REVIEW
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Redox and metabolic regulation of epigenetic modifications: an emerging toxic action mechanism

Wan-Qian Guo[1, 2], Wu-Sheng Xiao[1, 2], Wei-Dong Hao[1, 2*]

[1]Department of Toxicology, School of Public Health, Peking University, Beijing 100191, China. [2]Beijing Key Laboratory of Toxicological Research and Risk Assessment for Food Safety, Beijing 100191, China.

***Corresponding to:** Wei-Dong Hao. Department of Toxicology, School of Public Health, Peking University, 38 Xueyuan Road, Haidian District, Beijing 100191, China. E-mail: whao@bjmu.edu.cn.

Author contributions

Wan-Qian Guo: Conceptualization, Methodology, Data collection, Writing-original draft. Wu-Sheng Xiao: Conceptualization, Methodology, Data collection, Writing-Reviewing and Editing, Funding acquisition. Wei-Dong Hao: Conceptualization, Methodology, Writing-Reviewing and Editing, Supervision, Project administration, Funding acquisition.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

2-OGDH, 2-OG dehydrogenase; 5caC, 5-carboxyl cytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; 5-meTHF, 5-methyl-tetrahydrofolate; ACL, CAT, catalase; DEHP, di-2-ethylhexyl phthalate; DNMT, DNA methyltransferase; FAD, flavin adenine dinucleotide; GLUD, glutamate dehydrogenase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; HAT, histone acetyltransferase; HCY, homocysteine; HDAC, histone deacetylase; HMT, histone methyltransferase; IDH, isocitrate dehydrogenase; JmjC, Jumonji domain containing protein; LDH, lactate dehydrogenase; LSD, lysine-specific demethylase; MAT, methionine adenosyltransferase; MDH, malate dehydrogenase; MEHP, mono-(2-ethylhexyl) phthalate; MS, methionine synthase; NADH, reduced NAD^+; $NADP^+$, phosphorylated NAD^+; NADPH, reduced $NADP^+$; NAM, nicotinamide; NOX, NAD(P)H oxidase; NRF2, nuclear factor erythroid 2-related factor 2; OAADPr, 2′-O-acetyl-ADP-ribose; PAH, polycyclic aromatic hydrocarbon; PARP, poly (ADP)-ribosyl polymerase; PCB, polychlorinated biphenyl; PDH, pyruvate dehydrogenase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAHH, SAH hydrolase; SAM, S-adenosylmethionine; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TCA, tricarboxylic acid; TET, ten-eleven translocation demethylase; THF, tetrahydrofolate; TR, thioredoxin reductase.

Citation

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Abstract

Epigenetic modifications modulate conformational structure of chromatin and consequently gene expression by enzyme-mediated chemical modifications of DNA and histones. The activities of epigenetic modifying enzymes depend on many co-substrates and cofactors, such as 2-oxoglutarate (2-OG), iron, S-adenosylmethionine (SAM), nicotinamide adenine dinucleotide (NAD^+), flavin adenine dinucleotide (FAD), and acetyl-CoA. These factors are inter-connecting molecules that integrate cellular nutrient metabolism and redox homeostasis, two key regulators of cell proliferation, cell survival, and cell functions. Dysregulation of such delicate regulatory network has been implicated in many pathological conditions and also been increasingly recognized as an emerging mechanism responsible for environmental pollutant-induced adverse effects. In this review, we first summarize DNA and histone modifying enzymes and their essential factors, then discuss the metabolic sources and the redox regulatory roles of these enzymatic factors, and finally elaborate the mechanisms of how targeting such factors by environmental pollutants influences epigenetic regulation and perturbs cellular functions.

Keywords: epigenetic modifications; redox state; cellular metabolism

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Introduction

Epigenetic modifications, including mainly DNA methylation and histone modifications (e.g. methylation and acetylation), are the principal regulatory machinery that provide mammalian cells with pleiotropic plasticity to form organ systems during development and with adaptive capability to respond to environmental stress. Establishment of such epigenetic landscape depends on epigenetic code "writer" and "eraser" enzymes, such as DNA and histone methyltransferases and demethylases, histone acetylases and deacetylases. Their enzymatic activities and functions require many co-substrates and cofactors that are commonly shared by cellular redox couples and energy metabolism. For instance, the tricarboxylic acid (TCA) cycle intermediary metabolite 2-oxoglutarate (2-OG) is an essential co-substrate for DNA and histone demethylases. Nicotinamide adenine dinucleotide (NAD^+) is the precursor of redox molecules reduced NAD^+ (NADH), phosphorylated NAD^+ ($NADP^+$), and its reduced form (NADPH), and importantly is an indispensable cofactor for histone deacetylase Sirtuin proteins (SIRT1-7) [1]. Epigenetic modifications, cellular redox regulators, and nutrient metabolites thus form an intricate and elaborate network, which together dictates cellular gene expression and biological functions. As expected, dysregulation of such delicate regulatory network has been implicated in many disease conditions, such as diabetes, cardiovascular diseases, neurological diseases, and cancer [2–4], and in environmental pollutant-induced adverse effects [5]. A better understanding on such inter-regulatory mechanism helps to develop preventive and therapeutic strategies for various pathological and poisoning conditions. In this review, we highlight the principal roles of redox and metabolic regulations of epigenetics in modulating toxic effects induced by environmental pollutants.

Epigenetic modification enzymes

In eukaryotic cells, DNA is packaged into chromatin by histones, forming a compact spiral structure. A succession of DNA events, such as replication, transcription, repair, and recombination, all require an open structural conformation for the access of transcription factors and transcriptional coregulators. The mechanism mediating the transition of chromatin structure from a compact state to an open one is the central focus of epigenetic regulation. Such structural transformation of chromatin is primarily controlled by enzyme-catalyzed chemical modifications of DNA and histones.

DNA methylation enzymes

DNA methylation primarily occurs at CpG dinucleotide sites and is functionally transcriptional repression [6]. DNA methyltransferases (DNMTs) transfer the methyl group from its donor S-adenosylmethionine (SAM) to the 5^{th} carbon position of cytosine (C) forming 5-methylcytosine (5mC) and S-adenosylhomocysteine (SAH) (Figure 1) [7]. SAH is known as a potent inhibitor of DNMTs, serving as a negative feedback mechanism for DNA methylation [8].

The establishment of DNA methylation is catalyzed by de novo methyltransferases DNMT3A, DNMT3B, and DNMT3L [9]; and the maintenance of DNA methylation is mediated by DNMT1, which specifically recognizes the established semi-methylated CpG dinucleotides *via* its partner protein ubiquitin-like ring finger domains (UHRF1) [10]. The methyl group on methylated DNA can be removed by two mechanisms: passive demethylation and active demethylation (Figure 1). The former is a passive dilution process due to the absence of DNMT1, while the latter is mediated by ten-eleven translocation demethylases (TET1-3). TET enzymes oxidize 5mC to 5-hydroxymethylcytosine (5hmC), sequentially to 5-formylcytosine (5fC) and 5-carboxyl cytosine (5caC), all of which are poorly recognized by DNMT1 leading to a gradual loss of methylation during subsequent replication process [7]. The resulting 5fC and 5caC can also be converted back to cytosine through base excision repair by thymine-DNA glycosylase (TDG) [11].

Of note, these TET enzymes belong to the ferrous iron (Fe^{2+}) and 2-OG-dependent dioxygenase (2-OGDD) superfamily and require oxygen (O_2) and ascorbate as cofactors. In demethylation reactions, one oxygen molecule is used to produce a highly reactive ferryl-oxo species leading to hydroxylation of the methyl group on 5mC and oxidation of Fe^{2+} into ferric iron (Fe^{3+}); the second oxygen molecule is utilized for oxidative decarboxylation of 2-OG into succinate and CO_2 [12]. Ascorbate is required for reducing inactive Fe^{3+} back to its active Fe^{2+} form of TET enzymes (Figure 1). It is worth noting that 2-OG metabolites succinate, fumarate, and 2-hydroxyglutarate (2-HG) can compete with 2-OG for its binding sites due to structural similarity, and consequently inhibit 2-OGDD activity including TETs [13, 14]. Thus, changes in gene expression of these enzymes and the levels of their activity regulators are anticipated to affect DNA methylation patterns.

Histone modification enzymes

Histone modifications mostly at lysine (K) residues regulate gene transcription by alteration of chromatin accessibility. Trimethylation at K4 and K6 of histone H3 (H3K4me3 and H3K6me3) and acetylation at K27 of histone H3 (H3K27ac) typically activate gene transcription (also known as euchromatin modifications). Oppositely, trimethylation at K9 and K27 of histone H3 (H3K9me3 and H3K27me3) are often transcriptional inhibitory (also known as heterochromatin modifications) (Figure 1).

Similar to DNA methylation, histone methylation is mediated by histone methyltransferases (HMTs) and requires SAM as methyl group donor. Each HMT enzyme catalyzes methylation reaction at specific residues depending upon the conformation and accessibility of their substrate binding pockets [4]. For example, PR/SET domain 9 methyltransferase specifically catalyzes H3K4 trimethylation in germ cells [15].

Histone demethylases mainly include Jumonji domain containing proteins (JmjCs) and lysine-specific demethylases (LSDs) proteins (Figure 1). Like TET family, JmjC proteins belong to the 2-OGDD superfamily and require Fe^{2+} and 2-OG to demethylate mono-, di- and trimethylated K and arginine residues on histones [16]. By contrast, the LSD family proteins are flavin adenine dinucleotide (FAD)-dependent amine oxidases, which catalyze a two-electron oxidation of the C-N methylamine bond to form an imine group and a FADH2 molecule. The unstable imine group then quickly reacts with water, forming formaldehyde in combination with removal of the methyl group. The product $FADH_2$ is then oxidized to FAD by O_2 generating H_2O_2 as byproducts [17]. It should be noted that this process requires free electron pairs at methylated K residues and only mono- and di-methylated but not trimethylated histones are removable by LSD enzymes [18], indicating substrate selectivity of LSD-catalyzed demethylation.

Histone acetylation is coordinately regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Figure 1). CREB binding protein and p300 are two crucial HATs, which transfer an acetyl group from acetyl-CoA to K residues at histone tails and thus activate gene transcription [19]. The HDAC family includes class I (HDAC1, 2, 3 and 8), II (HDAC4, 5, 7 and 9), IIB (HDAC6 and 10), and IV (HDAC11), all of which utilize a redox-active metal (Zn^{2+} or Fe^{2+}) to catalyze hydrolysis of acetate on K residues. By contrast, the class III HDACs, or SIRTs, are solely NAD^+-dependent deacetylases [20]. NAD^+ reacts with acetyl group at K residues forming nicotinamide (NAM) and 2'-O-acetyl-ADP-ribose (OAADPr) [21]. Thus, cellular NAD^+ levels determine SIRT enzymatic activity and thereby histone acetylation levels.

Redox and metabolic regulation of epigenetic factors

The above-mentioned enzymatic co-substrates and cofactors are essentially either nutrient metabolites (e.g. NAD^+ and 2-OG) or derivatives of metabolic intermediates (e.g. SAM and acetyl-CoA), highlighting the integration of cellular metabolism and epigenetic modifications. Moreover, as we recently reviewed [22, 23], NAD^+ and its derivatives NADH, $NADP^+$, and NADPH are the principal redox

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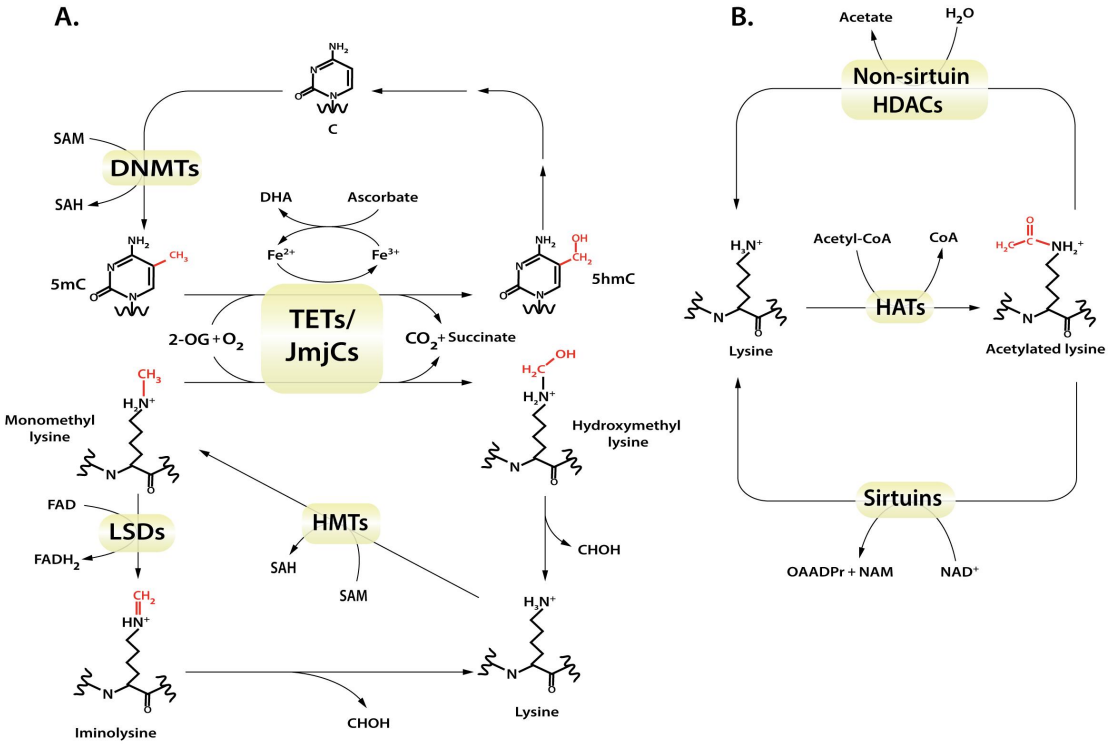

Figure 1 Epigenetic modifications. (A) DNA and histone methylation are mediated by DNMTs and HMTs using SAM as methyl group donor, respectively. Active demethylation of DNA is mainly catalyzed by TET proteins while histone demethylation is mediated by JmjC and LSD proteins. TETs and JmjC are Fe^{2+}- and 2-OG-dependent, while LSDs are FAD-dependent. **(B)** Histone lysine residues are acetylated by HATs using acetyl-CoA as acetyl moiety donor and then deacetylated by NAD^+-dependent Sirtuins and non-Sirtuin HDACs. 2-OG, 2-oxoglutarate; 5caC, 5-carboxyl cytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; DHA, dehydro-ascorbate; DNMTs, DNA methyltransferases; FAD, flavin adenine dinucleotide; FADH2, reduced FAD; HATs, histone acetyltransferases; HDACs, histone deacetylases; HMTs, histone methyltransferases; JmjC, Jumonji-domain containing proteins; LSDs, lysine-specific demethylases; NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; OAADPr, 2'-O-acetyl-ADP-ribose; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; TETs, ten-eleven translocation demethylases.

regulators, and their intracellular levels are closely modulated by many metabolic enzymes; the levels of reduced glutathione (GSH) and GSH disulfide (GSSG), another key redox regulatory couple, are also fine-tuned by metabolic activity and enzymes [22, 23]. Thus, cellular redox couples and energy metabolism are expected to significantly influence epigenetic enzymatic activity and modifications, and as a consequence govern various cellular functions and biological processes.

Metabolic sources and redox regulation of 2-OG

2-oxoglutarate (2-OG) is a five-carbon weak acid with two carboxyl groups and one carbonyl group. 2-OG is produced by oxidative decarboxylation of isocitrate via $NAD(P)^+$-dependent isocitrate dehydrogenases (IDH2-3) coupled with NAD(P)H generation in the TCA cycle, where it is further metabolized by NAD^+-dependent 2-OG dehydrogenase (2-OGDH) forming succinyl-CoA and NADH [22–24]. In addition, $NAD(P)^+$-dependent glutamate dehydrogenases (GLUD1-2) catalyze an oxidative deamination reaction of glutamate to generate 2-OG and NAD(P)H (Figure 2).

Besides as an intermediary metabolite of the TCA cycle, 2-OG is also a sensor and regulator of intracellular redox state. As a ketoacid, 2-OG directly reacts with H_2O_2 to produce succinate and CO_2, supporting its capability to counteract oxidative stress [25]. Indeed, 2-OG was shown to prevent lipid peroxidation and oxidative injuries in rats by enhancing the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) [26]. Furthermore, the 2-OGDH enzyme is sensitive to oxidative inhibition by reactive oxygen species (ROS), leading to suppression of 2-OGDH and thereby alterations of metabolic fates and cellular levels of 2-OG [27]. Together, these lines of evidence support that 2-OG is a key connector between cellular redox homeostasis and energy metabolism.

Metabolic sources and redox connection of SAM

SAM, the methyl donor for DNA and histone methylation, is a key metabolite of one-carbon metabolism, which links methionine cycle and folate cycle (Figure 2) [28]. In the methionine cycle, methionine adenosyltransferase (MAT) transfers the adenosyl moiety from ATP to methionine forming SAM, which then donates its methyl group to DNA and histones by methyltransferases and generates SAH [29]. The latter is further metabolized into homocysteine (HCY) catalyzed by NAD^+-dependent SAH hydrolase (SAHH). HCY can gain a methyl group from 5-methyl-tetrahydrofolate (5-meTHF) or betaine to regenerate methionine in the folate cycle under the catalysis of methionine synthase or betaine-homocysteine methyltransferase, respectively. Alternatively, HCY is shunted into the transsulfuration pathway forming cystathionine and then cysteine, an essential precursor amino acid for GSH biosynthesis [30].

Depletion of SAM was shown to induce oxidative stress by lowering GSH levels [28]. However, addition of SAM enhanced the transcriptional activity of nuclear factor erythroid 2-related factor 2 (NRF2), a known master regulator of oxidative stress response whose activation upregulates the transcription of many antioxidant enzymes and enhances GSH biosynthesis [31]. Therefore, SAM also links cellular redox niche with energy metabolism.

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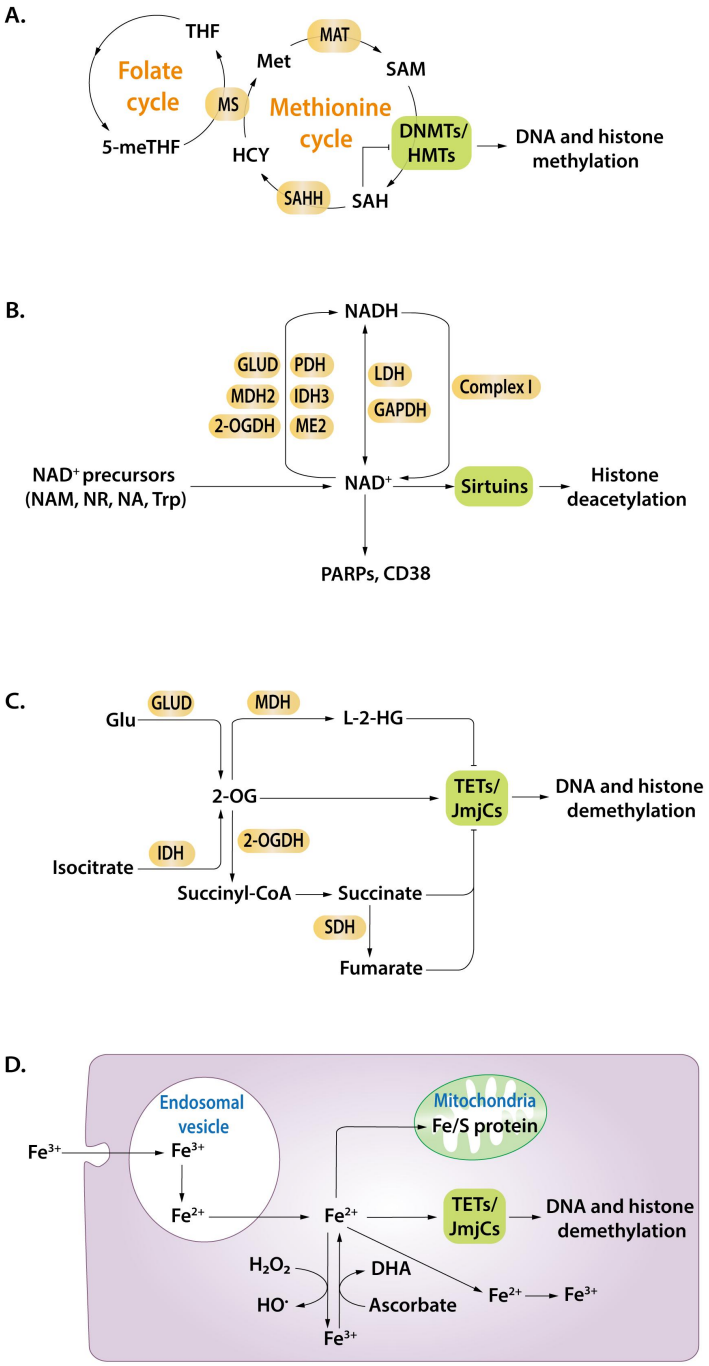

Figure 2 Inter-regulation of energy metabolism and epigenetic modifications. (A) Folate cycle and methionine cycle cooperatively maintain cellular SAM production and thereby DNA and histone methylation. SAH negatively modulates methyltransferase activity. **(B)** NAD^+ metabolic network including its biosynthesis, recycling, and consumption determines cellular NAD^+ levels and thus controls NAD^+-dependent Sirtuin deacetylase activity and histone deacetylation. **(C)** 2-OG generated by isocitrate and glutamate (Glu) is an essential co-substrate of DNA and histone demethylase TET and JmjC proteins. The 2-OG derivatives L-2-HG, succinate, and fumarate inhibit the activity of TETs and JmjCs through competitive inhibition of 2-OG binding due to structural similarity. **(D)** Iron homeostasis regulatory network including iron intake, transport, oxidation and reduction, storage, and utilization coordinately regulates DNA and histone demethylation through Fe^{2+}-dependent TETs and JmjCs. 2-OGDH, 2-oxoglutarate dehydrogenase; 5-meTHF, 5-methyltetrahydrofolate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUD, glutamate dehydrogenase; HCY, homocysteine; $HO^{\cdot}$, hydroxyl radical; IDH: isocitrate dehydrogenase; L-2-HG, L-2-hydroxyglutarate; LDH, lactate dehydrogenase; MAT, methionine adenosyltransferase; MDH, malate dehydrogenase; ME, malic enzyme; Met, methionine; MS, methionine synthase; NA, nicotinic acid; NADH, reduced NAD^+; $NADP^+$, phosphorylated NAD^+; NAM, nicotinamide; NR, nicotinamide ribose; OAA, oxaloacetate; PARP, poly (ADP)-ribosyl polymerase; PDH, pyruvate dehydrogenase; SAHH, S-adenosinehomocysteine hydrolase; SDH, succinate dehydrogenase; STEAP3, six-transmembrane epithelial antigen of prostate 3; THF, tetrahydrofolate; Trp, tryptophan.

Metabolic sources and redox regulation of NAD(H) and NADP(H)

In mammals, NAD^+ is synthesized by three pathways: the de novo pathway, the Preiss-Handler pathway, and the salvage pathway, among which the third pathway is the most efficient and principal route for maintaining cellular NAD^+ levels (as reviewed in details by Xiao et al [22]). Amino acid tryptophan is the precursor for de novo

synthesis of NAD^+ through multiple enzymatic reactions, where tryptophan 2,3-dioxygenase catalyzes the rate-limiting step [22, 32]. The Preiss-Handler pathway uses nicotinic acid (NA), and the salvage pathway utilizes NAM and nicotinamide riboside (NR) as the precursor(s) for NAD^+ biosynthesis [22, 33]. The phosphorylation of NAD^+ by NAD^+ kinases produces $NADP^+$, an important determinant of cellular $NADP^+$ levels [22]. Of note, the recycling and interconversion of NAD^+, NADH, $NADP^+$, and NADPH are mediated by multiple enzymes in glycolysis, the TCA cycle, and the pentose phosphate pathway (PPP) and by mitochondrial nicotinamide nucleotide transhydrogenase as detailed in our recent reviews (Figure 3) [22, 23].

The $NADP^+$/NADPH and NAD^+/NADH redox couples are crucial determinants of cellular redox homeostasis. Specifically, $NADP^+$ and NADPH are essential cofactors for neutralization of H_2O_2 and organic peroxides by GPXs and peroxiredoxins (PRXs) using GSH and reduced thioredoxins [TRX-$(SH)_2$] as co-substrates, respectively [22, 23]. The resultant GSSG and oxidized thioredoxins (Trx-S_2) are then recycled back their reduced forms by their corresponding enzymes glutathione reductase (GR) and thioredoxin reductase (TR) in conjunction with the oxidation of NADPH into $NADP^+$ (Figure 3), supporting their indispensable roles in detoxifying ROS [22, 23].

However, excessive levels of NADH and NADPH paradoxically lead to reductive stress, a state was defined as an excess accumulation of reducing equivalents (specifically NADH, NADPH, and GSH), exceeding the capacity of endogenous oxidoreductases [23]. This is evidenced by the facts that NADH and NADPH provide NAD(P)H oxidases (NOXs) with electrons to generate superoxide anions ($O2^{\cdot-}$) and H_2O_2 [34]. An increase of NADH also led to an elevated ROS production by complex I (NADH: ubiquinone oxidoreductase) [35, 36]. Thus, cellular metabolic activities are required to maintain the levels of NAD^+/NADH and $NADP^+$/NADPH redox couples at a delicate equilibrium condition. When such balance is disturbed, oxidative stress or reductive stress (collectively as redox stress) occurs.

Metabolic sources and redox functions of iron

Iron, a human essential trace element, enters the circulation from digestive tracts and is then transported by transferrin in its Fe^{3+} form [37]. Fe^{3+} is recognized by transferrin receptor on cell membrane, and then taken up via endocytosis, forming Fe^{3+}-containing endosomal vesicles (Figure 3). Subsequently, Fe^{3+} is reduced to Fe^{2+} by ferrireductase STEAP3 [38], and exported to the cytoplasm through solute carrier family 11 member 2 [39]. Fe^{2+} is critical for many metabolic processes, such as oxygen transport, energy metabolism, and assembly of iron-sulfur containing proteins in mitochondria. Excess intracellular iron is stored in light- and heavy-chain ferritins, and the latter can oxidize Fe^{2+} to Fe^{3+}as a safe storage mechanism of iron (Figure 3) [40]. Mitochondria autophagy and ferritin degradation could increase intracellular pools of free iron.

Free Fe^{2+}can readily react with H_2O_2 via the Fenton reaction generating a highly reactive species hydroxyl radical ($HO^{\cdot}$) (Figure 3), leading to oxidative damages of cellular macromolecules nucleic acids, proteins, and lipids. On known example is that Fe^{2+} initiates lipid peroxidation chain reactions of membrane phospholipids resulting in ferroptosis [37]. Inhibition of nuclear receptor coactivator 4, a cargo receptor for ferritin autophagy, increased cellular iron storage and limited ferroptosis in cancer cells [41]. Iron also increased the activities of lipoxygenases and egg-laying defective 9 prolyl hydroxylases causing redox stress [42]. Clearly, deficiency or overload of intracellular iron levels could significantly influence cellular metabolic functions and redox homeostasis.

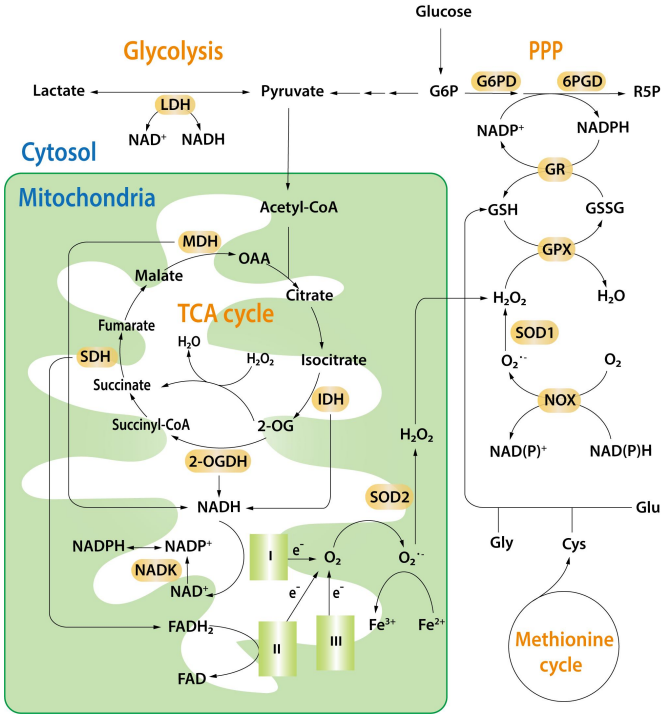

Figure 3 Metabolic sources of NAD(H), NADP(H), and GSH redox couples. NADH and NADPH are recycled by metabolic enzymes in glycolysis, the pentose phosphate pathway (PPP), and the TCA cycle. Mitochondrial NADH is oxidized into NAD^+ by respiratory complex I for ATP generation. The resultant NAD^+ is then phosphorylated by NAD^+ kinase (NADK) into $NADP^+$ and its reduced form NADPH. Cytosolic NADPH is mainly produced by the PPP shunt; and cytosolic GSH is biosynthesized from Glu, cysteine (Cys), and glycine (Gly). In the mitochondria, superoxide ($O_2^{\cdot-}$) is produced at respiratory complexes I–III due to leakage of electrons and then metabolized into H_2O_2 by manganese superoxide dismutase (SOD2). By contrast, in the cytosol, $O_2^{\cdot-}$ is generated by NAD(P)H oxidases (NOX) and then converted into H_2O_2 by SOD1. The neutralization of H_2O_2 is catalyzed by GPX enzymes, which utilize 2 molecules of GSH as co-substrates producing GSSG. The latter is recycled back to GSH by GR using electrons from cytosolic NADPH. 6PGD, 6-phosphogluconate dehydrogenase; G6P, glucose-6-phosphate; G6PD, G6P dehydrogenase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, GSH disulfide; H_2O_2, hydrogen peroxide; R5P, ribose-5-phosphate; SOD, superoxide dismutase.

Metabolic sources and functions of acetyl-CoA

Acetyl-CoA is a shared product of glucose metabolism and fatty acid oxidation in mitochondria. The glycolytic product pyruvate is transported into mitochondrial matrix, where it is decarboxylated by NAD^+-dependent pyruvate dehydrogenase (PDH) complex producing acetyl-CoA and NADH [22]. Acetyl-CoA is also the intermediary product of fatty acid beta-oxidation. In the TCA cycle, acetyl-CoA from both glucose and fatty acids condenses with oxaloacetate under the catalysis of citrate synthase to form a six-carbon molecule citrate [43]. In addition to its dedication to the TCA cycle, mitochondrial citrate can also be shuttled to the cytoplasm to generate acetyl-CoA by ATP citrate lyase (ACL) for fatty acid synthesis; whereas acetyl-CoA in the nucleus is utilized as a substrate for histone acetylation.

Under starvation, utilization of carbohydrates and synthesis of fatty acids were limited, fatty acid oxidation instead became the predominant metabolic pathway to supply acetyl-CoA for synthesis of ATP and ketone bodies in mitochondria [44]. Thus, maintenance of acetyl-CoA levels is a pivotal survival mechanism to support ATP production and also a vital regulatory mechanism to sustain histone acetylation.

Integrative regulation of redox, metabolism, and epigenetic modifications in cellular responses to environmental pollutants

Epigenetic dysregulations have been increasingly appreciated as common toxic mechanisms. Epidemiological evidence has indicated that environmental pollutants, such as metals, endocrine disruptors, and particulate matters ($PM_{2.5}$ and PM_{10}), perturb epigenetic modifications and thus alter gene expression and cell functions (Figure 4). Zhang and coworkers investigated DNA methylation and renal dysfunctions in Chinese residents from cadmium (Cd) polluted and unpolluted areas, and found that urinary and blood Cd levels positively correlated with hypermethylation of *RAS protein activator like 1* and *KLOTHO* promoter regions and negatively correlated with renal functions [45]. Janasik et al. found that urinary arsenic (As) levels were correlated with increased global DNA methylation levels in copper mill workers, and that As exposure increased NRF2 gene promoter methylation leading to inactivation of NRF2 signaling and downregulation of its target antioxidant genes [46]. Recently, an epigenome-wide association study showed that prenatal exposure of phthalates also correlated with variations of DNA methylation in many genes governing endocrine hormone activity, immune pathways, DNA damage, and neurodevelopment in venous buffy coat blood and buccal epithelial cells of infants [47]. In addition, in a birth cohort study in Belgium, each 5 $\mu g/m^3$ increment of gestational $PM_{2.5}$ and black carbon exposure positively associated with a 74.4% and a 38.4% increase in H3K4me3 levels in cord plasma DNA, respectively; and an inverse association was also found between gestational $PM_{2.5}$ exposure with H3K36me3 levels (-34.4%) [45]. Zheng et al found increase of PM_{10} and black carbon levels by a unit correlated with lower histone H3K27me3, H3K36me3, and H3K9ac levels in blood leukocytes of truck drivers than that of office workers in Beijing, China [48]. Mechanistically, these environmental pollutant-induced alterations of epigenetic modifications have been linked to changes in cellular levels of epigenetic modifying enzyme co-substrates and cofactors that integrate cellular redox and energy metabolism.

Targeting 2-OG- and Fe^{2+}-dependent demethylases

DNA demethylase TETs and histone demethylase JmjC proteins are dependent on 2-OG and Fe^{2+}, whose levels could be impacted by environmental pollutants leading to changes in their demethylase activities and cellular methylation levels (Figure 4). For instance, As exposure during gestation suppressed isocitrate dehydrogenase expression and reduced 2-OG levels in the liver of adult offspring mice, leading to inhibition of TET enzymatic activity. As a result, hepatic fatty acid β-oxidation related genes (e.g. PPARα and CPT1A) were hypermethylated and downregulated in As exposed mice at adulthood, which were associated with lipid accumulation in the liver, insulin resistance, and increased glucose tolerance [49]. Similarly, results from the same research group showed inhibition of isocitrate dehydrogenase expression, decrease in 2-OG levels, and thus reductions in TET enzymatic activity (despite no effects on their mRNA and protein expression) and total 5hmC levels in fetal brain tissues of As-treated pregnant mice. These changes in DNA methylation levels at early life were accompanied with an anxiety-like phenotype in adult offspring animals [50]. Furthermore, mRNA expression of TET1-3, DNMT1, and DNMT3a-3b as well as the levels of 2-OG were significantly lower in cortex tissues of rats when treated with As in drinking water for 6 months than that of control animals, which were associated with decreased 5mC and 5hmC levels as well as impaired learning and memory functions in treated animals [51].

In addition to 2-OG, depletion or competitive inhibition of Fe^{2+} by heavy metals could also inhibit Fe^{2+}-dependent demethylase activity and consequently DNA methylation levels (Figure 4). Nickel (Ni) chloride exposure reduced intracellular iron levels by 35%, resulting in increased H3K9me and H3K9me2 levels but decreased histone H3K9me3 levels in A549 cells, indicating perturbations of histone methylation [52]. Yin et al. reported that Ni significantly inhibited DNA demethylation by TET proteins both in somatic cells and embryonic stem cells leading to promotion of malignant transformation of somatic cells and disturbance of the naïve state of stem cells [53]. This perhaps was because Ni^{2+} has 7.5 times higher affinity for TETs than that of Fe^{2+} and binding of Ni^{2+} inhibited TET enzymatic activity [54]. Similarly, the binding of Ni^{2+} to JmjC protein active sites is 3 times stronger than that of Fe^{2+} leading to increases in global levels of H3K4me3, H3K9me2, and H3K9me1 after Ni exposure [55, 56]. In addition to Ni, other heavy metals, such as Cd, chromium (Cr), and antimony (Sb), were also able to significantly reduce TET activity and the levels of 5mC, 5fC, and 5caC in mouse embryonic stem cells probably by competitive inhibition of Fe^{2+} binding [57].

By sharp contrast, increase in cellular labile iron levels could enhance TET demethylase activity (Figure 4). For example, benzoquinone containing compounds stimulated the conversion of 5mC to 5hmC in cell-free system and increased 5hmC levels of 5,751 genes in cells leading to significantly altered expression of 3,414 genes involved in protein catabolic process, apoptosis, cell localization and transport process, and RNA processing. Mechanistically, quinone-induced 5hmC increase was dependent on upregulation of light-chain ferritin expression, elevation of labile Fe^{2+} levels, and increase in TET1/2 activity since iron chelator and TET1/2 knockout significantly abrogated these effects [58].

Of note, environmental pollutants could also directly influence demethylase activity. Liu and colleagues demonstrated that As^{3+} directly bound to cysteine residues of zinc finger domains of human TET protein peptides in HEK293T cells, resulting in inhibition of TET activity, decrease of 5hmC levels, and dysfunction of DNA repair in HEK293T cells and mouse embryonic stem cells [59]. In human vascular endothelial cells, treatment with polychlorinated biphenyl 77 (PCB77) and PCB126 upregulated Jumonji domain-containing protein 2B (JmjD2B) protein, which erased H3K9me3 modification on p65 promoter and as a result led to nuclear factor-κB (NF-κB) signaling activation, endothelial inflammation and dysfunctions [60].

Together, these lines of evidence suggest that environmental pollutants could target 2-OG and Fe^{2+} dependent demethylases through multiple layers of mechanisms leading to alterations of cellular DNA and histone methylation and eventually toxicity.

Targeting SAM-dependent methyltransferases

SAM is the exclusive methyl group source for DNA and histone methylation, and changes of intracellular SAM levels by environmental pollutants are expected to influence methylation states (Figure 4). One well-documented example is As, which consumed methyl moiety by As^{3+} methyltransferase (As3MT) forming mono- and di-methylated As and thus depleted SAM levels, leading to loss of global DNA and histone methylation as well as genomic instability [61]. Mice treated with As_2O_3 in drinking water for 6-month exhibited lower levels of testicular SAM, 5mC, H3K9me2, and H3K27me3, less germ cells in seminiferous tubules, worse sperm quality, and smaller

litter size than control animals, indicating impaired spermatogenesis and reproductive toxicity [62]. These effects were significantly attenuated in mice supplemented with vitamin B12 or in As3MT knockout mice. Consistently, treatment with As in mouse spermatocyte GC-2 cells in vitro led to dose-dependent reduction in SAM levels, DNA and histone hypomethylation, and apoptosis likely by upregulation of long interspersed element class 1 expression [62].

Other metals, such as tin, had similar effects. Tributyltin, triphenyltin, and their mixture significantly diminished SAM levels, increased SAH levels, and induced DNA hypomethylation in marine fish liver; and strong linear correlations between SAM levels, SAH levels, or the SAM/SAH ratio and global 5mC content were found in treated fish [63]. These changes in DNA methylation could explain their carcinogenic effects [63]. Likewise, $PM_{2.5}$ exposure also reduced intercellular SAM/SAH ratio and global DNA methylation levels, which were reversed by folate supplementation [64, 65]. Mechanistically, heavy metals or metalloids absorbed by these particles reacted with SAM to form methylated metabolites leading to depletion of intracellular SAM [64, 65]. Therefore, diminish of SAM levels and its resulting changes in DNA and histone methylation could explain the toxic effects induced by environmental pollutants, especially metals and metalloids.

Targeting NAD^+-dependent SIRT histone deacetylases

NAD^+ is an essential cofactor for SIRT deacetylases, and as anticipated, changes in NAD^+ levels by environmental pollutants perturb histone acetylation and gene expression (Figure 4). Tetrachlorodibenzo-p-dioxin (TCDD) exposure activated aryl hydrocarbon receptor (AHR) signaling and upregulated its target genes poly (ADP)-ribosyl polymerases (PARP) and CD38 expression, two NAD^+ consuming enzymes, leading to decrease in NAD^+ levels, increase in histone acetylation, and induction of sterile inflammatory responses in exposed human tissues [66, 67]. Likewise, PARP-induced consumption and depletion of NAD^+ levels by TCDD were also observed in primary chick embryo hepatocytes in vitro, which correlated with increased acetylation and ubiquitination-mediated proteasomal degradation of peroxisome proliferator-activated receptor γ coactivator 1 α, downregulation of gluconeogenic regulatory genes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, and decrease in hepatic glucose production [68]. Supplementation of NAM, the main substrate for NAD^+ synthesis by the salvage pathway, reversed these effects by recovery of NAD^+ levels [68, 69], supporting that NAD^+-dependent SIRT deacetylase activity regulates TCDD-induced toxicities.

Apart from its reliance on NAD^+ levels, the deacetylase activity of SIRTs could also be regulated by gene expression, oxidative stress, and others. For example, mono-(2-ethylhexyl) phthalate (MEHP) significantly and dose-dependently downregulated the expression and activity of SIRTs, despite no obvious change in NAD^+/NADH ratio in murine macrophage RAW264.7 cells [70]. Functionally, MEHP-induced inhibition of SIRTs was accompanied with elevation of ROS levels and activation of proinflammatory response in these cells [70]. Chen et al. examined histone acetylation in male rats after long-term exposure to acceptable “safe” doses of bisphenol A, and found that testicular SIRT1 protein expression was induced leading to decreases in H3K9ac, H3K27ac, and H4K12ac levels in treated animals [71]. Such gene expression profiling was correlated with intergenerational inheritance of these epigenetic markers and changes in male reproductive functions [71]. Upregulation of SIRT1 and Jarid1b expression and reduction of H4K16ac levels were also observed in the liver of rats exposed to PCBs during early life [72]. Furthermore, exposure of $PM_{2.5}$ lowered SIRT1 expression in the lung tissue of SD rats, which was corrected by addition of non-specific antioxidant N-acetyl-L-cysteine, suggesting the involvement of oxidative stress [73]. Treatment with Cr^{6+} in drinking water during gestational days 9.5-14.5 inhibited the interaction of SIRT1 and acetylated p53 leading to increase in acetyl-p53 levels, induction of pro-apoptotic BAX and caspase 3 proteins, and inhibition of anti-apoptotic Bcl2 and AKT proteins, which together caused germ and somatic cells apoptosis in ovaries of F1 offspring rats on postnatal day 1 [74]. Therefore, environmental pollutants influence the activity of SIRT deacetylases and consequently protein acetylation by NAD^+-dependent and -independent mechanisms.

Overloaded ROS generation as epigenetic regulators

Excessive ROS production, specifically $O2^{\cdot-}$ and H_2O_2, is a common toxic mechanism for numerous environmental pollutants through direct oxidation of macromolecules and/or indirect regulation of gene expression by mechanisms such as epigenetic modifications (Figure 4). $O_2^{\cdot-}$ often converts Fe^{2+} to Fe^{3+} by one-electron oxidation reaction leading to inactivation of Fe^{2+}-dependent epigenetic modifying enzymes, such as TET and JmjC proteins. By contrast, H_2O_2 preferably oxidizes cysteine residues into their disulfides leading to changes in conformational structure and thiol-dependent HDACs activity. For example, H_2O_2 inhibited the deacetylase activity of HDAC8 enzyme by directly but reversibly oxidizing its cysteine 102 (C102) and C153 residues into their disulfides [75]. Inhibition of H_2O_2 production by NOX inhibitor VAS2870 reduced the acetylation of structural maintenance of chromosomes 3 protein in BE2-C neuroblastoma cells [75]. Similarly, an intramolecular disulfide bond between Cys600 and Cys618 of LSD1 was formed by H_2O_2 stimulation and thus negatively regulated LSD1 demethylase activity, which was significantly recovered by thiol donor dithiothreitol [76].

Beyond oxidation of Fe^{2+} and cysteine at active centers, other posttranslational modifications are also involved in H_2O_2-induced changes of deacetylase activity. Treatment with H_2O_2 induced tyrosine nitration of HDAC2 leading to inhibition of its expression and activity and increase of proinflammatory interleukin 1β secretion in BEAS-2B cells [77]. Increased cellular H_2O_2 production by cigarette extracts also suppressed the expression and enzymatic activity of HDAC1-3 and SIRT1 in human monocyte-macrophage MonoMac6 cells and/or in lungs of SD rats. The inhibition of HDAC1-3 activity was possibly through protein nitro-tyrosine and aldehyde-adduct formation, despite the underlying mechanism of SIRT1 inhibition remains to be determined. Nevertheless, SIRT1 inhibition elevated p65 protein acetylation levels resulting in activation of NF-κB signaling and elevation of pro-inflammatory cytokine levels [78, 79]. Intriguingly, a recent study reported that maternal exposure of Cd in drinking water for 2 days significantly increased H_2O_2 production and HDAC1 activity in mouse embryos [80]. As a result, H4K8ac and H4K12ac levels were lower and DNA damage marker γ-H2AX levels were higher in embryos of Cd-treated animals than that of untreated controls, leading to hinderance of preimplantation embryo development and eventually embryo death [80].

Furthermore, DNA methylation was also influenced by elevated ROS levels. $PM_{2.5}$ stimulated H_2O_2 generation, TET1 mRNA expression and activity, and simultaneously increased global 5hmC levels and apoptotic cell death in SH-SY5Y human neuroblastoma cells, all of which were abrogated by generic antioxidant NAC or GSH pretreatment, suggesting H_2O_2 is the possible mediator for these epigenetic changes and cytotoxicity [81]. Similarly, increases in ROS production, TET1 activity, and global 5hmC levels were also found in benzene metabolite hydroquinone-treated HEK293 cells [82]. Di-2-ethylhexyl phthalate (DEHP) decreased p53 DNA methylation levels leading to upregulation of p53 protein and oxidation of proteins in the liver of ICR mice possibly through elevated ROS production [83]. Furthermore, Cd reduced DNA methylation of imprinted gene H19, which was accompanied by increases in ROS levels and DNA damage in mouse preimplantation embryos [80].

Taken together, these studies support that ROS are key regulators of DNA and protein epigenetic modifications by directly and indirectly controlling the expression and activity of epigenetic modifying enzymes.

Targeting Acetyl-CoA-dependent HATs

Cellular acetyl-CoA levels are determined by glucose and fatty acid metabolic activity and, in turn, control histone acetylation levels (Figure 4). Zhang et al. found that hyperglycemia and hyperlipidemia

promoted the nuclear translocation of mitochondrial PDH in vascular smooth muscle cells, increased acetyl-CoA and histone acetylation (H3K9ac) levels, and inhibited cell proliferation [84]. By contrast, metals such as zinc, As, Ni, and cobalt (Co) inhibited PDH activity, resulting in decreases of acetyl-CoA and thereby histone acetylation [52, 85, 86]. One possible explanation for these phenomena was that metals like Ni^{2+} and Co^{2+} induced "chemical" hypoxia and activated hypoxia-inducible factor 1 signaling leading to inhibition of PDH activity and consequently reduction of acetyl-CoA production under aerobic conditions [52, 87, 88]. Intriguingly, Clementino et al. recently showed that Cr^{6+}-transformed human bronchial epithelial BEAS-2B cells exhibited c-MYC activation-dependent enhancement of glycolytic activity, which led to increase in acetyl-CoA production via histone acetylation and activation of its key producing enzyme ATP citrate lyase [89]. This metabolic reprogramming eventually induced cancer stem cell-like features and tumorigenesis [89]. In summary, these lines of evidence support that alterations of acetyl-CoA levels by endogenous metabolic stress and exogenous metal exposure impact histone acetylation.

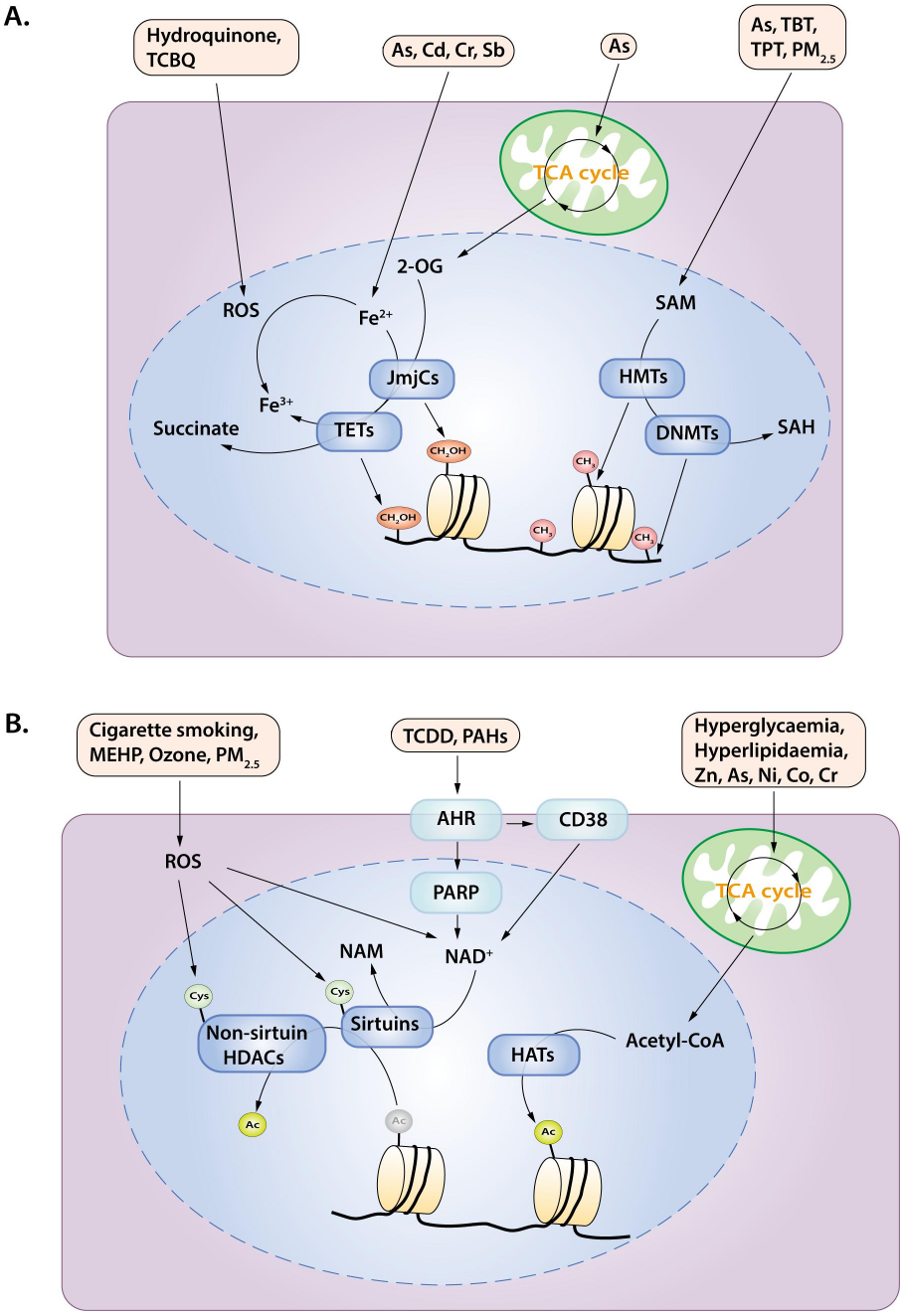

Figure 4 Redox and metabolic regulations of epigenetic modifications by xenobiotic toxicants. (A) Targeting key metabolic factors for DNA and histone methylation. Xenobiotic toxicants influence the activities of DNMTs and HMTs by changing SAM levels. 2-OG- and Fe^{2+}-dependent demethylase TETs and JmjCs are also the main targets of xenobiotic toxicants. **(B)** Targeting key metabolic and redox regulators for histone acetylation. Xenobiotic toxicants alter the production of acetyl-CoA in mitochondria and subsequently influence nuclear acetyl-coA levels and histone acetylation. Sirtuin deacetylase activity depends upon nuclear NAD^+ levels, which can be influenced by activation of its consuming enzymes and ROS-mediated oxidation under the treatments of xenobiotic toxicants. By contrast, ROS-mediated oxidation of active cystine residues is the principal regulatory mechanism for non-sirtuin HDACs. AHR, Aryl hydrocarbon receptor; MEHP, mono-(2-ethylhexyl)-phthalate; PAHs, polycyclic aromatic hydrocarbons; TBT, tributyltin; TCBQ, tetrachloro-1,4-benzoquinone; TCDD, tetrachlorodibenzo-p-dioxin; TPT, triphenyltin.

Conclusions

Epigenetic alterations have been widely recognized as crucial toxic mechanisms of numerous environmental pollutants. Exposure to these toxicants influences cellular epigenetic landscape by perturbing the expression of epigenetic modifying enzymes, and the availability of their functional co-substrates/cofactors including 2-OG, Fe^{2+}, SAM, NAD^{+}, FAD, and acetyl-CoA. These essential factors are also key nutrient metabolites and redox regulatory molecules, all of which together build an integrative triangular network among epigenetic modifications, energy metabolism, and redox homeostasis. Targeting such network by environmental pollutants leads to (epi) genotypic changes and thereby cellular dysfunctions, cytotoxicity, and eventually phenotypic alterations. The mechanisms of how environmental pollutants impact such network merit further investigations, which helps the development of effective counteraction measurements against environmental pollutant-induced adverse health outcomes.

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