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OVEREXPRESSION OF MDR-RELATED p170 GLYCOPROTEIN IN CHRONIC MYELOID LEUKEMIA

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ABSTRACT

Background and Methods. **Philadelphia (Ph) positive chronic myeloid leukemia (CML) cannot be induced into a true remission with conventional chemotherapy. Blast cells and precursors obtained from 51 Ph$^+$ CML cases were assayed for expression of the multidrug resistance (MDR)-associated glycoprotein (p170) by immunocytochemistry (APAAP) with the MRK-16 monoclonal antibody.**

Results and Conclusions. **Positive cells were found in 11/17 cases in chronic phase (65%), in 4/8 cases in accelerated phase, and in 23/26 cases in blastic phase (89%). The proportion of positive cells, which ranged between less than 1% and 95%, was higher in blastic phase (mean 32±29.9) than in chronic phase (mean 3±5.3) (p = 0.006). These findings show that p170 overexpression is common in Ph$^+$ CML, especially after progression to blastic phase, and suggest that p170-related MDR may contribute significantly to treatment failure.**

Key words: chronic myeloid leukemia, chemotherapy, drug resistance

A 170-Kd transmembrane glycoprotein (p170) acting as an efflux pump for hydrophobic compounds plays a key role in tumor cell resistance to a number of antitumor agents, including anthracyclines and anthracenedione derivatives, vinca alkaloids, epipodophylline derivatives and others.[1-5] p170 is coded by a gene referred to as mdr-1. It is known that this gene can be expressed to very different degrees in normal and tumor cells, and that in tumor cells it can also be amplified many times.[6]

Several independent studies showed that in leukemia and in malignant lymphoma many cells overexpress the mdr-1 gene and are actually multidrug resistant (MDR) even prior to any treatment, and that treatment outcome is negatively related to p170 expression.[7-15] Chronic myeloid leukemia (CML) is perhaps a unique model of a leukemia that cannot be induced into true remission by either conventional or intensive chemotherapy, especially after progression from chronic to accelerated or blastic phase.[16-17]

In this report we show that many leukemic cells in CML overexpress p170.

Materials and methods

Patients

This study included a total of 47 Philadelphia (Ph) positive CML patients admitted for treatment at the Division of Hematology, Udine University Hospital, between March, 1989 and December, 1993. Blastic phase was defined by

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more than 10% of non-granulated blast cells in the peripheral blood or more than 30% in the bone marrow. Accelerated phase was identified by an intermediate percentage of peripheral blood blast cells (5-10%), as well as by disease progression (including splenomegaly, or thrombocytosis, or anemia) during conventional treatment.

Thirteen patients were studied at diagnosis, prior to any treatment, and 2 of them were studied again after progression to blastic phase. Four patients were studied during treatment (hydroxyurea) for chronic phase. Eight patients were first studied in accelerated phase and 2 of these were again studied after progression to blastic phase; 22 patients were first studied during blastic phase. For these latter subjects time from diagnosis to blastic phase ranged between 5 and 98 months (median 21). In summary, the total number of studies was 51: 13 at onset, 4 during the first chronic phase, 8 in accelerated phase and 26 in blastic phase.

Cells

Leukemic cells were obtained for diagnostic or therapeutic purposes either from the marrow or from the peripheral blood, following the patients' informed consent. The samples were anticoagulated with heparin and mononuclear cells were separated on Ficoll-Hypaque, harvested, washed twice in 0.05 M Tris-buffered solution (TBS) at pH 7.4, and resuspended at a final concentration of 1×10^6 cells/mL. Mononuclear cells from blastic phase patients were predominantly (>90%) blastic. Mononuclear cells from chronic phase patients were a mixture of myeloblasts, promyelocytes and myelocytes. Lymphocytes were always less than 10%.

Controls included normal peripheral blood and marrow cells, as well as two MDR cell lines (LOVO DX and CEM VLB) and their respective non-MDR, parental cell lines (LOVO 109 and CCRF CEM).[14, 18]

Immunocytochemistry

Cells were prepared as described above and cytocentrifuge preparations were assayed by the akaline phosphatase anti-alkaline phosphatase (APAAP) technique as previously described,[14, 18] using the MRK-16 monoclonal antibody at a concentration of 10 μg/mL. MRK-16 is an IgG2a directed against extracytoplasmic p170 domains.[19] Many mononuclear cells reacted weakly with MRK-16 in normal blood and marrow samples, as was previously reported.[9,18,20] Weak reactivity was also occasionally detected in non MDR parental cell lines (LOVO 109 and CCRF CEM). Therefore, for the purposes of this study, leukemic cells were scored as positive only when their positivity was in the range of that of positive controls (Figure 1). Results were expressed as the median of the percentage of positive cells counted by three independent observers. In 7 samples all three observers

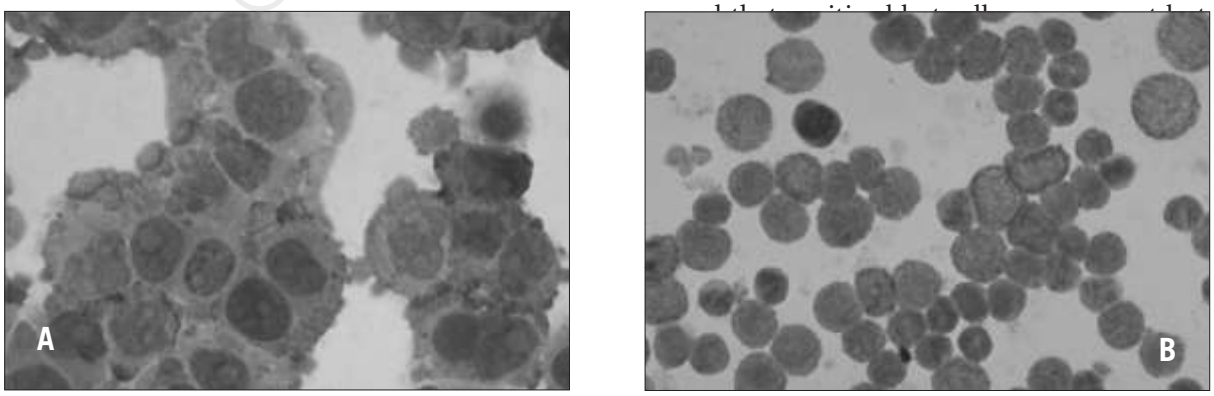

Figure 1. Immunocytochemistry (APAAP) with the MRK-16 monoclonal antibody: A. positive control (LOVO DX cell line); B. leukemic cells.

6/17 (35%) chronic phase samples and in 3/26 (11%) blastic phase samples. The frequency of positive cells was much lower (p=0.006) in chronic phase cases (3±5.3, mean±2 SD) than in the blastic phase ones (32±29.9, mean±2 SD). Only 8 cases were studied in accelerated phase: four of them were positive, and four were negative.

The percentage of positive cells and the intensity of the reaction to MRK-16 were identical in the 6 blastic phase samples with a lymphoid, CD10 positive phenotype, and in the 20 cases with a myeloid or a mixed phenotype.

Mononuclear cells were studied more than once in 4 patients: before and during or after progression from chronic or accelerated phase to blastic phase (Table 2). In all 4 of these cases, disease progression was clearly accompanied by a remarkable increase of MRK-16-positive mononuclear cells.

Table 1. Case distribution according to the presence of MRK-16 positive cells. The percentage of positive mononuclear cells was significantly higher in blastic phase.

	chronic phase	*blastic phase*	*p*
percentage of positive cells – mean ± 2SD	3±5.3	32±29.9	0.006
– median	0.5	23.0	
No. of cases with no positive cells	6/17 (35%)	3/26 (11%)	
< 1% positive cells	5/17 (30%)	2/26 (8%)	
> 1% positive cells	6/17 (35%)	21/26 (81%)	0.007

Discussion

Previous studies of MDR in CML pointed to gene overexpression but were not conclusive. Detectable amounts of p170 or mdr1-mRNA were found by Tsuruo et al.[21] in 3 of 6 cases, by Carulli et al.[22] in 4 of 6 cases, by Kuwazuru et al.[23] in 6 of 11 cases and by Sato et al.[24] in 4 of 12 cases. All these patients were studied in advanced, accelerated or blastic phase.

In contrast, Weide et al.[25] using the C219 monoclonal antibody and immunocytochemistry reported that positive cells were found in 11 of 27 chronic phase patients and in only 4/15 blastic phase patients. It is difficult to explain these data, also because it was reported that positivity was always restricted to mature granulocytes, while blast cells were negative. This is in contrast with all the data concerning mdr-1 expression in leukemic and normal hemopoiesis.[8-9, 11, 13-15, 18, 20, 26-29]

In this study we found that a variable percentage of leukemic cells in Ph+ CML, ranging between less than 1% and 90%, strongly reacted with a monoclonal antibody that recognizes p170. Positive cells were found in all phases of the disease, with a higher number of positive cells in the more advanced blastic phase (p= 0.006). We do not know if the different frequencies of positive cells between the chronic and accelerated or blastic phases was due to different p170 expression in the same cell types, or to different prevalences of different cell types (e. g. blast cells could be equally p170 positive in chronic and in blastic phase, but blast cells are more frequent in the blastic phase), or to

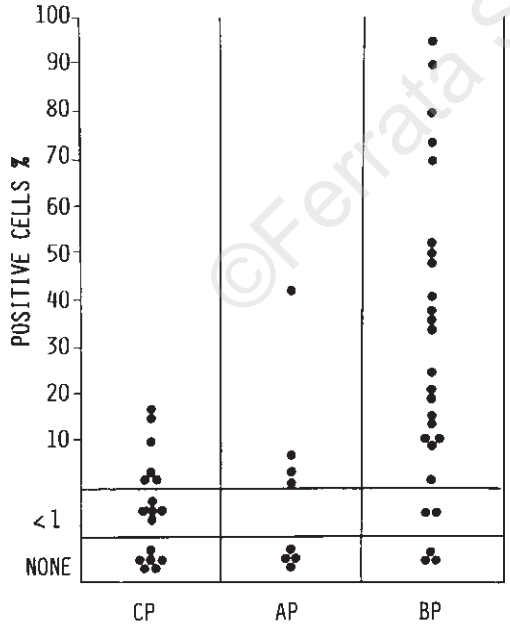

Figure 2. Case distribution according to the percentage of MRK-16 positive cells. Seventeen patients were studied in chronic phase (CP), 8 in accelerated phase (AP) and 26 in blastic phase (BP).

Table 2. Four CML patients were studied during or after progression from chronic phase (CP) or accelerated phase (AP) to blastic phase (BP). Disease progression was accompanied by a clear increase in MRK-16-positive mononuclear cells.

case	*study date (year/month)*	*phase*	*WBC ($\times 10^9$/L)*	*blast cells (%)*	*MRK-16+ cells (%)*
MAS	1991/06	CP	260	1	<1
	1992/12	BP	19	36	48
SPI	1991/01	CP	178	6	2
	1992/05	AP	37	87	<1
	1992/07	BP	3	90	25
REB	1992/07	AP	15	10	7
	1992/10	BP	55	39	50
	1992/12	BP	67	50	30
PIC	1992/12	AP	31	1	1
	1993/11	BP	62	6	80
	1993/12	BP	357	86	72

selection of MDR-positive cells during treatment. Nevertheless, these data clearly showed that there was either a selection or an accumulation of leukemic MDR cells in the blastic phase, as occurs in previously treated, relapsed or resistant acute leukemia.[8, 10-15]

Assessment of cell positivity may vary greatly depending on the method, the reagents and the scoring system. Using immunocytochemistry, we defined as positive only those cells which were as positive as the positive controls, namely, LOVO DX and CEM VLB cells. This degree of positivity has never been found in normal blood and marrow cells.[9, 18, 20] However, it should be remembered that small amounts of p170 can be present in all normal cells, including blood and hematopoietic cells, and these cells can give a slightly positive reaction with MRK-16 and other reagents.[9,18,20,26-29] It is not clear whether a weak positivity may be important, but this is unlikely because it has already been shown that in cell lines[30-31] and in leukemic cells[32-33] the amount of p170 was negatively related to cell sensitivity to anthracyclines and to anthracycline retention.

It is believed that Ph$^+$ CML originates from an early uncommitted hemolymphopoietic stem cell,[34] and recent studies suggested that mdr-1 gene expression is detectable in normal hematopoietic stem cells and can contribute to protecting these cells from chemical injuries.[26-28] Moreover, p170 overexpression in blastic phase can be a consequence of a loss of wild type p53 function leading to derepression of the mdr-1 promoter.[35] Although it is obvious that a number of other important mechanisms of drug resistance are operative in leukemic cells, the p170-related type is likely to be one of the major or more common forms of MDR, since several independent studies on acute leukemias showed a negative relationship between mdr-1 expression and the outcome of conventional chemotherapy.[8, 10-15] Therefore it is likely that the ability of Ph$^+$ cells to increase expression of the mdr-1 gene contributes substantially to the explanation of why conventional chemotherapy is unable to induce true remission in Ph$^+$ CML.

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