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The pattern of gene expression and gene dose profiles of 6-Mercaptopurine- and 6-Thioguanine-resistant human leukemia cells

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ABSTRACT

Exposure of MOLT4 human T-cell leukemia cells to 6-Mercaptopurine (6-MP) and 6-Thioguanine (6-TG) resulted in acquired resistance associated with attenuated expression of the genes encoding concentrative nucleoside transporter 3 (*CNT3*) and equilibrative nucleoside transporter 2 (*ENT2*). To identify other alterations at the RNA and DNA levels associated with 6-MP- and 6-TG resistance, we compared here the patterns of gene expression and DNA copy number profiles of resistant sublines to those of the parental wild-type cells. The mRNA levels for two nucleoside transporters were down-regulated in both of the thiopurine-resistant sublines. Moreover, both of these cell lines expressed genes encoding the enzymes of purine nucleotide composition and synthesis, including adenylate kinase 3-like 1 and guanosine monophosphate synthetase at significantly lower levels than wild-type cells. In addition, expression of the mRNA for a specialized DNA polymerase, human terminal transferase encoded by the terminal deoxynucleotidyl transferase (*DNTT*) gene, was 122- and 93-fold higher in 6-TG- and 6-MP-resistant cells, respectively. The varying responses to 6-MP- and 6-TG observed here may help identify novel cellular targets and modalities of resistance to thiopurines, as well as indicating new potential approaches to individualization therapy with these drugs.

1. Introduction

The thiopurine antimetabolites 6-Mercaptopurine (6-MP) and 6-Thioguanine (6-TG) are analogues of purine nucleosides widely used to obtain and maintain remission of acute lymphoblastic leukemia (ALL) and acute myelocytic leukemia (AML) [1].

They are inactive pro-drugs that exert their cytotoxicity only after being metabolized intracellularly to products that either inhibit *de novo* purine synthesis (DNPS) or are incorporated into DNA. In the case of both 6-MP- and 6-TG, activation is catalyzed by the hypoxanthine-guanine phosphoribosyl transferase (*HGPRT*), followed by multi-step conversion to thioguanine nucleotides (TGNs) that can be incorporated into DNA or RNA and/or, with 6-MP methylated products such as methyl-thioinosine monophosphate (Me-TIMP), that inhibit *de novo* purine synthesis (DNPS) [2,3].

There is competition between such activation by *HGPRT* and deactivation through methylation of thiopurines [3] by thiopurine methyltransferase (TPMT), which is characterized by several common genetic polymorphisms [4]. It is now well-established that reduction in TPMT activity, due to genetic polymorphism results in severe and sometimes fatal hematological toxicity in patients undergoing treatment with standard doses of thiopurines and, thus, for patients with heterozygous or homozygous polymorphisms in the *TPMT* gene the dose should be lowered. On the other hand, patients with very high levels of TPMT activity may be undertreated [2].

Indeed, since cellular TPMT activity is inversely related to intracellular concentrations of TGN, patients with ALL and non-functional variant alleles of the *TPMT* genes tend to respond more favorably to 6-MP therapy, while being at higher risk of developing undesirable side-effects such as hematopoietic toxicity [4], infections, stomatitis and secondary tumors. Conversely, patients with high enzyme activity can tolerate 6-MP better but also run an increased risk for relapse and hepatic toxicity caused by methylated metabolites. To avoid life-threatening side-effects in patients homozygous for two non-functional *TPMT* alleles, a 10- to 15-fold reduction in the standard dose is recommended [3].

Inosine 5′-monophosphate dehydrogenase (IMPDH) catalyzes the first and rate-limiting step in guanine nucleotide biosynthesis. Since thioinosine monophosphate (TIMP), the major intracellular metabolite of 6-MP, is a substrate for IMPDH, alterations in the

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activity of this enzyme should exert a significant impact on thiopurine metabolism, with elevated activity promoting toxicity and attenuated activity predicting a poor clinical response [2].

In addition, guanosine monophosphate synthetase (*GMPS*), which, like IMPDH, is a key enzyme in the *de novo* biosynthesis of guanine nucleotides, catalyzes the conversion of 6-thioxanthine-5′-monophosphate (TXMP) to 6-thioguanosine-5′-monophosphate (TGMP).

Inosine triphosphate pyrophosphatase (ITPase) is another actor in thiopurine metabolism, catalyzing the reconversion of inosine triphosphate (ITP) to inosine monophosphate (IMP) [4] and thereby preventing accumulation of ITP [2]. The higher frequency of toxicity (febrile neutropenia) observed in patients with a variant non-functional ITPase allele is thought to be caused by accumulation of methylated thiopurine nucleotide metabolites, which are known to have cytotoxic properties [4].

With regards to the uptake of thiopurines, the most extensively studied proteins that transport nucleosides and nucleobases and, thus, even the thiopurines are the nucleoside transporters, which can be subdivided into two major classes: equilibrative (facilitated) transporters (the SLC29 family), and concentrative or Na^+-dependent transporters (the SLC28 family) [2].

Multi-drug resistance protein 4 (*MRP4*/*ABCC4*), which is also thought to be involved in nucleoside drug transport, has been shown recently to protect against thiopurine-induced hematopoietic toxicity by actively exporting thiopurine nucleotides. Moreover, higher levels of *MRP4* mRNA levels in the leukemia cells of pediatric patients are associated with reduced TGN levels [4].

Despite their wide-spread use, intrinsic and acquired resistance to thiopurines has become a major problem in connection with their use in chemotherapy. The mechanisms underlying the acquired resistance of leukemic cells to 6-MP- and 6-TG are still poorly understood. The most extensively characterized mechanism is a reduction or lack of *HGPRT* activity [5]. In addition, altered TPMT activity can influence sensitivity to 6-MP- and 6-TG [6]. Furthermore, in MOLT4, CCRF-CEM and Jurkat cell lines, inactivation of the mismatch repair (MMR) system leads to pronounced thiopurine resistance.

We have recently characterized two separate MOLT4 cell lines that acquired resistance to 6-MP- and 6-TG, by reducing their uptake of these drugs via the third member of the family of concentrative nucleoside transporters, *CNT3*, and the second member of the equilibrative nucleoside transporter family, *ENT2* [7]. This resistance developed in response to the classic approach, involving exposure of wild-type MOLT4 cells to stepwise increasing concentrations of the drugs.

To elucidate the mechanisms underlying acquisition of resistance to thiopurines by leukemic cells in greater details we characterized here the pattern of gene expression profiles and aberrations in DNA copy number employing high-density microarrays.

2. Materials and methods

2.1. Culturing and initial characterization of the cell lines

The generation of sublines resistant to 6-MP- and 6-TG from the acute T-lymphoblastic leukemia MOLT4 cell line has been described previously [7]. In short, the parental MOLT4 cells were exposed to 6-MP or 6-TG in gradually increasing concentrations up to 5 μM. In the present study parental and resistant sublines were cultured for at least three passages in RPMI-1640 medium supplemented with 10% FCS, 100 U penicillin/ml, 100 μg streptomycin/ml, and 2 mM L-glutamine at 37.8 °C in a humidified incubator under 5% CO_2 prior to analysis. The cells were counted using a Coulter Multisizer (Coulter Electronics, Luton, United Kingdom) and harvested during the logarithmic phase of their growth.

The expression of selected genes i.e. concentrative nucleoside transporter 1 (*CNT1*; Hs00188418_m1, *CNT2* (Hs00188407_m1), *CNT3* (Hs00223220_m1), equilibrative nucleoside transporter 1 (*ENT1*; Hs00191940_m1), *ENT2* (Hs00155426_m1), multidrug resistance-associated protein 4 (*MRP4*; Hs00195260_m1), and *MRP5* (Hs00194701_m1) by these parental and resistant cells was determined previously employing commercially available quantitative real-time PCR analysis (Applied Biosystems, Stockholm, Sweden). The mRNA levels thus obtained were related to the level of glyceraldehyde-3-phosphate dehydrogenase mRNA (*GAPDH*; Hs99999905_m1) [7].

Both parental and resistant sublines were subjected to genotyping for determination of single nucleotide polymorphism (SNP) at the Mutational Analysis Facility (MAF), Karolinska Institutet, Stockholm, using the panel of 47 markers and analysis on a Sequenom™ mass 156 spectrometer as described by Hannelius and colleagues [8]. Pair-wise comparison revealed identical genotypes in parental and 6-MP-resistant cells and 88% identity between parental and 6-TG-resistant cells.

2.2. RNA extraction

Total RNA was extracted from cultured cells using the RNeasy Midi kit and the protocol recommended by the manufacturer (RNeasy Midi Handbook; Qiagen, KEBO Lab, Spånga, Sweden). RNA concentrations and quality were determined using a NanoDrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE), with extracts exhibiting absorbance on ratio of 1.99–2.0 at 260/280 nm being regarded as of acceptable purity.

2.3. Microarray evaluation of gene expression

The oligonucleotide microarrays and all other reagents required for these analysis were purchased from Affymetrix (Affymetrix Inc., Santa Clara, CA, USA). The wild-type, 6-TG-resistant and 6-MP-resistant MOLT4 cell lines were analyzed in triplicate in accordance with the manufacturer's instructions (Technical manual of Affymetrix GeneChip products). The experimental and analysis procedures have also been described in detail in a previous publication from our laboratory [9]. The data were analyzed using the GeneSpring software (Agilent) and subsequently categorized with the Ingenuity software for normalization and exclusion of probe sets that did not meet criteria for detection. Probe sets with low expression below 50 in intensity value were excluded. Statistical calculations were performed by Analysis of variance (ANOVA) with $p < 0.05$ as the cut-off for statistical significance. Selective functional categorization of differentially expressed genes was done using Ingenuity software. All of these microarray data are available at http://www.ncbi.nlm.nih.gov/geo/.

2.4. DNA isolation and array comparative genomic hybridization (array-CGH)

Array-CGH was carried out and analyzed essentially as described previously [10]. Genomic DNA was extracted using the GenElute™ kit (Sigma–Aldrich, Inc.) and quantified and subjected to quality control by NanoDrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Cellular DNA labeled with Cy3-dCTP and reference DNA (Promega, USA) labeled with Cy5-dCTP were pooled, mixed with human Cot-1 DNA, and hybridized to tiling 38 K BAC arrays (SCIBLU Genomics Centre at Lund University, Sweden; www.lu.se/sciblu) for 72 h at 37 °C. After washing and drying, the slides were scanned

Table 1
Comparision of fold changes detected by microarray and qRT–PCR for selected genes.

Gene symbol	Gene name	Microarray		qRT–PCR*	
		6-MP	6-TG	6-MP	6-TG
ENT1	Equilibrative nucleoside transporter 1	1.1	−1.2	1.0	1.0
ENT2	Equilibrative nucleoside transporter 2	−5.3	−2.8	−3.3	−5.0
CNT1	Concentrative nucleoside transporter 1	−1.5	1.5	1.0	1.0
CNT2	Concentrative nucleoside transporter 2	1.0	−1.6	1.0	1.0
CNT3	Concentrative nucleoside transporter 3	−1.3	−1.7	−1.7	−4.3
MRP4	Multidrug resistance-associated protein 4	1.1	1.1	1.0	1.0
MRP5	Multidrug resistance-associated protein 5	1.2	1.2	1.0	1.0

Fold changes refer to expression levels in resistant vs. parental MOLT4 cells.
* Data have been previously published by A.K. Fotoohi et al. [7].

in a GenePix 4200A (Axon Instruments Inc., Union City, CA), analyzed by GenePix Pro 6.0 (Axon Instruments, Wheatherford TX, USA), and uploaded into the BioArray Software Environment, BASE (http://www.base.thep.lu.se/) [11]. Normalization was done with the pin-based LOWESS algorithm [12] and relative copy numbers were identified with CGH plotter [13] applying:>0.25 (gain), >1.0 (amplification), <−0.25 (loss) and <−1.0 (homozygous loss) as cut-offs for log2 ratios. Mapping information and the cytogenetic localization of clones were achieved according to the UCSC genome browser (http://www.genome.ucsc.edu/; July 2004 freeze). The X and Y chromosomes were not included in these analysis and aberrations in telomeric regions were interpreted with caution.

3. Results

3.1. The expression profiles of 6-MP- and 6-TG-resistant cells

In addition to determining the profile of gene expression in each parental and resistant cell line, the methodology was assessed by comparing our microarray data with previously published qRT–PCR analysis [7] of *CNT1*, *CNT2*, *CNT3*, *ENT1*, *ENT2*, *MRP4* and *MRP5* mRNA, using *GAPDH* mRNA as an internal standard (Table 1). According to the qRT–PCR analysis, the level of the *CNT3* mRNA was reduced by 1.7- and 4.3-fold in 6-MP- and 6-TG-resistant cells, respectively. Similarly, the level of *ENT2* mRNA was 3.3- and 5.0-fold lower in these two type of cells, respectively. By microarray, we found 1.3- and 1.7-fold reductions in the level of expression of *CNT3*, as well as, 5.3- and 2.8-fold reductions in *ENT2* mRNA in 6-MP- and 6-TG-resistant cells, respectively. However, we observed no significant changes in expression of the *ENT1*, *MRP4*, *MRP5*, *CNT1* or *CNT2* genes.

Overall, there were 3298 genes whose levels of expression in the parental and one or both of the resistant cell lines differed by 1.3-fold or more. More specifically, 610 differences were seen between parental and 6-MP-resistant cells; 1082 differences between parental and 6-TG-resistant cells; and 1606 differences between the wild-type and both types of resistant cells. Functional categorization of selected genes revealed highly similar patterns for both 6-TG- and 6-MP-resistant sublines (Figs. 1 and 2). Application of a more stringent cut-off, i.e. 2-fold differences or more, resulted in 988 differences in gene expression between the parental and one or both of the resistant cell lines. The 50 probe sets whose expression in the parental and resistant cells lines differed to the greatest extent are documented in Supplementary Tables S1–S4. These include the *DNTT* gene encoding human terminal transferase, a nuclear enzyme highly up-regulated in both resistant sublines.

3.2. Determination of alteration in DNA copy number changes by array-CGH

When DNA copy numbers were determined for the parental and resistant cells using array-CGH (Table 2), aberrations detected included frequent gains and losses, but no amplifications. Homozygous deletions in the 14q11.2 region were detected in both parental and 6-TG-resistant cells. The 14q11.2 region encompasses the genes *OR4E2, OR10G2, TRAJ17, MGC40069, TRAV20, TRA@*, and 7q34 include genes *TRBV19, PRSS1, PRSS2*, *TRBV21-1*, *TRBV3-1*, and *TRBC1*. The chromosomal regions in which losses occurred included 1q, 3q, 6, 7p, 14q, 15q, 17p, 18q, 20 and 22q. Moreover losses within the 6p11.2-q21, 15q14, 17p11.2-p13.1, 20p12, 20q21.3 and 22qter chromosomal regions were also acquired by one of the resistant sublines (Table 2). Gains in copy number were detected on all chromosomes, with the exception of chromosome 15. The majority of these gains were identical or highly similar in the parental and resistant cells. Acquired gains occurred at loci within the 7q22.1, 7q21.11-qter, 9q33.3-qter, 10q11.1-q12, 14q32.2-qter, and 18qter chromosomal regions. Finally, we further clarified the alterations at RNA and DNA levels by comparing expression fold changes with copy number by array-CGH for some selected genes (Table 3).

4. Discussion

Even though treatment of ALL is presently associated with a success rate of as much as 80%, the remaining 20% of the patients risk relapse, resistance and chemotherapy-induced toxicity, which are thus still substantial problems. Like many cytotoxic agents, the therapeutic dose range for the thiopurine drugs, is relatively narrow with potential life-threatening side-effects primarily in the form of myelosuppression [2]. Since thiopurines remain the cornerstone in such therapy, understanding the mechanisms underlying the actions of and resistance to these drugs should help optimize treatment and improve the survival rate. Here, we employed high-through-put characterization of genetic aberrations, including both expression microarrays and array-CGH, to examine two resistant subclones of MOLT4 cells in an attempt to identify new markers and genes that may serve as valuable drug targets in the future.

The enzyme *GMPS* catalyzes the second step in the conversion of 6-MP to thioguanine nucleotides that can be incorporated into DNA. The 1.6-fold down-regulation of this enzyme in the 6-MP-resistant cell line may thus reduce the production of these highly toxic metabolites of this agent. However, no relationship between this activity and the efficacy of 6-MP as a cytotoxic agent has been reported.

Initial studies characterizing the MOLT4 cell line have reported activity of the terminal transferase (TdT) [14] also termed terminal deoxynucleotidyl transferase (DNTT). In the present study the *DNTT* gene was found to be expressed by the 6-TG- and 6-MP-resistant cells at levels that were 122- and 93-fold higher, respectively, than in the wild-type cells. TdT is a special intranuclear DNA polymerase which catalyzes the template-independent addition of deoxynucleotides to the 3′-hydroxyl terminus of oligonucleotide primers [15]. Normally, TdT is expressed only by lymphoid

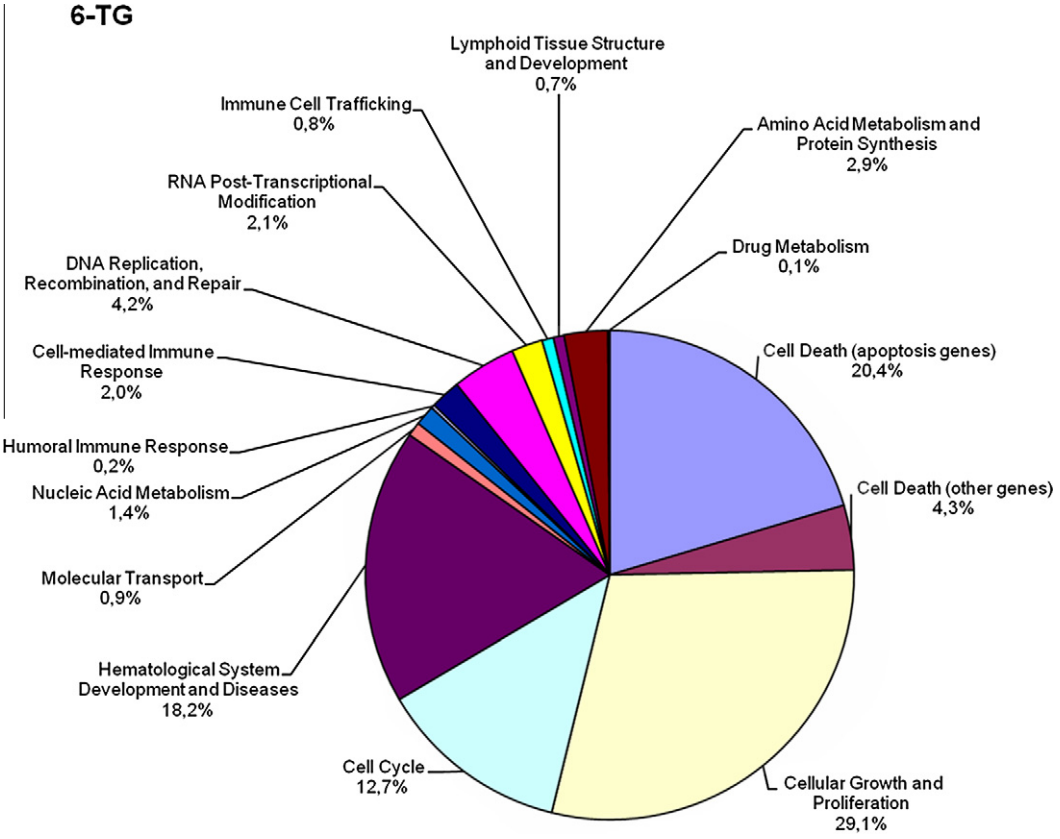

Fig. 1. Functional categorization of selected mRNA species whose levels in 6-TG-resistant and parental MOLT4 cells differ by at least 1.3-fold.

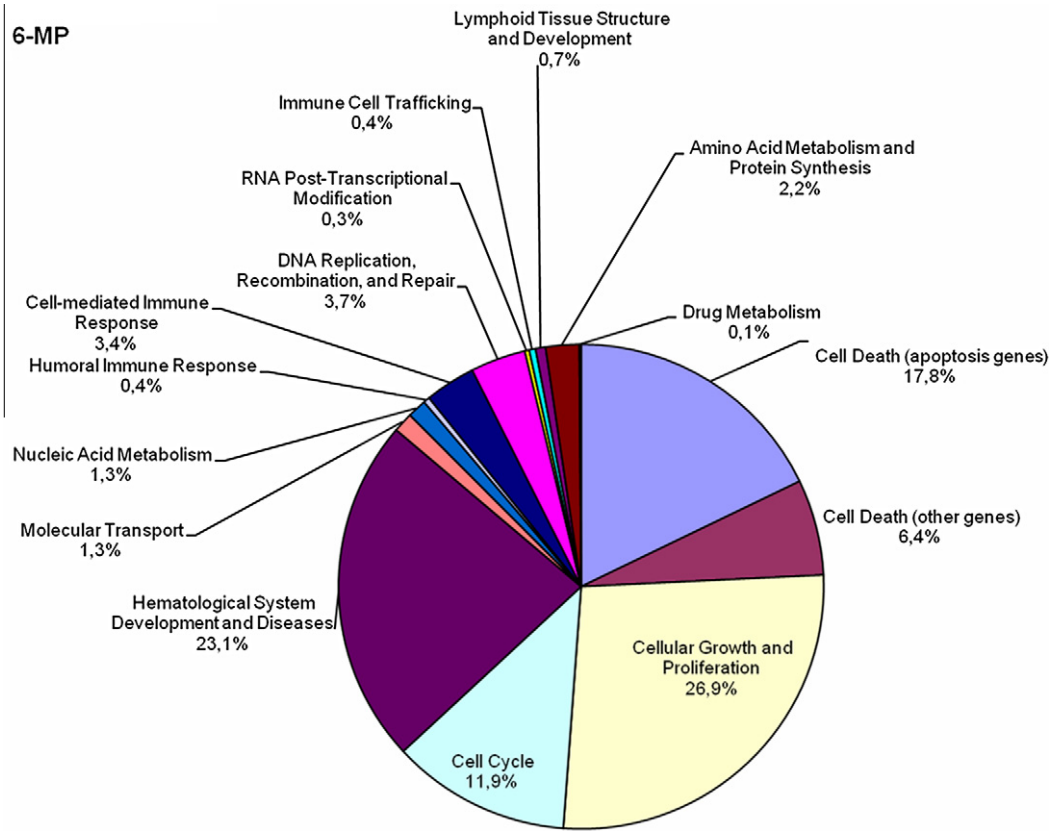

Fig. 2. Functional categorization of selected mRNA species whose levels in 6-MP-resistant and parental MOLT4 cells differ by at least 1.3-fold.

Table 2
Summary of copy number aberrations detected by array-CGH in parental and resistant cells.

Chr.	Parental	6-MP resistant	6-TG resistant
Copy number losses and homozygous deletions			
Chr 1	1q23.1-q31.3; 1q41	1q23.1-q41	–
Chr 3	3q13.31	3q13.31	–
Chr 6	–	–	6p11.2-q21
Chr 7	7p21.1-p22.1	–	7p21.1-pter
Chr 14	***14q11.2 hz***	***14q11.2 hz***	***14q11.2 hz***
Chr 15	–	–	15q14
Chr 17	–	–	17p11.2-p13.1
Chr 18	18q11.2-q23	18p11.22-pter; q11.2-23	–
Chr 20	–	20p12.2	20q21.3
Chr 22	–	–	22qter
Copy number gains			
Chr 1	1p32.3-pter	1p33-pter	1pter
Chr 2	2q35	2q35	2qter
Chr 3	3p21.1-p21.33	3p21.1-p21.33; q29	–
Chr 4	4p16.3-pter	4p16.3-pter	4p16.1-pter
Chr 5	5pter	5pter	5pter
Chr 6	6p21.1-p21.31; q21-qter	6p21.1-p21.31; q21-qter	6p21.1-p21.33
Chr 7	7q11.22-q12	7q22.1; q36.2-qter	7q21.11-qter
Chr 8	8pter; p21.3; q24.24-qter	8qter; p21.3, q24.24-qter	8pter-q23.1; q23.3-qter
Chr 9	–	9q33.3-qter	–
Chr 10	10qter	10qter	10q11.1-q12; q21.3-qter
Chr 11	11p15.5-pter; q12.2-14.1	11p15.5-pter; q12.1-13.5	pter
Chr 12	12q13.11-q14.1	12q13.11-q14.1	–
Chr 13	13q34-qter	13q34-qter	–
Chr 14	14q11.2	14q11.2; q32.2-qter	–
Chr 16	16p13.3-pter; q22.1; q23.2-qter	16p13.3-pter; q22.1; q23.2-qter	16p13.3
Chr 17	17q12-q21.32; q25.1-qter	17q11.1-q21.33; q22-qter	17q21.2-q21.33; q25.1-qter
Chr 18	–	18qter	18qter
Chr 19	19p13.11-pter; q13.31-q13.33	19p13.11-pter; q13.12-q13.33; q13.42-qter	19p13.3-pter
Chr 20	20pter-qter	20pter-qter	20pter-qter
Chr 21	21q22.11-22.2; q22.2-qter	21q22.11-22.2; q22.2-qter	–
Chr 22	22q11.1-q11.23; q12.3-q13.2; qter	22q11.1-q11.22; q12.3-q13.2; qter	22q13.33-qter

hz = homozygous losses; Chr. = chromosome.

Table 3
Comparison of expression fold changes with copy number by array-CGH for selected genes.

Gene symbol	Gene name	Fold change		Cytoband concerned	Altertion by array-CGH		
		6-MP	6-TG		Parental	6-MP	6-TG
IMPDH1	(Inosine 5′-monophosphate) dehydrogenase 1	1.3	1.3	7q32.1	n.a.	n.a.	Gain
IMPDH2	(Inosine 5′-monophosphate) dehydrogenase 2	1.3	1.0	3p21.31	Gain	Gain	n.a.
ENT1	Equilibrative nucleoside transporter 1	1.1	−1.2	6p21.1	Gain	Gain	Gain
ENT2	Equilibrative nucleoside transporter 2	−5.3	−2.8	11q13.2	Gain	Gain	n.a.
CNT1	Concentrative nucleoside transporter 1	−1.5	1.5	15q25.3	n.a.	n.a.	n.a.
CNT2	Concentrative nucleoside transporter 2	1.0	−1.6	15q21.1	n.a.	n.a.	n.a.
CNT3	Concentrative nucleoside transporter 3	−1.3	−1.7	9q21.33	n.a.	n.a.	n.a.
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase 1	−1.1	−1.2	Xq26.3	–	–	–
TPMT	Thiopurine S-methyltransferase	1.2	−1.2	6p22.3	n.a.	n.a.	n.a.
MRP4	Multidrug resistance-associated protein 4	1.1	1.1	13q32.1	n.a.	n.a.	n.a.
MRP5	Multidrug resistance-associated protein 5	1.2	1.2	3q27.1	n.a.	n.a.	n.a.
ITPA	Inosine triphosphatase	−1.1	−1.4	20p13	Gain	Gain	Gain
AK1	Adenylate kinase 1	−1.2	−1.1	9q34.11	n.a.	Gain	n.a.
AK2	Adenylate kinase 2	−1.3	−1.6	1p35.1	Gain	Gain	n.a.
AK5	Adenylate kinase 5	1.2	−1.4	1p31.1	n.a.	n.a.	n.a.
GMPS	Guanosine monphosphate synthetase	−1.6	−1.8	3q25.31	n.a.	n.a.	n.a.
BCL2L11	Bcl-2-like protein 11	−3.5	−4.9	2q13	n.a.	n.a.	n.a.
BCL2L10	Bcl-2-like protein 10	−1.4	−1.5	15q21.2	n.a.	n.a.	n.a.
FAS	TNF receptor superfamily, member 6	−1.9	−1.2	10q23.31	n.a.	n.a.	n.a.
STK17B	Serine/threonine kinase 17b	−1.7	−1.8	2q32.3	n.a.	n.a.	n.a.
STK17A	Serine/threonine kinase 17a	1.8	2.0	7p13	n.a.	n.a.	n.a.
CASP1	Caspase 1, apoptosis-related cysteine protease	3.3	−5.8	11q22.3	n.a.	n.a.	n.a.
AK3L1	Adenylate kinase 3-like 1	−3.5	−13.6	9p24.1	n.a.	n.a.	n.a.
SLC2A14	Solute carrier family 2, member 14	−4.3	−2.7	12p13.31	n.a.	n.a.	n.a.
SLC2A3	Solute carrier family 2, member 3	−17.5	−12.8	12p13.31	n.a.	n.a.	n.a.
GUCY1B3	Guanylate cyclase 1, soluble, beta 3	1.7	4.4	4q32.1	n.a.	n.a.	n.a.
OAS1	2′,5′-oligoadenylate synthetase 1, 40/46 kDa	−9.1	−3.4	12q24.13	n.a.	n.a.	n.a.

Cytoband location is according to information available in Ensembl [http://www.ensembl.org/Homo_sapiens].

precursors of the B- and T-cell lineage [15] and it serve as an useful marker for distinguishing ALL from mature lymphoid neoplasms [16]. Indeed, more than 90% of ALL cells and approximately 30% of chronic myelogenous leukemia cells exhibit elevated TdT

activity, which is associated with a poor prognosis and response to chemotherapy and reduced survival time [17]. Accordingly, specific inhibitors of this enzyme might be developed into a novel class of antitumor agents [17].

The adenylate kinase 3-like 1 gene (*AK3L1*) which encodes a member of the adenylate kinase family of enzymes was down-regulated in both resistant sublines. Adenylate kinases play major role in the regulation of adenine and guanine nucleotide compositions within cells through transferring of phosphate group among these nucleotides in a reversible manner, thus maintaining homoeostasis of these nucleotides in cells which is vital for performing various cellular functions [18].

We have demonstrated previously that impairment of the transport of 6-MP- and 6-TG as a result of attenuated expression of *CNT3* and *ENT2* can account for resistance to thiopurines. Interestingly, microarray analysis revealed that these two transporters were underexpressed in both resistant cell lines confirming their role in the uptake of thiopurines by cells.

In summary, the present characterization of the gene expression and gene dose profiles of 6-MP- and 6-TG-resistant cells provides valuable information concerning the pattern, categories and number of genes involved in their drug resistance.

Contributions

Study design: Freidoun Albertioni, Catharina Larsson, Alan K. Fotoohi.

Performed experiments: Hazhar Karim, Jamileh Hashemi, Ali Moshfegh, Alan K. Fotoohi.

Data analysis: Hazhar Karim, Jamileh Hashemi, Ali Moshfegh, Alan K. Fotoohi.

All co-authors listed contributed to the writing of the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.06.120.

References

[1] G.H. Elgemeie, Thioguanine, mercaptopurine: their analogs and nucleosides as antimetabolites, Current Pharmaceutical Design 9 (2003) 2627–2642.
[2] A.K. Fotoohi, S.A. Coulthard, F. Albertioni, Thiopurines: factors influencing toxicity and response, Biochemical Pharmacology 79 (2010) 1211–1220.
[3] N. Karas-Kuzelicki, I. Mlinaric-Rascan, Individualization of thiopurine therapy: thiopurine S-methyltransferase and beyond, Pharmacogenomics 10 (2009) 1309–1322.
[4] G. Stocco, K.R. Crews, W.E. Evans, Genetic polymorphism of inosine-triphosphate-pyrophosphatase influences mercaptopurine metabolism and toxicity during treatment of acute lymphoblastic leukemia individualized for thiopurine-S-methyl-transferase status, Expert opinion on drug delivery 9 (2010) 23–37.
[5] L. Lennard, The clinical pharmacology of 6-Mercaptopurine, European Journal of Clinical Pharmacology 43 (1992) 329–339.
[6] S.A. Coulthard, L.A. Hogarth, M. Little, E.C. Matheson, C.P. Redfern, L. Minto, A.G. Hall, The effect of thiopurine methyltransferase expression on sensitivity to thiopurine drugs, Molecular Pharmacology 62 (2002) 102–109.
[7] A.K. Fotoohi, M. Lindqvist, C. Peterson, F. Albertioni, Involvement of the concentrative nucleoside transporter 3 and equilibrative nucleoside transporter 2 in the resistance of T-lymphoblastic cell lines to thiopurines, Biochemical and Biophysical Research Communications 343 (2006) 208–215.
[8] U. Hannelius, L. Gherman, V.V. Makela, A. Lindstedt, M. Zucchelli, C. Lagerberg, G. Tybring, J. Kere, C.M. Lindgren, Large-scale zygosity testing using single nucleotide polymorphisms, Twin research and human genetics 10 (2007) 604–625.
[9] A.K. Fotoohi, Y.G. Assaraf, A. Moshfegh, J. Hashemi, G. Jansen, G.J. Peters, C. Larsson, F. Albertioni, Gene expression profiling of leukemia T-cells resistant to methotrexate and 7-hydroxymethotrexate reveals alterations that preserve intracellular levels of folate and nucleotide biosynthesis, Biochemical Pharmacology 77 (2009) 1410–1417.
[10] J. Hashemi, C. Worrall, D. Vasilcanu, M. Fryknas, L. Sulaiman, M. Karimi, W.H. Weng, W.O. Lui, C. Rudduck, M. Axelson, H. Jernberg-Wiklund, L. Girnita, O. Larsson, C. Larsson, Molecular Characterization of Acquired Tolerance of Tumor Cells to Picropodophyllin (PPP), PLoS ONE 6 (2011) e14757.
[11] L.H. Saal, C. Troein, J. Vallon-Christersson, S. Gruvberger, A. Borg, and C. Peterson, BioArray Software Environment (BASE): a platform for comprehensive management and analysis of microarray data, Genome Biology 3 (2002) SOFTWARE0003.
[12] M.C. Yang, Q.G. Ruan, J.J. Yang, S. Eckenrode, S. Wu, R.A. McIndoe, J.X. She, A statistical method for flagging weak spots improves normalization and ratio estimates in microarrays, Physiological genomics 7 (2001) 45–53.
[13] R. Autio, S. Hautaniemi, P. Kauraniemi, O. Yli-Harja, J. Astola, M. Wolf, A. Kallioniemi, CGH-Plotter: MATLAB toolbox for CGH-data analysis, Bioinformatics 19 (2003) 1714–1715.
[14] B.I. Sahai Srivastava, J. Minowada, Terminal deoxynucleotidyl transferase activity in a cell line (molt-4) derived from the peripheral blood of a patient with acute lymphoblastic leukemia, Biochemical and Biophysical Research Communications 51 (1973) 529–535.
[15] M. Onciu, R.B. Lorsbach, E.C. Henry, F.G. Behm, Terminal deoxynucleotidyl transferase-positive lymphoid cells in reactive lymph nodes from children with malignant tumors: incidence, distribution pattern, and immunophenotype in 26 patients, American Journal of Clinical Pathology 118 (2002) 248–254.
[16] L. Liu, L. McGavran, M.A. Lovell, Q. Wei, B.A. Jamieson, S.A. Williams, N.N. Dirks, M.S. Danielson, L.M. Dubie, X. Liang, Nonpositive terminal deoxynucleotidyl transferase in pediatric precursor B-lymphoblastic leukemia, American Journal of Clinical Pathology 121 (2004) 810–815.
[17] R. Di Santo, G. Maga, Human terminal deoxynucleotidyl transferases as novel targets for anticancer chemotherapy, Current Medicinal Chemistry 13 (2006) 2353–2368.
[18] T. Noma, K. Fujisawa, Y. Yamashiro, M. Shinohara, A. Nakazawa, T. Gondo, T. Ishihara, K. Yoshinobu, Structure and expression of human mitochondrial adenylate kinase targeted to the mitochondrial matrix, Biochemical Journal 358 (2001) 225–232.