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ORIGINAL RESEARCH REPORT

Prognosis biomarkers evaluation in chronic lymphocytic leukemia

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KEYWORDS
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Abstract
Objective/Background: From clinical and biological points of view, chronic lymphocytic leukemia (CLL) is a heterogeneous disease characterized by a progressive accumulation of lymphocytes in the peripheral blood, bone marrow, and lymphoid organs. New prognostic markers in CLL may be useful to clinicians for predicting outcome and in clinical decision-making. The aim of this study was to evaluate the potential prognostic value of the apoptotic/survival-controlling proteins and protein tyrosine kinase ZAP-70 gene expression in CLL patients and control individuals, correlating such findings with patients' clinical data.
Methods: Fifty-three patients diagnosed with CLL attending the hematology service of a clinical hospital, and 24 healthy individuals with no history of leukemia (Control group) were enrolled in this study. Analyses of apoptotic/survival-controlling proteins were performed by western blot and ZAP-70 gene expression was evaluated by real-time polymerase chain reaction.
Results: Significant differences were observed for the p-p38, Mcl-1 long, and Mcl-1 short proteins when patients were compared with CLL and controls. A positive correlation between the results for Mcl-1 short and Mcl-1 long and lymphocyte count was observed, corroborating the hypothesis of an imbalance between proteins of cell survival pathways/apoptosis in CLL.

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Conclusion: ZAP-70 gene expression was not detected as a discriminant biomarker in these CLL patients. An imbalance between apoptosis-related proteins was observed in the present study, corroborating the hypothesis of increased survival of lymphocytes in CLL patients.

Introduction

Chronic lymphocytic leukemia (CLL) is known as an indolent disease, with slow and progressive accumulation of lymphocytes, incident in elderly individuals, and with a shortened biological expectation of survival. However, this concept is no longer easily applied to all patients affected by this disease. Nowadays, CLL is seen as a heterogeneous disease with a variable clinical course [1]. The most important recent advance in the understanding of CLL pathogenesis was the identification of new prognostic factors in addition to clinical staging. Among them are the subgroup of cytogenetic abnormalities, the mutational status of the immunoglobulin, expression of ZAP-70 and CD38, and evaluation of proteins involved in apoptotic mechanisms.

CLL has been linked to an imbalance between proliferation of blood cells and their ability to undergo apoptosis [2]. Many chemotherapeutic agents kill target cells through protein activation of the bcl-2 family mitochondria-dependent apoptotic pathway. This family of cytoplasmic proteins is characterized by the presence of members that suppress apoptosis (e.g., Mcl-1, Bcl-2, Bcl-xL) or promote apoptosis (e.g., Bax, Bak, Bad, Bid, Bim, and Puma) [3]. The increased survival of B lymphocytes in CLL *in vivo* is considered primarily a result of inappropriate expression of proteins of the Bcl-2 family, particularly the increase of those that suppresses apoptosis and decrease of those that promote apoptosis. The imbalance between antiapoptotic and proapoptotic proteins seems to be one of the mechanisms of apoptosis resistance in CLL, and thus a key factor that determines the longevity of CLL B cells [4,5].

During B lymphocyte apoptosis, activation of B cell receptor (BCR) culminates in the activation of Cy2 phospholipase which, in turn, results in the release of intracellular calcium and activation of protein kinase C, essential for activation of mitogen-activated protein kinases, such as extracellular signal-regulated kinase (ERK), c-jun kinase, and p38, as well as transcription of nuclear factor κB and nuclear factor of activated T cells [6]. In CLL, B lymphocyte CD40 stimulation provokes activation of nuclear factor κB, ERK, and complex PI3K/Akt, also involved in apoptosis, leading to a reduction of both spontaneous and induced chemotherapeutic agent apoptosis. By contrast, proapoptotic protein p38 appears to have reduced phosphorylation, contributing to increased resistance to apoptosis, an important factor in the pathophysiology of CLL [7,8].

The zeta chain-associated protein (ZAP-70) is a 70-kDa protein associated with the T-cell receptor. ZAP-70 is a tyrosine kinase essential to initiate the signaling pathway promoted by activation of the T-cell receptor. Although not found in normal B-lymphocytes, ZAP-70 is highly expressed in most CLL cells in which the variable region of immunoglobulin heavy chain (IgVH) is not mutated. CLL lymphocytes with IgVH mutation rarely show expression of this protein [9]. The presence of ZAP-70 expression in patients without somatic damage in the IgVH gene is related to worse prognosis and shorter survival [10].

It should be noted that substantial changes already occur in the apoptotic process when the CLL is diagnosed, but most patients are asymptomatic at diagnosis and are therefore classified as Binet A. However, as the progression of the disease varies from one individual to another, it is extremely important to search for new biomarkers with potential prognostic implications, which would be important for the adoption of more individualized therapeutic measures. In this context, we evaluated the apoptotic/survival-controlling proteins and the protein tyrosine kinase ZAP-70 gene expression in CLL patients and in control individuals with no history of any hematology disorders. Prognostic evaluation of this kind of biomarker is still lacking in our CLL patient population.

Material and methods

Patients

Patients were selected by hematologists from the Hematology Unit of Clinical Hospital, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil. Clinical data on patients were obtained from their medical records. A total of 53 patients with confirmed chronic lymphocytic leukemia (CLL) rated by the Binet criteria [2] were included in the study: 38 with low risk, nine with moderate risk and six with high risk. Twenty-four clinically healthy individuals with normal blood counts and no history of blood disorders comprised the control group. The institutional Ethics Committee of Federal University of Minas Gerais approved this study, and informed consent was obtained from all participants. This study was carried out in accordance with the Declaration of Helsinki [11].

Blood samples

Whole blood samples were obtained by venipuncture using heparin and EDTA vacuum systems tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were processed immediately after collection and stored at −80 °C until further analysis.

Western blot for cell survival proteins

Mononuclear cells from whole blood were washed with phosphate-buffered saline (PBS) and whole cell extracts