR1 positive and MDR1 negative cases. This would suggest that factors other than MDR1 may be of relevance in Pediatric ALL.

Keywords Acute leukemia · AgNOR · Multidrug Resistance 1 · P-glycoprotein.

Abbreviations

ALL – Acute lymphoblastic leukemia
AML – Acute myeloid leukemia
MDR1 – Multidrug resistance 1
Pgp – P-glycoprotein
AgNOR – Nucleolar organizer regions

Introduction

The use of chemotherapeutic drugs in the treatment of leukemia has been plagued by the emergence of resistant cells, either at initial presentation or at the time of relapse [1]. The membrane transporter protein p-glycoprotein (P-gp or P 170) has been found to be a major contributory factor in many cases. P-gp is encoded by the Multidrug resistance 1 gene (MDR1) and acts as an ATP dependent efflux pump, transporting a wide variety of apparently unrelated organic...
compounds out of the cell. This protein is expressed in a wide variety of normal tissues and is said to play a role in the excretion of various substrates into bile, intestinal lumen and urine. P-gp also prevents the entry of toxic metabolites into cells [2]. Notably, the MDR1 encoded protein actively expels many drugs used to treat leukemias such as anthracyclines, vinca alkaloids and epipodophyllotoxins [3], thus decreasing intracellular drug accumulation. P-glycoprotein mediated efflux can be blocked by a number of chemosensitizing agents such as verapamil, cyclosporin and quinine.

In normal hematopoietic cells, MDR1 is expressed at very low levels [4]. However, overexpression is common on neoplastic cell membranes at diagnosis and at relapse of acute leukemias. Agents capable of modulating MDR-1 have been used for treating poor-risk AML including quinine, tamoxifen, calcium channel blockers, cyclosporine A, and its analog, PSC8335 [6].

Nucleolar organizer regions (AgNORS) are loops of DNA relating to the production of precursor of ribosomal RNA – AgNOR is a simple, inexpensive and reliable method of evaluating proliferative activity of tumors [7, 8].

In the present paper, we report our studies on MDR1 gene expression using immunohistochemistry and AgNOR count using the silver staining technique in 41 cases of acute leukemia in children.

Materials and Methods

Peripheral blood/bone marrow smears from 41 cases of acute leukemia in children were used in this study. Leishman stained smears were used to study morphology. The following cytochemical stains were carried out according to standard procedures [9]: Myeloperoxidase, Sudan black B, PAS and non specific esterase. The leukemias were classified according to FAB criteria [10, 11]. AgNOR staining was carried out on one smear from each case using the silver staining method of Crocker et al [8]. AgNORs are visualized as black intranuclear dots. Five hundred cells from each smear were examined under oil immersion objective, the number of AgNOR dots counted and the mean number per nucleus determined. The enumeration of AgNORS was done by method of Crocker et al [8]. MDR1 immunostaining [12] was carried out on smears using the APAAP technique. Briefly, the smears were fixed in cold acetone for 10 minutes and then rinsed in Tris buffer, 0.05M, pH 7.6. The smears were then incubated with prediluted primary mouse monoclonal antibody to P-glycoprotein (clone JSB1, Zymed) followed by rabbit antimouse secondary antibody (Dako, Denmark). Mouse APAAP (Dako) was then added followed by substrate solution containing Napthol AS MX phosphate and Fast Red TR with Levamisole. The slides were rinsed in TBS buffer (2 changes, 2 minutes each) in between the incubation steps. Positivity was indicated by red color at the sites of binding of primary antibody. Normal renal tissue was used as positive control. Patient samples were considered MDR positive if \( \geq 1\% \) of the blasts showed reactivity with MDR antibody as per standard criteria [13, 14]. Remission was assessed at the end of 1 month of induction therapy according to the established criteria [15]. The results were analyzed statistically using the SPSS package.

Results

Patient Characteristics

41 pediatric patients with acute leukemia were studied. The median age of the patients was 3 years (range ~36 days to 12 years) and sex ratio M: F was 1:4:1 (24 and 17 patients respectively). The mean WBC count was 25.5 x 10^9/l and mean blood blast percentage was 62.2%. The population studied included 36 newly diagnosed cases and 5 cases presenting at the time of relapse. The numbers of patients in different FAB categories is indicated in Fig. 1.

MDR Expression

The results of MDR immunostaining are given in Fig. 2. There was no statistically significant correlation between expression of MDR1 and known prognostic parameters - total WBC count, age and blast count at the time of presentation. Cytoplasmic positivity was observed in 12 cases and membrane positivity in 4 cases (Fig. 3). The intensity of MDR immunostaining was weak to moderate. Of the 41 cases enrolled in our study, 8 (5 ALL, 3 AML ) had to be excluded from evaluation of treatment results as 2 patients died due to sepsis and 6 patients were lost to follow up. Remission induction data for ALL is indicated in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>All – MDR1 positivity and response to treatment.</th>
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<tbody>
<tr>
<td>Presentation</td>
<td>MDR1 positive</td>
</tr>
<tr>
<td></td>
<td>No of cases</td>
</tr>
<tr>
<td>New cases</td>
<td>9</td>
</tr>
<tr>
<td>Presenting at relapse</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>10</td>
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* 5 MDR1 negative cases lost to follow up.
** CR= Complete remission