ABSTRACT

Previous studies showed that peripheral blood lymphocytes of B-cell chronic lymphocytic leukemia (B-CLL) displayed a high intracellular level of cell cycle inhibitor protein p27Kip1. It has been suggested that its high expression may confer them survival advantage and lead to unfavorable prognosis. However, the prognostic significance of p27Kip1 expression for previously untreated, non-advanced stage B-CLL was not established. We studied a relationship between the intracellular level of p27Kip1 of lymphocytes of early- and intermediate stage B-CLL patients and their spontaneous apoptosis in vitro, as well as the prognostic significance of p27Kip1 in B-CLL lymphocytes for the risk of disease progression. Intracellular p27Kip1 content of peripheral blood lymphocytes obtained from 48 previously untreated 0-II Rai stage B-CLL patients was determined by flow cytometry. The viability and apoptosis of those lymphocytes after 72-hours culture were also assessed. During the follow-up period (6-71 months, median 59.5), we recorded the time elapsed to the doubling of lymphocyte count, progression to a higher Rai stage and the appearance of indications for cytostatic treatment. p27Kip1 expression was not correlated with initial lymphocyte count, CD38 expression, cell viability nor spontaneous apoptosis ratio after 72-hours culture. Higher p27Kip1 level was related to the probability of earlier occurrence of each of those above-mentioned events. We did not find a prognostic significance of in vitro cell viability nor apoptosis as to the risk of disease progression. Our results indicate that elevated intracellular p27Kip1 level in leukemic lymphocytes of early- and intermediate stage B-CLL patients contributes to rapid progression of the disease.

RESULTS

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<th>Follow-up (months)</th>
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<th>p27Kip1high</th>
<th>p-value</th>
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<td>6 - 71 months</td>
<td>61.6 ± 20.93</td>
<td>70.8 ± 18.81</td>
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A 62.6 ± 20.93 B 70.8 ± 18.81

Table: Frequency of progression to higher Rai stage, doubling of peripheral lymphocyte count and appearance of indications for cytostatic treatment and expression of p27Kip1 in B-CLL lymphocytes with low (<270 AU) and high (>270 AU) expression of p27Kip1.

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CONCLUSION

We found that, in contrast to what is observed for solid tumors, increased intracellular expression of p27Kip1 in peripheral blood lymphocytes has a unfavorable prognostic value in early- and intermediate stage B-CLL patients as to the risk of disease progression. We did not find a relationship between p27Kip1 content and cell survival and apoptosis, nor between p27Kip1 content and other prognostic factors studied. Further studies are then necessary to elucidate the issue of the relationship between p27Kip1 expression and survival of leukemic cells, and to answer the question about the independency of its prognostic significance.

REFERENCES


MATERIAL AND METHODS

MATERNAL: Peripheral blood mononuclear cells (PBMC) separated from freshly drawn peripheral venous blood by density gradient centrifugation .

METHODS: Flow cytometry detemination of p27Kip1 expression: Mean intensity of fluorescence (MIF) expressed in arbitrary units (AU) as a semi-quantitative measure of intracellular p27Kip1 content in CD5/CD19-positive cells. Cell viability and apoptosis: of peripheral blood mononuclear cells after 72-hour culture in RPMI 1640 supplemented with 10% fetal bovine serum, glutamine 2 mmol/1 and gentamicin 0.16 mg/ml, at a final concentration of 1×10^6 cells/ml.

CONTROL POPULATION: peripheral blood CD5/CD19 leptocytes from 15 healthy age-matched subjects.

TUNNEL method: The cumulative probability of survival without lymphocytes doubling (lymphocyte-doubling free survival, LDFS), of survival without progression to a higher Rai stage (progression-free survival, PFS) and of survival without the appearance of an event justifying the cytostatic treatment (treatment-free survival, TFS) were calculated according to the Kaplan-Meyer method. We used as endpoints: doubling of PBL count, progression to a higher Rai stage and beginning of the cytostatic treatment, respectively. The observations, where cytostatic treatment was started before the attainment of the doubling of PBL count or the progression to a higher Rai stage, were considered as censored for the LDFS and PFS respectively. Kaplan-Meyer curves were compared using log-rank test.

CONCLUSIONS

We found that, in contrast to what is observed for solid tumors, increased intracellular expression of p27Kip1 in peripheral blood lymphocytes has a unfavorable prognostic value in early- and intermediate stage B-CLL patients as to the risk of disease progression. We did not find a relationship between p27Kip1 content and cell survival and apoptosis, nor between p27Kip1 content and other prognostic factors studied. Further studies are then necessary to elucidate the issue of the relationship between p27Kip1 expression and survival of leukemic cells, and to answer the question about the independency of its prognostic significance.