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THE EXPRESSION OF PROLIFERATION AND QUIESCENCE ASSOCIATED ANTIGENS IN ACUTE MYELOID LEUKEMIA CORRELATES WITH SURVIVAL DURATION: ANALYSIS OF 15 REFRACTORY CASES

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ABSTRACT

In this study, blast cells from 15 patients with acute myeloid leukemia resistant to induction therapy were examined with two monoclonal antibodies that identify, respectively, the nuclear protein specifically expressed in non proliferating cells (statin) and the proliferating cell nuclear antigen (PCNA). We found that statin values varied widely, ranging from 0.6% to 14.7% (mean value 6.4%). When the patients were subdivided according to the mean value, patients presenting with higher statin values survived for a shorter period of time than the ones characterized by lower levels (p=0.003).

We observed a wide variation in the range of PCNA values; however, if an agreement between survival duration and at least one of the proposed markers was considered, all but one case displayed concordance between survival duration and PCNA and/or statin values (in addition, 4/15 cases showed agreement for both markers).

These preliminary data could indicate a possible discriminating prognostic factor between categories of patients characterized by different aspects of resistance, perhaps susceptible to different salvage therapy approaches.

Key words: PCNA, acute myeloid leukemia, cell cycle, survival

Attempts to relate pretreatment leukemia cell kinetic patterns to the clinical outcome of the disease have led to contradictory results. Monoclonal antibodies are now available against several proteins expressed in a cell cycle-related manner; in particular, the proliferating cell nuclear antigen (PCNA or cyclin), a polypeptide antigen found in both normal and transformed proliferating cells,[1,2] is present throughout the cell cycle (although it is synthesized at a greater rate during the S-phase) and its amount is very low in non-proliferating cells.[3] On the other hand, statin is a 57KD envelope-associated nuclear protein expressed by quiescent cells in culture[4] and in tumors,[5] and this could make it possible to estimate noncycling cells in patients with acute leukemia, and more generally it may be useful in the investigation of cell cycle control and leukemogenesis.[6]

We therefore detailed the proliferative behavior of blast cells from acute myeloid leukemia, identified as percentage of statin- and PCNA-positive cells on the same bone marrow cell populations used for S-phase calculation the rough DNA flow cytometry.

Materials and Methods

The study was conducted retrospectively evaluating 15 AML patients refractory to induction therapy (for details, see Table 1). Preparation of bone marrow samples, immunofluorescence detection of PCNA and statin and the DNA flow cytometry were carried out as previously described.[7,8]

Leukemic cell doubling time (LDT) is defined as the period of time needed for peripheral blood leukemic cell counts to double their baseline value.

Data were analyzed by the Student's t-test and differences were considered significant when $p<0.05$ (two-tailed test).

Results

Good resolution of DNA profiles was obtained in all the samples examined, with a C.V. of the G0/G1 peak ranging from 2 to 4% (median 3%). All patients showed unimodal DNA distribution. The results of statin and PCNA immunodetection are summarized in Table 2. The statin values varied widely from case to case and ranged from 0.6% to 14.7%, with a mean value of 6.4%. However, subdividing the patients according to the leukemic cell doubling time (LDT) revealed two groups of cases: the first (7 cases) with statin levels higher than the mean value (5/7; 2 not evaluable), fast LDT, total insensitivity to therapy and very short survival (≤ 4 months); the second with statin values levels than

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Table 1. Patient characteristics.

Pt.	Sex	Age (years)	FAB	Survival (months)
1	M	29	M5	3
2	F	63	M5	3
3	M	56	M4	3
4	F	32	M4	3
5	F	47	M4	4
12	F	57	M5	3
14	F	50	M4	4
13	F	60	M5	4
6	M	17	M2	20
7	F	56	M2	24
8	F	68	M4	13
9	M	20	M2	14
10	F	72	M2	16
11	F	19	M5	20
15	F	69	M2	16

Chemotherapy: daunorubicin 45 mg/sqm/day for 3 days i.v., cytosine arabinoside 150 mg/sqm/day for 7 days continuous intravenous perfusion, etoposide 60 mg/sqm/day for 5 days intravenous perfusion (2 cycles).

Table 2. Statin and PCNA values in refractory acute myeloid leukemia cases (evaluation at diagnosis).

Pt.	LDT	statin (%)	PCNA (%)
1	fast	14.7	NE
2	fast	12.6	7.2
3	fast	8.0	5.3
4	fast	6.7	15
5	fast	8.72	13.4
12	fast	NE	5.3
14	fast	NE	1.86
13	slow	4.5	5.4
6	slow	4.3	21.5
7	slow	3.2	6.4
8	slow	0.8	12.95
9	slow	0.6	8.2
10	slow	NE	8.3
11	slow	NE	9.3
15	slow	NE	9.48
M±DS		6.4±4.7	9.2±5.0

Values are expressed as percentage of positive cells. NE: not evaluable.

the mean value (5/8 cases; 3 not evaluable) slow LDT, partial sensitivity to therapy and longer survival (more than 1 year). The difference between the two groups was significant (p=0.003).

The PCNA values varied greatly from patient to patient; the majority of those (4/7 cases) characterized by fast LDT were associated with levels lower than the mean value, whereas 4/8 patients with slow LDT showed values higher than the mean value (both concordant with proliferation characteristics). We found no statistical difference between the two groups of patients.

However, we observed that all but one case showed agreement between survival duration and at least one of the two markers. Cases with fast LDT and shorter survival displayed values of statin and PCNA, respectively, higher (5/7) and lower (2/7) than the mean values (in two cases, both: statin low and PCNA high); patients with slow LDT and longer survival were associated with values of statin and PCNA, respectively, lower (5/8) and higher (4/8) than the median (in two cases low statin and high PCNA were concomitantly observed).

Discussion

The availability of monoclonal antibodies against cell-cycle related proteins has increased interest in and opened new possibilities for the study of tumor cell kinetics. One of these proteins is statin, a 57 KD protein mainly expressed *in vitro* by cells in the G0 phase of the cell cycle.[9] Since the expression of statin declines when cells re-enter G1,[10] before the transition into S-phase, statin is thought to be a marker of cell quiescence, even though recent studies have demonstrated that statin expression may not be restricted to quiescent cells but may also be present in sub-populations of cycling cells.[4] Another nuclear protein expressed in a cell cycle-related manner is PCNA (proliferating cell nuclear antigen), a 36 KD nuclear protein that is upregulated in actively proliferating cells from a variety of tissues and species. Two populations of PCNA during S-phase have been recognized: one which is located in the cytoplasm and is easily extracted by detergents, and another that is insoluble and tightly bound to DNA replication sites. The procedure we used for PCNA fixation and immunostaining entailed treatment with detergents for cell permeabilization, so we may assume that insoluble PCNA was mainly measured in our cytometric analyses and the frequency of PCNA-positive cells should be strictly proportional to S-phase.

In this study we analyzed the proliferative activity of resistant AML cases by assessing, in addition to DNA content, statin and PCNA expression. We found that statin may be proposed as a useful marker related to the survival duration of patients with AML, since those with statin levels higher than the mean value developed resistance to chemotherapy that was associated with a very short period of survival; by contrast, those with lower statin values were associated with longer overall survival. A higher PCNA was, on the other hand, associated with longer overall survival. In addition, when considering the agreement of survival duration with at least one of the proposed markers, we observed that all but one case showed an agreement between survival duration and PCNA and/or statin values (4/15 cases did so for both markers).

In conclusion, these preliminary results suggest the clinical feasibility of a detailed study of

leukemia cell kinetics by means of immunodetection of cell kinetic markers, such as statin and PCNA. If confirmed, the data obtained with this approach could offer the possibility of separating at diagnosis or at evaluation of response to induction, a population of totally refractory cases from another group of patients who characterized by longer survival, could be evaluated for further salvage therapy.

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