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Influence of Arsenic on Global Levels of Histone Posttranslational Modifications: A Review of the Literature and Challenges in the Field

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Abstract

Arsenic is a human carcinogen, and also increases risk for non-cancer outcomes. Arsenic-induced epigenetic dysregulation may contribute to arsenic toxicity. Although there are several reviews on arsenic and epigenetics, these have largely focused on DNA methylation. Here, we review investigations of the effects of arsenic on global levels of histone posttranslational modifications (PTMs). Multiple studies have observed that arsenic induces higher levels of H3 lysine 9 dimethylation (H3K9me₂), and also higher levels of H3 serine 10 phosphorylation (H3S10ph), which regulates chromosome segregation. In contrast, arsenic causes a global loss of H4K16ac, a histone PTM that is a hallmark of human cancers. Although the findings for other histone PTMs have not been entirely consistent across studies, we discuss biological factors which may contribute to these inconsistencies, including differences in the dose, duration, and type of arsenic species examined; the tissue or cell line evaluated; differences by sex; and exposure timing. We also discuss two important considerations for the measurement of histone PTMs: proteolytic cleavage of histones and arsenic-induced alterations in histone expression.

Keywords

Arsenic; posttranslational histone modifications

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain primary data from any studies with animal or human subjects performed by the authors.

Conflict of Interest

Caitlin G. Howe and Mary V. Gamble declare they have no conflicts of interest.

Introduction

Arsenic Exposure

Inorganic arsenic (InAs) has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer [1]. Exposure to InAs has also been associated with numerous non-cancer health outcomes, including cardiovascular disease, nonmalignant lung disease, and neurodevelopmental outcomes (reviewed in [2]). The primary source of exposure is naturally contaminated drinking water [2]. More than 200 million individuals are exposed to arsenic concentrations which exceed the World Health Organization guideline for safe drinking water, which is 10 µg/L [3]. Dietary arsenic is also a major source of exposure and contributes to a larger fraction of total arsenic exposure when concentrations in drinking water are low, e.g., <50 [4] or <10 µg/L [5]. InAs is metabolized via two sequential methylation reactions, yielding monomethylarsonous acid (MMA) and dimethylarsinic acid (DMA), respectively [6]. The trivalent forms (MMA^{III} and DMA^{III}) are the most cytotoxic (reviewed in [7]). However, DMA^{III} is highly unstable, and it is unclear if this metabolite is present in large quantities *in vivo* [8].

Although eliminating exposure to arsenic remains the primary public health strategy, cancer risks remain elevated decades after arsenic exposure has been reduced [9]. However, the mechanisms underlying this are unclear and likely multifactorial. While arsenic is not a traditional mutagen [10], there is increasing evidence that arsenic induces epigenetic dysregulation, including alterations in both DNA methylation and histone posttranslational modifications (PTMs) (previously reviewed in [11]), which has been implicated in cancers and other adverse health outcomes [12]. There is also interest in the molecular actions of arsenic, because arsenic trioxide (As₂O₃), in combination with all-*trans* retinoic acid, is currently the standard of care for acute promyelocytic leukemia (APL) [13].

Arsenic and Epigenetics

Epigenetics is defined as the study of stable and heritable changes in gene expression or cellular phenotypes that occur without changes to the underlying DNA sequence [14]. Genomic DNA is approximately 2 m in length and must fit inside the nucleus, which on average has a diameter of 6 µm [12]. This is accomplished by packaging DNA into chromatin. The major unit of chromatin is the nucleosome, which is composed of approximately 146 base pairs of DNA, wrapped 1.65 times around a histone protein octamer, consisting of two copies of the four canonical core histones: H2A, H2B, H3, and H4 (Figure 1) [15]. A fifth histone (H1), known as the linker histone, helps to stabilize the nucleosome and facilitates the folding of nucleosomes into higher-order structures [15]. Together, several types of epigenetic modifications regulate the accessibility of DNA for different biological processes, such as gene transcription, DNA replication, and DNA repair. The best-studied modification is DNA methylation [16]. Histone proteins can also be modified, and these PTMs interact with DNA methylation to influence DNA accessibility [16].

Histones have two major domains: a globular core and a long, unstructured N-terminal tail. Although numerous globular core PTMs have been identified by mass spectrometry, PTMs in the N-terminal tails have been more thoroughly studied [17]. This is largely due to the fact

that histone PTMs were first identified by Edman degradation, a peptide sequencing technique which is limited to measuring the first 20 to 30 amino acids from the N-terminus [17]. The nomenclature of histone PTMs is based on the histone; the type of amino acid (indicated by its single letter abbreviation); the position of the modified amino acid in relation to the N-terminus (indicated by a number); the type of modification present; and, in the case of lysine methylation, the number of methyl groups present (0, 1, 2, or 3). Seventeen different types of PTMs on more than 30 amino acids have been identified for human H3 alone [18]. To date, the best described modifications include the acetylation (ac) and methylation (me) of lysine residues (K), and the phosphorylation (ph) of serine residues (S), particularly on H3 and H4. Histone PTMs can alter chromatin structure through both direct and indirect mechanisms. Since ac and ph groups are negatively charged, the addition of these moieties reduces the affinity of the positively charged histones for the negatively charged DNA, leading to a more open chromatin conformation [19]. Originally, it was proposed that the combinatorial patterns of simultaneously occurring PTMs formed a “histone code” [20]. More recently, a “language of histone marks” has been proposed, which is probably more appropriate given that there is significant crosstalk between distinct PTMs on the same, or different, core histones, which is important for the regulation of gene transcription and DNA damage repair (reviewed recently in [21]).

PTMs of histones can also indirectly alter chromatin structure by recruiting chromatin modifiers [19]. Since methyl groups have a neutral charge, histone methylation largely influences chromatin by recruiting or blocking the binding of chromatin modifiers, and each methylation state (i.e., me1, me2, or me3) can have distinct effects [19]. Gene-specific levels of histone PTMs are highly dynamic as they play active roles in regulating gene transcription. In contrast, global levels of histone PTMs are thought to be relatively stable over time. For example, global levels of histone PTMs remain unchanged in mouse mesenchymal stem cells during adipogenesis, despite alterations at specific genes [22]. Also, global levels of several histone PTMs measured in peripheral blood mononuclear cells (PBMCs) from human participants did not fluctuate measurably across three time points measured one week apart [23].

There have been several comprehensive reviews on arsenic and epigenetics that focus primarily on DNA methylation [11, 24–27]. Here we summarize the growing literature on arsenic and global levels of histone PTMs and discuss some of the major challenges in the field.

Influences of arsenic on global histone PTMs

The majority of studies on arsenic and histone PTMs have been conducted *in vitro* [28–47]. However, there are a few supporting studies in rodents [48, 49] and human populations [50–54]. Collectively, these studies provide evidence that arsenic alters many different PTMs at the global level. Details of each study are summarized in Table 1 and are also described below.

Total methylation of histone proteins

Two early studies observed that total methylation levels of H2B were increased by 50 μM trivalent InAs (As^{III}), while those of H3 and H4 were reduced, in cells derived from *Drosophila melanogaster* embryonic tissues [28, 29]. These studies used tritiated *S*-adenosylmethionine or methionine to measure methyl incorporation into histones and thus did not evaluate the methylation states of specific lysine residues, which are regulated by distinct enzymes and often have unique biological roles [19].

Methylation of H3K4

Methylation at H3K4 has been associated with actively transcribed genes [19]. H3K4me1 is a marker which best predicts enhancer regions, while H3K4me3 mainly localizes to transcription start sites [19]. For some genomic regions, all three methylation states of H3K4 show overlapping patterns [55–57]. All three methylation states have been examined in relation to arsenic exposure.

Two studies by Zhou et al. demonstrated that 1–5 μM As^{III} reduced global levels of H3K4me1 and increased global levels of H3K4me2 in A549 cells [32, 33], which were originally derived from a male human lung tumor. The finding for H3K4me2 was consistent with an occupational study of male steel workers ($n = 63$), which observed a positive association between inhalation of arsenic-contaminated particulate matter and H3K4me2, in peripheral blood leukocytes (PBLs) [50]. Zhou et al. also found that global levels of H3K4me3 were increased after exposure to As^{III} [32, 33], consistent with a study which utilized RWPE1 cells, which were originally derived from a healthy human prostate [39]. However, the latter finding was only observed when RWPE1 cells were simultaneously exposed to estradiol (E2) and the highest dose of arsenic examined (100 ng/mL) [39]. Interestingly, two studies have also observed that arsenic influences H3K4me3 differentially by sex [49, 51]. In an epidemiological study in Bangladeshi adults ($n = 40$), our group observed that the association between water arsenic (wAs) exposure (50–500 $\mu\text{g/L}$) and H3K4me3, in PBMCs, was negative among men, but positive among women [51]. In contrast, 50 $\mu\text{g/L}$ of pentavalent InAs (As^{V}) increased H3K4me3 in the dentate gyrus and frontal cortex of male mice and reduced this PTM in the dentate gyrus of female mice [49].

Methylation of H3K9

H3K9 methylation has generally been associated with transcriptional repression [58]. There is evidence that H3K9me1 primes certain regions of the genome for heterochromatin formation [58], although this PTM has also been associated with active genes [56]. In contrast, H3K9me3 is important for maintaining heterochromatin and genomic stability [58].

Zhou et al. examined the effects of 1–5 μM As^{III} on global levels of all three methylation states of H3K9 in A549 cells [32]. Although H3K9me1 levels were not altered by As^{III} , H3K9me2 was increased compared to controls [32]. Similarly, our group observed that wAs exposure was positively associated with H3K9me2 in PBMCs from Bangladeshi men and women [51]. In contrast with these findings, an epidemiological study in China ($n = 218$) observed a negative association between creatinine-adjusted urinary arsenic (uAs_{Cr}) and H3K9me2 in lymphocytes [53]. However, the same group also evaluated the effect of As^{III}

on H3K9me2 *in vitro*, using female-derived human embryonic kidney cells (HEK293T) and male-derived human keratinocytes (HaCaT); for both cell lines they observed that As^{III} initially reduced H3K9me2, but that longer durations of exposure (8 or 12 hours) increased this PTM, consistent with the findings by Zhou et al. and our epidemiological study in Bangladesh [53].

Similar to their findings for H3K9me2, Zhou et al. observed that As^{III} increased H3K9me3 [32]. Another group also observed that As^{III} increased H3K9me3 in CD4+ cell lines (Jurkat and CCRF-CEM); these cells were derived from male patients with acute lymphoblastic leukemia [54]. However, the same group observed an inverse association between uAs and H3K9me3, measured in sorted CD4+ cells, in a small epidemiological study of Argentinian women ($n = 28$) [54]. In contrast with these studies, alterations in H3K9me3 were not observed in either the dentate gyrus or the frontal cortex of adult male and female mice exposed to 50 µg/L As^V during the perinatal period [49]. None of these studies investigated the potential mechanisms by which arsenic might alter H3K9me3. However, one group speculated that differences in cell type, dose, or timing of exposure may have contributed to some of the inconsistencies across studies [49].

Methylation of H3K27

H3K27me3 is a repressive mark that is important for gene regulation and X chromosome inactivation [59]. Although several studies have examined the effects of arsenic on H3K27me3, the findings have been inconsistent; this is in spite of the fact that these studies all used antibody-based techniques to measure H3K27me3. One study did not observe that 7.5 µM As^{III} altered H3K27me3 in HepG2 cells, which were originally derived from a male human liver tumor [31]. However, Zhou et al. observed that 2.5 and 5 µM µg/L As^{III} reduced H3K27me3 in A549 cells [32]. Consistent with this, our group observed an inverse association between wAs exposure and H3K27me3 in PBMCs among Bangladeshi men [51]. In contrast, wAs was positively associated with H3K27me3 among women [51], similar to an *in vitro* study which utilized female-derived mouse embryonic fibroblasts and observed that 0.5 µM As^{III} increased this PTM [41].

Methylation of H3K36

H3K36 methylation is important for both transcriptional activation and transcriptional elongation [60]. There is also evidence that H3K36me2 and H3K36me3 play important roles in DNA repair [61], and that both PTMs are dysregulated in cancers; a global increase in H3K36me2 has been associated with oncogenic programming [62], while a global loss of H3K36me3 has been observed in many cancer types [63].

Although 2.5 and 5 µM As^{III} reduced H3K36me2 in A549 cells [32], we recently observed a positive association between uAs_{Cr} and H3K36me2, measured in PBMCs, among men in an epidemiological study of Bangladeshi adults ($n = 317$) [52]; since H3K36me2 antagonizes H3K27me3 [59], this finding was consistent with our previous finding that wAs was inversely associated with H3K27me3 among men from the same population [51]. Similarly, we found that uAs_{Cr} was positively, although not significantly, associated with H3K36me3 among Bangladeshi men [52], consistent with a previous finding that As^{III} increases

H3K36me3 in A549 cells [32]. An epidemiological study of Chinese adults also observed a positive association between uAs_{Cr} and H3K36me3, measured in lymphocytes [53]. However, in contrast with their population-based findings, the same group observed that As^{III} reduced H3K36me3 in both a dose- and time-dependent manner in two different cell lines (HEK293T and HaCaT) [53].

Methylation of H3K79

H3K79 is located in the globular core domain of histone H3. Of the PTMs located on this residue, only H3K79me2 has been examined in relation to arsenic exposure. This PTM plays important roles in transcriptional activation and elongation [57, 64]. It is also dysregulated in cancers and is currently a promising target for the treatment of MLL-fusion leukemia [65]. Two epidemiological studies in adults, one by our group in Bangladeshi adults and another in Chinese adults, observed null associations between uAs_{Cr} and H3K79me2, measured in peripheral blood [52, 53]. This is surprising, given that uAs_{Cr} was previously found to be inversely associated with H3K27me3, a PTM that is mutually exclusive with H3K79me2 [66].

H3 and H4 acetylation

The addition of acetyl groups to histone proteins reduces their affinity for DNA, leading to a more open chromatin conformation [67]. Acetylation at specific lysine residues can also recruit bromodomain-containing proteins, such as the SWI/SNF complex, which remodels the chromatin to a more open conformation to allow for active transcription [67].

Three studies have examined the effects of arsenic on pan-acetylation levels. Two early studies, which used *D. melanogaster* cell lines, found that 50 µM As^{III} reduced total acetylation levels of the core histones; these were determined by measuring the incorporation of acetyl groups derived from tritiated acetic acid [28, 29]. In contrast, 1 and 2 µM As^{III} increased H3 and H4 pan-acetylation levels, measured by Western blot, in human myeloid leukemia cell lines [37].

Several other studies have examined the influence of arsenic on specific histone PTMs and observed differences by lysine residue. Arsenic has not been shown to alter H4K5ac [44, 47] or H4K8ac [36, 44]. Furthermore, As^{III} did not alter H3K14ac in APL cell lines [30]. However, an epidemiological study in China observed a positive association between uAs_{Cr} and H3K14ac in lymphocytes [53].

The findings for H3K9ac have also been inconsistent. The same epidemiological study in China did not observe an association between uAs_{Cr} and H3K9ac in lymphocytes [53], and a small epidemiological study of Argentinian women did not observe a significant association between uAs and H3K9ac, measured in sorted CD4⁺ and CD8⁺ cells. However, 7.5 µM As^{III} increased H3K9ac in HepG2 cells [31] and 0.1–100 µM As^{III} also increased this PTM in a dose-dependent manner in Jurkat and CCRF-CEM cells. In contrast, As^{III} (0.5–5 µM) decreased this PTM in female-derived human embryonic kidney cells and in UROTsa cells, which were derived from a female human ureter [36]. Similarly, an inverse association between wAs and H3K9ac, measured in PBMCs, was observed in both Bangladeshi men and women [51]. Furthermore, 100 µg/L As^{III} reduced genome-wide levels of H3K9ac,

measured by ChIP-seq, in both the hippocampus and cortex of mice (males and females combined) [48]. However, importantly, As^V (50 µg/L) has also been shown to alter H3K9ac in both a region- and sex-dependent manner in the mouse brain [49].

Only two studies, both of which utilized UROTsa cells, have evaluated the effect of arsenic on H4K12ac. One group observed that 50 nM MMA^{III} reduced H4K12ac [44], while the other group did not observe alterations in this PTM after exposure to 0.5–2.5 µM As^{III} [36]. Both studies measured H4K12ac by Western blot, using antibodies purchased from Millipore. It is therefore unlikely that differences in laboratory methods explain these inconsistencies. Alternatively, it is possible that MMA^{III} and As^{III} differentially influence certain PTMs, such as H4K12ac. However, this will need to be confirmed in future studies, which examine both arsenic species under identical laboratory conditions.

The effects of arsenic on H4K16ac have been more consistent. In addition to its role in regulating nucleosome-level interactions, which are essential for the formation of 30 nm chromatin fibers, H4K16ac influences interactions between chromatin fibers, which determine the higher order tertiary structure of the chromatin [68]. Four studies, three of which utilized UROTsa cells, measured this PTM by either Western blot or mass spectrometry, and observed that As^{III} and MMA^{III}, across a range of doses (0.2–10 µM) induced a global loss of H4K16ac [34, 40, 44, 47], which is a hallmark of most human cancers [68]. However, two studies, both of which measured H4K16ac by Western blot, have been less consistent. One of these studies used primary human neonatal keratinocytes (donor sex unspecified) and observed that As^{III} increased H4K16ac [35], and another study, which used UROTsa cells and female-derived human embryonic kidney cells, did not observe alterations in this PTM after treatment with As^{III} at doses ranging from 0.5–2.5 µM [36].

Only one group has examined the effects of As^{III} on H3K18ac, which were found to be both dose- and time-dependent, with short durations (4 or 8 hours) increasing H3K18ac in HEK293T and HaCaT cells, but longer durations (84 days) reducing this PTM [53]. The same group also observed that H3K18ac was associated with the expression of several oxidative stress response genes and inversely associated with urinary 8-hydroxy-2-deoxyguanosine, a biomarker of oxidative stress [53]. It was therefore hypothesized that arsenic induces an adaptive response to oxidative stress which may be mediated by H3K18ac [53]. However, this response appears to be transient and may ultimately be inhibited by long-term exposure to arsenic [53]. Consistent with this, the same group observed an inverse association between uAs_{Cr} and H3K18ac in lymphocytes from Chinese adults who had been chronically exposed to arsenic [53].

Histone phosphorylation and phosphoacetylation

Histone phosphorylation is important for transcriptional activation and chromatin compaction during mitosis and meiosis [69]. One study observed that a very high dose of As^{III} (10 µM) reduced total phosphorylation levels of both H1 and H3 in Chinese hamster ovary cells [46]. However, multiple studies using various cell lines, have consistently observed that different doses and durations of arsenic, including both As^{III} and DMA^{III}, induce higher global levels of H3S10ph [30, 38, 42, 43, 45], which is critical for regulating chromosome segregation during mitosis [70]. One study also observed that As^{III} induces

H3S10phK14ac in APL cells; this phosphoacetylation mark is thought to play an important role in regulating As₂O₃-induced apoptosis in this cell type [30].

Challenges in the field

Although several studies have examined the effects of arsenic on global levels of histone PTMs, the findings have been largely inconsistent. These discrepancies may be attributed to many factors, including differences in the dose, duration, and type of arsenical examined; the particular tissues or cell lines utilized; the timing of exposure; the potential modifying effects of sex; and possible measurement error.

Dose, duration and type of arsenical examined

Although three studies have examined MMA^{III} or DMA^{III} in addition to As^{III} and observed consistent effects [34, 40, 42], the majority have solely evaluated As^{III} [28–33, 35–39, 41, 43, 45–47]. Additional studies will therefore be needed to evaluate whether arsenic metabolites differentially influence histone PTMs. Furthermore, while both As₂O₃ and NaAsO₂ have been used to generate As^{III} in experimental studies, there is evidence that As₂O₃ is more toxic [71]. Thus, the source of arsenic may be another important consideration. However, there may also be inconsistencies across studies which use the same source of arsenic, since cell lines differ in their capacity to metabolize arsenic ([72] and reviewed in [10]). Similarly, interspecies differences in arsenic metabolism may contribute to inconsistencies between *in vivo* studies [73].

The impact of arsenic on histone PTMs may also vary based on the dose and duration used. Two studies observed that certain PTMs were only altered at the higher doses of arsenic examined [30, 34], and a third study found that arsenic had completely opposite effects at a lower (100 pg/mL) versus higher (100 ng/mL) dose [39]. Furthermore, while six experimental studies examined exposure durations of weeks or months [39, 41, 44, 48, 49, 53], the majority evaluated durations of 24 hours or less [28–33, 38, 42, 43, 45, 46]. These shorter durations may have profoundly different effects than the chronic exposures experienced by most human populations [50, 51, 53, 54]. In support of this, one group found that a single treatment of As^{III} for 8 hours increased H3K18ac, while repeated exposure to As^{III} for 84 days led to an eventual reduction in this PTM; the latter finding was consistent with their epidemiological study of Chinese adults who had been chronically exposed to arsenic [53].

Tissue Differences

While some studies have examined the effects of arsenic on multiple cell lines or in different tissue types and observed similar effects [32, 37, 45, 47, 48, 53], the majority have evaluated a single cell line or tissue [28–31, 33–35, 38–44, 46, 50, 51]. Furthermore, one study observed that arsenic influenced H3K4me3 and H3K9ac in a region-dependent manner in the mouse brain [49], and another observed that arsenic was associated with lower levels of H3K9me3 in CD4+, but not CD8+, lymphocytes [54]. These findings suggest that arsenic may have tissue-specific effects, at least for some histone PTMs. Arsenic may also have differential effects in normal vs. transformed/cancer cell lines. However, this has not been

well-studied. One group did observe that arsenic increased H3K9me2 in cells derived from both normal human lung tissue (BEAS-2B) and a human lung tumor (A549) [32]. However, the BEAS-2B cells were found to be much more sensitive to arsenic and were therefore exposed to lower doses [32].

Timing of exposure

Thus far, epidemiological studies have exclusively examined the effects of arsenic on histone PTMs during adulthood [50–54]. However, the prenatal period is thought to be particularly susceptible to epigenetic dysregulation [74]. While two studies in mice have examined the effects of pre- or perinatal arsenic exposure, respectively, on histone PTMs in the brains of pups [48] or adult animals [49], this was not compared with postnatal or adulthood exposures, which may also impact histone PTMs.

Potential modifying effects of sex

There is evidence that susceptibility to arsenic toxicity differs by sex (reviewed in [2]). For some outcomes, such as skin lesions and skin cancer, males are more susceptible [75, 76], while for other outcomes, such as certain developmental outcomes and, potentially, cardiovascular disease, females may be more susceptible [77–79]. All three of the studies which stratified by sex observed that arsenic influenced histone PTMs in a sex-dependent manner [49, 51, 52]. This is consistent with previous studies which have demonstrated sex-specific effects of arsenic on DNA methylation patterns [80–83]. Although many of the systemic sex differences that exist *in vivo* cannot be easily replicated *in vitro*, potential contributions from hormones and sex chromosomes can at least be considered. One *in vitro* study demonstrated that arsenic influences histone PTMs differently based on the presence or absence of E2 [39]. Androgens have not been similarly evaluated. However, many histone modifying enzymes bind androgen receptors, and this can alter their function [84]. For example, histone demethylase LSD1 has been shown to demethylate H3K4 in the absence of androgen, but demethylates H3K9 in the presence of androgen [85]. Although few studies have systematically evaluated potential interactions between arsenic and sex hormones, numerous *in vitro* studies may have been influenced by such interactions, as cell culture media is commonly supplemented with fetal bovine serum, which contains sex steroid hormones [86]. Genetic sex differences between tissues and cell lines may also be important considerations, as some histone demethylases are X-encoded, and others are Y-encoded [87].

Potential sources of histone PTM measurement error

There are many possible causes of histone PTM measurement error. However, two particular sources of concern include: 1) enzymatic cleavage of histone proteins and 2) arsenic-induced alterations in histone expression.

Histone cleavage has been the topic of several recent reviews, and is a phenomenon that occurs in many cell types and species [88–90]. Although the causes and consequences of histone cleavage remain largely unclear, many potential biological functions have been hypothesized (reviewed in [88–90]). Importantly, H3 cleavage has been shown to influence the measurement of certain histone PTMs [91]. This is likely true for other histone proteins, which can also be clipped [88]. While some studies have screened samples for histone

cleavage by Western blot before measuring affected PTMs [49, 52], the majority have not considered this phenomenon.

Importantly, arsenic may also increase expression of the canonical histones [92]. Therefore, histone PTM measures may be overestimated in tissues exposed to higher concentrations of arsenic, unless PTM measures are normalized to total expression levels of the respective histone protein.

Other considerations

Histone variants, which replace the canonical histone proteins, pose an additional challenge, as these variants often harbor distinct PTMs [93, 94]. The effects of arsenic on specific histone variants remain largely unknown. However, in multiple cell types, arsenic has been shown to induce γ -H2AX [95–97], a phosphorylated variant of H2A which is an established mark of double-strand breaks [94]. There is also evidence that arsenic induces aberrant polyadenylation of H3.1, leading to increased expression of this canonical histone and potential displacement of the H3.3 variant [92, 98]. Furthermore, a recent study demonstrated that mRNA and protein levels of 10 different H2B variants were altered in HeLa and BEAS-2B cells transformed by As^{III} (0.5 and 1 μ m for 5 weeks) [99]. Interestingly, two of the H2B variants were highly downregulated (H2B1B and H2B10), while two were highly upregulated (H2B1C and H2B1K), compared with controls [99].

Importantly, folate and other nutritional methyl donors may differentially influence particular histone PTMs [100–104], and nutritional status has been shown to modify the effects of arsenic on DNA methylation [82, 105, 106]. Therefore, differences in the nutritional composition of cell culture media and mouse chow, as well as inter-individual differences in nutritional status, may also contribute to inconsistencies across studies.

Conclusions

Numerous studies have observed that arsenic alters global levels of histone PTMs, which are dysregulated in cancers and other outcomes. For example, there is sufficient evidence that As^{III} increases global levels of H3S10ph, a mark that is important for chromosome segregation. There is also substantial evidence that arsenic causes a global reduction in H4K16ac, which is a hallmark of many human cancers. However, the findings for other PTMs have been inconsistent or sparsely studied. Inconsistencies are likely due in part to the complexity of the relationship between arsenic and PTMs, which may vary based on the type, dose, and duration of exposure; the particular tissue examined; differences by sex; and the importance of exposure timing, but may also be due to potential sources of measurement error, including enzymatic cleavage of histone proteins and arsenic-induced alterations in histone expression. Moving forward, these factors will need to be considered to better understand and reduce some of the discrepancies between studies. Since histone PTMs are reversible and are promising targets of epigenetic therapeutics, a more complete characterization of the effects of arsenic on PTMs, and the factors which modify these relationships, may improve the ability to design interventions which reduce disease burden in arsenic-exposed populations. Future studies which examine the downstream implications of arsenic-induced alterations in histone PTMs, including changes in other epigenetic

modifications and gene expression, and their potential effects on clinical outcomes, will also be critical for a better overall understanding of the mechanisms underlying arsenic-induced health outcomes.

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Figure 1.

Nucleosome structure. The basic unit of chromatin is the nucleosome, which is composed of 147 base pairs of DNA wrapped around a histone octamer. The histone octamer is comprised of two copies of each of the four core histone proteins: H2A, H2B, H3, and H4. Histones H2A and H2B form two dimers, while two copies of histones H3 and H4 form a tetramer. (Adapted from [107] by permission from Macmillan Publishers Ltd.)

Table 1
Summary of studies examining the effects of arsenic on global levels of histone PTMs

Reference	Cell Line, Mouse Strain, or Population	Sex ^d	Exposure; Dose ^b	Dose converted to $\mu\text{g/L}^c$	Duration	Technique	PTMs examined	Findings
<i>Cell Culture Studies</i>								
Arrigo (1983) [28]	Kc 161 (<i>D. Melanogaster</i> , embryonic)	?	NaAsO ₂ (As ^{III}); 50 μM	6496 $\mu\text{g/L}$	4 hours	Radiolabeling	H3, H4, H2A, H2B methylation and acetylation	Methylation: $\downarrow$ H3, H4, $\uparrow$ H2B Acetylation: $\downarrow$ H3, H4, H2A, H2B
Destrosiers and Tanguay (1986) [29]	Schneider and Kc III cells (<i>D. Melanogaster</i> , embryonic)	?	NaAsO ₂ (As ^{III}); 50 μM	6496 $\mu\text{g/L}$	4 hours	Radiolabeling	H3, H4, and H2B methylation and acetylation	Methylation: $\downarrow$ H3, H4, $\uparrow$ H2B Acetylation: $\downarrow$ H3, H4, H2B
Cobo et al. (1995) [46]	CHO (Chinese hamster, ovary)	F	NaAsO ₂ (As ^{III}); 10 μM	1299 $\mu\text{g/L}$	2 hours	Radiolabeling	H1, H2A, H3, H4 phosphorylation	Phosphorylation: $\downarrow$ H1 and H3 No effects on H2A or H4
Perkins et al. (2000) [37]	HL-60 (human, APL) and K562 (human, CML)	F	As ₂ O ₃ (As ^{III}); 1, 2 μM /2 μM	198, 396 $\mu\text{g/L}$ /396 $\mu\text{g/L}$	7 days/24 hours	Western blotting	H3 and H4 acetylation	$\uparrow$ Acetylation for all doses and durations
Li et al. (2002) [30]	NB4 (human, APL)	F	As ₂ O ₃ (As ^{III}); 0.4, 0.8, 1.6 μM	79, 158, 317 $\mu\text{g/L}$	24 hours	Western blotting	H3S10phK14ac, H3S10ph, H3K14ac, H3K9acK14ac	0.8 and 1.6 μM $\uparrow$ H3S10ph and H3S10phK14ac No effect on H3K14ac or H3K9acK14ac
Kannan-Thulasiraman et al. (2006) [45]	KT-1 (human, CML)/NB4 (human, APL)	M/F	As ₂ O ₃ (As ^{III}); 2 μM	396 $\mu\text{g/L}$	20 minutes	Western blotting	H3S10ph	$\uparrow$ H3S10ph
Ramirez et al. (2008) [31]	HepG2 (human, liver cancer)	M	NaAsO ₂ (As ^{III}); 7.5 μM	974 $\mu\text{g/L}$	24 hours	Western blotting	H3K4me2, me3, H3K9ac, H3K9me2, me3, H3K27me3, H4K20me3	$\uparrow$ H3K9ac No effects on methylation marks
Zhou et al. (2008) [32]	A549 (human, lung cancer)/BEAS-2B (human, healthy lung, SV40-transformed)	M/M	NaAsO ₂ (As ^{III}); 2.5, 5 μM /1, 2 μM	325, 650 $\mu\text{g/L}$ /130, 260 $\mu\text{g/L}$	24 hours	Western blotting	H3K4me1, me2, me3, H3K9me, me2, me3, H3K27me3, H3K36me3, H3K36me2, me3, H3K9me2	$\uparrow$ H3K9me2, me3, no effect on H3K9me1, $\uparrow$ H3K4me2, me3, $\downarrow$ H3K4me1, $\downarrow$ H3K27me3, $\uparrow$ H3K36me3, $\downarrow$ H3K36me2/ $\uparrow$ H3K9me2
Zhou et al. (2009) [33]	A549 (human, lung cancer)	M	NaAsO ₂ (As ^{III}); 1, 5 μM	130, 650 $\mu\text{g/L}$	24 hours	Western blotting	H3K4me1, me2, me3	$\uparrow$ H3K4me2, me3 $\downarrow$ H3K4me1

Reference	Cell Line, Mouse Strain, or Population	Sex ^a	Exposure; Dose ^b	Dose converted to $\mu\text{g/L}^c$	Duration	Technique	PTMs examined	Findings
Jo et al. (2009) [40]	UROTsa (human, healthy urothelium, SV40-transformed)	F	NaAsO ₂ (As ^{III}); 3 μM MMA ^{III} O (MMA ^{III}); 1 μM	390 $\mu\text{g/L}$	7 days	Western blotting	H4K16ac	$\downarrow$ H4K16ac for both As ^{III} and MMA ^{III}
Suzuki et al. (2009) [42]	HepG2 (human, liver cancer)	M	NaAsO ₂ (As ^{III}); 60 μM C ₂ H ₇ AsI (DMA ^{III}); 0.5 μM	7795 $\mu\text{g/L}$	0.5, 1.5, 3, 5, 7 hours	Western blotting	H3S10ph	$\uparrow$ H3S10ph
Chu et al. (2011) [34]	UROTsa (human, healthy urothelium, SV40-transformed)	F	NaAsO ₂ (As ^{III}); 1 nM, 3 and 10 μM /MMA ^{III} O (MMA ^{III}); 0.3, 1, 3 μM	0.1, 390, 1299 $\mu\text{g/L}$	24 hours, 7 days	Mass spectrometry	H3 and H4 acetylation	10 μM As ^{III} $\downarrow$ H4K16ac, $\downarrow$ H3 acetylation/ 3 μM MMA ^{III} $\downarrow$ H4K16ac, $\downarrow$ H3 acetylation
Treas et al. (2012) [39]	RWPE1 (human, healthy prostate)	M	NaAsO ₂ (As ^{III}); 100 pg/mL +/- E2, 100 ng/mL +/- E2	0.1, 100 $\mu\text{g/L}$	Every 6 days for 6 months	Western blotting	H3ac, H3K4me3	$\uparrow$ H3ac with As ^{III} or E2 alone, even greater $\uparrow$ for As ^{III} + E2 (100 ng/mL) $\downarrow$ H3ac for combination of As ^{III} + E2 (100 pg/mL) $\uparrow$ H3K4me3 for combination of As ^{III} + E2 (100 ng/mL)
Kim et al. (2012) [41]	3T3 cells (BALB/c mouse, embryo fibroblasts)	F	As ₂ O ₃ (As ^{III}); 0.5 μM	99 $\mu\text{g/L}$	2, 4 weeks	Western blotting	H3K27me3	$\uparrow$ H3K27me3
Suzuki et al. (2013) [43]	HepG2 (human, liver cancer)	M	As ₂ O ₃ (As ^{III}); 0.5 μM	99 $\mu\text{g/L}$	0.5, 1, 2, 5 hours	Western blotting	H3S10ph	$\uparrow$ H3S10ph
Ge et al. (2013) [44]	UROTsa (human, healthy urothelium, SV40-transformed)	F	CH ₃ AsI ₂ (MMA ^{III}); 50 nM	NA	12 weeks	Western blotting	H4K5ac, H4K8ac, H4K12ac, H4K16ac	$\downarrow$ H4K12ac and H4K16ac No effects on H4K5ac or H4K8ac
Herbert et al. (2014) [35]	Primary human neonatal keratinocytes	?	Arsenic source unspecified (As ^{III}); 0.5 μM	Source unspecified	1, 12, 24, 48 days	Western blotting	H4K16ac	$\uparrow$ H4K16ac for all durations
Liu et al. (2015) [47]	HeLa (human, cervical cancer)/HEK293T (human, embryonic kidney)	F/F	As ₂ O ₃ (As ^{III}); 0.2–0.8 μM	40–158 $\mu\text{g/L}$	24, 48, 72 hours	Western blotting	H4K16ac for both cell lines	$\downarrow$ H4K16ac in both cell lines

Reference	Cell Line, Mouse Strain, or Population	Sex ^a	Exposure; Dose ^b	Dose converted to $\mu\text{g/L}^c$	Duration	Technique	PTMs examined	Findings
Rahman et al. (2015) [36]	HEK293T (human, embryonic kidney)/UROtsa (human, healthy urothelium, SV40-transformed)	F/F	As ₂ O ₃ (As ^{III}); 1, 5 μM As ₂ O ₃ (As ^{III}); 0.5, 2.5 μM	198, 989/99, 495 $\mu\text{g/L}$	72 hours 3 hours	Western blotting	H3K9ac, H4K12ac, H4K16ac	↓H3K9ac (UROtsa only after 72 h, both doses) No effect at shorter duration or for other PTMs
Ray et al. (2015) [38]	HaCaT (human, keratinocytes from healthy skin, SV40-transformed)	M	NaAsO ₂ (As ^{III}); 0, 1, 5, 10, 25 μM /10 μM	0, 130, 650, 1299, 3248 $\mu\text{g/L}$ /1299 $\mu\text{g/L}$	8 hours/0-24 hours	Western blotting	H3S10ph	↑H3S10ph in dose- and time-dependent manner
Ma et al. (2016) [53]	HEK293T (human, embryonic kidney)/HaCaT (human, keratinocytes from healthy skin, SV40-transformed)	F/M	NaAsO ₂ (As ^{III}); Single treatment: 0, 1.56, 3.13, 6.25 μM /0, 2.5, 5, 10 μM Repeated treatment: 0.3 μM (HEK293T only)	0, 203, 407, 812 $\mu\text{g/L}$ /0, 325, 650, 1299 $\mu\text{g/L}$ 39 $\mu\text{g/L}$	Single treatment: 8 hours/4 hours Single treatment different durations (6.25 μM /10 μM); 0, 2, 4, 8, 12 hours Repeated treatment: 84 days	Western blotting	H3K18ac, H3K9me2, H3K36me3	Single treatment: ↑H3K18ac in dose-dependent manner ↓H3K36me3 and H3K9me2 in dose-dependent manner (both cell types) Single treatment multiple durations: Initial ↑H3K18ac then decrease Initial ↓H3K9me2 then increase ↓H3K36me3 in time-dependent manner (both cell types) Repeated treatment: ↑H3K18ac after 1 week of treatment and ↓ after that
Pourmora et al. (2016) [54]	Jurkat (human, acute T cell leukemia)/CCRF-CEM (human, ALL)	M/M/F	NaAsO ₂ (As ^{III}); 0.1, 1, 100 $\mu\text{g/L}$	0.1, 1, 100 $\mu\text{g/L}$	48 hours, 72 hours	Western blotting	H3K9me3, H3K9ac	↑H3K9me3 at 1 $\mu\text{g/L}$ and ↑H3K9ac with increasing doses of arsenic, beginning with 0.1 $\mu\text{g/L}$ /↑H3K9me3 at 100 $\mu\text{g/L}$ and ↑H3K9ac with increasing doses of arsenic beginning at 1 $\mu\text{g/L}$
<i>Mouse Studies</i>								
Cronican et al. 2013 [48]	C57BL/6J (brain, cortex and hippocampus)	Both, combined	NaAsO ₂ (As ^{III}); 100 $\mu\text{g/L}$	100 $\mu\text{g/L}$	(1 week before conception until birth)	ChIP-seq	H3K9ac	↓H3K9ac
Tyler et al. 2015 [49] ^e	C57BL/6 (brain, dentate gyrus and frontal cortex)	Both, separate	Na ₃ AsO ₄ (As ^V); 50 $\mu\text{g/L}$	50 $\mu\text{g/L}$	(10 days prior to pregnancy –weaning)	Western blotting	H3K4me3, H3K9ac, H3K9me3	M dentate gyrus: ↑H3K4me3 and ↑H3K9ac F dentate gyrus: ↓H3K4me3 and ↓H3K9ac M frontal cortex: ↑H3K4me3 and ↓H3K9ac F frontal cortex: No change in

Reference	Cell Line, Mouse Strain, or Population	Sex ^a	Exposure; Dose ^b	Dose converted to $\mu\text{g/L}^c$	Duration	Technique	PTMs examined	Findings
<i>Population Studies</i>								
Cantone et al. 2011 [50]	Adults, occupationally exposed via inhalation, Italy (PBLs) ($n = 63$)	M	Arsenic in particulate matter (0.01 – 0.31 $\mu\text{g}/\text{m}^3$)	NA	Chronic (years)	Sandwich ELISA	H3K4me2, H3K9ac	$\uparrow$ H3K4me2
Chervona et al. 2012 [51]	Adults, exposed via contaminated drinking water, Bangladesh (PBMCs) ($n = 40$, 50% M)	Both, separate	Water As (primarily As ^{III}) (50 – 500 $\mu\text{g}/\text{L}$)	50–500 $\mu\text{g}/\text{L}$	Chronic (years)	Sandwich ELISA	H3K4me3, H3K9ac, H3K9me2, H3K18ac, H3K27ac, H3K27me3	Whole sample: $\uparrow$ H3K9me2, $\downarrow$ H3K9ac, $\downarrow$ H3K4me3 and H3K27me3. M: $\downarrow$ H3K9me2, $\uparrow$ H3K27ac. E: $\uparrow$ H3K4me3 and H3K27me3, $\downarrow$ H3K27ac
Howe et al. 2016 [52]	Adults, exposed via contaminated drinking water, Bangladesh (PBMCs) ($n = 317$, 50% M)	Both, separate	uAs _{Cr} and bAs	NA	Chronic (years)	Sandwich ELISA	H3K36me2, H3K36me3, H3K79me2	M: $\uparrow$ H3K36me2 No significant associations with H3K36me3 or H3K79me2 in either sex
Ma et al. 2016 [53]	Adults with and without arsenicosis, exposed via diet and inhalation due to use of arsenic-contaminated coal for indoor cookstoves, China (lymphocytes) ($n = 215$, 44% M)	Both, combined	uAs _{Cr} and hair arsenic	NA	Chronic (years)	Sandwich ELISA	H3K9ac, H3K14ac, H3K18ac, H3K9me2, H3K36me3, H3K79me2	$\downarrow$ H3K18ac and H3K9me2 $\uparrow$ H3K14ac and H3K36me3
Pournara et al. 2015 [54]	Adults, exposed via contaminated drinking water, Argentina (sorted CD4+ and CD8+ cells) ($n = 28$)	F	uAs	NA	Chronic (years)	Western blotting	H3K9ac, H3K9me3	$\downarrow$ H3K9me3 in CD4+ cells No significant association with H3K9me3 in CD8+ cells No significant association with H3K9ac in either cell type

Abbreviations used: APL, acute promyelocytic leukemia; ALL, acute lymphoblastic leukemia; bAs, blood arsenic; CML, chronic myelogenous leukemia; E2, estradiol; PBL, peripheral blood leukocyte; PBMC, peripheral blood mononuclear cell; uAs, urinary arsenic; uAs_{Cr}, urinary arsenic adjusted for urinary creatinine; wAs, water arsenic

^aWith respect to cell culture studies, sex refers to the biological sex of the animal or patient from which the cell line was derived

^bUnits are reported as they were in the original reference.

Units were only converted to $\mu\text{g/L}$ for inorganic arsenic sources, since these are the predominant arsenic species in drinking water

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