Published in final edited form as:

Free Radic Biol Med. 2012 September 1; 53(5): 1041–1047. doi:10.1016/j.freeradbiomed.2012.07.020.

The control of histone methylation and gene expression by oxidative stress, hypoxia and metals

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Abstract

The harmful consequences of carcinogenic metals, such as nickel, arsenic and chromium, are thought to be in part due to their ability to induce oxidative stress. The ubiquity of oxidative stress in biological systems has made it a fairly obvious culprit in causing cellular damage and/or development of disease. However, the full extent of oxidative stress-induced damage is not limited to its direct effects on cellular components, such as lipids, proteins and DNA, but may extend to its ability to alter gene expression. Gene expression regulation is an important component of cellular and/or tissue homeostasis, and its alteration can have detrimental consequences. Therefore, a growing amount of interest is being paid to understanding how oxidative stress can influence gene expression. Oxidative stress-induced epigenetic dysregulation in the form of post-translational histone modifications, in particular, is a popular topic of research. This review will therefore primarily focus on discussing the role of oxidative stress and hypoxia on histone methylation and/or gene expression alterations. The sources of oxidative stress discussed here are carcinogenic metals, such as, nickel, arsenic and chromium.

Keywords

Metals; histones; hypoxia; oxidative stress; gene expression

Introduction

Oxidative stress is a widely occurring phenomenon in biological systems that can be principally described as an imbalance between the production of reactive oxygen species (ROS) and the system's ability to promptly detoxify the generated reactive intermediates and/or repair the subsequent cellular damage [1]. The disruption of the normal redox potential in a cellular environment via the production of ROS, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), can have detrimental effects on all cellular components, including proteins, lipids and DNA [2–4]. Oxygen concentration plays an important role in the manifestation and maintenance of oxidative stress. Increasing oxygen concentration can markedly influence the production of free radicals, while decreasing it can lead to hypoxia [5, 6].

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Oxidative stress is thought to play a role in the development of toxicity and/or carcinogenicity of a number of metals, such as nickel, chromium, and arsenic [3, 7–10]. However, since the majority of carcinogenic metals are either weak or non-mutagens, such as nickel, and with the exception of hexavalent chromium, which is a mutagen, do not directly interact with DNA (i.e. form DNA adducts), the induction of epigenetic dysregulation through oxidative stress may be a significant factor in governing metal-induced carcinogenicity [11–13]. Post-translational histone modifications, such as methylation of lysines, have been shown to play an important role in chromatin architecture, X-inactivation, gene expression, development, and carcinogenesis [14–18]. This review will therefore primarily focus on the role of oxidative stress and hypoxia on histone methylation and/or gene expression alterations. The sources of oxidative stress discussed here are carcinogenic metals, such as nickel, arsenic and chromium.

Histone Methylation

Given that post-translational modifications of histone tails are essential components of cellular epigenetic machinery, their respective functional consequences can bring about structural changes to chromatin and/or serve to include or exclude protein complexes from coming in contact with DNA, thereby influencing the transcriptional pattern of genes, altering their activity, and plausibly contributing to the emergence of disease [18–22]. As noted earlier, methylation, which can be found on arginine and/or lysine residues of histone tails, is involved in regulating a wide range of processes, including: gene expression, chromatin structure, dosage compensation and epigenetic memory [18, 23, 24]. Histones can become mono-, di- or tri methylated, and the functional consequences of methylation depend on the number of methyl groups, the residue itself, and its location within the histone tail. For example, histone 3 lysine 4 (H3K4) di- and trimethylation and histone 3 lysine 9 (H3K9) monomethylation is correlated with open chromatin and active gene expression, while histone 3 lysine 27 (H3K27) di- and trimethylation and H3K9 di- and trimethylation is associated with inactive chromatin and repression of gene expression [18]. Unlike other post-translational histone modifications (i.e. acetylation), methylation of histones does not regulate gene expression by changing the charge of the modified amino acid. Instead, it is thought to regulate gene expression by antagonizing or augmenting the effects that other histone modifications have on gene expression and is often transduced by a family of binding proteins that bind to histones with methylated lysines (chromodomains) [14].

Methylation of histone tails is a fairly dynamic process and is maintained by histone modifying enzymes, such as methyltransferases (HMTs) and demethylases. The majority of HMTs belong to a family of enzymes with a conserved catalytic domain called SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) and their activity towards the lysine and arginine residues can result in mono-, di-, or tri methylation state of the amino acids. There are 48 known SET domain methyltransferases in the human genome [25]. Histone demethylases, on the other hand, can be classified in to two classes: KDM1 (Lysine (K) Demethylase1) family, also known as LSD1, are FAD-dependent amine oxidases, and the Jumonji C (JmjC) domain containing demethylases (JHDMs), which are iron and 2-oxoglutarate-dependent enzymes [26]. Given that LSD1 and JmjC histone demethylases both require oxygen to function it is plausible to consider that the status of histone methylation is also influenced by oxygen concentration.

Hypoxia

Cells and/or tissues often become hypoxic when the demand for cellular growth and metabolism surpasses that of the oxygen supply. Hypoxia is an important factor in the pathology of a number of human diseases, including cancer, diabetes, ageing, and stroke/

ischemia [6, 27]. Hypoxic regions can often be found in cancer tissue due to high cellular proliferation rate coupled with the development of abnormal vasculature. Solid tumors, for example, often become hypoxic because normal tissue vasculature can only support tumor growth within a diameter of ~2 mm [28].

When oxygen tension is low, a family of transcription factors called Hypoxia Inducible Factors (HIF), become activated. This family is comprised of three members, HIF-1 α , HIF-2 α , and HIF-3 α , all of which can form a heterodimer of an oxygen labile subunit, HIF- α , and an oxygen insensitive HIF-1 β , also known as aryl hydrocarbon nuclear translocator (ARNT) [6]. Under normal oxygen tension, normoxia, HIF- α is targeted for degradation by the von Hippel-Lindau (VHL) binding protein, which then recruits an E3 ubiquitin ligase complex. VHL recognizes HIF in the presence of oxygen via the hydroxylated proline residues in the oxygen-dependent domain of the latter [29]. The hydroxylation of these prolines is carried out by a family of dioxygenases, called Prolyl-Hydroxylases (PHDs) [30]. Another dioxygenase that can influence HIF activity is the Factor Inhibiting HIF (FIH). FIH mediates the hydroxylation of asparagine residues within the C-terminus trans-activation domains of HIF- α , preventing binding to co-activators such as p300 or CBP, and thus limiting HIF transcriptional activity[31]. It is also important to note that the enzyme activities of PHDs and FIH both require oxygen, α -ketoglutarate, iron (Fe²⁺), and ascorbate as cofactors and that a depletion of any of these cofactors can potentially impair/inhibit the function of these enzymes.

Histone demethylases, such as LSD1 and JmjC domain containing iron and 2-oxoglutarate-dependent enzymes also require oxygen as a cofactor [23]. LSD1, or KDM1A, is believed to demethylate lysine 4 of histone 3 (the mono and di form only) by using oxygen as an electron acceptor to reduce methylated lysine to form lysine, formaldehyde, and hydrogen peroxide (Fig. 1A) The demethylation of lysines by JHDMs on the other hand, occurs by catalyzing the generation of oxidized iron in the presence of oxygen, reactive oxygen species, α -ketoglutarate, and ascorbate. These resultant species attack the methyl groups on histone lysines and produce unstable oxidized intermediates that spontaneously release formaldehyde, resulting in the removal of methyl groups from histone lysines [32] (Fig. 1B). While the functional significance of these enzymes with respect to hypoxic response has yet to be fully elucidated, a number of studies have already demonstrated their involvement. Hypoxia was shown to epigenetically down-regulate *BRCA1* and *RAD51*, by decreasing the levels of H3K4 methylation in their promoters via LSD1 demethylation [33]. VHL inactivation, which was dependant on the constitutively active HIF-2 α and Jumonji/ARID Domain Containing Protein 1C (JARID1C), or KDM5C, was also shown to decrease levels of H3K4me3 in clear-cell renal cell carcinoma cells. In VHL^{-/-} cells, HIF appeared to induce *JARID1C* expression, which in turn altered the expression of hypoxia-responsive genes (HRGs) and reduced the levels of H3K4me3 levels at the promoters of insulin-like growth factor-binding protein 3 (*IGFBP3*), *COL6A1*, *DNAJC12* and *GDF15* [34]. Moreover, HIF-1 α also appeared to be involved in the enrichment of four other 2-oxoglutarate dioxygenases. JARID1B (KDM5B), JMJD1A (KDM3A), JMJD2B (KDM4B), and JMJD2C (KDM4C) appeared to be direct HIF-1 target genes with robust HIF-1 binding within their promoters and up-regulated expression under hypoxic conditions[35]. Furthermore, JMJD1A was shown to regulate a subset of hypoxia-induced genes, including *ADM* and *GDF15*, by maintaining a lower level of H3K9me2) at their promoter regions [36]. Moreover, in addition to up-regulating the expression of certain demethylases, hypoxia also appears to inhibit them. Hypoxic conditions resulted in an elevation of H3K4me3 through the inhibition of JARID1A dimethylation activity, both on a global (Fig. 2) and gene specific level in lung epithelial cells. The knock down of JARID1A increased H3K4me3 at the promoters of *HMOX1* and *DAF* genes [37].

Inhibition of Jumonji histone demethylases, JMJD1A-C (KDM3A-C), and JMJD2A-D (KDM4A-D), with hypoxia, as well as with the treatment of dioxygenase-inhibitors, such as DMOG (N-(methoxyoxoacetyl)-glycinemethyl ester, DETA-NO (2,20-(hydroxynitrosohydrazono)bis-ethanimine) and ROS, resulted in increased methylation levels of H3K9me2/me as well as H3K36me3. An increase of H3K9me2 and H3K9me3 was markedly apparent at the promoter regions of chemokine Ccl2 and the chemokine receptors Ccr1 and Ccr5 [38]. Hypoxia and nickel exposure also increased the level of H3K9me2 at the promoter of *SPRY2*, a key regulator of receptor tyrosine kinase/extracellular signal-regulated kinase (ERK), by inhibiting JMJD1A [39]. While, elevated levels of H3K9me2 at the promoter regions of *MHL1* and *DHFR* genes correlated with the repression of these two genes during hypoxic stress, indicating that the hypoxia-induced H3K9me2 might play an important role in gene silencing during tumor progression [40]. Furthermore, hypoxia and hypoxic mimetics, such as deferoxamine (DFX) and dimethyloxalylglycine, increased the protein and enzymatic activity of G9a, a histone 3 lysine 9 methyltransferase, as well as inhibited the histone demethylation processes, resulting in increasing global levels of this histone mark [40]. Up regulation of G9a by hypoxia also resulted in an increase of H3K9me2 at the promoter region of neprilysin (*NEP*), an enzyme important in the degradation of amyloid b-peptide (Ab) plaques [41].

Nickel

Nickel (Ni) is one of the most abundant elements in the earth's crust amounting to roughly 3% of the earth's composition. Hundreds of metric tons of nickel are released into the environment from natural (i.e. volcanic dusts and rock weathering) and anthropogenic sources (i.e. fossil fuel combustion, and industrial use/disposal of nickel compounds and alloys) [42, 43]. Exposure to nickel can occur via several different pathways: ingestion, dermal contact, and inhalation. In addition to being abundant, nickel compounds are also carcinogenic and have been implicated in the occurrence of a number of human cancers [44–48]. However, nickel, which is a hypoxia mimetic, is either a non- or weakly-mutagenic [11, 12]. A number of studies have suggested that nickel-induced carcinogenesis is not a consequence of its mutagenicity, but rather of its ability to alter chromatin structure and induce epigenetic effects [49]. Exposure to nickel can result in the distortion of a number of different post-translationally modified histone marks. However, for the purpose of this review only the methylation marks will be discussed. A non-toxic dose of nickel was shown to localize and significantly increase global levels of H3K4me3 and H3K9me2 (Fig. 3) [50]. Nickel ion exposure also resulted in an increase of H3K9me2 levels in A549 lung carcinoma cells [51]. Moreover, acute Ni ion exposure also resulted in an increase in global H3K9me1 and H3K9me2, both of which are critical marks for DNA methylation and long-term gene silencing, in several different cell lines [52, 53]. Moreover, recently it has been demonstrated that nickel exposure could alter the global levels of post-translational histone modifications in a human population. Global levels of H3K4me3 were found to be elevated and H3K9me2 were decreased in nickel exposed refinery workers. Furthermore, results indicated that temporal variability within individuals was relatively small, compared to variability between subjects, suggesting that global H3K4me3 and H3K9me2 histone modifications are relatively stable over time in human peripheral blood mononuclear cells (PBMC)s from both nickel-exposed and referent subjects [54]. In steel workers, global levels of H3K4me2 were also positively correlated with particulate matter (PM) exposure, which contained nickel and arsenic, [55].

With respect to the mechanisms of the nickel-driven changes in histone methylation levels, it appears that interference/inhibition of the iron- and 2-oxoglutarate- dependent dioxygenase family of enzymes is the likely mechanism. The JmjC domain containing histone demethylases have a His/His/carboxylic acid facial triad that binds iron in their

catalytic center [56]. Nickel was shown to selectively inhibit this family of enzymes, because it is able to displace the iron that is bound to the active site of this enzyme [30, 57]. Nickel has about 3 times higher affinity for the iron binding site than iron itself. Exposure to nickel ions resulted in an increase of H3K9me2 via inhibition of the histone demethylation process (Fig. 4) [53]. Furthermore, JHDM2A/JMJD1A (KDM3A) and ABH3, human homologues of bacterial DNA repair enzymes Alkb (ABH1-9), activities were highly sensitive to nickel inhibition [32, 39]. Once bound, the nickel ions replaced the ferrous iron in the catalytic center of these enzymes and inhibited their activity in intact cells [58]. The use of purified JHDM2A and ABH3 recombinant proteins demonstrated that in the presence of iron, nickel was able to inhibit the demethylase activities of both JHDM2A and 69His-ABH3 in a dose-dependent manner. Nickel was also shown to preferentially bind to ABH2 with a dissociation constant of 1.7 μM , compared to that of 4.5 μM for iron. Furthermore, x-ray absorption spectroscopy studies have shown that nickel binds to the dioxygenases at the same sites as iron, but unlike iron, nickel assumes a 6 coordinate geometry preventing oxygen binding, while iron binds with a 5 coordinate geometry, allowing -oxygen to bind [59]. The consequences of nickel's ability to interact with the active sites of these enzymes and inhibit their activity can, as in the case of JHDM2A/JMJD1A, result in the accumulation of the silencing mark H3K9me2 in promoters of genes and loss of their transcription [39, 53].

Arsenic

Arsenic (As) is one of the most widely distributed and pervasive biological toxicants in the world. It is a naturally occurring toxin, but is also released into the environment through anthropogenic sources such as mining, smelting of ores, burning of fossil fuels, and industrial applications, including glass manufacturing and strengthening of alloys [60]. Given its natural abundance and industrial use, arsenic's environmental impact is felt by nearly 150 million people in at least 70 countries [61]. Arsenic has been identified as a causal agent in human skin, lung, and bladder cancers and some epidemiological studies have also implicated As in the development of liver and prostate cancers [62–67]. Furthermore, arsenic exposure has also been associated with non-carcinogenic health outcomes, including cardiovascular disease, neurologic deficits, neuro-developmental deficits in childhood, and hypertension [68–72]. Arsenic is an excellent generator of oxidative stress. It does so by cycling between its oxidation states or by interfering with antioxidants and increasing inflammation, resulting in the accumulation of free radicals in cells. Methylated arsenic species can also release redox-active iron from ferritin. Free iron plays a central role in generating harmful oxygen species by promoting the conversion of superoxide and hydrogen peroxide to the highly reactive hydroxyl radical through the Haber–Weiss reaction [8]. Major arsenic-induced ROS include superoxide anion ($\text{O}_2^{\bullet-}$), hydroxyl radical ($\bullet\text{OH}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1\text{O}_2$) [8]. Although arsenic produces large amounts of ROS, it is a very poor mutagen [73]. A substantial body of evidence has accumulated proposing that the mechanism(s) of arsenic induced carcinogenesis may stem from its ability to interfere with or disrupt epigenetic homeostasis [49]. One can further postulate that the observed disturbance of epigenetic homeostasis may be linked to arsenic-induced oxidative stress.

Altered histone methylation patterns were observed in arsenic-transformed human urothelial cells. The transformed cells experienced a loss of H3K27me3 and H3K9me2, both of which are marks of transcriptional repression, and an increase in H3K4me2 in the promoter region of *WNT5A* gene, which is important in cellular development and differentiation and is often improperly regulated in numerous cancers [74]. In human lung carcinoma A549 cells, exposure to inorganic trivalent As (arsenite) increased H3K9me2 and decreased H3K27me3, while increasing the global levels of H3K4me3, a gene-activating mark. The increase in

H3K9me2 was mediated by an increase in the histone methyltransferase G9a protein and messenger RNA levels (Fig. 5) [75]. Moreover, sodium arsenite treatment also resulted in a significant increase in H3K4me3 after 24-hour or 7 day exposure in A549 cells, which was maintained a week after the removal of arsenite, suggesting that this epigenetic effect was inherited through cell division [76]. Arsenic trioxide (As₂O₃) treatment also affected the levels of another histone H3 lysine 9 (H3K9)-specific methyltransferase, Setdb1/Eset. Specifically, arsenic treatment, which induces the degradation of promyelocytic leukemia (Pml) protein, resulted in the disappearance of Sedb1 signals from the nuclei of mouse embryos [77].

Chromium

Hexavalent chromium (Cr(VI)) compounds are well established human carcinogens [78–80]. The toxic and carcinogenic properties of hexavalent chromium originate from its chemical reactivity and cellular uptake. Like arsenic, it is a very potent generator of oxidative stress. Upon entering the body, Cr(VI) is readily absorbed by a number of different tissues due to the fact that the chromate anion (CrO₄²⁻) structurally resembles a phosphate ion (PO₄²⁻) and is erroneously transported across the cellular membrane through the anion sulphate/phosphate uptake channels. Once inside the cell, Cr(VI) undergoes a sequence of reducing reactions, producing two unstable and highly reactive intermediates, Cr(V) and Cr(IV), and is then reduced to Cr(III), a stable species that is formed at high levels inside the cell and has a high binding-affinity to cellular ligands. Cr(V) is formed when glutathione is the reductant, whereas Cr(IV) is reduced by two electron reduction when ascorbate is the reductant. The reduction of hexavalent chromium via Fenton-like and/or Haber-Weiss reactions leads to the generation of reactive oxygen species (ROS) and can cause substantial cellular damage [81].

Until recently, DNA damage (i.e. double- and single-stranded break(s), DNA-protein, DNA intra-strand cross links, Cr adducts, and oxidized nucleotide bases), genomic instability (i.e. centrosome abnormalities, aneuploidy, and microsatellite instability), and gene expression changes (i.e. repression of *hMHL1*, a DNA mismatch repair gene) were considered to be the underlying mechanisms of Cr(VI) carcinogenesis [82–84]. However, an emerging body of evidence is suggesting that epigenetic effects may in part be responsible for the genotoxicity and carcinogenicity of Cr(VI). Dysregulation of post-translational histone modifications, in particular, appears to play an important role in Cr(VI) induced carcinogenesis. Short-term treatment of A549 cells with Cr(VI) significantly increased the levels of H3K4me3 [50]. Furthermore, Cr(VI) exposure of human lung A549 cells resulted in an increase in the global levels of H3K9me2 and H3K4me3, but decreased the levels of H3K27me3 and dimethylated histone H3 arginine 2 (H3R2me2). Most interestingly, the observed H3K9 dimethylation was enriched in the human *MLH1* gene promoter and was correlated with decreased *MLH1* mRNA expression. In addition, chromium exposure also increased both the protein and mRNA levels of the histone methyltransferase, G9a, which specifically methylates H3K9. Interestingly, supplementing cells with ascorbate, the primary reductant of hexavalent chromium and an essential cofactor for the histone demethylase activity, partially reversed the observed H3K9me2, suggesting that Cr(VI) may deplete reduced ascorbic acid, thereby inhibiting histone demethylation (Fig. 6) and consequently affecting global and promoter specific histone methylation and silencing of tumor suppressor genes, such as *MLH1* [85].

Conclusion

Epigenetic modifications are indispensable drivers of many cellular components and processes, including development, differentiation, chromatin architecture, gene expression,

and X chromosome inactivation [18, 20–22, 86]. Disruption of their homeostasis can result in numerous detrimental consequences, such as genomic instability, altered gene expression, and ultimately carcinogenesis [17, 21, 87]. The reviewed literature suggests that although, oxidative stress is not the only process that can induce epigenetic dysregulation, it is a formidable one. Oxygen is an important cofactor in the activities of the dioxygenase enzymes and its concentration can markedly affect the levels of histone methylation, both on a global and gene specific level, thereby altering gene expression. The availability of oxygen to mammalian species is so essential that it makes sense that there are direct links to gene expression regulation for different levels of oxygen tension. The Jumonji C domain containing demethylases appear to be particularly vulnerable to changes in oxygen levels (i.e. hypoxia) and carcinogenic metals, such as nickel, chromium and possibly arsenic. Nickel's ability to replace the iron from its catalytic center and chromium and/or arsenic's ability to affect the ascorbate levels could attenuate the demethylase enzymatic activity in cells, resulting in the accumulation of the histone marks in promoters of genes that could either repress or activate their transcription. Furthermore, hypoxia and/or carcinogenic metals also appear to influence histone methyltransferase activity in cells, thereby also affecting the methylation status of histones. Although the effect of oxidative stress on epigenetic dysregulation is already being explored, as evidenced by the literature reviewed here, future research should aim to further elucidate the exact mechanisms by which oxidative stress alters epigenetic homeostasis and explore the biological and clinical consequence associated with it.

Acknowledgments

This work was supported by grants ES000260, ES010344, ES014454, and ES005512.

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Highlights

- Oxidative stress-induced epigenetic dysregulation alters gene expression.
- Carcinogenic metals can induce oxidative stress and/or mimic hypoxia.
- Histone modifying enzymes are affected by metal exposure.
- Post-translational methylation of histone tails influences expression of genes.

Figure 1A

Figure 1B

Figure 1.

Figure 1A: Model for the demethylation of lysine residues by FAD-dependent amine oxidases.

Figure 1B: Model for iron and 2-oxoglutarate-dependent dioxygenase demethylation of lysines.

Figure 2.

A, Beas-2B cells were seeded with DMEM complete medium. The following day, cells were pre-incubated with complete DMEM or methionine-deficient DMEM for 4 h, and were placed under hypoxic conditions for 24 h. Histones were extracted and immunoblotted with anti- H3K4me3 antibody. As loading control, the gel was stained with coomassie blue. The results were repeated with additional independent experiments; one representative blot is shown here. B, Hypoxia inhibited the activity of histone H3K4 demethylase *in vitro*. The histone H3K4 demethylation assay was performed as described in Materials and Methods. The reaction mixture was incubated on ice, at 37°C in normoxia, or hypoxia (1% oxygen) and in the presence of 1 mM deferoxamine, DFX, overnight. The same membrane was stripped and re-blotted with H3 antibody to verify the loading. Each condition was used in duplicate. The intensity of the bands was quantified, and values were normalized to the samples that were incubated on ice and were plotted in the graph. Standard deviation is represented with error bars.

Figure 3.

Distinct localization of H3K9 di-methylation (H3K9me2) and H3K4 tri-methylation (H3K4me3) in nickel-exposed cells. A549 cells were exposed to 1mM NiCl₂ for 24 h. After exposure, cells were co-stained with di-methylated H3K9 (red) and tri-methylated H3K4 (green) antibodies. The nucleus was counterstained with DAPI (blue). Merge images show a merge of the red, green and blue staining. The pictures were taken using a confocal microscope. From “Effects of nickel, chromate, and arsenite on histone 3 lysine methylation” vol. 236(1) by Zhou X. et al. Copyright 2009 Toxicology and Applied Pharmacology. Reproduced with permission of Toxicology and Applied Pharmacology via Copyright Clearance Center.

Figure 4.

Nickel ions increased H3K9 dimethylation by inhibiting the histone demethylation process. (a) A549 cells were seeded with F-12-K complete medium. On the second day, cells were replenished with complete DMEM or methionine-deficient DMEM. After incubation for 4 h, cells were then exposed to 1 mM NiCl₂ for 24 h. Histones were extracted and immunoblotted for dimethyl-H3K9. BT represents the histones extracted from A549 cells in DMEM before the administration of NiCl₂ treatment. (b) Nickel ions decreased the removal rate of histone methylation. A549 cells were radiolabeled with L-[methyl-3H] methionine in methionine-deficient DMEM for 24 h and then replenished with F-12-K complete medium supplemented with 1 mM hydroxylurea. After incubation for 24 h, cells were exposed to 1 mM NiCl₂ for an additional 48 h in the presence of hydroxylurea. Histones were extracted, and the radioactivity was measured with a scintillation counter. The experiment was conducted in triplicate. From “Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing” by Chen H. et al. vol. 26(10) Copyright 2006 Molecular and Cellular Biology. Reproduced with permission of Molecular and Cellular Biology via Copyright Clearance Center.

Figure 5.

Arsenite-induced histone methyltransferase G9a expression. A549 cells were treated with arsenite (2.5, 5, and 10 μ M) for 24 h. A set of representative results is shown from three independent experiments. (A) G9a and JHDM2A protein levels were analyzed by western blotting with antibodies as described in the Materials and Methods. The same membrane was reblotted with α -tubulin to assess protein loading. (B) Arsenite exposure increases G9a mRNA in A549 cells. Total RNA was extracted and subjected to northern blotting. The bottom panel shows the total RNA in the formaldehyde-agarose gels detected by ethidium bromide staining. The numbers below the figure represent the relative intensity of the bands. (C) Increased level of G9a in As-treated A549 cells. A549 cells were treated with arsenite at different concentrations for 24h, followed by immunofluorescent staining with G9a antibody as described in Materials and Methods. From "Arsenite alters global histone H3 methylation" by Zhou X. et al. vol. 29(9). Copyright 2008 Carcinogenesis. Reproduced with permission of Carcinogenesis via Copyright Clearance Center

Figure 6.

JHDM2A demethylated H3K9me2 in an ascorbate-dependent manner. Purified recombinant Flag-JHDM2A was incubated with core histones in a buffer containing 100 μM Fe^{3+} , 1 mM 2-oxoglutarate, and increased concentrations of ascorbate as indicated. After reaction, the loss of H3K9me2 in histones was assessed using Western blot. The same membrane was blotted with anti-histone H3 antibody to assess the loading of histones. The bottom figure shows the amount of recombinant Flag-JHDM2A added into each reaction. From "Modulation of histone methylation and MLH1 gene silencing by hexavalent chromium" vol. 237(3) by Sun et al. Copyright 2009 Toxicology and Applied Pharmacology. Reproduced with permission of Toxicology and Applied Pharmacology via Copyright Clearance Center.