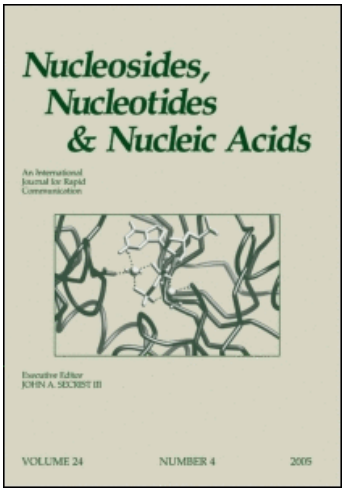

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Purine Metabolism in B-Cell Lymphocytic Leukemia: A Microarray Approach

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PURINE METABOLISM IN B-CELL LYMPHOCYTIC LEUKEMIA: A MICROARRAY APPROACH

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□ *B-cell chronic lymphocytic leukemia (B-CLL) is an adult-onset highly heterogeneous malignancy characterized by a cells resistance to apoptosis rather than to highly proliferative cells. In previous research, we evidenced an imbalance of purine metabolism in B-CLL cells. Since the extracellular adenosine has been proved to induce apoptosis via A2b receptor, enzymes involved in adenosine metabolism could play an important role in apoptosis resistance of B-CLL cells. We prepared a microarray chip for the analysis of 50 selected genes that could be of interest in B-CLL: enzymes of purine de-novo, salvage and catabolic pathway, oxidative stress enzymes, and apoptotis-related proteins. Preliminary results identify many genes of purine metabolism that exhibit low or high expression, while genes involved in signal transduction and apoptosis exhibit lower alterations even if of remarkable interest. This application of microarray technique seems promising and at least a subset of these genes will be valid candidates for further studies.*

Keywords Leukemia; Apoptosis; Gene expression; Microarray; Purine metabolism

INTRODUCTION

B-cell chronic lymphocytic leukemia (B-CLL), the most common leukemia, is an adult-onset heterogeneous malignancy characterized by the presence of elevated numbers of circulating clonal leukemic B cells, that typically express CD19, CD23, CD5. These cells exhibit resistance to apoptosis rather than high proliferation.

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In previous studies, we found imbalanced purine metabolism in B-CLL lymphocytes with reduced nucleotide content, decreased kinetic of nucleotide formation, block of inosinic branch point and low activity of salvage-pathway enzymes.[1] We also found decreased activity of three 5′-nucleotidase isoforms: ecto 5′-NT (e-N or CD73), e-Ns derived from e-N and cytosolic c-N-II. By FACS, we found that most B-CLL cells were CD73-negative.[2] CD73 regulates adenosine availability, influencing cell energy, signal transduction and cell maturation.[3] Since extracellular adenosine induces apoptosis via A2b receptors, enzymes such as CD73 involved in extracellular adenosine release could play a role in B-CLL cell resistance to apoptosis. Gene expression profiling by microarray is proving to be very useful in understanding tumor cell biology and it has been used successfully by a variety of investigators also in B-CLL[4] to a better understanding of the disease. Microarrays, therefore, are useful to assay gene expression within a single sample or to compare gene expression in two different cell types or tissue samples, such as in healthy and diseased tissue. We set up a chip to analyse 50 selected genes that could be of interest in B-CLL: enzymes of the purine de-novo, salvage and catabolic pathways, oxidative stress enzymes, signal transduction, and apoptosis-related proteins.

MATERIALS AND METHODS

Patients

Two B-CLL patients, who had not received treatment, were analyzed after informed consent; diagnosis was confirmed by clinical and immunological criteria. Peripheral blood lymphocytes were isolated by gradient density centrifugation and B cells were separated by immunomagnetic procedure. As control we used peripheral blood B cells from 3 healthy, age-matched (>60 years) subjects.

Total RNA was extracted and microarray analysis was performed by MWG-service (Ebersberg, Germany).

Data Analysis

The intensity of each spot and the corresponding background from the individual scanner tiff image were calculated using the ImaGene software (Biodiscovery, Inc., Los Angeles, CA). The same software was used to create a color overlay image of the microarray. The overlay images can provide an overview about the level of differential expression (up- and downregulated genes) dependent on the intensities in the Cy5 and Cy3 channel. The genes represented by the yellow spots do not show differences in expression. The ImaGene result files are processed further using the MAVI-Pro software (MWG). MAVI-Pro combines the ImaGene data from the multiple scans at

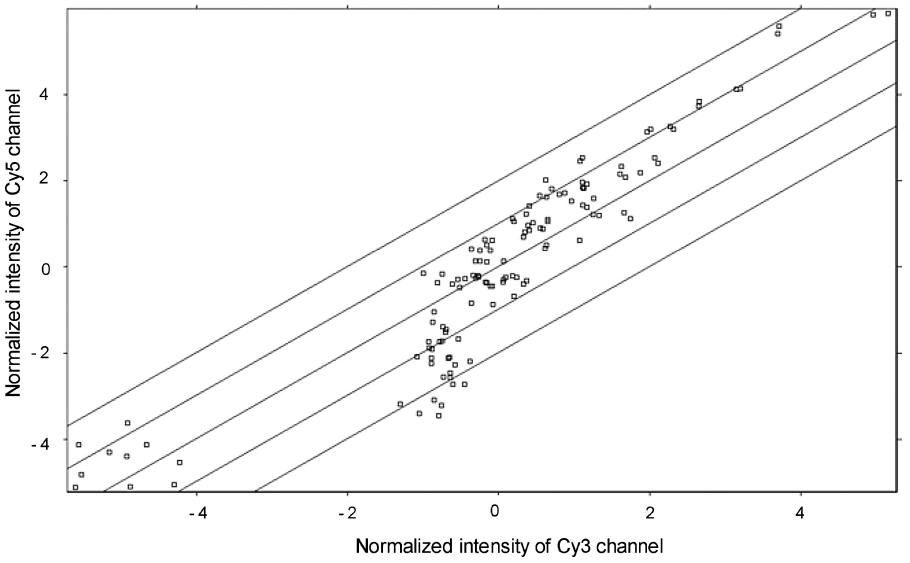

FIGURE 1 The scattar plot image of the intensities displays the distribution of the normalized intensities of both channels. Represented genes with ratio 1 (log transformed = 0; not regulated) are along the central diagonal line. The other lines parallel to the central diagonal mark 2-fold (log transformed = 1/−1) and 4-fold (log transformed = 2/−2) up- or downregulation, respectively.

different PMT gain setting to one dataset per slide using linear regression analysis; this extend the dynamic range while at the same time avoiding saturation problems. MAVI-Pro also calculates the background subtraction and normalization of intensity data. The normalized and background corrected intensities were used to calculate a Cy3/Cy5 ratio value. Data visualization of experiments was performed by GeneSight (Biodiscovery, Inc., Los Angeles, CA), for better distribution in histogram and scatter plots data were log transformed.

RESULTS

Data analysis pointed out 17 genes (34% of genes in the chip) whose expression varied at least 2-fold (Figure 1) and confirmed their altered expression level on additional B-CLL samples not included in the present microarray analysis.

Some genes of purine metabolism with different expression versus controls were identified. Among de novo enzymes, the Gars-Airs-Gart (phosphoribosylglycinamide synthetase–phosphoribosylaminoimidazole synthetase-phosphoribosylglycinamide formyltransferase) complex was overexpressed and IMP dehydrogenase seemed underexpressed. Regarding the enzymes of salvage pathway, APRT (Adenine phosphoribosyltransferase)

TABLE 1 Gene with Expression Varied at Least 2-Fold, in Replicate

Underexpressed gene		
IMP-dehydrogenase 1	0.44	0.45
APRT	0.47	0.48
Adenylosuccinate lyase	0.55	0.59
Adenylate kinase 1 (cytosolic)	0.60	0.61
Adenosine A1 receptor	0.51	0.52
Caspase 6	0.54	0.56
Caspase 8	0.56	0.56
G-prot-coupled receptor kinase 6	0.27	0.31
Overexpressed gene		
Adenosine A3 receptor	2.16	2.44
Gars-Airs-Gart	7.13	6.71
Adenylate kinase 3 (mitochondrial)	3.44	4.82
Myodenylate deaminase	1.97	2.57
NMN adenylyltransferase	5.82	4.85
CD26	2.76	2.37
CD38	3.10	2.36
Interleukin 18	3.58	3.29
Interleukin 4	4.90	3.74

revealed underexpression but HGPRT (Hypoxanthin-Guaninphosphoribosyltransferase) was unchanged.

An imbalance in adenosine related protein gene expression has been also pointed out, with an overexpression of Adenosine Deaminase Complexing Protein (CD26), cyclic ADP-ribose hydrolase (CD38) and mitochondrial adenylate kinase 3, while adenosine receptor A1 (Adora1) and cytosolic adenylate kinase 1 were underexpressed. We also identified some interesting alterations in apoptosis related proteins, namely caspases 6 and 8 and in interleukin 4 and 18 (both overexpressed) (Table 1). Conversely, no alteration has been observed in the expression of some proapoptotic or antiapoptotic factor like Bcl-10, DNA fragmentation factor alpha polypeptide (dffa), Bcl-2 associated x protein- Bax and Bcl-2 isoform 1, respectively.

DISCUSSION

The analysis above described suggest that the set of 50 unique genes considered in this study could be of interest in the investigation of apoptosis resistance of B-CLL cell and in the biology of normal versus transformed B cell. The behavior of the altered gene evidenced in this research could be evaluated in experimental data in relation to apoptotic stimuli or apoptosis-inducing drugs. A simultaneous gene profiling of apoptosis related factors and purine metabolism enzymes is of particular interest for the following reasons: 1) Inhibitors of purine metabolism are immunosuppressive drugs commonly used in therapeutic procedures during organ transplantation,

in particular mycophenolate which has been demonstrated to be effective in inducing apoptosis. 2) Fludarabine, a acytotoxic agent used in the treatment of B-CLL, is administrated as the 5′-nucleoside monophosphate and is converted to the nucleoside by the activity of serum phosphatase and ecto-5′-nucleotidase (CD73). Very little is known on the involvement of pro- and anti-apoptotic factors and their gene expression and regulation in the pathogenesis of B-CLL. Further microarray investigations will be helpful in clarifying if B-CLL lymphocyte resistance to apoptosis is governed by a general mechanism or is rather multifactorial as in Burkitt's lymphoma; nevertheless, microarray studies could give interesting informations regarding the correlation between gene expression signature and disease progression.

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