Frequent Deletion in the Methylthioadenosine Phosphorylase Gene in T-Cell Acute Lymphoblastic Leukemia: Strategies for Enzyme-Targeted Therapy

By Ayse Batova, Mitchell B. Diccianni, Tsutomu Nobori, Thai Vu, John Yu, Louis Bridgeman, and Alice L. Yu

Methylthioadenosine phosphorylase (MTAP), an enzyme essential for the salvage of adenine and methionine, is deficient in a variety of cancers, including acute lymphoblastic leukemia (ALL). Because the MTAP gene is located adjacent to the tumor-suppressor gene p16 on chromosome 9p21 and more than 60% of T-cell ALL (T-ALL) patients have deletion in the p16 gene, we examined the status of the MTAP gene in T-ALL patients. Quantitative polymerase chain reaction amplification of exon 8 of MTAP showed a deletion in 16 of 46 (33.3%) patients at diagnosis and in 13 of 33 (39.4%) patients at relapse. Southern blot analysis showed that, in addition to deletion of the entire MTAP gene, a common breakpoint was between exons 4 and 5, resulting in deletion of exons 5 through 8. The finding of frequent deficiency of MTAP in T-ALL offers the possibility of an enzyme targeted therapy for T-ALL. MTAP(−) T-ALL-derived cell line, CEM cells were very sensitive to methionine deprivation, with cell viability at 50% of control as early as 48 hours after methionine deprivation. In contrast, methionine deprivation had little effect on the viability of normal lymphocytes or on their proliferative response to phytohemagglutinin. Alanosine, an inhibitor of AMP synthesis, inhibited the growth of both MTAP(+) (Molt-4 and Molt-16) and MTAP(−) (CEM and HSB2) cell lines. However, the addition of methylthioadenosine, the substrate of MTAP, protected the MTAP(+) cells but not the MTAP(−) cells from alanosine toxicity. These findings suggest the possibility of targeting MTAP for selective therapy of T-ALL.

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METHYLTIOADENOSINE phosphorylase (MTAP), is an important salvage enzyme for both adenine and methionine. Specifically, 5′-deoxy-5′-methylthioadenosine (MTA) generated during the synthesis of polyamines is rapidly cleaved by the ubiquitous enzyme, MTAP, into adenine and 5-methylthiobutyrate. MTAP is efficiently salvaged to form AMP by adenine phosphoribosyltransferase, and MTR-1-P is converted to methionine by a complex set of oxidations via the intermediate 2-keto-4-methylthiobutyrate.

MTAP is abundant in all normal tissues and cells derived thereof, but deficient in some murine and human tumor cells. We previously reported a deficiency of MTAP activity in one patient with T-cell acute lymphoblastic leukemia (T-ALL) and another with common acute lymphoblastic leukemia in a study of 20 leukemia patients. More recently, a deficiency of MTAP activity has been documented in acute nonlymphoid leukemia, melanoma, lung cancer, rectal adenocarcinoma, glioma, and breast cancer. The MTAP gene has been mapped to chromosome 9p21, in close proximity of the tumor-suppressor genes p15 and p16 that encode inhibitors for cyclin-dependent kinases 4 and 6. A high frequency of p16 gene deletion and mutations have been found in a wide variety of tumor cell lines and primary tumors. We recently found that more than 60% of T-ALL patients have deletion in chromosome 9p21 involving the p15 and/or p16 gene(s) (Diccianni et al, manuscript submitted). Given the close proximity of the MTAP gene to the p15 and p16 genes, it is likely that MTAP may be frequently codeleted with the p15 and p16 genes in T-ALL and other cancers. In MTAP(−) cells, the salvage of methionine and adenine from MTA would be blocked, thus resulting in an increased dependency on an exogenous supply of these nutrients. It is therefore conceivable that an enzyme-selective chemotherapy may be developed in which MTAP-deficient cancer cells would be killed with drugs that deplete purine nucleotides and/or methionine under conditions in which MTAP(+) normal cells would be rescued with MTA as a source for purines and methionine.

The present study is the first to examine the prevalence of MTAP gene deletion in primary T-ALL and to determine whether MTAP(−) T-ALL cells could selectively be killed by inhibitors of de novo purine synthesis or methionine deprivation. Our results show frequent MTAP deletions in primary T-ALL and suggest that MTAP-targeted chemotherapy may be an effective treatment for T-ALL.

MATERIALS AND METHODS

Materials. Alanosine was a generous gift from the Drug and Synthesis Branch/Developmental Therapeutic Program/Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). MTA was purchased from Sigma (St Louis, MO). The random prime labeling kit was from Boehringer Mannheim (Indianapolis, IN). Hybrid membrane was from Amersham (Arlington Heights, IL).

Cell lines. The cell lines studied included those derived from T-ALL (CEM, HSB2, Molt-4, and Molt-16) and chronic myelogenous leukemia (K562). All cell lines were obtained from American Type Culture Collection (Rockville, MD), except for Molt-16 (generous gift from Dr M.O. Diaz, University of Chicago, Chicago, IL) and maintained in minimum essential medium (MEM) or RPMI supplemented with 10% dialyzed horse serum, 2 mmol/L glutamine, and 1% penicillin/streptomycin (complete media).

Patient population and isolation of T-ALL cells. Heparinized venous blood was obtained from 14 patients with T-ALL (10 male, 4 female; age, 2 to 18 years) at the time of diagnosis. Freshly isolated T lymphocytes were cultured in 10 mL of complete media (MEM or RPMI) containing 100 U/mL of human recombinant interleukin-2 (interleukin-2) (Genzyme, Cambridge, MA) in roller bottles at 37°C in 5% CO2 atmosphere. Cell suspensions were harvested after 5 days and washed in complete media, and cell viability was determined by trypan blue staining (2000 to 3000 cells/mL).

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bone marrow or peripheral blood samples were obtained from T-ALL patients in first relapse enrolled in Pediatric Oncology Group (POG) protocol no. 8862. “Treatment of first relapse of childhood T-ALL with combination chemotherapy including 2′-deoxycoformycin.” Diagnosis samples were obtained from those who enrolled in POG no. 9000 and no. 9400 (ALL Biology Study). To safeguard patient confidentiality, each patient was assigned a code number. The patient population consisted of 25% female and 75% male patients. There were 70% Caucasian, 13% Black, 12% Hispanic, 1.5% Filipino, 1% American Indian, 1% Chinese, and 1.5% others.

Mononuclear cells from bone marrow or peripheral blood were isolated by isopycnic sedimentation through Ficoll-Hypaque (Pharmacia, Piscataway, NJ) at 2,000 rpm for 30 minutes. The content of lymphoblasts in these patient samples, as determined by Wright stain, was greater than 90%.

**Polymerase chain reaction (PCR) amplification.** Genomic DNA was isolated using a genomic DNA isolation kit from GIBCO BRL (Gaithersburg, MD). PCR amplification of exon 8 of MTAP was performed using 50 ng of genomic DNA in a 20 µL reaction volume containing 10 mmol/L Tris-HCl, pH 8.3, 25 mmol/L KCl, 2 mmol/L MgCl₂, 50 µmol/L dNTP, 1% dimethyl sulfoxide (DMSO), 1 U Taq DNA polymerase (GIBCO BRL), and 10 pmol each sense and antisense primer. After an initial denaturation at 95°C for 3 minutes, amplification proceeded for 1 minute each at 95°C, 50°C, and 72°C for 28 cycles. Multiplex PCR amplification of MTAP exon 1 and the MTAP pseudogene was performed using 50 ng of genomic DNA in a 25 µL reaction volume containing 10 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 200 µmol/L dNTP, 0.6% DMSO, 2.5 U Taq polymerase, and 100 ng of each sense and antisense primer. After an initial denaturation at 94°C for 4 minutes, amplification proceeded for 1 minute each at 94°C, 63°C, and 72°C for 25 cycles. Taq polymerase was added during a 4-minute pause during the first cycle. Because the lymphoblast count in the T-ALL patients was generally greater than 90%, there was little contamination by normal cells. However, to accurately determine the presence or absence of the MTAP gene in patient samples, control PCR amplifications were used to detect deletions of normal lymphocyte DNA with MTAP(-) CEM cell DNA. The standards generated from these reactions were used to quantify MTAP DNA in patient samples.

Oligonucleotide primers for MTAP exon 8 were 5′-GTCATATCAGCTGCCTCTCT-3′ (sense) and 5′-GGGGAGGAAGAGGAGTCAAG-3′ (antisense). Oligonucleotide primers for MTAP exon 1 were 5′-AAGAAGAATCGGGCAGGGCGAACC-3′ (sense) and 5′-GGGGAGGAAGAGGAGTCAAG-3′ (antisense). Oligonucleotide primers for MTAP pseudogene were 5′-ATATGTGGA-TACCTCATTGGC3′ (sense) and 5′-TACAGACCCCCGGAAGAAAATGCT-3′ (antisense).

**Southern analysis.** Ten micrograms of genomic DNA was digested with EcoRI and resolved on a 1% agarose gel. Southern blot analysis was performed according to established procedures using p15, p16, MTAP, or actin cDNA probes (p15 and p16 cDNAs were generous gifts from Dr. D. Beach, Howard Hughes Medical Institute, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) labeled with [32P]dCTP to a specific activity of 1 to 3 × 10⁸ cpm/µg by random priming. The p15-, p16-, and MTAP-specific signals were visualized by a phosphorimager (Molecular Dynamics, Sunnyvale, CA) and normalized to that of actin cDNA or the MTAP pseudogene.

**Determination of sensitivity to methionine deprivation.** Cells were plated at a density of 0.5 × 10⁶ cells/mL in 24-well plates containing complete media (MEM or RPMI) with or without methionine. Viable cell number was determined by trypan blue exclusion on the days indicated. Stimulation of peripheral blood lymphocyte proliferation by phytohemagglutinin (PHA; 1 to 5 mg/mL) was determined by 3H-thymidine incorporation. Cell cycle distribution was determined by flow cytometry after cells were fixed with ice-cold 70% ethanol and stained with propidium iodine on the days indicated.

**RESULTS**

**Incidence of MTAP deletion in T-ALL.** The structure of the MTAP gene has been delineated by Nobori et al. and consists of eight exons and seven introns. Exon 8 of MTAP is adjacent to the p16 gene and exon 1 is adjacent to the interferon gene cluster. In a recent study (Dicianni et al., manuscript submitted), we found frequent deletions, mostly homozygous, and mutations of the p15 and p16 genes in T-ALL patients (Table 1 and Fig 1). The p16 gene was deleted in 68.8% (33/48) and 69.7% (23/33) of patients at diagnosis and relapse, respectively. The p15 gene was deleted in 53.2% (25/47) of patients at diagnosis and 57.6% (19/33) of patients at relapse. Given the close proximity of MTAP to these genes on chromosome 9p21, it appears very likely that the MTAP gene may be codelleted with p15 and p16. We examined this possibility among 48 diagnosis and 33 relapse T-ALL patient samples. Examination of the status of the MTAP gene as determined by quantitative PCR amplification of exon 8 of MTAP showed that 33.3% (16/48) of patients at diagnosis and 39.4% (13/33) of patients at relapse have a deletion of exon 8 of the MTAP gene (P = 0.64; Table 1 and Fig 2A, lanes 6 and 7). These findings closely agree with those of Travek et al., who found a deficiency in MTAP enzyme activity in 38% (3/8) of T-ALL patient samples examined. Southern blot analysis of 28 patient DNA samples showed that the entire MTAP gene was deleted in 4 patients (representative blot shown in Fig 3A, lanes 6 and 7). In 5 others, a common deletion site exists between exons 4 and 5, resulting in the deletion of exons 5 through 8 (representative blot shown in Fig 3A, lanes 2, 8, and 10). Because exon 1, like exon 8, was very difficult to detect by Southern Blot analysis, it was analyzed by PCR amplification. The results indicated that exon 1 was intact in all DNA samples analyzed, except in those in which the entire MTAP gene was deleted (Fig 2B, lanes 6 and 7). Among 81 samples analyzed, the MTAP gene deletion was always homozygous, with the exception of one sample that was hemizygous (Table 1, patient no. 29). The MTAP gene deletion was always accompanied by homozygous deletion of the p16 gene (29/29 [100%]) and, in most cases, also of the p15 gene (25/29 [86%]), indicating deletion of the entire region of the chromosome encompassing these genes (Table 1 and Fig 3A and B). On the other hand, among patients with homozygous p16 deletion, MTAP was deleted in 50.0% (15/30) and 72.2% (23/31) of patients at diagnosis and relapse, respectively (P = 0.23; Table 1).

**Differential sensitivity of normal lymphocytes and MTAP(-) T-ALL cell lines to methionine deprivation.** MTAP cleaves MTA into adenine and MTR-1-P. The latter is salvaged to form methionine by a complex set of oxidation reactions. It is thus anticipated that MTAP(+) cells would be protected from adenine or methionine deprivation by MTA, whereas MTAP(-) cells would not.
Table 1. Alterations of the p15, p16, and MTAP Genes in T-ALL

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All results are based on a consensus of at least two quantitative PCR experiments and/or Southern blot analysis. Gene rearrangements have been determined by Southern blot analysis.

Abbreviations: +, intact gene; -, homozygous deletion; Hem, hemizygous deletion; +/-R, heterozygous gene rearrangement with remaining allele intact; -/R, heterozygous gene rearrangement with one allele deleted; 2R, independent rearrangement of both alleles; ND, not determined.

* Shifted band observed on Southern blot suggestive of gene rearrangement.

Sensitivity of MTAP(-) leukemic cells (CEM) and MTAP(+) normal cells to methionine deprivation. CEM (MTAP-) and normal lymphocytes (MTAP+), which consists largely of T cells, were cultured in complete medium with or without methionine for 7 days. As shown in Fig 4, CEM cells were very sensitive to methionine deprivation, with viable cell number at 50% of control as early as after 48 hours and at 15% after 6 days of methionine depletion. Flow cytometric analysis of CEM cells showed a progressive G1 accumulation from the control of 42% to 60% after 7 days and to 78% after 15 days of methionine starvation (data not shown). With G1 accumulation, there was a concomitant decline in S-phase from the control of 49% to 23% and 19% on days 8 and 15, respectively. In contrast, viability of normal lymphocytes was not significantly affected by methionine deprivation (Fig 4) for up to 7 days. Such methionine independence of normal lymphocytes cannot simply be attributed to the fact that they are mostly resting cells, because when they were stimulated to proliferate by PHA, methionine depletion had only a slight effect (<21%) on their stimulation index (P < .05), although the absolute levels of [3H]-thymidine incorporation were less than in the presence of methionine (Fig 5).

Selective rescue of MTAP(+) T-ALL-derived cells by MTA. To compare the ability of MTA to rescue MTAP(+) and MTAP(-) cells from methionine starvation, MTAP(+) T-ALL-derived cell lines, Molt-4 and Molt-16, and MTAP(-) cells, CEM and HSB2, were cultured in methionine-free complete media containing increasing concentrations of MTA. Although depletion of methionine was...
growth-inhibitory for all cell lines, only the $MTAP^+$ cells were partially (up to 35% of control) rescued by the addition of MTA (Fig 6). Although only partial rescue of $T-ALL^+$-derived Molt-4 and Molt-16 ($MTAP^+$) was obtained with MTA, normal cells that are less sensitive to methionine depletion, as suggested by our data, would be protected by MTA.

Selective chemotherapy of $MTAP^-$ $T-ALL^+$-derived cells with purine synthesis inhibitors. Because malignant cells lacking $MTAP$ are not able to recycle adenine from MTA, they are more dependent on de novo synthesis. Therefore, by blocking de novo purine synthesis and providing MTA as the only source of adenine, it should be possible to selectively kill $MTAP^-$ malignant cells. When $MTAP^+$ (Molt-4 and Molt-16) and $MTAP^-$ (CEM and HSB2) $T-ALL^+$ cells were cultured in medium containing alanosine, which inhibits conversion of IMP to AMP, all cell lines were growth-inhibited. The $IC_{50}$ ranged from 6 to 10 $\mu$mol/L for these four cell lines (data not shown). However, when MTA was added to the medium, only the $MTAP^+$ cells, Molt-16 and Molt-4, were rescued from alanosine cytotoxicity, with cell numbers ranging from 66% to 80% of control (Fig 7A and B). Under conditions of the assay, MTA concentrations greater than 20 $\mu$mol/L resulted in some toxicity, and thus complete rescue was not observed. On the other hand, complete rescue (107% ± 19%) was obtained with normal fibroblasts treated with 30 $\mu$mol/L alanosine and 5 $\mu$mol/L MTA (data not shown). Overall, our data indicate that de novo purine synthesis inhibitors such as alanosine can selectively inhibit the growth of $MTAP^-$ $T-ALL^+$ cells, whereas $MTAP^+$ cells will be protected when MTA is provided as a source of purines.

**DISCUSSION**

The finding of frequent deletions of chromosome 9p21 in a number of malignancies prompted an intensive search for putative tumor-suppressor genes mapping to this region. Two genes that were mapped to this region, $p15$ and $p16$, were found to be deleted or mutated in a wide variety of tumor cell lines and primary tumors. Recently, we found that the $p15$ and $p16$ genes were frequently deleted in $T-ALL^+$ patients at both diagnosis and relapse (Diccianni et al, manuscript submitted). In the present study, we report that the $MTAP$ gene harbors deletions in 33.3% and 39.4% of $T-ALL^+$ patients at diagnosis and relapse, respectively. In addition to deletion of the entire gene, deletions of exons 5 through 8 were observed in some patient samples analyzed by Southern blot analysis. This same observation was made by Nobori et al in $T-ALL^+$-derived cell lines. These data indicate that a break point exists between exons 4 and 5 of $MTAP$.

**Fig 3.** Southern blot analysis of $MTAP$, $p15$, and $p16$ in $T-ALL$. EcoRI-digested genomic DNA (10 $\mu$g) was hybridized first with the $MTAP$ cDNA probe (A) and then with $p15$ and $p16$ cDNA probes (B). Lane 1, normal lymphocyte DNA (+ control); lane 2, CEM DNA (+ control); lanes 3 through 10, $T-ALL^+$ patient DNA. The $MTAP$ pseudogene (P) identified by Nobori et al was used as a control for DNA loading. The exons (E) of $MTAP$ are identified according to Nobori et al.
MTAP GENE DELETION IN T-ALL THERAPEUTICS

Fig 4. Effect of methionine depletion on the viability of normal lymphocytes and CEM cells. CEM cells (—) and normal lymphocytes (--) were plated at a density of 0.5 × 10^6 cells/mL in 24-well plates containing complete MEM with or without (●) methionine. The number of viable cells was determined by trypan blue exclusion on the days indicated. The results are an average of data from two independent experiments. Each experimental condition was performed in triplicate and the data are reported as the mean ± SD.

Fig 5. Effect of methionine depletion on PHA stimulation of normal lymphocytes. Normal lymphocytes were plated at a density of 0.5 × 10^6 cells/mL into a 96-well plate containing complete RPMI with (●) or without (●) methionine. Cells were treated with increasing concentrations of PHA for 3 days before 3H-Thymidine uptake. The results of two independent experiments are shown. (—) Experiment 1; (—,-) experiment 2. Each experimental condition was performed in triplicate and the data are reported as the mean ± SD. Stimulation index = cpm with PHA/cpm without PHA. (+) methionine control cpm = 646, 965; (+ methionine) maximum stimulation cpm = 58199, 35197; (—) methionine control cpm = 298, 404; (— methionine) maximum stimulation cpm = 22609, 20033, for experiments 1 and 2, respectively.

Fig 6. Rescue of MTAP(+) T-ALL-derived cells from methionine depletion by MTA. Cells were plated at a density of 0.25 × 10^6 cells/mL in complete RPMI with or without methionine and increasing concentrations of MTA. The viable cell number was determined by trypan blue exclusion after 2 to 4 days. The results are an average of data from two independent experiments. Each experimental condition was performed in triplicate and the data are reported as the mean ± SD. (●) MTAP(+) cells; (●) MTAP(-) cells. (●) Molt-4, (●) CEM, (●) Molt-16, (●) HSB2. In two independent experiments, the control (+ methionine) cell numbers per milliliter were 1.09 × 10^6, 1.24 × 10^6 (Molt-4), 1.25 × 10^6, 1.24 × 10^6 (CEM), 1.20 × 10^6, 2.26 × 10^6 (Molt-16), 0.50 × 10^6, 1.15 × 10^6 (HSB2).

the MTAP gene and that exon 8 is always deleted in T-ALL samples with MTAP gene abnormalities. Among the DNA samples analyzed thus far, deletion of the MTAP gene does not appear to occur independently of the p16 gene. Because the p16 gene is deleted more frequently than the p15 or the MTAP gene, the main target of deletion in T-ALL is most likely p16. In addition, like the p15 and p16 genes, MTAP gene deletion is not limited to relapse patients and thus is an early event in T-ALL.

Because MTAP is required for the salvage of adenine and methionine during polyamine synthesis, malignant cells deficient in MTAP would be more dependent on an exogenous source of adenine and methionine than their normal counterparts. Past studies have attempted to take advantage of this metabolic difference between normal and malignant cells using drugs that inhibit de novo purine synthesis, such as azaserine and methotrexate, to selectively inhibit growth of MTAP-deficient malignant cells.2 These studies showed that the addition of MTA (substrate of MTAP) in combination with de novo purine synthesis inhibitors enabled normal cells to proliferate by allowing them to salvage adenine, unlike the MTAP(-) cancer cells. Unfortunately, because methotrexate and azaserine affect not only purine synthesis, but also pyrimidine synthesis, and because azaserine is too toxic for clinical use, MTA will not completely protect normal cells from these drugs. A more specific de novo purine synthesis inhibitor, 5,10-dideazatetrahydrafolic acid (DDATHF), was used in combination with MTA in a recent study that showed that MTAP(-) cells could be killed selectively in medium depleted of exogenous purines.7 In phase I studies, DDATHF had significant antitumor activity even though patients received purines from their diet.25,26 It is important to note that, although human serum contains MTAP, under physiologic conditions, the serum enzyme activity is very low and cannot cleave MTA to generate sufficient levels of adenine to rescue MTAP(-) cells.28 More-
over, MTA can be measured in plasma and urine, suggesting that the levels of MTAP in blood are not sufficient to cleave all endogenous MTA. In the present study, we used alanosine in combination with MTA to selectively kill MTAP(−) CEM and HSB2 cells. Alanosine inhibits the conversion of IMP to AMP and therefore specifically blocks the synthesis of ATP, unlike DDATHF, which also blocks the synthesis of GTP. Our results showed that, even though both MTAP(+) (Molt-4 and Molt-16) and MTAP(−) (CEM and HSB2) cells were killed by alanosine with similar IC50, only the MTAP(+) cells were rescued by MTA. These findings suggest that alanosine would specifically target MTAP(−) tumor cells because normal cells can be rescued by using MTA as a source of adenine. Previously, little success was obtained in phase I and phase II clinical trials of alanosine in patients with leukemia and various solid tumors. Unfortunately, these patients were not evaluated for MTAP status, and patients with malignancies known to have a high incidence of MTAP deficiency, such as glioma and T-ALL, were not included in these trials.

The absolute requirement of some malignant cells for methionine, in contrast to normal cells, has been well documented. Our data indicated that the viability of normal lymphocytes was not significantly affected by methionine deprivation, in contrast to the immediate growth arrest and gradual decline of viability of MTAP(−) CEM cells. Furthermore, methionine depletion had only a slight effect on the proliferation of normal lymphocytes when they were stimulated by PHA. Although we cannot rule out the presence of low levels of methionine in the dialyzed serum, our data indicate that the mitogenic response of normal lymphocytes can proceed under methionine-deficient conditions that arrest growth and greatly reduce viability of MTAP(−) malignant cells.

To exploit the increased methionine dependence of tumors in vivo, two strategies, including dietary restriction of methionine and use of a methionine degrading enzyme, methioninase, have been proposed and are under active investigation. Guo et al showed that a methionine-free diet can result in tumor-selective cell cycle arrest, regression of tumor, and prolonged survival of Yoshida tumor-bearing rats. Kreis and Hession observed that methioninase slowed the growth of W-256 carcinoma-sarcoma in rats. It is conceivable that MTAP-deficient tumors will be more sensitive than normal cells because of their inability to salvage methionine from MTA. To explore this, we determined whether MTAP(+) cells, unlike MTAP(−) cells, can be rescued from methionine starvation by providing MTA as a source of methionine. Our results showed that MTAP(+) Molt-4 cells were partially (up to 35%) rescued by MTA, whereas MTAP(−) CEM cells were not. The lack of complete rescue of Molt-4 and Molt-16 cells may, in part, be due to a defect in biochemical reactions subsequent to MTA cleavage by MTAP that convert MTR-1-P to methionine. It has been speculated that this type of defect is present in malignant cells and would further augment the much increased requirement for methionine over normal cells. Furthermore, in our studies, concentrations of MTA greater than 20 μmol/L, depending on the assay conditions and cell type, were toxic to cells, presumably due to feedback inhibition of polyamine synthesis by MTA. Our findings are consistent with those of a report by Nobori et al that MTAP(+) non-small cell

![Fig 7. Rescue of MTAP(+) Molt-4 and Molt-16 cells from alanosine toxicity by MTA. Cells were plated at a density of 0.25 × 10⁶ cells/mL in complete media. (A) Molt-4 (□) and CEM cells (□) were treated with 10 μmol/L alanosine and increasing concentrations of MTA for 3 days. The viable cell number was determined by trypan blue exclusion and compared with controls. (B) Molt-4 (□) and CEM cells (□) were treated with 10 μmol/L alanosine, and Molt-16 (□) and HSB2 (□) were treated with 15 μmol/L alanosine, in addition to increasing concentrations of MTA for 3 to 4 days. The viable cell number was determined by trypan blue exclusion and compared with controls. (——) MTAP(+) cells; (—–) MTAP(−) cells. The results for Molt-4 and CEM cells presented in (A) and (B) represent data from two independent experiments. Control (− alanosine) cell numbers per milliliter were 1.00 × 10⁶, 1.18 × 10⁶ (Molt-4), 0.79 × 10⁶, 1.08 × 10⁶ (CEM) for experiments A and B, respectively. The results for Molt-16 and HSB2 (□) are data averaged from two independent experiments. In these two experiments, the control (− alanosine) cell numbers per milliliter were 1.00 × 10⁶, 1.53 × 10⁶ (Molt-16), 0.98 × 10⁶, 0.82 × 10⁶ (HSB2). Each experimental condition was performed in triplicate and the data are reported as the mean ± SD.](image URLs)
lung cancer cells were rescued by MTA to levels 70% of the control, whereas the MTAP(−) counterparts were not. Thus, the level of rescue by MTA of MTAP(+) malignant cells may depend on their sensitivity to MTA toxicity as well as on defects in the metabolic pathway of methionine.

Taken together, our results, along with those of others, suggest that methionine depletion or use of specific de novo purine synthesis inhibitors can selectively kill MTAP(−) cancer cells under conditions in which normal cells can be rescued by MTA as a source of methionine and adenine. Thus, the presence of a common metabolic defect such as MTAP deficiency can facilitate development of selective chemotherapy for several types of cancer, including T-ALL.

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Frequent deletion in the methylthioadenosine phosphorylase gene in T-cell acute lymphoblastic leukemia: strategies for enzyme-targeted therapy

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