Title
Involvement of N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) in arsenic biomethylation and its role in arsenic-induced toxicity.

Permalink
https://escholarship.org/uc/item/2r12k83t

Journal
Environmental Health Perspectives, 119(6)

Authors
Ren, Xuefeng
Aleshin, Maria
Jo, William
et al.

Publication Date
2011-06-01

DOI
10.1289/ehp.1002733

Peer reviewed
Involvement of N-6 Adenine-Specific DNA Methyltransferase 1 (N6AMT1) in Arsenic Biomethylation and Its Role in Arsenic-Induced Toxicity

Xuefeng Ren,* Maria Aleshin,† William J. Jo,‡ Russel Dills,§ David A. Kalman,∥ Christopher D. Vulpe,‡ Martyn T. Smith,† and Luoping Zhang†

†Genes and Environment Laboratory, Division of Environmental Health Sciences, School of Public Health, and ‡Department of Nutritional Sciences and Toxicology, University of California–Berkeley, Berkeley, California, USA; §Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington, Seattle, Washington, USA

Inorganic arsenic (iAs) compounds are considered known human carcinogens that target multiple sites, including the lung, skin, and urinary bladder. In humans, inorganic arsenic (iAs) is metabolized to methylated arsenical species in a multistep process mainly mediated by arsenic (+3 oxidation state) methyltransferase (AS3MT). Among these metabolites is monomethylarsonous acid (MMAIII), the most toxic arsenic species. A recent study in A3mt-knockout mice suggests that unidentified methyltransferases could be involved in alternative iAs methylation pathways. We found that yeast deletion mutants lacking MTQ2 were highly resistant to iAs exposure. The human ortholog of the yeast MTQ2 is N-6 adenine-specific DNA methyltransferase 1 (N6AMT1), encoding a putative methyltransferase.

Objective: We investigated the potential role of N6AMT1 in arsenic-induced toxicity.

Methods: We measured and compared the cytotoxicity induced by arsenicals and their metabolic profiles using inductively coupled plasma–mass spectrometry in UROtsa human urothelial cells with enhanced N6AMT1 expression and UROtsa vector control cells treated with different concentrations of either iAsIII or MMAIII.

Results: N6AMT1 was able to convert MMAIII to the less toxic dimethylarsinic acid (DMA) when overexpressed in UROtsa cells. The enhanced expression of N6AMT1 in UROtsa cells decreased cytotoxicity of both iAsIII and MMAIII. Moreover, N6AMT1 is expressed in many human tissues at variable levels, although at levels lower than those of AS3MT, supporting a potential participation in arsenic metabolism in vivo.

Conclusions: Considering that MMAIII is the most toxic arsenical, our data suggest that N6AMT1 has a significant role in determining susceptibility to arsenic toxicity and carcinogenicity because of its specific activity in methylating MMAIII to DMA and other unknown mechanisms.

Key words: arsenic methylation, arsenic toxicity, arsenite, monomethylarsonious acid, N6AMT1.

†Environ Health Perspect 119:771–777 (2011). doi:10.1289/ehp.1002733 [Online 30 December 2010]
purchased from Invitrogen (Carlsbad, CA), and the MTQ2 deletion strain has the same background as the wild-type strain. Growth was conducted in rich media (yeast extract-peptone-dextrose (YPD)) at 30°C with shaking at 200 rpm. UROtsa cells (generously provided by P. Simeonova, National Institute for Occupational Safety and Health, Morgantown, WV) were cultured at a starting cell density of 4–5 × 10^6 cells/mL in RPMI 1640 (Mediatech, Inc., Manassas, VA) with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin (Omega Scientific, San Diego, CA), under standard culturing conditions.

Arsenical exposures. We purchased sodium arsenite [NaAsO2 (iAsIII); purity > 99%] from Sigma-Aldrich (St. Louis, MO), Diiodomethylarsine [MMAIII] and MMAIII [MMIII] was a generous gift from J. Gandolfi (University of Arizona, Tucson, AZ). iAsIII and MMAIII solutions were freshly prepared using sterile water (Milli-Q; Millipore, Billerica, MA) set to 30°C with intermittent shaking, and OD 595 measurements were taken at 15-min intervals for 24 h. The culture medium was then removed and 200 μL dithiothreitol was added and thoroughly mixed for 10 min. Spectrophotometric absorbance at 570 nm was measured in a microplate reader.

Yeast growth assay. Yeast strains were pregrown in YPD media to mid-log phase, diluted in fresh media to an optical density of 595 nm (OD595) of 0.0165, and inoculated into a 48-well microplate. Stock solutions of arsenicals were added to each culture with at least three replicate wells per dose.

Plates were incubated in a Tecan GENios spectrophotometer (Tecan Systems Inc., San Jose, CA) set to 30°C with intermittent shaking, and OD595 measurements were taken at 15-min intervals for 24 hr. Raw absorbance data were averaged for all replicates, corrected for background, and plotted as a function of time. The area under the curve (AUC) was calculated for the cultures in each well using Prism software (version 5.01; GraphPad Software, Inc., La Jolla, CA), and the treatments were averaged and expressed as a percentage of the control.

Human tissue array and real-time quantitative polymerase chain reaction (PCR) assay. We used TaqMan-based real-time quantitative polymerase chain reaction (rt-qPCR) to quantify N6AMT1 and AS3MT expression on a panel of 48 normal human tissues using the Human Rapid-Scan Plate (OriGene Technologies, Inc., Rockville, MD). The human tissues were selected from multiple individuals of different ethnicity and pooled together. We obtained the primers and probes used for amplification of N6AMT1, AS3MT, and ACTB (β-actin; control) from Applied Biosystems (Foster City, CA). Gene expression of N6AMT1 and AS3MT was calculated relative to ACTB using the ΔΔCT method.

N6AMT1 gene expression vector constructs and stable cell lines. Human N6AMT1 cDNA (GenBank accession no. NM_013240; National Center for Biotechnology Information 2011) was PCR amplified with primers 5′-AACCGAGCGAAGGACTAT-3′ and 5′-CAGTAGTTCTGGGCACAC-3′. The PCR product was gel purified (Qiagen, Valencia, CA) and cloned into pCDNA 2.1 vector (Invitrogen) according to the manufacturer’s instructions, and the sequence was confirmed. The pCDNA 2.1 vector containing the N6AMT1 gene was excised using NotI/BamHI restriction enzymes (New England Biolabs, Ipswich, MA) and subjected to gel purification. The nucleotides of the N6AMT1 gene containing BamHI and NotI overhangs were annealed and ligated to a linearized pRetro X-IRES-ZsGreen vector (Clonetech, Mountain View, CA) digested with BamHI and NotI (New England Biolabs). The pRetro X-IRES-ZsGreen vector is a fluorescent retroviral expression vector that allows both a gene of interest and the ZsGreen gene to be expressed. The resultant constructs were amplified, purified, and sequenced. UROtsa cells were transfected with this constructed vector or a control vector using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions. After incubation at 37°C for 8 hr, the supernatant fraction containing the retroviral vector was removed and replaced with normal growth medium. Cells grown for 48–72 hr were assessed by fluorescence microscopy. The ZsGreen fluorescent marker yields a bright green fluorescence, permitting direct monitoring of the delivery efficiency. Finally, the cell populations were sorted by the DAKO-Cytomation MoFlo High Speed Sorter (Dako North America, Carpinteria, CA) and the green fluorescent cells were purified and sequenced. UROtsa cells with vector were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics. Culture medium was collected after exposure to iAsIII or MMAIII for 24 hr or 3 days and stored at −80°C until analysis. Cells treated with iAsIII or MMAIII for 3 days were collected, lysed in RIPA buffer, and extracted in methanol by incubating overnight at 4°C in a rotational shaker. After centrifugation at maximum speed for 5 min at 4°C, supernatants were transferred to microcentrifuge tubes and stored at −80°C until analysis. Before analysis, samples were diluted 1:5 with water–methanol to bring the methanol concentration to 2.5%, incubated at 5°C to precipitate poorly soluble material, and filtered (0.45 μm). Analysis was performed by HPLC-ICP-MS (Agilent 1090 HPLC and Agilent 7500CE ICP-MS run in normal mode; both from Agilent Technologies, Santa Clara, CA) under conditions that resolved neutral, trivalent, and pentavalent iAs species. The ion-pairing method (Le et al. 2000) was used with major modifications to improve the resolution of the species. Briefly, calibrators were prepared from neat materials [As2O3 (Aldrich) and As2O5 (Acros), Sigma-Aldrich; DMAV and MMAV·6H2O, Chem Service, West Chester, PA) in deionized water (≥ 18 MΩ). For speciation, we used a Phenomenex Gemini-NX column (3 μm, C18, 110Å, 150 × 6.6 mm; Torrance, CA) with a corresponding guard column at 40°C. Concentrations of arsenic species in stock solutions were standardized against NIST traceable commercial ICP-MS standards (VWR BDH Aристар Plus; Ultra Scientific, Kingstown, RI). Serial dilutions were made into deionized water. iAsIII and iAsV species were quantified by separate calibration series, and iAsIII concentration in the
calibrants was corrected for any conversion to iAs\textsuperscript{V}. The HPLC conditions were isocratic (5 mM tetrabutylammonium hydroxide, 10 mM ammonium carbonate, 2.5% methanol, pH 9.2, 1 mL/min) for 5 min; then a step gradient (5 mM tetrabutylammonium hydroxide, 30 mM ammonium carbonate, 2.5% methanol, pH 8.75, 1.2 mL/min) for 5 min to elute iAs\textsuperscript{V} was followed by step gradients (5 mM tetrabutylammonium hydroxide, 30 mM ammonium carbonate, 2.5% methanol, pH 9.2, 1.2 mL/min) for a 5-min equilibration to the initial pH and finally to the initial mobile phase for 5 min (1.2 mL/min). The data were analyzed by LC ChemStation A.09.03 and ICP-MS ChemStation B.03.03 software (Agilent Technologies).

**Data analysis.** Statistical analyses were performed using one-way analysis of variance. Data represent mean ± SE of at least three independent experiments.

**Results**

**Deletion of yeast MTQ2 leads to increased resistance to arsenic treatment.** We evaluated the growth phenotype of the MTQ2-deletion mutants in the presence of either iAs\textsuperscript{III} or MMA\textsuperscript{III} (Figure 1). Deletion strains and their isogenic wild-type counterpart, BY4743, were treated with equioxic doses equivalent to the concentrations that resulted in 20% growth inhibition (IC\textsubscript{20}) and 2 × IC\textsubscript{20}, which were 300 and 600 μM for iAs\textsuperscript{III} and 150 and 300 μM for MMA\textsuperscript{III}, respectively. iAs\textsuperscript{III} treatments had no effect on the growth of MTQ2-deletion mutants but significantly decreased growth of the wild-type strain. In comparison, the growth of both MTQ2-deletion mutants and wild-type yeast treated with MMA\textsuperscript{III} decreased to the same degree despite the higher toxicity of MMA\textsuperscript{III}.

**Differential level of N6AMT1 mRNA expression in human tissues.** A direct search for sequence homology and conserved functional domains revealed that the human N6AMT1 gene is orthologous to the yeast MTQ2 gene. We used the web-based online tool Protein Function Prediction (PFP; Kihara Bioinformatics Laboratory 2010), to analyze and predict its potential functions (Hawkins et al. 2009). The suggested molecular functions of N6AMT1 include protein heterodimerization and methionine S-methyltransferase activity, with an almost certain (100%) predicted probability of being a methyltransferase. Given N6AMT1’s suggested function as a methyltransferase, we were interested in exploring its potential involvement in arsenic biomethylation. Considering that the primary methyltransferase responsible for arsenic metabolism in human cells is AS3MT, we did pairwise alignment analysis of N6AMT1 and AS3MT using EMBOSS Pairwise Alignment Algorithms, an online tool (European Bioinformatics Institute 2010). The two proteins shared about 25% similarity. Of the three sequence motifs found in most AS3MT homologs, only motif ILDLGSGSG is highly conserved in N6AMT1 [LEVGSN; see Supplemental Material, Figure 1 (doi:10.1289/ehp.1002733)], whereas [D/N]FFY is present in N6AMT1 but not in AS3MT. These differences suggest that the mechanism by which N6AMT1 methylates arsenic may differ from that of AS3MT, if N6AMT1 is, in fact, involved in the methylation of arsenicals.

A search of the Expressed Sequence Tags (EST) Database (National Center for Biotechnology Information 2010) revealed sequences matching the cDNA of N6AMT1 in many human tissues with varied expression levels. To experimentally measure the mRNA expression of N6AMT1 across tissues, we performed rt-qPCR analysis using cDNA from a panel of 48 human tissues contained in a tissue array (Figure 2). We confirmed the amplification products to be N6AMT1 by DNA sequencing. Using the liver as a reference, the expression of N6AMT1 was normalized to the expression level of ACTB [with cycle threshold (C\textsubscript{T}) values ranging from 18 to 20] and found to be relatively highly expressed in tissues such as the parathyroid, pituitary, adrenal gland, and kidney, and weakly expressed in tissues such as the skin, lung, and mammary gland. We also measured AS3MT mRNA levels using the same tissue panel in order to compare N6AMT1

**Figure 1.** Deletion of the MTQ2 gene in yeast results in increased resistance to arsenite (iAs\textsuperscript{III}), shown as the growth phenotype of MTQ2 mutant yeast cells (B) and the wild-type BY4743 cells (A) treated with 300 or 600 μM iAs\textsuperscript{III} or 150 or 300 μM MMA\textsuperscript{III}. Growth curves show the OD\textsubscript{595} for each treatment as a function of time for 24 hr. Bars represent the mean ± SE AUC for three technical replicates. At the doses tested, iAs\textsuperscript{III} treatment did not alter the growth pattern of MTQ2 mutants but led to a dose-dependent reduction in growth of the wild-type strain; the growth patterns of both yeast strains were similar after MMA\textsuperscript{III} exposure.

*p < 0.001, compared with control.*

---

Environmental Health Perspectives • VOLUME 119 | NUMBER 6 | June 2011 | 773
expression in each tissue and AS3MT expression [see Supplemental Material, Figure 2 (doi:10.1289/ehp.1002733)]. The data showed that the level of N6AMT1 mRNA was relatively low in most measured tissues compared with AS3MT mRNA. The presence of detectable levels of N6AMT1 expression in all tissues analyzed suggests that this enzyme could be involved in methylating arsenicals. N6AMT1 may act in parallel to AS3MT and may be functional only under certain conditions or in certain tissues.

Figure 2. N6AMT1 mRNA expression in human tissues, quantified by rt-qPCR analysis a panel of 48 normal human tissues; transcript levels of N6AMT1 were normalized to ACTB expression and are shown as the fold change relative to liver.

Figure 3. Enhancing expression of human N6AMT1 in UROtsa cells increases resistance to both iAsIII and MMAIII treatment. (A) Representative images of UROtsa cells with either plasmid alone or plasmid with N6AMT1; bars = 10 μm. (B) Semiquantitative RT-PCR analysis shows dramatically increased N6AMT1 expression in UROtsa cells with N6AMT1 relative to control cells or cells with plasmid. AS3MT mRNA was not detected in either cell line; ACTB was used as the loading control. UROtsa cells with vector and with N6AMT1 were treated with increasing concentrations of iAsIII (C) or MMAIII (D) for 24 hr, and cell viability was evaluated with MTT. Bars represent the mean ± SD of three independent experiments. Treatment with either iAsIII or MMAIII resulted in a dose-dependent decrease in viability. However, increased expression of N6AMT1 in UROtsa cells led to resistance to both iAsIII (C) and MMAIII (D); this effect was more significant when cells were treated with MMAIII than with iAsIII.

**p < 0.01, compared with control and vector control.
Overexpression of N6AMT1 in UROtsa cells increases resistance to arsenic treatment. We also measured and compared the level of N6AMT1 mRNA in several cell lines, including 293 (human embryonic kidney cells), HeLa, UROtsa, and HL60 (human promyelocytic leukemia cells). N6AMT1 expression in UROtsa cells is relatively low, with a Ct value of about 33 (data not shown). This cell line also has almost no detectable level of AS3MT, making it an excellent model to study the role of N6AMT1 in arsenic toxicity and metabolism in mammals. We enhanced N6AMT1 expression in UROtsa cells using a retrovirus-based vector (Figure 3A) and found the level of N6AMT1 mRNA in UROtsa cells to be significantly increased by approximately 5-fold in clone 2, as measured by semiquantitative RT-PCR (Figure 3B). We also measured AS3MT gene expression in these two cell lines and found no detectable mRNA level in either cell line (Figure 3B). We further confirmed these PCR results by real-time PCR analysis (data not shown). Unfortunately, we could not detect N6AMT1 protein levels in these cells using two commercially available antibodies. Transfected UROtsa cells did not have an altered doubling time or morphology in culture. The UROtsa cells with N6AMT1 (N6AMT1-enhanced cells) and the UROtsa cells with vector cells (vector control) were treated with either iAsIII or MMAIII at a series of concentrations for 24 hr. Arsenical treatments induced a dose-dependent decrease in viability of both cell lines. However, increased expression of N6AMT1 in UROtsa cells resulted in higher viability after iAsIII and MMAIII treatment at almost all concentrations tested, compared with the UROtsa vector control cells (Figure 3C,D). This increased arsenic resistance was more apparent in cultures treated with MMAIII than with iAsIII, approximately 2- and 1.3-fold, respectively.

Enhanced N6AMT1 in UROtsa cells methylates MMAIII to DMA. We collected medium and cell extracts from cultures of UROtsa cells with N6AMT1 and UROtsa cells with vector treated with different concentrations of either iAsIII or MMAIII for up to 3 days and then measured and analyzed arsenic metabolic profiles using ICP-MS [for representative chromatograms, see Supplemental Material, Figure 3 (doi:10.1289/ehp.1002733)]. Methylated metabolites were undetectable either in the media (Table 1) or in the cell extract (Table 2) from cultures of UROtsa cells with N6AMT1 or of UROtsa cells with vector, after treatment with iAsIII, suggesting that N6AMT1 does not methylate iAsIII. When UROtsa cells with vector control were treated with MMAIII, levels of MMAV but not dimethylarsenic acid (DMAV) increased in the media (Table 3) and cells (Table 2). Treatment with MMAIII of UROtsa cells increases resistance to arsenic treatment. MMAIII is the most toxic arsenical (Drobná et al. 2005; Ferrario et al. 2008; Kligerman et al. 2003; Petrick et al. 2001). In cultured human cells, MMAIII is the most toxic arsenical (Drobná et al. 2005; Ferrario et al. 2008; Petrick et al. 2001) and inhibits several key cellular proteins, such as glutathione reductase (Styblo et al. 1997) and thioredoxin reductase (Lin et al. 1999, 2001). Several studies have shown that MMAIII is capable of inducing genetic damage and changes in signal transduction by either direct or indirect mechanisms (Ahmad et al. 2002; Kligerman et al. 2003; Nesnow et al. 2002). In addition, exposure to MMAIII for 52 weeks induced malignant transformation of UROtsa cells (Bredfeldt et al. 2006). Epidemiological studies have suggested that individuals who especially MMAIII, may be more toxic than iAs both in vivo and in vitro (Drobná et al. 2005; Ferrario et al. 2008; Kligerman et al. 2003; Petrick et al. 2001).

Discussion

MMAIII is the most toxic arsenic metabolite in vivo and in vitro. In humans, iAs is metabolized to methylated arsenical species in a multistep process. Methylated arsenicals, N6AMT1 methylates MMAIII, in a dose-dependent manner. Moreover, the amount of DMAV in the culture medium increased 5-fold after 3 days of treatment (1 μM MMAIII) compared with 1 day of treatment.

Table 1. Arsenic metabolic profile in cell culture medium after iAsIII treatment of UROtsa cells with vector and UROtsa cells with N6AMT1 (ng/mL; mean ± SE).

| Treatment (μM) | iAsIII (μM) | MMAIII | MMAV | DMAV | iAsIII (μM) | MMAIII | MMAV | DMAV |
|---------------|-------------|--------|------|------|-------------|--------|------|------|
| 0             | 0           | 0      | 0    | 0    | 0           | 0      | 0    | 0    |
| 1             | 0           | 0      | 0    | 0    | 0           | 0      | 0    | 0    |
| 10            | 0           | 0      | 0    | 0    | 0           | 0      | 0    | 0    |

Table 2. Arsenic metabolic profile in cell extract after 3-day iAsIII and MMAIII treatment of UROtsa cells with vector and UROtsa cells with N6AMT1 (ng/mL; mean ± SE).

| Treatment (μM) | iAsIII (μM) | MMAIII | MMAV | DMAV |
|---------------|-------------|--------|------|------|
| 0             | 0           | 0      | 0    | 0    |
| 1             | 0           | 0      | 0    | 0    |
| 10            | 0           | 0      | 0    | 0    |

Table 3. Arsenic metabolic profile in cell culture medium after MMAIII treatment of UROtsa cells with vector and UROtsa cells with N6AMT1 (ng/mL; mean ± SE).

| Treatment (μM) | MMAIII | MMAV | DMAV |
|---------------|--------|------|------|
| 0             | 0      | 0    | 0    |
| 1             | 0      | 0    | 0    |
| 10            | 0      | 0    | 0    |
excrete a higher proportion of ingested arsenic as MMAIII are more susceptible to arsenic-related cancer (Steinmaus et al. 2006). MMAIII has been proposed as the ultimate genotoxic form of arsenic (Kligerman et al. 2003), and the existing evidence indicates that biomethylation of iAs to MMAIII is likely to alter the adverse effects of environmental arsenic exposure on human health.

**AS3MT is primarily responsible for methylating iAs to MMAII and DMA in humans.** The arsenic methyltransferase AS3MT is recognized as the primary enzyme responsible for conversion of iAs to its methylated metabolites MMAII and DMA (Lin et al. 2002; Wood et al. 2006). Studies have shown that single-nucleotide polymorphisms in AS3MT lead to different urinary arsenical profiles (Agusa et al. 2009; Schlawécke Engström et al. 2009; Wood et al. 2006), some of which are associated with increased risk of premalignant skin lesions (Valenzuela et al. 2009). Although these data suggest that AS3MT plays a critical role in arsenic metabolism and toxicity, a recent study showed that AS3MT-knockout mice retain some ability to methylate arsenicals, suggesting the existence of other methyltransferases that could be involved in alternative arsenic metabolism pathways (Drobna et al. 2009).

**N6AMT1 is capable of methylating MMAIII to DMA.** In this study, we found that N6AMT1 has the capacity to methylate MMAIII to DMA. The expression of N6AMT1 is generally low compared with the expression level of AS3MT in most human tissues. In addition, the low sequence homology shared between these two proteins, about 25%, supports differences in substrate specificity and, possibly, in mechanisms of arsenic methylation. In contrast to AS3MT, which methylates iAs to the more toxic MMAIII, N6AMT1 metabolites MMAIII to the less toxic DMA. This is consistent with the increased resistance to MMAIII of UROtsa cells overexpressing N6AMT1 compared with vector control cells, but other mechanisms likely also contribute to this increased resistance. Thus, our results suggest that N6AMT1 may play a role in modulating arsenical-induced toxicity and that decreased N6AMT1 expression or activity could have a significant impact on arsenic-induced toxicity and perhaps carcinogenicity under certain conditions or in certain tissues.

**N6AMT1 in human cells responded to arsenicals differently from MTQ2 in yeast.** We noted differences in response to arsenical treatments between yeast and human cells. Specifically, the MTQ2-deletion yeast strain is resistant only to iAsIII. We did not find evidence of iAsIII methylation in yeast wild-type; that is, DMA and MMA levels in cells and culture media were below the limit of quantitation (data not shown). Therefore, the function of MTQ2 in arsenic toxicity may not be related to iAsIII methylation. In contrast, overexpression of N6AMT1 in UROtsa cells leads to resistance to both iAsIII and MMAIII, which provides evidence of its involvement in protection from these arsenicals. These results indicate that the orthologous genes—MTQ2 in yeast and N6AMT1 in humans—have different roles in the cellular response to arsenic toxicity.

**Conversion of MMAIII to DMA by N6AMT1 needs to be further confirmed biochemically.** Our analyses showed that enhanced expression of N6AMT1 in UROtsa cells converts MMA to DMA. However, purified recombinant N6AMT1, in the presence of SAM and other cofactors, was unable to methylate iAsIII or MMAIII (data not shown). N6AMT1 dimerizes with tRNA methyltransferase 11-2 homolog (TRMT112), which appears to be necessary for proper N6AMT1 activity (Figaro et al. 2008) and is consistent with heterodimerization activity as predicted with PFP. Thus, the lack of N6AMT1-dependent MMAIII methylation in this test tube may be due to the absence of TRMT112 or another unknown protein. In addition, N6AMT1 overexpression increased resistance to iAsIII, although UROtsa cells were not able to methylate iAsIII to MMAIII or to other methylated species, suggesting that mechanisms other than participation in arsenic methylation might be involved. It is not clear whether interaction between N6AMT1 and TRMT112 has a role in arsenic toxicity, but it is certainly worthy of further investigation.

**Conclusions**

Our data suggest an important potential role of N6AMT1 in modulating arsenical-induced toxicity by methylating MMAIII to the less toxic DMA. However, further investigation is warranted to determine whether N6AMT1 can methylate MMAIII in vivo and also to identify the genetic and environmental factors that can alter N6AMT1 expression and/or activity. Our ongoing experiments are focused on the biochemical characterization of N6AMT1, specifically its capacity to methylate MMAIII, as well as its ability to modulate arsenic toxicity and carcinogenicity.

**REFERENCES**

Abernathy CG, Liu YP, Longfellow D, Aposhian HV, Beck B, Fowler B, et al. 1999. Arsenic: health effects, mechanisms of actions, and research issues. Environ Health Perspect 107:593–597.

Agusa T, Iwata H, Fujihara J, Kunito T, Takeshita H, Minh TB, et al. 2009. Genetic polymorphisms in AS3MT and arsenic metabolism in residents of the Red River Delta, Vietnam. Toxicol Appl Pharmacol 236(2):131–141.

Ahmad S, Kitchin KT, Cullen WR. 2002. Plasma DNA damage caused by methylated arsenicals, arsenic acid and human liver thiol. Toxicol Lett 132(1):47–57.

Bentley R, Chasteen TG. 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. Microbial Mol Biol Rev 66(2):250–271.

Bredfeldt TG, Jagadish B, Eblin KE, Mash EA, Gandolfi AJ. 2009. Comparative functional genomic analysis identifies distinct and overlapping sets of genes required for arsenic toxicity. J Environ Sci Health B 41(10):2399–2428.

Chen F, Fowler B, et al. 1999. Arsenic: health effects, mechanisms of actions, and research issues. Environ Health Perspect 107:593–597.

Drobna Z, Waters SB, Devesa V, Harmen AW, Thomas DJ. 2005. Metallothionein and other human urothelial cells expressing rat arsenic (+3 oxidation state)-methyltransferase. Toxicol Appl Pharmacol 207(2):147–159.

Eblin KE, Bredfeldt TG, Gandolfi AJ. 2008. Immunolocalized human urothelial cells as a model of arsenic-induced bladder cancer. Toxicology 248(2–3):79–89.

European Bioinformatics Institute. 2010. EMBOSS Pairwise Alignment Algorithms. Available: http://www.ebi.ac.uk/Tools/emboss/alignment/index.html (accessed 15 October 2010).

Ferrario D, Coera C, Brustio R, Collota A, Bowe G, Vaiter M, et al. 2008. Toxicity of inorganic arsenic and its metabolites on hamster alveolar macrophages: "in vitro" comparison between species and sexes. Toxicology 248(2–3):102–108.

Figueroa S, Scrima N, Buckingham RH, Heurges-Hamard V. 2008. HemK2 protein, encoded on human chromosome 21, methylates translation termination factor eRF1. FEBS Lett 582(16):2352–2356.

Hall EL, George SE, Kohn MJ, Styblo M, Thomas DJ. 1997. In vitro methylation of inorganic arsenic in mouse intestinal cecum. Toxicol Appl Pharmacol 147(1):101–109.

Hawkins T, Chitale M, Luban S, Khara D. 2009. PFP: automated protein function prediction Job Submission. Available: http://dragon.bio.purdue.edu/pfp/ (accessed 15 October 2010).

Kligerman AD, Doerr CL, Tennant AH, Harrington-Brock K, Allen JW, Winkfield E, et al. 2003. Methylated trivalent arsenicals as candidate ultimate genotoxic forms of arsenic: induction of chromosomal mutations but not gene mutations. Environ Mol Mutagen 43(3):192–205.

Le XC, Ma M, Cullen WR, Aposhian HV, Xu L, Zheng B. 2000. Determination of monomethylarsenous acid, a key arsenic methylation intermediate, in human urine. Environ Health Perspect 108:1015–1018.

Lin S, Cullen WR, Thomas DJ. 1999. Methylarsenicals and arsinothiols are potent inhibitors of mouse liver thioredoxin reductase. Chem Res Toxicol 12(10):924–930.

Lin S, Del Razo LM, Styblo M, Beck MA, Herbin-Davis KM, et al. 2002. A novel 5'-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. J Biol Chem 277(13):10795–10803.

National Center for Biotechnology Information. 2010. Expressed Sequence Tags Database. Available: http://www.ncbi.nlm.nih.gov/dbEST/ (accessed 15 October 2010).

National Center for Biotechnology Information. 2011. GenBank. Available: http://www.ncbi.nlm.nih.gov/entrez?db=nucleotide (accessed 15 October 2010).

Nesnow S, Roop BC, Lambert G, Kadiiska M, Mason RP, Cullen WR, et al. 2002. DNA damage induced by methylated trivalent arsenicals as candidate ultimate genotoxic forms of arsenic (+3 oxidation state) and arsenite (AsIII) in yeast. Toxicol Sci 74(3):566–582.

Petrick JS, Jagadish B, Mash EA, Aposhian HV. 2001. Mono methylarsenic acid (MMAII) and arsenite (AsIII) in human blood: a comparison of Methods and Contaminants. Microbiol Mol Biol Rev 66(2):250–271.

Pershagen G. 1981. The carcinogenicity of arsenic. Environ Health Perspect 40(3):100.

Petrick JS, Jagadish B, Mash EA, Aposhian HV. 2001. Monomethylarsenic acid (MMAII) and arsenite (AsIII) in hamst ers and in vitro inhibition of pyruvate dehydrogenase. Chem Res Toxicol 14(8):851–856.

Polevoda B, Span L, Sherman F. 2006. The yeast translation
release factors Mrf1p and Sup45p (eRF1) are methylated, respectively, by the methyltransferases Mtq1p and Mtq2p. J Biol Chem 281(5):2562–2571.

Ratel D, Ravanat JL, Charles MP, Platet N, Breuillaud L, Lunardi J, et al. 2006. Undetectable levels of N6-methyl adenine in mouse DNA: cloning and analysis of PRED28, a gene coding for a putative mammalian DNA adenine methyltransferase. FEBS Lett 580(13):3179–3184.

Schlüsselwicke Engström K, Nermell B, Concha G, Strömberg U, Vältber M, Broberg K. 2009. Arsenic metabolism is influenced by polymorphisms in genes involved in one-carbon metabolism and reduction reactions. Mutat Res 667(1–2):4–14.

Sens DA, Park S, Gurel V, Sens MA, Garrett SH, Somji S. 2004. Inorganic cadmium- and arsenite-induced malignant transformation of human bladder urothelial cells. Toxicol Sci 79(1):56–63.

Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, et al. 1992. Cancer risks from arsenic in drinking water. Environ Health Perspect 97:259–267.

Smith AH, Steinmaus CM. 2009. Health effects of arsenic and chromium in drinking water: recent human findings. Annu Rev Public Health 30:107–122.

Steinmaus C, Bates MN, Yuan Y, Kalman D, Atallah R, Rey OA, et al. 2006. Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. J Occup Environ Med 48(5):478–488.

Stephens C, Reisenauer A, Wright R, Shapiro L. 1996. A cell cycle-regulated bacterial DNA methyltransferase is essential for viability. Proc Natl Acad Sci USA 93(3):1210–1214.

Straif K, Benbrahim-Tallaa L, Baan R, Grosse Y, Secretan B, El Ghissassi F, et al. 2009. A review of human carcinogens—part C: metals, arsenic, dusts, and fibres. Lancet Oncol 10:453–454.

Styblo M, Del Razo LM, Vega L, Gernoele DR, LeCluyse EL, Hamilton GA, et al. 2000. Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch Toxicol 74(6):289–299.

Styblo M, Serves SV, Cullen WR, Thomas DJ. 1997. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenui. Chem Res Toxicol 10(1):27–33.

Thomas DJ, Li J, Waters SB, Xing W, Adair BM, Drobna Z, et al. 2007. Arsenic (+3 oxidation state) methyltransferase and the methylation of arsenicals. Exp Biol Med (Maywood) 232(1):3–13.

Valenzuela OL, Drobna Z, Hernández-Castellanos E, Sánchez-Peña LC, García-Vargas GG, Borja-Aburto VH, et al. 2009. Association of AS3MT polymorphisms and the risk of premalignant arsenic skin lesions. Toxicol Appl Pharmacol 236(2):200–207.

Wood TC, Salvagione DE, Mukherjee B, Wang L, Klumpp AF, Thomas BA, et al. 2006. Human arsenic methyltransferase (AS3MT) pharmacogenetics: gene resequencing and functional genomics studies. J Biol Chem 281(11):7364–7372.