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Review

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Epigenomic reprogramming in inorganic arsenic-mediated gene expression patterns during carcinogenesis

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Abstract: Arsenic is a ubiquitous metalloid that is not mutagenic but is carcinogenic. The mechanism(s) by which arsenic causes cancer remain unknown. To date, several mechanisms have been proposed, including the arsenic-induced generation of reactive oxygen species (ROS). However, it is also becoming evident that inorganic arsenic (iAs) may exert its carcinogenic effects by changing the epigenome, and thereby modifying chromatin structure and dynamics. These epigenetic changes alter the accessibility of gene regulatory factors to DNA, resulting in specific changes in gene expression both at the levels of transcription initiation and gene splicing. In this review, we discuss recent literature reports describing epigenetic changes induced by iAs exposure and the possible epigenetic mechanisms underlying these changes.

Keywords: alternative splicing; chromatin; DNA methylation; epithelial-to-mesenchymal transition; histones.

Introduction

Inorganic arsenic (iAs) is a naturally occurring toxic metalloid to which humans are routinely exposed to the environment. It is widely distributed in the earth's crust and is ubiquitous in soil, water, and air. Humans are chronically exposed to iAs through contaminated food and drinking water (1, 2). It is estimated that contaminated wells, formed

from proximity to iAs-rich geological formations, expose 160 million individuals worldwide to excessive levels of iAs (1) which is over the US environmental protection agency's guideline of 10 ppb (3). The long-term effects of low-dose iAs exposure from contaminated drinking water continue as mining efforts, which release high amounts of iAs into the groundwater, persist in many regions of the world (1, 4). While not genotoxic, arsenic and iAs compounds are listed as known human carcinogens by the National Toxicology Program (2). Epidemiological studies demonstrate a strong relationship between environmental iAs exposure and an increased cancer incidence. Such exposure correlates with an increased risk of lymphatic (5), hematopoietic (6), skin, lung (5–7), digestive tract (5), liver (6), urinary tract (6, 8, 9), and prostate (5, 6) cancers. Although the precise mechanisms by which iAs causes cancer remain elusive, several mechanisms have been proposed. These mechanisms include iAs-induced oxidative stress, inhibition of DNA repair, micronuclei formation, chromosomal aberrations, and changes to the epigenome. This review will focus on proposed epigenetic mechanisms that contribute to iAs-induced carcinogenesis; however iAs generally, might be effecting similar epigenetic mechanisms during pathogenesis.

Proposed mechanisms of iAs-mediated carcinogenesis and toxicity

A variety of mechanisms may contribute to the carcinogenicity caused by iAs. One mechanism involves the iAs-induced production of reactive oxygen species (ROS) which has been reported in multiple cell models including human lung bronchial epithelial BEAS-2B cells, human vascular smooth muscle cells, vascular endothelial cells, U937 cells, NB4 cells, human-hamster hybrid cells, CHO-K1 cells, and HEL30 cells (10–16). The impact of iAs on ROS production is hypothesized to occur in two steps. First,

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iAs initiates production of high ROS levels, which chemically react with DNA. This reaction damages the DNA and disrupts its structural integrity; thus, contributing to cellular transformation and tumor proliferation (17). NADPH oxidase (NOX) has been implicated as the primary source for the generation of O_2 (18, 19). Though iAs is not able to induce the expressions of p47, p67, p91, and several scaffolding proteins of the NOX complex, it is, however, able to stimulate enzyme activity of NOX by inducing the phosphorylation and translocation of p47 (18, 20). Second, cells react to the increased amounts of ROS by overexpressing the antioxidant enzymes superoxide dismutase, catalase, and glutathione, which protect cells against apoptosis. The decreased apoptosis and accumulation of damaged cells increases carcinogenic potential (17).

Chromosomal instability and epigenetic modifications may also play roles in the carcinogenicity of iAs. At low doses, iAs does not cause DNA base pair mutations; instead, it is known to generate double-stranded breaks (21), which results in large-scale chromosomal aberrations (22). Interestingly, iAs exposure results in the impairment of proteins involved in base excision repair such as apurinic/apyrimidinic (abasic) endonuclease (APEX1), DNA ligase 1 (LIG1), DNA ligase 3 (LIG3), oxo-guanine glycosylase (OGG1), poly(ADP) ribose polymerase (PARP1), and DNA polymerase β (POLB) [reviewed in (23)]. The iAs-mediated chromosomal instability occurs frequently at centromeres, leading to the formation of acentric chromosomes or the fusion of centromeres between two chromosomes (24). Fusion of two chromosomes at their centromeres can cause improper chromosome segregation, which results in aneuploidy or in micronuclei formation (21, 25). However, a fusion that occurs at chromosomal ends may result in the formation of ring-like structures and/or participate in abnormal sister chromatid exchanges (24, 26); both of which are deleterious to the cell. While these are large-scale chromosomal rearrangements, iAs also effects changes to chromatin structure at the nucleosome level; these changes alter local chromatin conformation that ultimately fine-tune the iAs-mediated gene expression profile.

Eukaryotic DNA is packaged in the form of chromatin, which has a basic repeating unit, the nucleosome. Each nucleosome consists of 147 DNA base pairs wrapped around two molecules each of four canonical histones (histones H2A, H2B, H3, and H4). The tails of these histones are subject to a variety of post-translational modifications (PTMs) (27, 28), which are dynamically regulated to control the accessibility of chromatin to factors that direct gene expression. Histone PTMs, other epigenetic marks (e.g. methylated DNA or distinctly-combined histone variants), and microRNAs (miRNAs) are collectively known as

epigenetic regulators. The remainder of this review will focus on the most recent studies that investigate the role of iAs exposure in reprogramming these epigenetic marks, and how this reprogramming may mediate iAs toxicity.

DNA methylation

DNA methylation is one of the several epigenetic mechanisms that cells use to control gene expression. The effect of DNA methylation on a gene's expression depends on the type of regulatory element at which the methylation occurs. For instance, methylation of a promoter is associated with gene repression. However, methylation within the gene body is generally associated with gene expression and splice regulation, although the latter processes are poorly understood.

The results of several studies have implicated aberrant DNA methylation in many cancers but our understanding of the impact of iAs on DNA methylation is just emerging (29–31). For instance, Zhao et al. showed that chronic, low-dose exposure of cells to iAs for 18 weeks caused global hypomethylation. Other studies revealed that iAs exposure to human skin led to global hypomethylation of some tissues (leukocytes), and iAs exposure in rodent liver instigated hypomethylation (32, 33). However, other research indicates that iAs exposure leads to hypermethylation at the promoters of specific tumor-suppressor genes, resulting in their repression (34, 35). For example, in iAs-exposed human hepatocytes, significant hypermethylation of the promoters for genes involved in DNA repair such as excision repair cross-complementation group 2 (*ERCC2*) and replication protein A1 (*RPA1*), and of genes associated with the Wnt pathway like c-MYC (*MYC*) and Wnt family member 2B (*WNT2B*), were observed (34). Additionally, significant hypermethylation of the promoters for the tumor suppressor *p16* (36, 37), and the DNA repair gene, mutL homolog 1 (*MLH1*) (36), were observed in whole blood obtained from humans chronically exposed to iAs. Conversely, iAs-induced hypomethylation of promoters for genes involved in processes such as endocytosis and RNA transport were also observed (34). Interestingly, another study involving chronic exposure of a human population to arsenic demonstrated hypomethylation at the promoter of the DNA repair gene, *ERCC2* (38).

While these findings may at first seem incongruous, it is now clear that, although global hypomethylation occurs, modifications at specific genetic loci can also be either hypo or hypermethylated. Furthermore, it is possible that some of these disparities may be explained by the use of different model systems, different stages of

malignant transformation, and different experimental conditions (e.g. the dose of iAs, the duration of iAs exposure, and the presence or absence of other carcinogens). For instance, Pilser et al. suggested that folate can influence iAs-mediated DNA methylation profiles in peripheral blood leukocytes taken from Bangladeshi adults (33). Overall, though, these studies suggest that iAs-mediated malignant transformations result from multiple changes in DNA methylation patterns and that these altered patterns can have positive or negative effects on the expression of specific genes. One should be cautious when drawing general conclusions about observed iAs-induced changes in DNA methylation patterns from any one study. Most importantly, profiling should be performed together with analyses of the functional consequences of such changes in DNA methylation.

Researchers have proposed several possible mechanisms for iAs-mediated changes in DNA methylation. In humans, DNA methylation is initiated by the *de novo* DNA methyltransferase 3 (DNMT3A and B) and maintained by DNA methyltransferase 1 (DNMT1) (39). Following low-dose iAs exposure, the expression of the DNMTs is reduced, which results in less methylation at target sites (40–42). Because DNMTs also participate in DNA repair, inactivation and/or reduction in the expression levels of DNA methyltransferases will also impede DNA repair efficiency (35, 41, 43, 44). Another possible explanation for iAs-mediated alterations in DNA methylation involves the depletion of methyl groups. When cells metabolize arsenic, the arsenic methyltransferase, AS3MT, transfers a methyl group from S-adenosylmethionine (SAM) to the arsenite (34), depleting the available methyl groups needed by the DNMTs for DNA methylation (41). This competition for methyl groups affects DNA methylation supported by DNMTs, but also the histone methyltransferases. As the name implies, these enzymes methylate histones and, likewise, are important components of epigenetic regulation. Interestingly, there is interplay between DNA methylation and histone modification; thus, an imbalance in one epigenetic mark could also trigger other epigenetic changes at specific gene regulatory regions.

Histone modifications

PTMs of the N-terminal tails of histone proteins change the chromatin structurally and functionally, thereby altering gene expression. Histone PTMs include methylation, acetylation, phosphorylation, glycosylation, carbonylation, ubiquitylation, biotinylation, sumoylation, citrullination, ADP-ribosylation, N-formylation, crotonylation,

propionylation, and butyrylation, as well as proline and aspartic acid isomerization. The sum of all of these histone PTMs is known as the histone code (45). Histone PTMs permit, increase or restrict access to chromatin by gene regulatory factors. Among the many that have been identified, the most extensively studied and best-understood histone PTMs in the context of transcriptional competency are phosphorylation, methylation, acetylation, and ubiquitination (46).

Several *in vitro* studies have demonstrated that iAs exposure can result in global changes in histone PTMs. These changes include increases in H3K9me2, H3K4me3, and H3S10, decreases in H2B ubiquitination, and either increases or decreases in H3K27me3 (47–52). In addition, following iAs exposure in humans, blood cells exhibited an overall decrease in H3K9me3 and H3K9ac, with an increase in H3K9me2 (47, 48). Interestingly, these studies also showed that the iAs-induced changes in histone PTMs can be gender-specific. For instance, a decrease in H3K27ac and H3K18ac and increase in H3K4me3 and H3K27me3 was observed in iAs-exposed females, while the inverse trend was observed in iAs-exposed males (47).

The majority of PTMs that affect chromatin accessibility are modifications to histone H3 (e.g. H3K4, H3K9, H3K27, and H3K36). Methylation of H3K4 is performed by a series of type 2 lysine methyltransferases (KMT2) (53) and occurs in a stepwise manner; methylation proceeds from the mono- to the di- and, finally, to the tri-methylation state. These different H3K4me states vary in their genomic distributions: H3K4me1 occurs at the 3' end of genes, H3K4me2 occurs within the gene body, and H3K4me3 occurs at gene promoters (53). Deposition of H3K4me3 at gene promoters and coding regions correlates with transcriptional activation (50, 54, 55). Exposure of cells to iAs results in a global decrease in monomethylated H3K4, with a corresponding increase in H3K4's di- and tri-methylated states (50). This shift in the global H3K4 methylation status occurs quickly; exposing cells to 1 μ M iAs led to the changes in H3K4 methylation states in as little as 24 h (50). Furthermore, Tyler et al. found that perinatal arsenic exposure resulted in changes to H3K9Ac and H3K4me3 levels in the brains of adult mice (56, 57).

Analogous to H3K4 methylation, H3K9 is first monomethylated and then dimethylated by euchromatic histone-lysine N-methyltransferase 2 (KMT1C, also known as G9a). Suppressor of variegation 3–9 homolog 1 (KMT1A, also known as SUV39h1) then converts the di-methylated state further to the tri-methylated state by (49, 58). These methylation events are offset by lysine demethylase 3A (KDM3A), a histone demethylase that reverses the mono- or di-methylation of H3K9 (49). Like methylated H3K4, the

di- and tri-methylated states of H3K9 are typically found in different chromatin locations; H3K9me2 tends to localize at the edges of the nucleus, where heterochromatin is more common, while H3K9me3 is found in the center of the nucleus, where euchromatin predominates (49, 50). Exposure of A549 lung carcinoma cells to iAs increased expression of KMT1C, leading to an increase in H3K9me2 and H3K9me3 levels (49). Furthermore, Chervona et al. observed a variety of correlations between iAs exposure and this type of modification in peripheral blood mononuclear cells using a population-based study in Bangladesh. This study further revealed a positive correlation between urinary iAs and H3K9me2 (47, 59).

H3K27 methylation is another important PTM for histone H3. This repressive PTM is mediated by the polycomb repressive complex 2 (PRC2); PRC2 is composed of three core components: Enhancer of Zeste 1 or 2 (EZH1/2), suppressor of Zeste 12 (SUZ12), and embryonic ectodermal development (EED). The methylation state conferred by PRC2 is determined by the homolog of EZH present in the complex – EZH1 mediates the monomethylation of H3K27, while EZH2 mediates its di- and tri-methylation. H3K27me3 is associated with inactive promoters and gene silencing and, importantly, it marks chromatin for further compaction by protein regulator of cytokinesis (PRC1) via ubiquitylation of H2AK119 (60). The iAs exposure greatly increases the expression of the EZH2 homolog and of other PRC2 core components, resulting in a global increase in H3K27me3 levels (51). A persistent increase in EZH2 and the subsequent incorporation of H3K27me3 into chromatin silences the promoters of target genes, which is enhanced by the crosstalk that occurs between epigenetic marks. A clear example of this cross-talk is seen by the reduced expressions of p19ARF and p16INK4a due to the incorporation of H3K27me3 and H3K9me2, and to the methylation of DNA at the *INK4/ARF* regulatory locus in iAs-exposed cells (37, 61, 62).

It is well known that many changes in histone marks are mediated by histone kinases. The kinase-mediated phosphorylation of histone H3 is one example that is associated with cell proliferation and transformation (63, 64). With regard to arsenic exposure, it is likely that iAs activates a cell signaling pathway that modifies histones. For example, iAs induces the activation of the nuclear mitogen and stress-activated protein kinase 1 (MSK1) via the p38 MAPK pathway (29, 65), and this process was necessary for iAs-induced transformation (66). Interestingly, in response to stress, MSK1 phosphorylates KDM3A (p-KDM3A) at Ser264, resulting in the enrichment and recruitment of signal transducer and activator of transcription 1 (STAT-1) and p-KDM3A to target gene promoters.

These events promote the localized demethylation of H3K9 with consequential increased transcriptional activity (67). Additionally, MSK1 can directly phosphorylate H3S10 (68, 69), which recruits scaffolding proteins to the promoters of immediate-early genes, including those of proto-oncogenes *FOS* and *JUN*, to induce their activation (70).

Only a few studies have demonstrated a connection between histone phosphorylation and arsenic-induced carcinogenicity. Studies have suggested that arsenic-induced H3 phosphorylation might be responsible for the up-regulation of the oncogenes *c-fos* and *c-jun* (65). Most recently, Ray et al. reported that iAs induces a coordinated regulation of Nrf2 and histone H3S10 phosphorylation, which activates the human heme oxygenase-1 gene (*HMOX1*) (52). These results reinforce the notion that iAs modulates gene expression by operating through the JNK and p38/Mpk2 kinase pathway to promote cancer.

The iAs may also modulate histone PTMs by activating the epidermal growth factor (EGF) pathway (71). Upon activation of this pathway, pyruvate kinase, muscle 2 (PKM2) – a histone kinase – translocates to the nucleus, where it binds and phosphorylates Thr11 on histone H3 (H3T11). This phosphorylation not only accelerates the demethylation of H3K9me3 by the trimethylation-specific H3 methylase KDM4C but also leads to the dissociation of HDAC3, allowing the acetylation of H3 with subsequent transcription of oncogenes *MYC* and cyclin D1 (*CCND1*) (72). By influencing this pathway, iAs may indirectly control the phosphorylation and acetylation state of histones, and therefore the expression patterns of target genes.

Histone variants

In addition to the four canonical histones, there are also highly conserved variants of these histones. The variants differ by only a few amino acids, and their expression is generally lower than the canonical histones. Each histone variant has a specific gene expression, and a distinct chromatin localization/incorporation that confers, within the chromatin structure, information specifying the cell-type, the stage of differentiation and tissue. However, compared to the canonical histones, studies on histone variants are limited, and so the role these chromatin-incorporated histone variants play in normal cells and even in diseased states is only beginning to emerge. Our understanding of the impact these histone variants have on chromatin biology is hindered by a lack of high-resolution and sensitive assays to differentiate these proteins, as they have a very similar amino acid composition to canonical histones. However, using high-resolution Top-down mass

spectrometry, recent studies from our laboratory showed that some histone variants were dynamically altered during iAs-mediated malignant transformation. Specifically, we showed that histone H2B variants were abnormally expressed following iAs exposure, and during the subsequent iAs-mediated epithelial-mesenchymal transition (EMT) (73). We identified a total of 16 H2B variants, 13 somatic and three testis-specific; of the somatic variants, seven were upregulated while three were downregulated (73–77). The most significant changes observed in our study were the upregulation of histones H2B1K and H2B1C, and downregulation of histones H2B1D and H2B1B. The results demonstrated that although histones are highly conserved, changes in single amino acids can influence chromatin dynamics that lead to transcriptional reprogramming critical for cell-type specificity and differentiation (77). Interestingly, removal of iAs returned expression levels for some variants to normal levels while others remained abnormal, suggesting that some epigenetic changes are transient (73). This iAs removal and its associated reversed gene expression, correlated with a reversal in the variant histones expressed during EMT. Therefore, it is possible that the carcinogenic potential seen in these cells is driven by expression patterns of histone variants that did not revert following iAs removal.

The iAs is known to disrupt the balance between canonical histone H3.1 and variant histone H3.3. Histone H3.1 transcription levels are highest during S phase, while H3.3 expression levels are consistently low throughout the cell cycle (78–83). Functionally, histone H3.1 is ubiquitously present in genomic chromatin, while histone H3.3 is incorporated into DNA promoter regions to specifically facilitate transcriptional activation (84, 85). The iAs treatment increases histone H3.1 stability, hindering the interaction of histone H3.3 with chromatin and changed nucleosome composition; these effects likely change the gene expression patterns (86). Specifically, the irregular incorporation of H3.1 could silence tumor-suppressor genes and abnormally activate cell-cycle genes. The proposed mechanism for this iAs-mediated increased influence by H3.1 involves an associated inhibition of stem-loop binding protein (SLBP) expression (87, 88). Under normal conditions, SLBP binds to the stem-loop on histone transcripts preventing their degradation. With iAs exposure, SLBP expression levels are reduced allowing more proteasomal degradation of the protein (80, 86). With less SLBP present, canonical histone transcripts with the stem loop are degraded, leaving those with a polyadenylated tail (H3.1) intact. Indeed, the addition of a poly(A) tail to histone transcripts increases their stability so they may be present in higher levels outside of

S phase. This processing ultimately increases H3.1 levels and blocks potential histone H3.3 interactions at strategic sites, causing aberrant gene expression (86). Knockdown of SLBP caused increased cell growth and transformation, indicating that this mechanism may be implicated in arsenic-induced carcinogenesis (86).

Other histone variants are implicated in carcinogenesis. For example, abnormal expression of histone H2A.X, a histone variant involved in double-stranded break repair, genome stability, and tumor suppression (89–93) has been associated with progression of EMT in colon and lung cancer (94, 95). When histone H2A.X levels are reduced, the variant is removed from the gene loci for Slug and ZEB1 (EMT markers) creating a relaxed chromatin structure and increased expression (94). Upregulation of histone H2A.Z, another histone H2A variant, is implicated in colorectal and breast cancer (96–100). Histone H2A.Z normally maintains the stability and integrity of the genetic material within the cell (101, 102). When this variant is upregulated, cell growth and proliferation increase by an activation of cell cycle regulators and EMT markers such as E-cadherin and fibronectin (94, 96, 103). Yet another set of histone H2A isoforms, H2A.1 and H2A.2, are repressive and reduce tumorigenesis (104). These histone variants typically act as tumor suppressors by hindering the efficiency of transcription factor binding and chromatin remodeling at strategic sites (105–108). Finally, upregulation of CENP-A, a centromere-specific H3-like histone, is thought to increase the incidence of aneuploidy and dysregulation of cell cycle-associated genes, and also is implicated in cancer (109). Further research is needed to determine if iAs-induced carcinogenesis and toxicity employ any of these cancer-related histone variants.

MicroRNAs

MicroRNAs (miRNAs) constitute another epigenetic mechanism of gene regulation affecting development, growth, and the response to stress. Several *in vitro* and human studies demonstrated arsenic-induced alterations in miRNA gene expression. For instance, Marsit et al. showed global increases in miRNA expression in response to iAs exposure (110). In addition, miR-222 and miR-21 expressions were increased in the peripheral blood of steel factory workers (111). In contrast, a study of Hong Kong children aged 12–19 years, found decreased expression of both miR-21 and miR-221 associated with increased urinary arsenic and lead levels (112). Furthermore, in a pregnancy cohort from Mexico, maternal total urinary iAs was associated with the increased expression of 12 miRNAs

in infant cord blood. This study utilized genome-wide miRNA analysis and suggests that miRNA alterations can lead to gene expression changes in progeny (113). Using transcriptome-wide next generation sequencing, Yu et al. identified expression changes for 36 new miRNAs from iAs-tainted rice (114). Another study in plants showed that iAs altered the expression of miR167, miR319, and miR854 (115). Investigations have pursued the function and potential mechanism of these microRNAs in iAs toxicity. For example, upregulation of microRNA-21 can enhance the transformation potential of a cell by targeting programmed cell-death 4 (PDCD4) (116). Another functional mechanism identified is the iAs-mediated transformation in p53 (low) HBECs, which can be reversed by zinc finger E-box binding homeobox 1 (ZEB1 and ZEB2)-mediated increases in the levels microRNA 200b. Also identified, iAs-mediated autophagy is supported by microRNA-21-induced PTEN-ERK signaling, and iAs-induced angiogenesis occurs through a microRNA425-5p-regulated CCM3 (117). With the help of new technology, additional iAs-associated miRNAs functions and processes will be discovered, revealing the regulatory network that mediates iAs toxicity and carcinogenesis.

Alternative splicing

Epigenetic regulation of gene expression occurs at the level of transcriptional activation, as discussed above, but also through the less investigated process of splicing. Alternative splicing (AS) of pre-mRNA occurs in the majority of multi-exon genes and greatly increases the diversity of the proteome. Aberrant splicing is known to occur in human cancers (118–120), and a proposed mechanism is the substitutions of isoforms associated with carcinogenesis (121), angiogenesis (122), and EMT (123, 124). Epigenetic modifications are thought to regulate AS by two non-mutually exclusive means: 1) by affecting the kinetics of polymerase elongation and 2) through recruitment of splicing regulatory factors. Two types of epigenetic marks, DNA methylation (125–128) and histone PTMs (120, 129–132), have been implicated in the selection of exon-candidates. Exposure to iAs significantly alters DNA methylation (133) and histone PTMs (48–50, 62, 134–136), and so it is reasonable to expect that this exposure can induce changes in alternative splicing. Indeed, we recently showed that low-dose iAs exposure results in changes in AS (133), though the mechanism remains unknown.

One possible mechanism by which iAs influences AS may be through an inhibition of DNA binding by alternative

splicing regulators such as CCCTC-binding factor (CTCF) and poly (ADP) ribose polymerase (PARP1) (132, 137, 138). Interestingly, PARP1 co-localizes with CTCF on chromatin; this complex, with CTCF-dependent automodification of PARP1, permits PARylation activity in the absence of DNA damage (137, 139). Importantly, many splicing factors are regulated by PARylation (140–144) and any iAs-mediated inhibition of PARP1 binding to DNA not only affects the structural properties of chromatin but also the PARylation activities, which indirectly affect splicing decisions. In addition, the binding of proteins to DNA can be altered by the presence of iAs due to the high binding affinity of this metalloid for cysteine residues that are found in C4 and C3H1 zinc finger motif-containing proteins such as PARP1 (145–147). Other DNA-binding proteins with zinc finger motifs inhibited by iAs are the methylcytosine dioxygenases (TET1/2), needed to oxidize 5-methylcytosine to 5-hydroxymethylcytosine and 5-carboxylcytosine (148). Inactivation of TET1/2 by iAs allows 5-methylcytosine to accumulate at CTCF target sites and prevents CTCF from binding to its target sites, resulting in exon exclusion (126). In summary, if iAs blocks the binding of PARP1 or CTCF to DNA, the chromatin-associated functioning of these proteins is altered, which includes splicing decisions.

A second possible mechanism by which iAs may alter AS is by increasing the presence of p52 through activation of the non-canonical NF- κ B pathway (149). P52 co-localizes and interacts with the splicing factor SRSF1 to modulate splicing (129). The role of SRSF1 in carcinogenesis is well studied (150), and its expression is upregulated by MYC (151), which in itself is deregulated in iAs exposure (34, 152). MYC also directly upregulates core pre-mRNA machinery during carcinogenesis and maintains appropriate splicing of alternative exons (153).

The dominant isoform expressed for any given gene is tissue-specific and may result from differentially expressed splicing regulatory factors among tissue types (154–156). As changes in AS are likely to vary from tissue to tissue, our ability to dissect and understand the complicated issue of what drives iAs-mediated carcinogenesis is exacerbated.

Conclusion

A large body of research has implicated low-dose arsenic exposure in carcinogenesis and EMT. This review examines the impact of epigenetic processes, including DNA methylation, histone PTMs, histone variants, and alternative splicing, on carcinogenesis mediated through iAs

exposure. While all of these epigenetic marks are associated with iAs-induced carcinogenesis, additional research is required to clarify the mechanism(s) driving each system. Expanded research in this area will delineate the role of iAs exposure in the initiation and development of cancer. Finally, while this review is on arsenic, it is possible that other heavy metals act through similar epigenetic mechanisms. However, a comprehensive genome-wide analysis comparing the effects of toxic metals in a single study will help delineate whether similar epigenetic mechanisms are targeted by heavy metals.

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