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Apoptotic gene expression under influence of fludarabine and cladribine in chronic lymphocytic leukemia-microarray study

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Abstract:
Background: A deep insight into gene expression profiling (GEP) is a key to understanding the background of disease. It can lead to identification of diagnostic and prognostic factors and then to a selection of the most appropriate therapy. The aim of this study was to evaluate differences in apoptotic gene expression in chronic lymphocytic leukemia (CLL) cells influenced by fludarabine (FA) or cladribine (2-CdA).
Methods: GEP was performed in cells obtained from 10 untreated CLL patients and cultured *in vitro* with FA or 2-CdA. Ninety-three selected apoptotic genes were analyzed using 384 TaqMan® Low Density Arrays in pooled RNA.
Results: Relevant results were found in a set of 27 genes, however, the most striking differences between FA and 2-CdA were observed in the following 5 genes: *BAD*, *TNFRSF21*, *DAPK1*, *CARD 6* and *CARD 9*.
Conclusion: We have found some differences in apoptotic gene expression between FA and 2-CdA. These findings give prominence to genes qualifying for further studies currently conducted in our Department.

Key words:
chronic lymphocytic leukemia, microarrays, gene expression profiling, fludarabine, cladribine

Introduction

The initial cause of chronic lymphocytic leukemia (CLL) remains an open question, but it is currently well accepted that accumulation of CD5/CD19/CD23 positive cells in bone marrow and peripheral blood due to inhibition of their apoptosis is a key event of the disease [4, 6]. Additionally, a recent study suggests that CLL is not solely an accumulation disease but also a proliferative one [25].

Several chemotherapies have been used for the treatment of CLL, such as alkylating agents, antracyclines, corticosteroids, purine nucleoside analogues (PNAs) and monoclonal antibodies [23]. Among the class of PNAs an important role is played by fludarabine (FA) and cladribine (2-CdA), which currently are the most frequently used in CLL therapy. The chemical structure of both drugs is similar to adenosine. They require phosphorylation to activate metabolites, and the induction of apoptosis, as well as cytotoxicity, depends on their accumulation of their triphosphate form (PNA-TP). The mechanism of action of both drugs action is similar, however, minor differences are observed [36].

It is known that PNA-TP causes DNA strand breaks and inhibition of DNA repair, leading to an increase of the expression of *P53* and other proapoptotic genes, and thus results in mitochondrial dysfunction. FA primarily inhibits DNA polymerases and its active metabolite (FA-TP) is incorporated into an elongating DNA chain in competition with dATP and leads to inhibition of DNA repair and accumulation of DNA breaks. In contrast, 2-CdA-TP mainly inhibits ribonucleotide reductase (RR) activity. FA-TP and 2-CdA-TP have also been shown to be incorporated into RNA and affect its synthesis, although by different mechanisms [36].

PNAs taken up by leukemic cells cause the cancer cells to die by two different apoptotic pathways, an intrinsic and an extrinsic one [13]. By the intrinsic pathway, the *P53*-dependent or *P53*-independent influence on mitochondrial membrane should be considered. 2-CdA-TP, in contrast to FA-TP, can induce apoptosis also by *P53*-independent binding with proteins located in the mitochondrial membrane and probably therefore it exerts action at concentrations 5–10-fold lower than FA [36]. Changes in mitochondrial membrane potential lead to a release of either cytochrome c or apoptosis-inducing factor (AIF). The binding of cytochrome c to both procaspase-9 and apoptotic protease activating factor-1 (APAF-1) results in the formation of apoptosome and sequential activation of caspase-9 and caspase-3, whereas the activation of the intrinsic apoptotic pathway by AIF can occur in a caspase-independent manner. Both these pathways lead to DNA condensation and fragmentation resulting in apoptosis [8]. Nevertheless, the extrinsic apoptotic pathway should also be taken into consideration [19, 28]. In this mechanism an important role is played by the death receptor FAS/CD95 [19, 28].

For the last few years, microarray technology is one of the most innovative tools used to study gene expression. It provides a lot of information in a very short time. A deep insight into the gene expression profile (GEP) of apoptosis is a key to understanding the background of CLL, the mechanism of changing gene expression and how this expression is connected with apoptotic pathways. The information obtained with the microarray method can lead to identification of diagnostic and prognostic factors and then to a selection of the most appropriate therapy.

There are only a few reports indicating apoptotic gene expression modifications in CLL cells obtained from patients treated with PNAs [24, 32, 38]. There are also a few *in vitro* studies focused mainly on the set of genes affected by FA or 2-CdA [12, 17, 30, 33, 40]. There is an absence of data comparing the effects of the above-mentioned PNAs on gene expression involved in apoptosis. As it is known, however, that they do act in quite different ways, the undertaking of this study was entirely justified.

The aim of this study was to evaluate differences in apoptotic gene expression in CLL cells influenced by FA and 2-CdA in *in vitro* cultures by means of the microarray method.

Materials and Methods

Patients

Fresh peripheral blood samples were collected from 10 untreated CLL patients who were diagnosed and followed at the Hematology Department, Medical University, Łódź, Poland. The CLL diagnosis was based on IWCLL criteria [14]. The Ethics Committee