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REVIEW ARTICLE

Advantages of omics technology for evaluating cadmium toxicity in zebrafish

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Abstract

In the last decade, several advancements have been made in omics technologies and they have been applied extensively in diverse research areas. Especially in toxicological research, omics technology can efficiently and accurately generate relevant data on the molecular dynamics associated with adverse outcomes. Toxicomics is defined as the combination of toxicology and omics technologies and encompasses toxicogenomics, toxicoproteomics, and toxicometabolomics. This paper reviews the trend of applying omics technologies to evaluate cadmium (Cd) toxicity in zebrafish (*D. rerio*). Cd is a toxic heavy metal posing several environmental concerns; however, it is being used widely in everyday life. Zebrafish embryos and larvae are employed as standard models for many toxicity tests because they share 71.4% genetic homology with humans. This study summarizes the toxicity of Cd on the nerves, liver, heart, skeleton, etc. of zebrafish and introduces detailed omics techniques to understand the results of the toxicomic studies. Finally, the trend of toxicity evaluation in the zebrafish model of Cd based on omics technology is presented.

Keywords Cadmium · Zebrafish · Toxicogenomics · Toxicoproteomics · Toxicometabolomics

Introduction

Cadmium (Cd) is widely used in objects such as batteries and in processes such as plating and can also leak around factories manufacturing these products. In our daily lives, we may be exposed to Cd through food and air, but the actual amounts are negligible and have little effect on the human body. However, if harmful metals from factory by-products or household waste pollute the atmosphere, soil, and water and accumulate in animals and plants, they may be absorbed in large amounts by humans and cause deleterious health effects. For example, the Itai-Itai disease ranks among the four major pollution-related diseases in Japan; its symptoms are pain and osteomalacia, and it was caused by the consumption of rice contaminated with Cd from a factory leakage [1]. In Korea, high concentrations of Cd in groundwater and rivers have been reported; the source was a smelter located upstream of the Nakdong River [2]. Water pollution with Cd leads to soil accumulation, and finally affects agricultural and marine products consumed by humans. Fine dust, an air pollution source, can also be a source of exposure to Cd [3]. In addition to environmental problems, exposure to Cd is caused by problems in the processing of pharmaceuticals, cosmetics, toys, and household goods or because of occupational reasons [4].

Omics technologies produce large-scale datasets with information on genes, proteins, metabolites and/or protein modification by measuring the global, qualitative, and quantitative changes at the molecular, cell, tissue, and individual levels [5, 6]. In the last decade, omics technologies have advanced tremendously and have been applied extensively in research. From a toxicological perspective, omics can efficiently and accurately generate relevant data on

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molecular dynamics associated with adverse outcomes [7]. Compared with previous approaches to precisely measure toxicant-induced molecular alteration, omics technologies have the potential to improve chemical safety assessment and reduce animal testing in regulatory toxicology [8].

The term toxicomics is not defined in the existing literature, but this study proposes it to refer to the omics applied to toxicology. Specialized omics terms exist for toxicology research fields based on a single omics technology, such as toxicogenomics, toxicoproteomics, and toxicometabolomics [7–9]. These three terms can be collectively defined as toxicomics, just as omics includes genomics, epigenomics, transcriptomics, proteomics, and metabolomics (Fig. 1). Toxicogenomics generally refers to transcriptomics, the techniques used to study genomic-scale changes in RNA, which are mainly detected via a known set of differentially expressed target genes [5, 10]. Toxicoproteomics applies global protein expression technologies to toxicology testing and clinical research [11]. Toxicometabolomics is the systematic study of endogenous metabolites and biochemical processes in the cell, tissue, or organism to identify and characterize the end products of toxic reactions [12]. Genomics is based on the sequence information of genes and proteins, while transcriptomics, proteomics, and metabolomics provide information about the biological function of genetic information.

Zebrafish (*Danio rerio*) is a standard model animal for omics-based toxicity assessment. Its embryos and larvae are used as models in many toxicity tests. Embryos can be obtained in large quantities, and the fact that they are huge and transparent enables the easy visualization of toxicity-related changes. Zebrafish is a vertebrate that shares 71.4% genetic homology with humans. Besides, the major organs such as the heart, liver, and kidneys are comparable between both species [10]. This study describes and compares the omics- and zebrafish model-based Cd toxicity assessments. Based on the research findings from the zebrafish model, a new, promising research trend that uses the omics technology in the identification of the toxicity mechanism is emerging.

Cadmium toxicity in zebrafish

The toxicity results of Cd in zebrafish, which were identified without using omics technology, are summarized in Table 1. Cd mainly causes developmental abnormalities in zebrafish embryos and damages the nervous system. In particular, Cd inhibits the expression of glial fibrillary acidic protein; induces the expression of *mpz*, a specific myelin gene, which

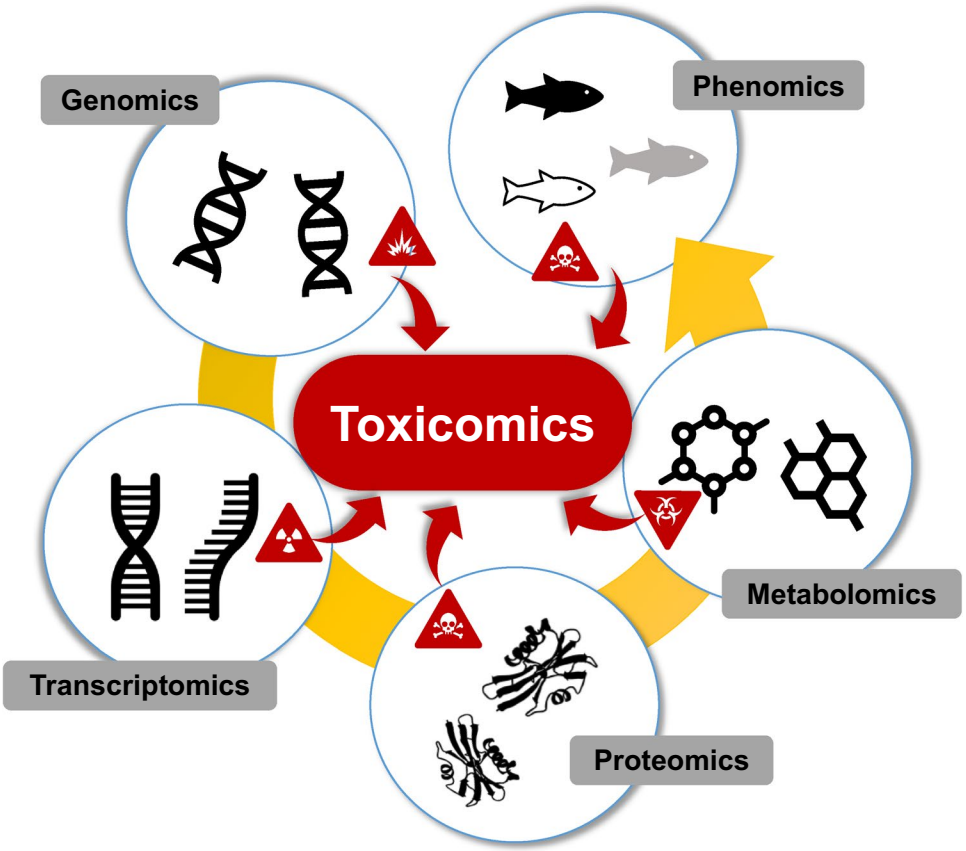

Fig. 1 Overview of harmonized toxicomics. Molecules that change according to the exposure to toxic substances are collected based on each omics technology and the toxic mechanism is identified through integrative omics analysis

Table 1 Summary of toxic effects of cadmium in zebrafish

Toxicity		Ref.
Embryos		
Liver	Hepatic lipid accumulation	[20]
Nerve	Neuroglia alterations	[12]
	Increased ATPase activity in brain	[15]
	Reduction of neuronal differentiation and axonogenesis	[14]
	Interference of neural development	[13]
	Anti-estrogen in brain	[16]
	Abnormal somite patterning	[19]
Myoskeletal retina	Eye hypoplasia and hypopigmentation	[18]
Cardiovascular organ	Heart edema and increased pericardial area	[17]
	Activation of cell death pathway in olfactory epithelium	[40]
Olfactory organ	Delay in hatching time	[17]
Others	Tail and axis malformation	[17]
Larvae		
Nerve	Circadian rhythms disruption	[22]
Others	Cell death and structural alterations in olfactory epithelium	[21]
Adults		
Liver	Carcinogenesis	[45]
	Hepatic lipid accumulation	[23]
	Oxidative damage	[24, 25]
Nerve	Oxidative damage	[25, 26]
Myoskeletal	Structural disorganization, disassembly of muscular myofibrils	[28]
Reproductive organ	Pair spawning reduction and teratogenicity	[27]
	Ovary: oxidative damage	[25]
Retina	Nerve fiber thickening and vacuolating	[29]

changes the glial cells; inhibits the expression of neurexin protein; and inhibits neuronal development [12, 13]. In the differentiation process, proneural gene transcription is reduced, thereby inhibiting neuron differentiation, which affects the activity of specific enzymes such as ATPase in the brain, and inhibits the estrogen signaling pathway [14–16].

Besides provoking neurological disorders, Cd delays hatching and damages the other organs [17]. The heavy metal inhibits the development of neural functions in pigment cells, causing abnormalities in the eyes. Furthermore, it leads to an abnormal expression of the genes necessary for the formation of the musculoskeletal system, causing larvae morphology [17–19]. It also induces apoptosis in the olfactory organs and damages the olfactory epithelium. In the liver, it alters the structure and function of HDL3, which is required for lipid metabolism [20].

Cd also inhibits metabolism and may damage the cardiovascular system [17]. In larvae, Cd affects the olfactory organs (in the same way it affects those of the embryo) and also causes an immune response which alters the circadian rhythm [21, 22]. In adults, the site of toxic reactions is similar to that of embryos, and the liver shows similar abnormalities in lipid metabolism [23]. The typical toxicity seen in adults is oxidative damage, which occurs in the liver, nerves, and ovaries [24–26], and exposure to Cd lowers the spawning success rate of female zebrafish and decreases the fertility of the born larvae [27]. It also causes structural abnormalities of the musculoskeletal system and retina [28, 29]. Reviewing the literature, we found that Cd exerts various toxic effects, including developmental disorders in several organs of zebrafish, and is involved in areas from transcription to enzyme activity. We then attempted to relate the omics result to the traditional Cd toxicity mechanisms.

Research technologies in omics

Omics research is subdivided into various fields such as genomics, transcriptomics, proteomics, and metabolomics (Fig. 1). Omics research emerged as a new tool in environmental toxicity assessment to identify the adverse outcome pathways, point of departure (PoD), etc. [6, 30]. Omics analysis is an effective technical tool for the qualitative and quantitative analysis of biological molecules which require sensitive analytical techniques [31]. When compared with conventional toxicity assessment, the advantage of omics technology is the ability to understand changes at the molecular level based on the abundance of useful information.

Genomics has provided fundamental information by identifying the nucleotide sequence, structure, and function of the genome [32]. Especially, the research applications of genomics in zebrafish are broad, and functional genomics plays a central role. At the DNA level, studies on mutagenesis typically modify zebrafish genes to produce specific diseases, such as Parkinson's disease and cancer, or to utilize them in toxicity assessment models [33–35]. Epigenomics, (emerging toxicological indicator), investigates the inheritance phenomenon by altering the expression pattern of genes without changing the nucleotide sequence after birth [36]. It confirmed that DNA alterations (methylation, histone modifications, and miRNA expression) could be induced by external stimuli such as toxic chemical exposure [37].

In the translation process, mRNA is involved as a transcript, and the abundance of the transcriptome can be confirmed by analyzing the nucleotide sequence of the expressed mRNA through transcriptomics [38]. Microarray or new generation sequencing (NGS), such as RNA-sequencing technology, can provide information about the transcriptome [39].

NGS determined the mRNA expression of over 20,000 genes related to embryonic development in zebrafish much faster than any other methods [40]. Ontological validation methods, such as quantitative polymerase chain reaction, are commonly used to reinforce the reliability of volumonous data [41–44]. The function of genes corresponding to the expressed mRNAs or the relationship between genes can be associated with variations in metabolic pathways using databases such as the Kyoto Encyclopedia of Genes and Genomes pathways and Gene Ontology (GO) [45]. Meanwhile, progressive research is going on to derive PoD from the reduced zebrafish transcriptome approach and apply it to regulatory toxicology [46].

An example is an experiment that evaluated the toxicity in a zebrafish model through transcriptomics. Dibenzazepine, one of the representative polyhalogenated carbazoles that are structurally similar to dioxin, disrupted the aryl hydrocarbon receptor activation genes, such as *AhR1* and *CYP1A* [47]. As a result, the metabolic pathway related to protein processing in the endoplasmic reticulum (ER) and taste transduction was disrupted. Bisphenol A, a well-known environmental hormone, was identified for the adverse effects of retinol and glutathione metabolism and lipid transport by the upregulation of steroid hormone biosynthesis genes (such as *cyp19a1b*) and lipid transport protein genes (*apoa1a*, *apobb1*, and *apoa4a*) [48].

The proteome encompasses all the products of gene translation, which have a plethora of structures and functions [49]. The goal of proteomics is to identify the structure and function of proteins and their modifications [50, 51]. Toxicoproteomics applies global protein expression analysis technologies to toxicological and clinical research [11]. Especially, mass spectrometry (MS)-based toxicoproteomics has measured the quantitative changes in proteins, which are the adverse effects of toxicants, by identifying the protein through MS [52]. To apply MS, the samples must be digested to denature the protein and label the specific peptide site. Three labeling methods are available: (1) stable isotope labeling with amino acids in cell culture (SILAC), (2) isobaric tags for relative and absolute quantitation (iTRAQ), and (3) tandem mass tag (TMT) [53–55]. MS-based methods can identify thousands of proteins within hours using a single analysis by overcoming the limitations of the classical methods such as Western blot assay [56, 57]. These techniques can help identify differentially expressed proteins, and the quantitative results can be analyzed using bioinformatics tools to obtain information on the motif and protein–protein interaction networks [58].

There are previous reports in which the mechanism of toxicity has been elucidated through proteomic studies in a zebrafish model. In 3,4-dichloroaniline toxicity, non-detachment of the tail, lack of somite formation, absence of heartbeat, pericardial edema, abnormal curvature of the spine, and yolk sac edema were observed. These effects were presumed to be due to the disruption of hormone-related pathways and lipid metabolism [59]. Following proteomic analysis, β-methyl-amino-L-alanine showed increased protein biosynthesis and RNA processing proteins, such as eIF3a/c and CPSF5, and decreased 40S ribosomal protein S21 and phenylalanine-tRNA ligase α, which are linked to the disruption of pathways for glutamate receptor activity/recycling, ER stress, protein biosynthesis, and neural cell death [60].

Metabolites (endogenous small-molecule substances) are products of physiological processes. Metabolomics aims to obtain meaningful information on metabolic processes by analyzing changes in organisms at the metabolome level [61]. The method for metabolite analysis is either called target analysis or non-target analysis depending on whether the specific metabolite is targeted [62, 63]. Based on the research objectives, the metabolites are extracted by proper pretreatment methods, such as liquid–liquid extraction or solid-phase extraction, and then identified through MS-based analysis [64]. Since even very low concentrations ($< \text{pg ml}^{-1}$) of metabolites can be detected, changes in the molecular level can be understood using a single analysis without the need for several detection kits [65]. Changes in the identified metabolite levels are visualized by statistical methods such as hierarchical clustering analysis, principal component analysis, and partial least squares discriminant analysis [66]. Similar to the other omics described thus far, toxicometabolomics analyzes the metabolic pathways and summarizes the results to obtain clear information on changes due to toxicity [67].

In previous studies, the mechanism of toxicity was identified through metabolomic analysis. Haloperidol, a common butyrophenone-derived antipsychotic drug, was identified to have adverse effects on vitamin B12 metabolism, neurotransmission, insulin signaling, and mammalian target of rapamycin pathway, which are linked to pericardial edema, curvature of the spinal cord, and heart sac edema [68]. Perfluorooctanoic acid, an alternative of perfluorooctane sulfonate, showed similar adverse effects on peroxisome proliferator-activated receptor-γ-regulated signaling pathways and mitochondrial pathways, and hepatoxicity and neurotoxicity were observed in exposed subjects [69].

Since changes in biological substances are closely related to biological processes, results derived from each omics need to be correlated with those from other omics [70, 71]. Hence, the multi-omics approach is gaining attention; it can satisfy this need and provide more reliable biomarkers, thanks to a wide field of view encompassing several layers of omics [72]. The overlapping goal of this multi-omics approach is to identify the levels of RNA, proteins, and metabolites through non-target analysis and link them to physiological changes [73]. Besides, a study employing multi-omics obtained significant information even at low concentrations, a level at which embryos do not show lethality [74]. Even at low concentrations, substances such as environmental hormones, which have similar toxic mechanisms, showed disruptive effects on transcriptomic, proteomic, and metabolomic profile in zebrafish in similar patterns. Therefore, it is reasonable to expect that detecting in vivo changes at the level of omics will allow the evaluation and prediction of toxicity in the near future [75, 76].

Omics-based cadmium toxicity

We confirmed the results from previous studies on zebrafish exposed to Cd with omics technologies, namely a detoxification mechanism in addition to the toxic mechanism of Cd (Table 2). The transcription of proteins mainly involved in metal homeostasis was increased. Exposition to Cd increases the expression of the *mt2* gene, which codes for the metallothionein-2. This protein plays a role in homeostasis and detoxification of heavy metals and is a molecular marker for metal contamination [77]. Besides, *hsp70.1* and *hsp70l*, whose expression is increased, induce the synthesis of heat shock protein 70, a chaperone [78]. Furthermore, exposure to Cd increases the expression or activity of antioxidant glutathione-*S*-transferase (GST), catalase (CAT), and glutathione reductase (GR) [79, 80]. Specifically, concerning GST, the expression of *gstm3* at 48 hpf, and *gstp1*and *gstp2* at 96 hpf increased depending on the $CdCl_2$ concentration (at 0.9, 1.8, and 3.3 mg/L) and decreased when exposed to 4 µM for 5 days. CAT also showed a complex pattern. When exposed to 4 µM $CdCl_2$ for 5 days, the expression decreased and the activity was reported to increase, but when exposed to 1.78 µM Cd for 7 days, the activity decreased [80, 81]. These omics approach results confirmed that additional studies on GST and CAT are needed.

The omics approach results on the toxic effects of exposure to Cd are as follows: in zebrafish, Cd inhibits the

Table 2 Toxicity of Cd in zebrafish based on omics analysis

Toxicity		Omics	Ref.
Detoxification	Metal ion binding gene (*mt2*, *klf11a*, *klf11b*) upregulation	Transcriptomics	[79, 88]
	Matrix metalloproteinases gene (*mmp9*, *mmp13a*) upregulation		
	Detoxication gene (*gstm3*, *gstp1*, *gstp2*) upregulation		
	Oxidant stress gene (*hsp70.1*, *hsp70l*, *mt2*) upregulation		
	Metallothionein, glutathione reductase upregulation	Proteomics	[80]
Nerve	*uqcrfs1 and rpsa* upregulation	Proteomics	[84]
	Related to neuromast gene (*cldnb*, *stat3*) upregulation	Transcriptomics	[88]
Liver: oxidative stress	Catalase downregulation	Transcriptomics	[81]
	ATP7A and metallothionein downregulation		
	Downregulation of gene expression because of GpG methylation of HSP70 upregulation		
	GSH, SOD, catalase downregulation	Proteomics	[80]
	GSH, SOD downregulation		
Immune	IL-1β, iNOS, TNF-α downregulation	Transcriptomics	[81]
Metal homeostasis	Calcium homeostasis gene (stc1l) downregulation	Transcriptomics	[79]
Skeletal muscle	Pro-apoptotic gene (*bax*, *mt1*) upregulation Cytoglobin gene (*cyt*) upregulation Pyruvate carboxylase gene (*pyc*) upregulation ABC transporter gene (*tap*) upregulation	Transcriptomics	[87]

activity and synthesis of antioxidant enzymes and proteins. Consequently, reactive oxygen species (ROS) in various organs cause several problems. For instance, the liver accumulates excessive fat through lipid peroxidation [82]. Cardiac toxicity of Cd is associated with the decreased expression of stanniocalcin 1, which is involved in the regulation of calcium and phosphate homeostasis [79]. Since stanniocalcin 1 protects the cells from ventricular dysfunction and ROS hyperplasia, its downregulation by Cd leads to heart edema or increased pericardial area [83]. *Uqcrfs1,* which is highly expressed in the Cd-induced zebrafish brain and encodes ubiquinol-cytochrome C reductase, Rieske iron-sulfur polypeptide I, constitutes an electron transport system and is involved in ATP synthesis. Abnormal expression of *uqcrfs1* affects the electron transport system, and it is also used as a biomarker because it is related to cancerous conditions [52, 84, 85]. In the musculoskeletal system, the pro-apoptotic gene *bax* is upregulated by Cd [66]. Indeed, exposure to Cd causes abnormal apoptosis in zebrafish embryos [86]. Considering these two results together, the abnormal apoptosis caused by Cd may affect the development of nerves and muscles in embryos.

More toxicity can be inferred based on what was learned through omics research. We believe that additional studies can be conducted on immune adverse reactions based on the decreased expression of IL-1β, iNOS, and TNF-α and on blood coagulation disorders based on the overexpression of the cytoglobin gene (cyt) [81, 87].

Perspectives

In this paper, we investigated the toxicity that occurs when zebrafish are exposed to Cd and linked it to gene and protein expression. Cd mainly causes abnormalities in the development of embryos and larvae, and toxicity was also observed in the liver and nervous system. Omics research provided additional information about the toxicity of Cd. Regarding Cd-induced oxidative stress, toxicity was traditionally determined by evaluating the expression of only designated markers related to ROS after exposure to Cd [24, 25], whereas proteomics-based studies suggested proteins that respond to the 'Response to Stress category' even with designated markers [60]. A detailed mechanism of Cd-derived oxidative stress was suggested, providing insights for further research.

Although this study compared the Cd toxicity in the zebrafish model, the toxicity assessment in adults, larvae, and embryos was subdivided, and due to the difference in detailed methods in the omics-based and non-omics-based experiments, accurate comparisons could not be made. Since the two approaches have their own strengths and weaknesses and research goals, it may not be appropriate to consider comparative advantage. Overall, the technical basis of omics research is a great advantage when exploring detailed mechanisms for toxic phenomena. It goes beyond exploring a few mechanisms at a time and allows the quantification of several unspecified markers simultaneously. Since the accuracy and reproducibility of the omics research technology has rapidly increased in recent years, its utility in toxicity studies will surely receive attention. This study presents the results of Cd toxicity evaluation in the zebrafish model and the trend of toxicity evaluation research based on Omics technology. Although the number of omics-based studies is still insufficient, we expect the development of omics technologies to soon allow further clarification of the Cd toxicity mechanisms.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to disclose.

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