Deciphering epigenetic regulation in cardiac developmental toxicity: Mechanisms and implications (Review)

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Abstract. As the first functional organ to form during vertebrate embryogenesis, the heart exhibits heightened susceptibility to developmental toxicity. Epigenetic regulatory mechanisms, including DNA methylation, histone modifications, non-coding RNAs, N6-methyladenosine methylation and chromatin accessibility alterations, mediate cardiac developmental toxicity induced by exogenous compounds including environmental chemicals and pharmaceuticals. The present review comprehensively summarizes the current understanding of the molecular mechanisms through which these compounds exert cardiac developmental toxicity through epigenetic regulation. An in-depth analysis of research progress and technical challenges across diverse epigenetic pathways is provided. By summarizing recent evidence, the present review proposes candidate epigenetic biomarkers for cardiac developmental toxicity monitoring and explores potential intervention strategies targeting these pathways. Future research should prioritize multi-omics integration technologies and clinical translation system development. These advances are anticipated to foster innovation in both mechanistic research and preventive strategy development for cardiac developmental toxicity.

Contents

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1. Introduction

Cardiac developmental toxicity refers to harmful effects induced by external factors, including chemical substances, pharmaceuticals, environmental pollutants or infectious agents, which impair normal cardiac morphogenesis, cellular differentiation or functional maturation during key phases of embryonic or fetal heart development (1). This disruption may result in structural cardiac anomalies (such as ventricular septal defects or patent ductus arteriosus) or functional impairments (such as cardiac arrhythmias and impaired cardiac function). Epidemiological studies demonstrate that congenital heart disease (CHD) affects >1% of live births and accounts for the majority of prenatal miscarriages (2-4). In the United States alone, ~500,000 adults currently live with CHD (5). Notably, although >80,000 chemical substances are commercially available in the United States, the cardiac teratogenic potential of the majority of these substances remains uncharacterized, creating notable gaps in prenatal exposure risk assessment. Beyond genetic predispositions, maternal exposure to cardiac teratogens during the key cardiac morphogenesis period, spanning 3 months pre-conception to gestational weeks 2-7, markedly elevates the risk for developmental cardiac defects (6). Therefore, elucidating the molecular mechanisms of xenobiotic-induced cardiac developmental toxicity is important for developing targeted clinical surveillance programs and formulating evidence-based preventive public health measures.

Epigenetic regulation controls transcriptional activity through stable but reversible chromatin modifications that occur independently of DNA sequence alterations (7). Key epigenetic mechanisms, including DNA methylation, histone modifications (acetylation and methylation), regulatory

non-coding RNAs (ncRNAs), RNA methylation and chromatin remodeling, act synergistically to precisely modulate gene expression while preserving the underlying genetic sequence (8,9). These modifications demonstrate plasticity in response to diverse extrinsic factors, encompassing dietary composition, physical activity, pathogenic challenges, environmental toxicants, senescence processes and psychosocial stressors (10,11). Maternal exposure to pharmaceuticals or environmental contaminants during pregnancy may disrupt cardiac development through epigenetic mechanisms, leading to dysregulation of cardiac morphogenesis-related genes and impaired cardiac differentiation. Such perturbations ultimately contribute to congenital heart defects. The present review summarizes emerging evidence regarding epigenetic regulation of cardiac developmental toxicity (Fig. 1), offering potential avenues for early detection and prevention of chemical-induced cardiac malformations.

2. DNA methylation

DNA methylation represents a key epigenetic mechanism involving the addition of methyl groups to the fifth carbon position of cytosines, generating 5-methylcytosine (5mC) (12,13). This reaction is catalyzed by DNA methyltransferases (DNMTs), with DNMT3A and DNMT3B primarily mediating *de novo* methylation at unmethylated CpG sites (14,15). DNMT1, as the principal maintenance methyltransferase, serves a key role in preserving epigenetic patterns during DNA replication. This enzyme specifically recognizes hemimethylated CpG dinucleotides, ensuring accurate methylation inheritance from parent to daughter strands and thereby maintaining epigenetic memory throughout cellular proliferation (16). The enzymatic transfer of methyl moieties to the fifth carbon of cytosines induces conformational constraints in genomic DNA, creating steric occlusion that competitively inhibits transcription factor docking. This biochemical blockade constitutes a fundamental paradigm of epigenetic gene silencing (17,18).

DNA demethylation proceeds through two mechanistically distinct pathways. Namely, passive replication-dependent dilution, followed by active enzymatic removal. Passive demethylation results from the gradual loss of methylation marks during successive rounds of DNA replication, whereas active demethylation involves direct enzymatic erasure of methyl groups independent of DNA synthesis (19). The ten-eleven translocation (TET) family of dioxygenases (TET1, TET2 and TET3) drive active DNA demethylation through iterative oxidation reactions, progressively modifying 5mC to form 5-hydroxymethylcytosine, subsequently advancing to 5-formylcytosine and concluding with the generation of 5-carboxylcytosine (20). Following oxidative modification, thymine DNA glycosylase selectively binds and cleaves these epigenetic intermediates, enabling their replacement with canonical cytosine through base excision repair-mediated nucleotide substitution (21). Alternatively, non-oxidative demethylation pathways exist whereby deaminases such as activation-induced deaminase/apolipoprotein B mRNA editing catalytic polypeptide family enzymes can directly convert 5mC to thymine (22). This initiates distinct repair processes that ultimately result in demethylation.

With regard to DNA methylation dysregulation-induced cardiac developmental toxicity, recent methodological advances in whole-genome bisulfite sequencing (WGBS), coupled with newly developed single-cell methylome analysis technologies, now facilitate genome-wide DNA methylation profiling with unprecedented resolution and comprehensiveness (23,24). Mounting evidence indicates that DNA methylation serves as a key epigenetic regulator governing embryonic development, cellular differentiation and responses to environmental toxicants (25-27). Notably, aberrant alterations in DNA methylation are associated with a number of birth defects and developmental disorders, such as cardiac malformations and abnormal heart rates, highlighting its importance in developmental toxicity. Table I summarizes the mechanisms by which potential toxic substances induce cardiac developmental toxicity through abnormal DNA methylation.

Phthalates, a class of ubiquitous industrial chemicals, are recognized endocrine-disrupting compounds associated with potential cardiovascular impairment, reproductive dysfunction and developmental toxicity (28,29). Mu *et al* (30) discovered through methylated DNA immunoprecipitation sequencing (MeDIP-Seq) that exposure to di-(2-ethylhexyl) phthalate and di-butyl phthalate could alter DNA methylation patterns of cardiac development-related genes, thereby modulating gene expression and subsequently inducing cardiac developmental defects in zebrafish embryos. This study observed that reduced DNA methylation at the natriuretic peptide A (NPPA) and cardiac troponin T2 (cTnT) gene loci was frequently associated with higher transcriptional activity. By contrast, increased methylation levels in the T-box transcription factor 5 (TBX5)-b promoter region tended to be associated with decreased expression, suggesting transcriptional repression. These methylation-expression relationships support the possibility that DNA methylation serves as an important epigenetic mechanism influencing cardiac developmental toxicity following phthalate exposure.

Particulate matter (PM) represents a heterogeneous mixture of airborne contaminants comprising chemical components derived from numerous emission sources, including vehicle emissions, industrial processes and biomass burning, with PM2.5 specifically referring to fine particulate matter with an aerodynamic diameter of $\leq$2.5 μm (31). Accumulating epidemiological evidence demonstrates that prenatal exposure to particulate matter is associated with adverse pregnancy outcomes, particularly preterm delivery, reduced birth weight and impaired neurodevelopment (32-34). Emerging research highlights the role of DNA methylation alterations as a key mechanism underlying PM-induced cardiac developmental toxicity. Genome-wide DNA methylation profiling revealed that exposure to PM2.5 extracts induces widespread methylation dysregulation (both hyper- and hypomethylation) in zebrafish embryonic hearts, leading to developmental defects (25). This process is associated with abnormal folate metabolism, suggesting that PM2.5 may disrupt folate metabolic homeostasis through DNA methylation modifications, thereby contributing to cardiac developmental toxicity.

Selenium, while recognized as a key micronutrient important in maintaining normal physiological functions, such as development and immune responses, poses marked

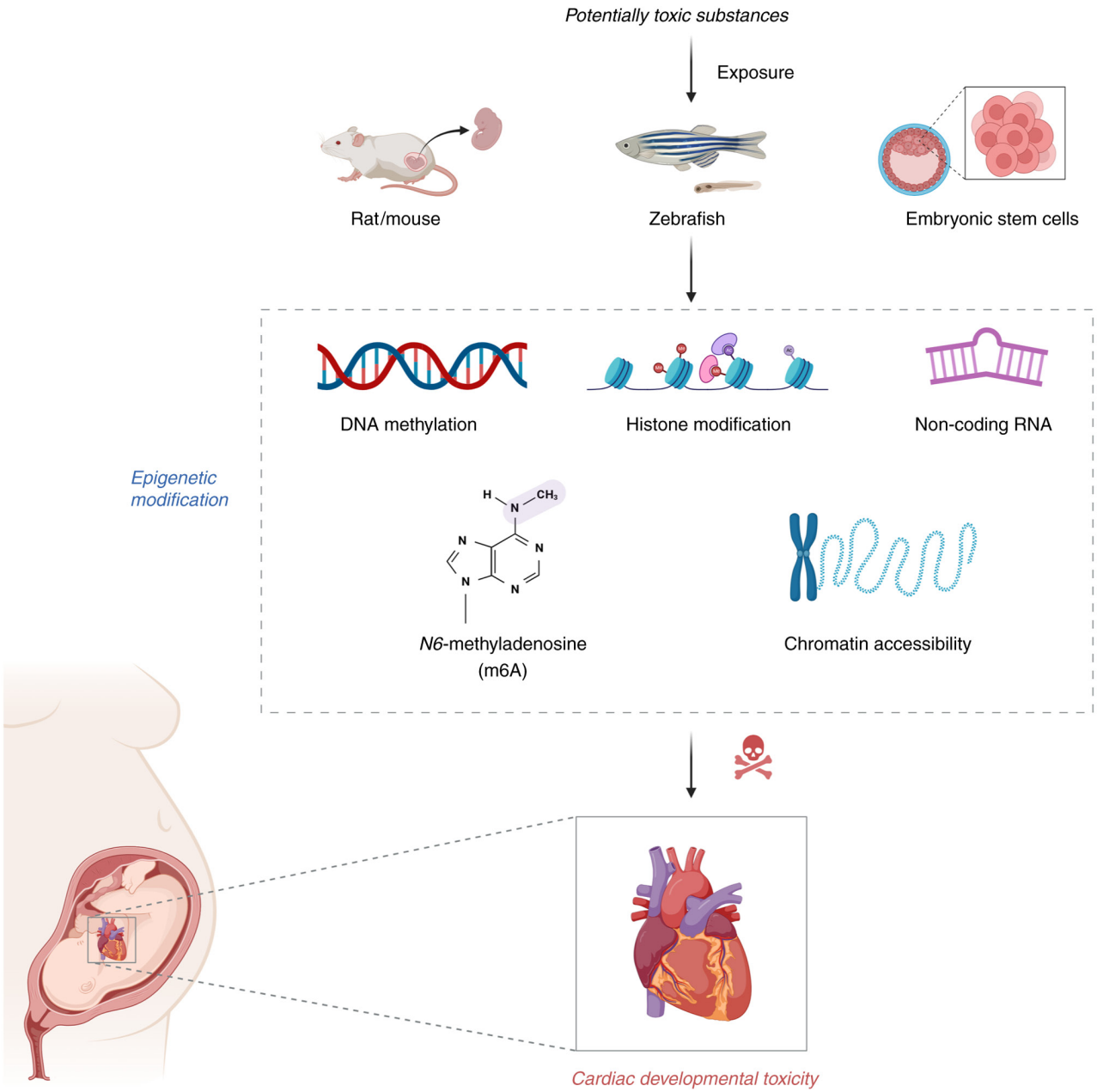

Figure 1. Exposure to potential toxicants induces cardiac developmental toxicity through epigenetic regulatory mechanisms in experimental models. The figure was created using BioRender.com.

toxicological risks due to the narrow therapeutic margin it exhibits between nutritional adequacy and toxicological thresholds, thereby warranting serious consideration of environmental exposure hazards (35,36). As the primary selenium source for selenoprotein biosynthesis in mammalian cells, selenite has been demonstrated to induce developmental toxicity and neurobehavioral abnormalities in zebrafish embryos (37,38). Ma *et al* (39) revealed that selenite induces cardiac developmental toxicity in zebrafish embryos by disturbing DNA methylation dynamics, manifesting as aberrant early-stage hypomethylation followed by late-stage hypermethylation, concomitant with ventricular/atrial morphological defects and impaired cardiac function. In addition, folate notably alleviated cardiac defects by rescuing methylation imbalance, demonstrating that selenite-induced cardiac developmental toxicity depends on the disruption of DNA methylation dynamics. Similarly, arsenic is a widely distributed naturally occurring element that poses hazards to living organisms (40). Particularly in its trivalent form as arsenite, arsenic has been demonstrated to cause notable harm to humans through multiple biochemical pathways, including mitochondrial reactive oxygen species (ROS) generation, due to its potent carcinogenicity and toxicity (41). A previous study demonstrated that exposure to 2.0 mM arsenite induced cardiac developmental defects in zebrafish embryos at 48 h post-fertilization (hpf), characterized by reduced ventricular volume, morphological alterations and a markedly increased atrioventricular angle (42). Concurrent global DNA hypermethylation was observed, suggesting that this epigenetic dysregulation may contribute to arsenite-mediated cardiotoxicity. These findings provide experimental evidence for understanding

Table I. DNA methylation dysregulation-induced cardiac developmental toxicity.

First author, year	Toxicant	Embryo model	Methylation status	Potential mechanism	Cardiac toxicity manifestations	(Refs.)
Jiang *et al*, 2019	PM2.5	Zebrafish	Widespread methylation dysregulation (both hyper- and hypomethylation)	Dysregulated cardiac developmental gene expression	Increased incidence of cardiac malformations and decreased heart rate	(25)
Mu *et al*, 2020	Phthalates	Zebrafish	Hypomethylation of NPPA and cTnT, hypermethylation of TBX5b	Dysregulated cardiac developmental gene expression	Abnormal heart rate and pericardial edema	(30)
Ma *et al*, 2012	Selenite	Zebrafish	Early hypomethylation, late hypermethylation	Dysregulated proliferation-apoptosis homeostasis	Bradycardia, pericardial edema and reduced cardiac chamber volume with morphological abnormalities	(39)
Li *et al*, 2009	Arsenite	Zebrafish	Early hypomethylation, late hypermethylation	Dysregulated proliferation-apoptosis homeostasis	Bradycardia and altered ventricular shape	(42)
Jiao *et al*, 2023	Cyhexatin	Zebrafish	Global DNA hypomethylation	Apoptosis, oxidative stress and endocrine disruption	Abnormal heart rate and pericardial edema	(44)
Chatterjee *et al*, 2021	CMIT/MIT	Zebrafish	Global DNA hypermethylation	Dysregulated cardiac developmental gene expression	Abnormal heart rate and pericardial edema	(48)

NPPA, natriuretic peptide A; cTnT, cardiac troponin T2; TBX5b, T-box transcription factor 5b; CMIT/MIT, 5-chloro-2-methyl-4-isothiazoline-3-one/2methyl-4-isothiazolin-3-one; PM2.5, particulate matter 2.5.

the DNA methylation mechanisms underlying the cardiac developmental toxicity of heavy metals/metalloids.

Cyclohexylltin (CYT), an organotin compound, is often employed as a broad-spectrum pesticide in modern agricultural practices for pest management (43). However, its potential cardiotoxic effects during embryonic development remain to be systematically elucidated. In a zebrafish model, CYT exposure was shown to induce genome-wide DNA hypomethylation, characterized by decreased S-adenosylmethionine (SAM)/S-adenosylhomocysteine (SAH) ratios and suppressed DNMTs expression, along with cardiac malformations such as bradycardia and pericardial edema (44). These findings suggest that CYT disrupts DNA methylation dynamics, potentially resulting in aberrant transcriptional regulation of genes key to cardiac development.

5-Chloro-2-methyl-4-isothiazolin-3-one/2-methyl-4-isothiazolin-3-one (CMIT/MIT) is an isothiazolinone-based biocide that is commonly formulated in numerous aqueous consumer products, with extensive applications in disinfectants and cosmetics (45,46). Previous research has indicated the potential neurotoxic effects of CMIT/MIT (47), and due to its extensive commercial applications and consequent exposure risks, research has shifted focus toward investigating its developmental toxicity. Chatterjee *et al* (48) investigated the developmental toxicity of CMIT/MIT in zebrafish embryos and found that exposure to this compound markedly upregulated the expression of DNMTs, including DNMT1, DNMT3a2, DNMT3b1 and DNMT3b4, leading to global DNA hypermethylation The observed cardiac malformations, including pericardial edema and bradycardia, were associated with concurrent epigenetic modifications, indicating that CMIT/MIT-induced developmental cardiotoxicity may occur through DNMT-dependent dysregulation of DNA methylation.

Studies utilizing advanced sequencing technologies have demonstrated that multiple toxicants can disrupt DNA methylation patterns, inducing gene-specific alterations or genome-wide epigenetic instability. These modifications impair key developmental processes such as cardiogenesis, potentially culminating in developmental cardiotoxicity (25,30). However, existing research has predominantly identified associative relationships between DNA methylation changes and developmental toxicity, without direct experimental validation of causality between specific epigenetic modifications

Table II. Histone modification dysregulation-induced cardiac developmental toxicity.

First author, year	Toxicant	Embryo model	Histone modification	Potential mechanism	Cardiac toxicity manifestations	(Refs.)
Zhang *et al*, 2024	Fenbuconazole	Zebrafish	H3K9Ac↓ H3K14Ac↓	Dysregulated cardiac developmental gene expression	Cardiac arrhythmia and cardiac morphological defects	(68)
Cheng *et al*, 2016	Cigarette smoke	mESCs	Global H3 hypoacetylation	Dysregulated cardiac developmental gene expression	Decreased cardiac contraction rate	(75)
Wu *et al*, 2022	Cadmium	Two-dimensional cardiac differentiation model and three-dimensional EBs and cardiac organoid models derived from hESCs	H3K27me3 H3K4me3↓	Inhibition of mesoderm formation and suppression of cardiomyocyte differentiation and cardiac induction	Decreased cardiac contraction rate	(79)

↑ indicates upregulation and ↓ indicates downregulation. mESCs, mouse embryonic stem cells; EBs, embryoid bodies; hESCs, human embryonic stem cells; H3K, histone H3 lysine K; miR, microRNA.

and toxicological outcomes. In addition, the mechanistic pathways through which aberrant DNA methylation influences downstream gene expression to confer toxicity remain only partially characterized. Despite intricate crosstalk between DNA methylation and other epigenetic modifications such as histone modifications and ncRNAs, the precise regulatory mechanisms governing these interactions remain incompletely characterized (49,50). Integrating multi-omics approaches and employing targeted DNA methylation editing technologies to validate the functional roles of key methylation sites could advance the understanding of how DNA methylation influences cardiac developmental toxicity (51).

3. Histone modification

Histones constitute the key protein subunits that form octameric nucleosome complexes, creating the structural scaffold necessary for DNA compaction. These include four core histone variants (H3, H4, H2A and H2B), which coordinately assemble to establish the basic repeating unit of chromatin (52). In addition to their structural function, histones undergo diverse covalent post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination and small ubiquitin-like modifier (SUMO)-ylation, that serve as key epigenetic regulators modulating transcriptional activity (53,54). Such modifications frequently target flexible histone N-terminal domains, whereby genetic activity would be modulated through the dual mechanisms of directly changing histone-DNA interaction dynamics or serving as docking sites for chromatin-associated regulatory proteins (55).

Among the diverse number of histone modifications, methylation and acetylation constitute two of the most extensively characterized epigenetic marks. The addition of methyl moieties to specific lysine or arginine amino acids is enzymatically facilitated by histone methyltransferases, utilizing S-adenosylmethionine as the methyl donor molecule (56). Counteracting this process, histone demethylases catalyze the elimination of these methyl groups through oxidative reactions. Simultaneously, the acetylation process introducing acetyl functional groups through histone acetyltransferases induces chromatin decondensation, creating a permissive environment for transcriptional machinery (57). This modification is dynamically reversible through the enzymatic activity of histone deacetylases, which leads to chromatin compaction associated with gene silencing (58). These reversible modifications collectively constitute a sophisticated regulatory network of histone modifications.

With regard to histone modification dysregulation-induced cardiac developmental toxicity, existing evidence has demonstrated that dysregulated histone modifications can destabilize genomic integrity and dysregulate gene transcription, ultimately driving multiorgan toxicity manifestations, particularly hepatotoxicity, neurotoxicity and reproductive toxicity (59-62). Furthermore, histone modification-mediated regulatory mechanisms have become a key focus in developmental toxicity research, as their dynamic alterations may influence embryonic development and organogenesis, garnering notable scientific interest (63-65). The experimental evidence linking toxicant-induced histone modifications to cardiac developmental toxicity is summarized in Table II.

Fenbucoonazole (FBZ), a triazole-class fungicide, is extensively employed in agricultural and horticultural applications (66). A previous study has demonstrated that FBZ exposure induces abnormal transcription of specific genes, thereby compromising cardiac development and function in larvae (67). However, the involvement of histone modifications

in this process remains poorly understood. Zhang *et al* (68) revealed that zebrafish embryos exposed to FBZ showed markedly decreased acetylation levels of histone H3K9 and H3K14 (H3K9Ac and H3K14Ac) in the hearts of both filial (F)0 and F1 generation adult fish. This epigenetic repression directly downregulated the transcription of core cardiac developmental genes [GATA binding protein 4 (GATA4), TBX5 and NK2 homeobox 5 (NKX2-5)] and calcium homeostasis-related genes, resulting in cardiac abnormalities including arrhythmia and morphological defects. Notably, these adverse effects were transgenerationally transmitted to the F2 generation. These findings offer mechanistic insights into histone acetylation-mediated cardiotoxicity during embryogenesis, while providing evidence to support transgenerational epigenetic inheritance.

According to official reports from the U.S. Surgeon General, tobacco smoke exposure represents a notable environmental health hazard, accounting for ~480,000 annual mortalities and constituting one of the most notable pathogenic environmental factors (69,70). Prenatal exposure to tobacco pollutants induces embryotoxicity, characterized by growth restriction, structural anomalies including craniofacial malformations, congenital heart defects and skeletal abnormalities, as well as embryonic lethality (71-74). Cheng *et al* (75) demonstrated that both mainstream smoke and sidestream smoke (SS) exposure during cardiac differentiation of mouse embryonic stem cells (ESCs) markedly reduced global histone H3 acetylation (H3ac) levels in developing cardiomyocytes. This study revealed that SS specifically diminished H3ac enrichment at the promoter region of GATA4 (a key cardiac transcription factor), leading to its transcriptional downregulation. The observed epigenetic modifications suppressed (bone morphogenetic protein (BMP)-SMAD family member 4 signaling, a key pathway regulating cardiac morphogenesis. These results demonstrate that dysregulated histone acetylation constitutes a primary molecular mechanism driving developmental cardiotoxicity induced by tobacco smoke exposure.

Cadmium (Cd), a highly toxic heavy metal, poses escalating global health risks due to its environmental persistence and good aqueous solubility (76). Studies have demonstrated that Cd exposure exhibits both direct embryogenic regulatory disruption and association with multiple pathologies, including developmental cardiotoxicity, establishing its developmental toxicity as a key concern in mechanistic toxicology (77,78). Wu *et al* (79) employed human ESCs (hESCs) in two-dimensional differentiation models, three-dimensional embryoid bodies and cardiac organoids as experimental systems. Cd exposure was found to markedly elevate the levels of repressive histone mark H3K27me3 while reducing the levels of active histone mark H3K4me3. This epigenetic dysregulation was associated with the downregulation of mesoderm markers (including heart and neural crest derivatives expressed 1 and HOP homeobox) and cardiac-specific genes (including NKX2-5 and GATA4), ultimately impairing mesoderm-to-cardiomyocyte differentiation. The experimental results demonstrate that Cd-induced cardiac developmental toxicity is regulated by histone methylation, particularly during mesoderm specification, a developmental phase exhibiting heightened vulnerability.

Histone ubiquitination regulates diverse chromatin-associated processes, including transcriptional regulation, DNA repair and chromatin structure remodeling, with this regulatory mechanism having attracted growing research attention in recent years (80-82). Although direct evidence linking toxicant-induced developmental cardiotoxicity to histone ubiquitination pathways remains limited, existing studies have provided a number of theories (83,84). The cullin-RING E3 ubiquitin ligase (CRL) family has been demonstrated to regulate cardiac morphogenesis and tissue maturation through ubiquitin-dependent modifications (83). As a key member of the CRL family, cullin 4A (CUL4A) serves a key role in heart development by mediating ubiquitin-dependent degradation of target proteins. A previous study has shown that CUL4A deficiency induces pericardial edema, abnormal cardiac looping and pectoral fin defects in zebrafish embryos, phenotypes associated with impaired cardiomyocyte proliferation and increased apoptosis (84). These findings suggest that dysregulation of ubiquitination pathways may contribute to developmental cardiotoxicity. Given the role of histone ubiquitination in DNA damage response and cell fate determination, the present review proposes it may regulate cardiac developmental toxicity, representing a promising research avenue.

Aberrant histone modifications disrupt cardiac developmental gene expression through alterations in methylation and acetylation marks, demonstrating direct mediation of teratogenic effects. Current research has primarily focused on histone acetylation and specific methylation events, whereas other key modifications, including phosphorylation, ubiquitination and SUMOylation, remain poorly characterized. This knowledge gap limits comprehensive understanding of the diverse mechanistic contributions of histone modifications to developmental toxicity (68,75,79). While numerous current studies demonstrate associative relationships between histone modification alterations and toxic phenotypes, the key molecular mechanisms, particularly how toxicants precisely regulate histone-modifying enzyme activity within upstream regulatory networks, remain inadequately elucidated (68,75,79). Subsequent investigations should aim to focus on delineating temporal alterations in histone marks and their interactions with additional epigenetic regulators to elucidate underlying pathological mechanisms (55).

4. NcRNAs

NcRNAs constitute transcriptionally active, yet untranslated, genetic elements predominant in eukaryotic systems, with three principal subtypes prevailing, namely long ncRNAs (lncRNAs), microRNAs (miRNAs) and circular RNAs (circRNAs) (85,86). Despite their protein-coding deficiency, these RNA molecules exert comprehensive regulatory control over important biological processes, including chromatin remodeling, transcriptional modulation, post-translational modifications and signal transduction (87,88). As key regulatory molecules, ncRNAs have been demonstrated to participate in the mechanisms underlying toxicity induced by numerous environmental pollutants and pharmaceutical compounds, such as crude oil and ribavirin (89,90).

With regard to ncRNA dysregulation-induced cardiac developmental toxicity, emerging scientific evidence has

Table III. NcRNAs dysregulation-induced cardiac developmental toxicity.

First author, year	Toxicant	Embryo model	ncRNAs	Target	Potential mechanism	Cardiac toxicity manifestations	(Refs.)
Ye *et al*, 2020	Ribavirin	hiPSCs	lncRNA GAS5↑ lncRNA HBL1↑	p53	ROS accumulation and DNA damage	Pericardial edema, incomplete cardiac looping and ventricular/atrial enlargement	(96)
Xu *et al*, 2019	Crude oil	Red drum (*Sciaenops ocellatu*)	MiR-18a↓ miR-27b↑ miR-203a↓	-	Disruption of cardiac developmental pathways	Pericardial edema	(101)
Magnuson *et al*, 2022	Phenanthrene	Zebrafish	miR-203a↓	VEGFA	Dysregulated cardiac developmental gene expression	Abnormal heart rate and pericardial edema	(110)
Guo *et al*, 2024	MC-LR	Mice	miR-377-3p	NR6A1	Macrophage polarization imbalance	Ventricular dilation and myocardial thinning	(111)

↑ indicates upregulation and ↓ indicates downregulation. hiPSCs, human induced pluripotent stem cells; MC-LR, microcystin-leucine arginine; VEGFA, vascular endothelial growth factor A; NR6A1, nuclear receptor subfamily 6 group A member 1; miR, microRNA.

established ncRNAs as key molecular mediators orchestrating toxicological responses throughout embryogenesis (91,92). These discoveries not only offer promising biomarker candidates for developmental toxicity assessment but also improve the mechanistic understanding of the epigenetic regulatory circuitry underlying developmental toxicity. The dysregulation of ncRNA-mediated cardiac developmental toxicity is summarized in Table III.

LncRNAs represent a distinct class of ncRNAs characterized by lengths of >200 nucleotides, with certain transcripts extending to hundreds of kilobases. These RNA molecules regulate key cellular processes such as proliferation, apoptosis and cellular migration through multiple molecular mechanisms (93-95). Notably, dysregulation of lncRNAs has been implicated in drug-induced developmental toxicity, as exemplified by antiviral drugs (96). Ribavirin, as a broad-spectrum antiviral drug, may induce adverse effects in clinical applications, such as rashes, hemolytic anemia and teratogenicity (97). However, its potential cardiotoxicity and underlying mechanisms during cardiac development remain elusive. Ye *et al* (96) found that ribavirin upregulates the expression of lncRNAs growth arrest specific 5 and Heart Brake lnRNA 1, which inhibits the differentiation of human induced pluripotent stem cells into cardiomyocytes, triggering DNA damage and p53 pathway activation. This finding establishes novel perspectives on the teratogenic mechanisms of ribavirin while decoding the functional importance of lncRNAs in heart development.

MiRNAs constitute a class of endogenous small ncRNAs (~22 nucleotides in length) that post-transcriptionally regulate gene expression by binding to complementary mRNA sequences, ultimately inducing mRNA degradation or translational repression (98). The integration of high-throughput sequencing technologies with sophisticated bioinformatics tools has advanced miRNA research, driving developments in mechanistic studies and translational applications within this field (99). Increasing evidence suggests that environmental toxicant exposure can disrupt normal developmental processes by interfering with epigenetic regulatory mechanisms, particularly through modulating miRNA expression profiles. These findings provide novel insights into toxicological pathway mechanisms (100-102).

Trichloroethylene (TCE), a volatile organic solvent, is distributed in environmental media including soil, groundwater and air, posing marked contamination risks (103). A growing body of evidence indicates that TCE exposure may induce cardiac developmental abnormalities in humans and other organisms (103-106). Huang *et al* (91) found that TCE exposure downregulates miR-133a in zebrafish embryonic hearts, leading to heart developmental defects by increasing ROS generation and excessive cell proliferation, yet miR-133a agonists effectively mitigated this toxic effect. This study demonstrates that miR-133a serves a key mediating role in TCE-induced cardiac developmental toxicity, suggesting its potential as a biomarker for cardiotoxicity assessment.

Crude oil and its chemical components represent common environmental contaminants in aquatic systems, with their long-term toxicological impacts remaining an active area of investigation (107-109). Xu *et al* (101) employed red drum (*Sciaenops ocellatus*) larvae to investigate miRNA-mediated cardiotoxicity through integrated mRNA-miRNA sequencing analysis. This study revealed that Deepwater Horizon crude oil exposure altered the expression of miR-18a, miR-27b and miR-203a and this was concentration-dependent. Specifically, miR-27b upregulation promoted physiological/pathological cardiac hypertrophy, while downregulation of miR-18a and miR-203a impaired heart rate regulation and cardiac valve morphogenesis, respectively. Mechanistically, these dysregulated miRNAs disrupted key cardiac developmental pathways, including calcium signaling and hypertrophic responses, by modulating their target genes, thereby providing a novel miRNA-dependent mechanism underlying polycyclic aromatic hydrocarbon (PAHs)-induced developmental cardiotoxicity in marine fish. Similarly, another study conducted on larvae mahi-mahi revealed that exposure to PAHs in crude oil resulted in dose-dependent upregulation of miR-34b and miR-23b, as well as downregulation of miR-203a (102). These miRNAs dysregulated cardiac developmental genes, including components of calcium signaling and regulators of cardiac hypertrophy, leading to pericardial edema, bradycardia and other morphological abnormalities. Additionally, the activation of p53 signaling further exacerbated developmental cardiac impairments. These findings provide evidence for the central regulatory role of miRNA-mRNA interactions in crude oil-induced cardiotoxicity during fish development. The developmental toxicity mediated by miR-203a in PAHs has garnered notable research attention. Magnuson *et al* (110) demonstrated that inhibition of miR-203a in zebrafish embryos led to heart rate reduction and pericardial edema, cardiac defects that mimic the developmental toxicity induced by typical PAHs found in crude oil. This study elucidated the molecular mechanism wherein miR-203a contributes to cardiac developmental impairment by regulating key factors such as VEGFA and fibrosis-associated genes including Krüppel-like factor 4.

As a key metabolic and immunomodulatory organ at the maternal-fetal interface, the placenta serves indispensable roles in mediating developmental toxicity through xenobiotic metabolism and cytokine signaling networks. A study by Guo *et al* (111) revealed that the environmental toxin microcystin-leucine arginine can deliver miR-377-3p through trophoblast cell-derived extracellular vesicles, targeting and suppressing the nuclear receptor subfamily 6 group A member 1 gene in macrophages. This suppression activates the mammalian target of the mTOR/ribosomal protein S6 kinase β-1/sterol regulatory element-binding protein signaling pathway, leading to M1 polarization and metabolic disruption in placental macrophages, ultimately resulting in CHDs in future offspring. To the best of our knowledge, this study was the first to elucidate the role of miRNA-mediated placenta-heart axis signaling in environmentally induced cardiac developmental toxicity, providing novel evidence for miRNA-regulated transgenerational mechanisms in heart development.

NcRNAs have been demonstrated to serve as key regulatory molecules in cardiac developmental toxicity induced by numerous toxicants, exhibiting marked potential as biomarkers and therapeutic targets (96,101,110,111). However, current research exhibits notable limitations. As on the one hand, studies have predominantly focused on miRNAs, while investigations into the mechanisms of lncRNAs, circRNAs, small interfering RNAs and Piwi-interacting RNAs in cardiac developmental toxicity remain markedly insufficient. On the other hand, the interaction networks and synergistic regulatory mechanisms among different ncRNA classes are yet to be elucidated, hindering a comprehensive understanding of ncRNA-mediated developmental toxicity. Future studies should aim to broaden the scope of ncRNA research and deepen the exploration of regulatory relationships among numerous ncRNAs to achieve a more systematic mechanistic understanding.

5. m6A methylation

N6-methyladenosine (m6A), initially identified in eukaryotic messenger RNAs in 1974, represents the methylation of adenine at the N6 position (112). As the primary internal RNA modification in eukaryotes, m6A occurs at an average frequency of 1-2 modifications per 1,000 nucleotides (113). Among >170 identified RNA modifications, m6A is the most abundant and dynamically reversible epigenetic mark in eukaryotic mRNAs, constituting approximately one-half all methylated ribonucleotides (114).

The m6A modification system achieves dynamic and reversible regulation through three functionally distinct classes of regulatory proteins, namely writers, erasers and readers (115). Methyltransferases such as the methyltransferase-like (METTL)-3/METTL14 complex act as 'writers', catalyzing m6A RNA modifications (116). In 2011, two demethylases, fat mass and obesity-associated protein (FTO) and AlkB homolog 5 (ALKBH5), were discovered as 'erasers' that remove these methyl groups (117,118). This finding revealed the dynamic reversibility of m6A modifications. Notably, both FTO and ALKBH5 belong to the α-ketoglutarate-dependent dioxygenase AlkB homolog family, highlighting their conserved role in regulating RNA methylation plasticity (119). Simultaneously, m6A reader proteins, functioning as recognition factors, regulate RNA metabolism processes (including splicing, folding, transport, degradation and translation) through their specific recognition of m6A-modified sites (114). Together, these core regulatory components form an integrated m6A modification network, serving not only as key tools for studying RNA methylation mechanisms but also emerging as a key research focus in molecular biology due to their important roles in diverse physiological and pathological processes.

With regard to m6A methylation dysregulation-induced cardiac developmental toxicity, the developmental toxicity mediated by m6A methylation has gradually attracted researchers' attention (120,121). m6A methylation dynamically regulates post-transcriptional processes (such as mRNA stability and translation efficiency) of myocardial development genes. Dysregulation of this epigenetic mechanism may contribute to congenital heart malformations. Bisphenol A (BPA) is a representative industrial chemical used in the production of polycarbonate plastics and epoxy resins (122). It has been established that BPA exposure is associated with numerous health disorders, including metabolic dysregulation,

reproductive impairment, cardiovascular abnormalities, developmental malformations and mammary tumorigenesis (123). Given the potential hazards of BPA, bisphenol C (BPC) has been adopted as an alternative in industrial applications. The presence of BPC is commonly identified in multiple human biospecimens, particularly infant urine, indicating a potential exposure hazard for young children (124). However, whether BPC exerts toxic effects on embryonic development remains poorly characterized, highlighting the need for further toxicological evaluations. Su *et al* (125) demonstrated that BPC disrupts m6A modification homeostasis by suppressing the expression of m6A methyltransferase METTL3. This leads to decreased m6A methylation levels in mRNAs encoding key cardiac developmental regulators, including acyl-CoA oxidase 1 involved in fatty acid metabolism and troponin T2d, key in myocardial contraction. The impaired m6A modification compromises transcript recognition and stabilization by m6A reader protein insulin-like growth factor 2 mRNA-binding protein 2b, ultimately causing structural and functional cardiac abnormalities in zebrafish. This work reveals environmental pollutants can interfere with cardiac development by subverting m6A-mediated epitranscriptomic regulation, identifying novel molecular targets for evaluating developmental toxicity of bisphenol analogs.

In addition to the aforementioned DNA methylation alterations discussed, PM2.5 exposure-induced cardiac developmental toxicity may also involve dysregulation of m6A methylation. A study by Ji *et al* (126) using a zebrafish larval model revealed that extractable organic matter from PM2.5 activates the aryl hydrocarbon receptor (AHR), leading to direct suppression of m6A methyltransferase transcripts METTL14 and METTL3. This resulted in global reduction of cardiac m6A methylation levels, consequently decreasing m6A modifications while increasing expression of apoptosis-related genes (TNF receptor associated factor 4 and BCL2 binding component 3), ultimately triggering excessive ROS production, apoptosis and cardiac developmental malformations. These results establish that AHR-mediated disruption of m6A methylation constitutes a central mechanism underlying PM2.5-induced cardiac developmental toxicity. Importantly, supplementation with the methyl donor betaine or overexpression of METTL14 and METTL3 was found to restore m6A homeostasis and ameliorate PM2.5-induced cardiotoxicity, thereby providing key experimental evidence for therapeutic interventions against environmental pollutant-associated cardiac developmental defects.

Notable knowledge gaps persist in understanding the functional mechanisms of m6A RNA methylation during cardiac developmental toxicity. Although existing studies have established the critical roles of 'writers' such as METTL3/METTL14 in toxin-induced cardiac malformations, the target-specific recognition mechanisms of 'readers' (such as the insulin-like growth factor 2 mRNA binding protein family) and the dynamic regulatory functions of 'erasers' (such as FTO/ALKBH5) remain poorly understood (125,126). Furthermore, the upstream molecular mechanisms by which environmental pollutants (including BPC and PM2.5) specifically interfere with m6A-modifying enzyme activities are yet to be elucidated, although proteomics and co-immunoprecipitation techniques may help identify their direct molecular targets. Additionally, the interactions between m6A and other RNA modifications, such as 5-methylcytosine (m5C) and N7-methylguanosine (m7G), and their synergistic effects in cardiac developmental toxicity warrant further investigation (127). Future research should aim to integrate high-throughput sequencing technologies to comprehensively delineate the spatiotemporal dynamics of m6A methylation in cardiac developmental toxicity.

6. Chromatin accessibility

Chromatin is a dynamic nucleoprotein complex residing in eukaryotic nuclei, comprising three core components, namely genomic DNA, histone proteins and non-histone proteins, with the additional potential association of small RNA molecules (128). As the organizational form of eukaryotic genetic material, chromatin facilitates genetic information storage and transcriptional regulation through its distinctive three-dimensional architecture, while demonstrating dynamic spatiotemporal variations in both molecular composition and structural organization. Chromatin accessibility allows genomic DNA regions to be recognized and bound by nuclear factors. This biochemical characteristic is principally governed by local nucleosome positioning dynamics and competitive binding interactions among DNA-associated proteins (129-131). Key cellular activities, including transcription factor-dependent gene regulation, scheduled DNA replication and repair of genomic damage, all depend on proper chromatin accessibility (132,133). Research findings demonstrate that fluctuating chromatin accessibility patterns serve key regulatory functions across multiple biological pathways, encompassing senescence, immune system regulation and embryogenesis (134-136). The Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) enables efficient detection of cell type-specific chromatin accessibility features and their dynamic alterations under perturbations or disease conditions, while simultaneously facilitating genome-wide analysis of transcription factor binding sites and comparative assessment of chromatin landscapes across biological contexts (137).

With regard to chromatin accessibility dysregulation-induced cardiac developmental toxicity, as advances in epigenetics have occurred, research on the association between chromatin accessibility and cardiac developmental toxicity is gradually emerging. A previous study demonstrated that parental zebrafish exposure to 10 μg/l perfluorobutane sulfonate (PFBS) disrupts the maternal transmission of transcripts related to histone-DNA interactions in offspring oocytes, suggesting that this contaminant may impair transcription factor accessibility to DNA by altering chromatin compaction (138). Notably, exposure to 100 μg/l PFBS further results in reduced embryonic heart rates, providing evidence for PFBS-induced dysregulation of chromatin accessibility to be associated with cardiac developmental toxicity. Unlike the mechanistic inference from zebrafish models, experiments in human stem cells directly demonstrated the chromatin accessibility alterations that lead to cardiac developmental defects through ATAC-seq analysis. Liu *et al* (139) employed ATAC-seq in human induced pluripotent stem cells and hESCs, demonstrating that 13-cis-retinoic acid (isotretinoin;

INN) disrupts cardiac development by altering chromatin accessibility. This perturbation leads to aberrant enhancement of transcription factor binding activities, including hepatocyte nuclear factor 1β, SRY-box transcription factor 10 and nuclear factor I C. This subsequently disrupts the normal function of TGF-β and Wnt signaling pathways and dysregulates the expression of key mesodermal differentiation genes, such as Eomesodermin, Mix paired-like homeobox 1 and Dickkopf WNT signaling pathway inhibitor 1. These findings systematically elucidate the epigenetic mechanisms underlying INN-induced cardiac developmental toxicity at the human stem cell level, providing key evidence for drug safety evaluation.

Investigations into toxin-induced perturbations of cardiac development mediated through chromatin accessibility are only just emerging. Contemporary research proposes that exposure to environmental toxicants can disrupt physiological transcription factor binding to chromatin, potentially inducing aberrant expression of cardiac developmental gene programs (139). However, these findings only indicate associations, lacking direct experimental validation regarding the functionality of open chromatin regions or the regulatory roles of upstream mechanisms, such as DNA methylation and histone modifications, in shaping chromatin accessibility. Furthermore, existing studies predominantly rely on static, *in vitro* models at single time-points, failing to capture the dynamic progression from cardiac mesoderm to cardiomyocyte differentiation. Limitations in multi-omics approaches [such as underutilization of WGBS, chromatin immunoprecipitation followed by sequencing (ChIP-seq)] further hinder systematic dissection of the epigenetic regulatory network (140). Integrating gene-editing technologies with model organisms such as zebrafish will enable more precise elucidation of the mechanisms by which potential toxicants disrupt cardiac development through chromatin remodeling.

7. Epigenetic crosstalk in cardiac developmental toxicity

Advancements in epigenetic research, particularly regarding DNA methylation and histone modifications, have highlighted their intricate interplay and well-coordinated regulatory networks (141-143). Although direct evidence concerning the crosstalk among epigenetic modifications in cardiac developmental toxicity remains scarce, emerging studies provide key mechanistic theories (144-146). Epidemiological data demonstrate a dose-dependent association between maternal caffeine intake and adverse pregnancy outcomes, including spontaneous abortion, low birth weight and congenital cardiac/genital anomalies (147). Experimental investigations consistently reveal that *in utero* caffeine exposure disrupts embryonic cardiac function, leading to developmental cardiotoxicity (144,145). Of note, Fang *et al* (146) demonstrated that gestational caffeine exposure markedly altered both DNA methylation patterns and the expression of histone modification regulators in murine embryonic ventricles, concurrent with the dysregulation of cardiac-specific miRNAs (miR-208a/b and miR-499). These findings collectively suggest that synergistic interactions among DNA methylation, histone modifications and ncRNAs may drive cardiac maldevelopment through disruption of epigenetic homeostasis. However, further systems-level investigations integrating multi-omics profiling and functional validation are key for delineating precise molecular interactions underpinning this regulatory hierarchy and establish a comprehensive epigenetic framework for cardiac developmental toxicity.

8. Advances in epigenetic research methods and models for cardiac developmental toxicity

Integrative multi-omics studies: From single-modification analysis to multi-omics convergence. Advancements in high-throughput sequencing now permit epigenetic evaluation of cardiac developmental toxicity through multi-omics integration, overcoming the previous limitations of single-omics analyses. By contrast with traditional single-epigenetic-marker detection, multidimensional omics technologies integrating DNA methylation (WGBS/MeDIP-Seq), histone modifications (ChIP-Seq), chromatin accessibility (ATAC-Seq), m6A methylation (methylated RNA immunoprecipitation followed by sequencing) and ncRNAs [RNA sequencing (RNA-Seq)] enable systematic elucidation of toxicity-induced epigenetic reprogramming networks and their regulatory effects on cardiac development (Fig. 2A). For instance, the integrated multi-omics analysis of RNA-Seq and MeDIP-Seq not only enables simultaneous detection of DNA methylation modifications and gene expression changes, but also effectively establishes their causal associations (30). This methodology conclusively establishes that toxin-induced cardiac developmental toxicity primarily arises through DNA methylation-directed modulation of gene transcription processes. Furthermore, in combined ChIP-seq and ATAC-seq analyses, researchers can first map the binding sites of cardiac developmental transcription factors using ChIP-seq, and then identify functionally active regions within open chromatin at these binding sites through ATAC-seq (148). This strategy accurately identifies core regulatory elements disrupted by toxicants, demonstrating that cardiac developmental impairment occurs through direct interference with transcription factor binding and diminished chromatin accessibility. This comprehensive approach provides novel mechanistic insights into cardiac developmental toxicity. Furthermore, it enables the advancement of epigenetic biomarkers from individual markers to diagnostic signature profiles, thereby potentially improving both the specificity and sensitivity of toxicity risk assessment.

Cardiac developmental toxicity models: From zebrafish to human cell-based systems. In cardiac developmental toxicity research, zebrafish and human cell-based models represent widely utilized experimental systems (Fig. 2B). In contemporary developmental biology research, zebrafish serve as a primary animal model for heart formation studies (149). Notably, the cardiac structure in zebrafish demonstrates marked conservation with mammalian hearts, displaying ~70% genetic homology to human cardiac genes (150). Embryonic heart development follows a well-defined chronological sequence. First, cardiac progenitor cells begin to differentiate by 5 hpf, forming a linear heart tube by 16 hpf (151-153). Subsequently, key morphological changes occur, including cardiac looping into an S-shape (~33 hpf), initiation of

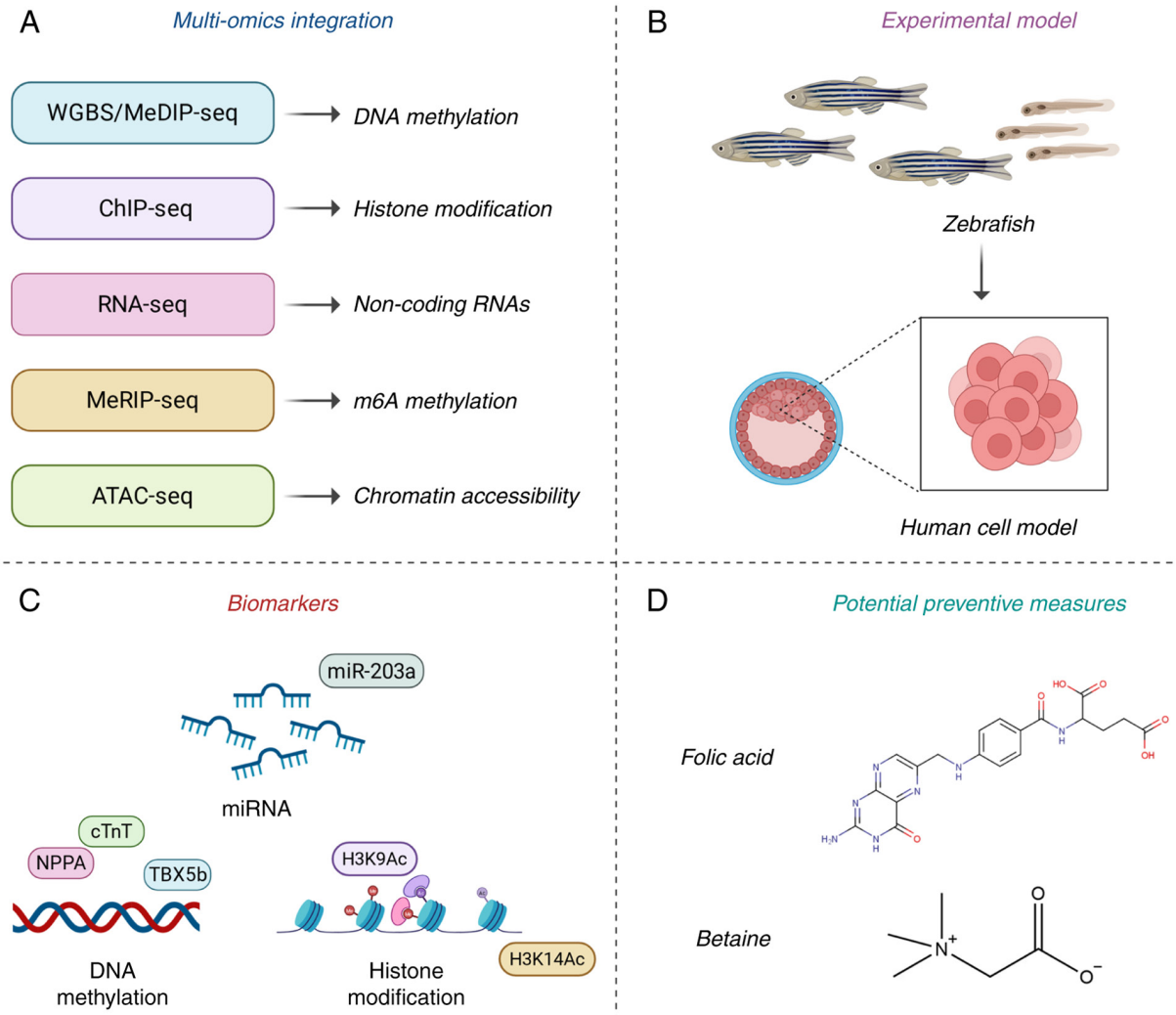

Figure 2. Integrative application of multi-omics technologies and models in cardiac developmental toxicity studies. (A) Multi-omics integration of epigenetic regulation in cardiac developmental toxicity research. (B) Research progress in experimental models of cardiac developmental toxicity. (C) Epigenetic biomarkers identified in cardiac developmental toxicity research. (D) Preliminary measures for preventing cardiac developmental toxicity through epigenetic regulation. WGBS, whole-genome bisulfite sequencing; MeDIP-Seq, methylated DNA immunoprecipitation sequencing; ChIP-Seq, chromatin immunoprecipitation followed by sequencing RNA-Seq, RNA sequencing; MeRIP-Seq, methylated RNA immunoprecipitation followed by sequencing; ATAC-Seq, Assay for Transposase-Accessible Chromatin using sequencing; NPPA, natriuretic peptide A; cTnT, cardiac troponin T2; TBX5b, T-box transcription factor 5b; miR, microRNA; H3K, histone H3 lysine K. The figure was created using BioRender.com.

regular contractions (~36 hpf) and formation of primitive valves (~40 hpf). By 2 days post-fertilization (dpf), distinct inner and outer curvatures become evident in the ventricular region (154). Additionally, zebrafish embryos remain optically transparent until 3-4 dpf, enabling non-invasive real-time visualization and dynamic monitoring of chemical effects on heart development. These features, combined with high-throughput screening technologies, markedly enhance the efficiency of developmental toxicity assessments. Zebrafish exhibit good reproductive capacity, with adult individuals capable of year-round cyclical spawning, producing <200 eggs per female weekly (155). From an ethical standpoint, the early life stages of zebrafish are considered to experience minimal pain or distress upon chemical exposure, more closely aligning with animal welfare guidelines and reducing ethical concerns in experimental studies (156). However, this model also presents a number of limitations. Compared with mammalian models (including mice), zebrafish possess fewer biological resources such as antibodies and gene-editing tools, restricting their application in protein biochemistry and related fields. The cardiac architecture of zebrafish is composed of two distinct chambers (an atrium and ventricle), differing from the four-chambered organization typical of mammals. This structural disparity restricts its effectiveness as a model for investigating cardiac septation processes or sophisticated hemodynamic parameters (152). Additionally, zebrafish embryos primarily absorb test compounds through cutaneous exposure, differing from common human exposure routes such as oral ingestion or inhalation (157). Furthermore, the absence of distinct sex differentiation markers during early developmental stages hinders the assessment of sex-specific toxicological effects (158).

Overall cardiac organogenesis relies on animal models to investigate morphogenesis and systemic integration, yet early key events (such as stem cell differentiation and cardiac progenitor fate determination) can be effectively studied

using pluripotent stem cells (PSCs) and *in vitro* models (159). Through directed differentiation of ESCs or induced PSCs (iPSCs), researchers can systematically recapitulate key stages of cardiac development. Specifically, BMP/Wnt signaling activation initially induces mesoderm formation and then, under defined growth factors (such as activin A and BMP4) combined with Wnt pathway inhibition, drives further differentiation into cardiac progenitor cells, ultimately yielding functionally contractile cardiomyocytes (159). This process closely mimics the spatiotemporal features of embryonic heart development, providing an ideal platform for exploring cardiac developmental mechanisms and drug toxicity screening (79). However, this technology exhibits inherent limitations. First, the differentiation efficiency of ESCs/iPSCs into cardiomyocytes remains relatively low (typically 1-3%) (159), often resulting in heterogeneous cellular populations containing undesired non-cardiac lineages that may compromise experimental reliability (160). Second, the *in vitro* culture system cannot fully recapitulate the dynamic regulatory network governing cardiac development *in vivo*, thereby constraining its applicability for comprehensive organ-level studies. Consequently, this model system may be more suited towards mechanistic investigations at cellular and molecular levels rather than integrated organ-scale analyses.

Human cardiac organoids (hCOs) represent a biomimetic three-dimensional culture model that effectively recapitulates key aspects of heart development and drug toxicity responses. These systems are composed of multiple cell types, including cardiomyocytes, endothelial cells and stromal cells, establishing a physiologically relevant microenvironment that surpasses conventional two-dimensional cultures in functional evaluation (161). hCOs exhibit mature functional properties, such as spontaneous contractility and stable electrophysiological signals, enabling simultaneous assessment of drug effects on cardiomyocyte proliferation and function. However, it should be noted that hCOs more closely mimic the postnatal cardiac physiology, limiting their applicability for toxicity prediction during early embryonic development (162). Additionally, challenges remain, including prolonged culture duration, technical complexity and insufficient standardization, which warrant further optimization to enhance the reproducibility and scalability of the hCO model.

9. Epigenetic discoveries elucidating cardiac developmental toxicity

Identification of epigenetic biomarkers in cardiac developmental toxicity. Biomarkers represent measurable biological parameters that objectively indicate physiological processes, pathological conditions or pharmacological responses, making them important tools for disease screening, accurate diagnosis and reliable prognostic assessment (163). Advances in omics technologies, particularly high-throughput genomic profiling, have enabled systematic identification of candidate biomarkers while providing novel approaches to elucidate disease mechanisms. Epigenetic markers have gained particular prominence as promising next-generation biomarkers, given their key role in regulating disease pathogenesis (7).

In the investigation of cardiac developmental toxicity, a number of epigenetic biomarkers have been preliminarily identified as serving as early warning indicators of toxicity (Fig. 2C). Notably, miR-203a has been consistently shown to be downregulated during cardiac toxicity events and zebrafish embryos treated with miR-203a inhibitors exhibit characteristic cardiac malformations, suggesting its potential as a biomarker for assessing developmental cardiotoxicity induced by numerous toxicants (101,102,110). At the DNA methylation level, hypomethylation of NPPA and cTnT genes, along with hypermethylation at the TBX5b promoter region, may serve as epigenetic signatures for phthalate-induced cardiac defects (30). Regarding histone modifications, reduced H3K9Ac and H3K14Ac levels appear to represent key epigenetic biomarkers for triazole fungicide (FBZ-induced) transgenerational cardiotoxicity (68). However, current limitations include insufficient mechanistic validation of these biomarkers and their specificity to particular toxicants. This further compromises their general applicability. Therefore, establishing standardized epigenetic toxicity assessment systems will markedly facilitate the identification and application of developmental cardiotoxicity biomarkers.

Preliminary exploration of potential preventive measures. Based on current research findings, numerous candidate drugs have been identified that may exhibit protective effects against cardiac developmental toxicity (Fig. 2D). Folic acid, a key water-soluble B vitamin (vitamin B9), cannot be synthesized endogenously and must be obtained through dietary sources such as leafy green vegetables and citrus fruits (164). This biological component has key functions in important cellular activities such as nucleotide biosynthesis and DNA methylation processes (165). Research demonstrates that folic acid, as a vital nutritional factor, exhibits notable efficacy in preventing cardiac and neurological developmental defects during embryogenesis (166). Additionally, folic acid has shown promising potential in exploring epigenetic mechanisms for preventing cardiac malformations, particularly through DNA methylation-related pathways. Research has demonstrated that folic acid supplementation corrects methylation imbalances by restoring the SAM/SAH ratio and modulating DNMT expression, consequently ameliorating cardiac malformations (25). This discovery provides a potential intervention strategy against cardiac developmental toxicity induced by environmental pollutants such as PM2.5. Similarly, evidence shows that folate effectively alleviates selenite-induced genomic methylation disorders and markedly reduces the incidence of cardiac developmental anomalies, indicating its preventive role against developmental toxicity through methylation reprogramming regulation.

Betaine, also known as trimethylglycine, derives its name from its structural similarity to glycine but with three additional methyl groups (167). As a safe and stable natural compound, betaine primarily serves as a methyl donor in biological systems, participating in transmethylation reactions and serving a key role in SAM synthesis (168). SAM, a key cellular methyl donor, is involved in diverse epigenetic modification processes, including m6A RNA methylation. By promoting SAM production, betaine indirectly modulates m6A methylation levels, thereby influencing gene expression, RNA metabolism, and cellular functions regulating glucolipid metabolism (168-170). Previous research has demonstrated

that betaine supplementation restores global m6A methylation levels in the heart, mitigating PM2.5-induced cardiac developmental toxicity by reducing ROS overproduction, mitochondrial dysfunction and cell death (126). These findings provide important evidence for preventing cardiac developmental toxicity through epigenetic regulatory mechanisms, although the discussed evidence primarily focuses on DNA methylation and m6A RNA methylation regulation. Future investigations should aim to examine more thoroughly the therapeutic implications of addressing supplementary epigenetic regulators, such as histone marks, ncRNA-mediated control and DNA accessibility patterns, in developmental cardiotoxicity contexts. Currently, the majority of evidence originates from preclinical studies (cell-based and animal models), whereas clinical validation in humans remains limited. Unresolved issues, such as optimal dosage, administration timing and potential adverse effects, require systematic investigation before this approach can progress to clinical application.

10. Conclusion

Environmental pollutants and pharmaceuticals have become a growing concern owing to their widespread potential to induce embryonic cardiac developmental toxicity. Epigenetic marks demonstrate notable dynamic plasticity during embryonic heart development, with their modification patterns subject to regulation by numerous environmental factors such as nutritional status, maternal exposure to environmental contaminants and pharmacological agents. The present review focuses on epigenetic regulatory mechanisms and elucidates the molecular pathways through which specific pollutants and chemicals mediate cardiac developmental toxicity through epigenetic modifications. In addition, potential epigenetic biomarkers and preventive pharmaceuticals are summarized, which may contribute to monitoring embryonic cardiac developmental toxicity and implementing preventive interventions (25,68,110,126).

The current understanding of epigenetic mechanisms underlying cardiac developmental toxicity remains limited, with numerous key knowledge gaps yet to be resolved. First, the establishment of causality remains inadequate. The majority of studies have only observed associations between epigenetic modifications (such as DNA methylation) and cardiac malformations, lacking direct experimental evidence demonstrating that specific modifications drive the toxic phenotypes (42,44,48). This gap in causality markedly restricts the translational applicability of existing findings. Second, systematic research into epigenetic networks is lacking. Although crosstalk exists among numerous epigenetic modifications, the mechanisms by which potential toxicants regulate these interactions to induce cardiac developmental defects remain poorly understood. In addition, cardiac development is a continuous and precisely timed process, yet current studies tend to employ static analytical approaches, examining epigenetic changes at isolated time-points only. Consequently, they fail to elucidate how pollutant-induced epigenetic reprogramming dynamically evolves across different developmental stages (such as cardiac tube formation and atrioventricular septation) or its stage-dependent impact on developmental progression. Furthermore, the number of identified epigenetic biomarkers remains limited and exhibits insufficient specificity, hindering their utility in clinical diagnosis and environmental monitoring (30,68,110). Drug development targeting epigenetic regulation for the prevention of cardiac developmental defects is notably underdeveloped. Although methyl donors such as folate and betaine have demonstrated protective effects in a number of studies, their actions lack specificity and may inadvertently interfere with normal epigenetic regulation during development (23,119).

Future research should aim to integrate multi-omics and epigenomic profiling technologies with CRISPR-based functional validation to systematically decipher the molecular mechanisms by which environmental exposures (such as pharmaceuticals and pollutants) perturb cardiac development through epigenetic regulation. Additionally, it is important to strengthen the 'exposure-epigenetics-phenotype' evidence chain by precisely defining key time windows and dose-effect relationships. The development of AI-driven high-throughput biomarker screening platforms may help identify diagnostic molecular signatures and facilitate the exploration of targeted epigenetic interventions. These strategies will enhance early detection and prevention of embryonic cardiac developmental toxicity, providing a strong scientific foundation for translational applications.

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ZQ and RC drafted the manuscript and prepared the figures and tables. RC and DS edited and revised the manuscript. All the authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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