



Cadmium Through Disturbing MTF1-Mediated Metal Response Induced Cerebellar Injury

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Abstract

Cadmium (Cd) is a toxic environmental contaminant, which bio-accumulate in animals through the food chain. Cerebellum is one of the primary target organs for Cd exposure. In this study, we established a chronic Cd exposure model; 60 chickens were treated with Cd (0 mg/kg, 35 mg/kg, 70 mg/kg) for 90 days. Clinical manifestations indicated that the chicken was depressed and has unstable gait under Cd exposure. Histopathological results indicated that Cd induced neuronal shrunken and indistinct nucleoli, and the number of Purkinje cells decreased significantly. Cerebellar metal contents were analyzed by ICP-MS. We found that Cd caused Cd and Cu accumulation and decreased the content of Se, Fe, and Zn, suggesting that Cd disturbed metal homeostasis. Besides, Cd treatment group also showed high levels of malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) content and inhibited selenoprotein transcriptome, suggesting that Cd exposure resulted in oxidative stress. Notably, low-dose Cd exposure activated MTF1 mRNA and protein expression and its target metal-responsive genes, including MT1, MT2, DMT1, ZIP8, ZIP10, TF, and ATP7B which indicate cellular adaptive response against Cd-induced damage. On the other hand, 70 mg/kg Cd downregulated MTF1-mediated metal response, which was involved in Cd-induced cerebellar injury in chicken. In conclusion, our data demonstrated that molecular mechanisms are associated with Cd-induced cerebellar injury due to disturbing MTF1-mediated metal response.

Keywords Cadmium · Selenoprotein transcriptome · MTF1-mediated metal response · Chicken · Cerebellar injury

Introduction

Cadmium (Cd) is a widespread hazardous pollutant that has a long biological half-life in humans (20–30 years) (Satarug et al. 2010). Metallurgy and the plastic industry, mining, and

battery production released Cd into the environment that polluted agricultural land and food (Anetor 2012). Cd through the food chain accumulates into the kidney, liver, and other organs and resulted in irrevocable injury to the target organs; moreover, Cd exposure also caused certain carcinogenic and increased non-carcinogenic health risk (Wang et al. 2021). China paid high attention to food security issues that caused

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by Cd (Feng et al. 2020). Notably, Cd has been linked to the development of neurological diseases via triggering oxidative stress, which caused cellular damage and altered gene transcription, which ultimately led to cell apoptosis (Jomova and Valko 2011; Lopez et al. 2003). Moreover, it has been confirmed that Cd can cause nervous system injury and neurological disorders that lead to learning disabilities and cognitive dysfunction (Karri et al. 2016). It is worth noting that selenium (Se) can reduce the accumulation of Cd in the brain of chickens and reduce oxidative stress and histopathological damage (Liu et al. 2014). Previous study has shown that exogenous Se addition inhibits the absorption of Cd (Costa et al. 2020), suggesting Se might reduce the concentration of Cd and thereby reduce the toxicity of Cd. Importantly, selenoprotein was associated with antioxidant roles and promote the growth of nerve cells (Adedara et al. 2020). However, the effect of Cd toxicity on the nervous system has not been comprehensively investigated, especially in cerebellum.

It is worth noting that metal transcription factor 1 (MTF1) is the indicator that can respond higher and lower levels of metal content and protects cells against oxidative and hypoxic stresses (Park and Jeong 2018). MTF1 regulates expression of its target genes in response to heavy metal load, through binding metal response elements (MREs) in the respective enhancer/promoter regions; in addition, it plays a protective role in the oxidative stress response (Wimmer et al. 2005). Former researchers have proposed that low affinity interactions between zinc and specific zinc fingers in MTF-1 reversibly regulate its binding to the metal response elements in the mouse metallothionein-I promoter. Besides, MTF1 can activate metallothionein expression in response to the heavy metals Cd (Smirnova et al. 2000). Previous study indicated that the sturdy induction of metallothionein-1 and 2 (MT1 and MT2) genes by Zn and Cd requires the specific transcription factor MTF1 (Wimmer et al. 2005), and MTs bind and sequester metals that are involved in zinc, ferrum, and copper homeostasis and alleviating oxidative stress and adjusting metal homeostasis (Chen et al. 2020; Forcella et al. 2020).

As stated above, although some signaling pathways have been involved in Cd-induced neurotoxicity, however, the role of MTF1-mediated metal response in the underlying mechanism of Cd-induced cerebellar injury is still unclear. The present study explored the novel mechanism of Cd-induced cerebellar injury in chicken via adjusting MTF1-mediated metal response.

Materials and Methods

Study Approval and Reagents

The animal protocol was approved by the Animal Care and Use Committee of Northeast Agricultural University. All

procedures were performed in accordance with the ethical standards of the institution. In addition, the study was in accordance with Good Laboratory Practice (Trial) published by the China Food and Drug Administration (CFDA). Furthermore, CdCl₂ was provided by Tianjin Zhi-yuan Chemical Reagent Company, China; the assay kits of hydrogen peroxide (H₂O₂), catalase (CAT), glutathione peroxidase (GSH-PX), total superoxide dismutase (T-SOD), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) contents were supplied by Nanjing Jian-cheng Bioengineering Institute, P.R. China.

Animals and Treatment

Chicks were obtained from Xian Feng Chick Farm (Harbin, P.R. China). The chicks were equally divided into 3 groups (20 chickens/group) including the control group (basic diet), low dose group (35 mg/kg CdCl₂), and high dose group (70 mg/kg CdCl₂). Furthermore, chicks were caged separately. All treatments were given access to water and standard chow and given by oral gavage for 3 months. Cerebellum from each chicken was cautiously divided and stored at −80 °C for subsequence experiments.

Assessments of Oxidative Stress–Related Markers

This study detected the MDA, H₂O₂, T-AOC, T-SOD, CAT, and GSH-PX according to the manufacture's instruction; the optimal operating conditions were described by Zhao and Talukder et al. (Talukder et al. 2021; Zhao et al. 2018).

Histopathological Analysis

Cerebellums were fixed in 10% neutral buffered formalin solution and then processed for paraffin. Five-μm-thick sections were prepared for staining with hematoxylin and eosin (H&E) for microscopic observation. All slides of the kidneys were examined under an optical microscope at 200× and 400× magnifications.

Detection of Trace Elements

The contents of 5 metals, including Cd, Fe, Cu, Se, and Zn in the cerebellum, were detected using inductively coupled plasma mass spectrometry (ICP-MS). The optimal operating conditions were described by Jin et al. (2018). Procedures are described in Supplementary 1.1.

Western Blot Analysis

Protein samples were extracted from the cerebellum tissues and quantified using commercially available kits (Beyotime institute of biotechnology, P.R. China). Detailed treatment

methods were performed as previously described (Zhang et al. 2019). MTF1 (1:700) antibody was purchased from Nan-Jing AnYan Biotechnology Co., Ltd; SepSecS (1:500) antibody was synthesized from Jin-Long Li Lab; GPX4 (1:800) antibody was purchased from Abclonal, USA; β -actin (1:1000, Beijing Biosynthesis Biotechnology Co., Ltd); and secondary antibody against rabbit IgG was purchased from Santa Cruz, CA.

RNA Extraction and Quantitative Real-time PCR Analysis

An animal RNAout commercial kit (3070, Tiandz, Inc, China) was used to extract total mRNA from cerebellum tissues (50 mg). The specific oligonucleotide primers sequence was designed by Oligo 7.0 and listed in Table S1. qRT-PCR program was performed by LineGene 9600 (Bioer Technology Co. Ltd). The value indicated relative mRNA expression level compared to Con by the $2^{-\Delta\Delta CT}$ method normalized to β -actin (1 and 2).

Statistical Analysis

Statistical analysis of all data was performed using GraphPad Prism 5.1 (GraphPad Software, Inc., USA). Results were analyzed using one-way ANOVA and Tukey's post hoc pairwise comparison. Asterisks (*) indicates statistical significance compared to the control group, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Each experiment was performed in triplicate and data are means \pm standard deviation (SD). PCoA analysis was using CANOCO 5.0 software. Correlation analysis was accomplished using R Programming Language version 4.0.2. Heatmaps were plotted using R Programming Language version 3.4.1.

Results

Effect of Cd on the Histopathological Changes in the Cerebellar Tissues

The histopathological observation of the cerebellum is shown in Fig. 1A. Cerebellar sections stained with H&E exhibited normal histological structure in the Purkinje cell layer and regular morphology in control group. Some neurons were slightly shrunken; the number of Purkinje cell decreased significantly in Cd 35 mg/kg group ($P < 0.05$) (Fig. 1B). On the other hand, Cd induced neuronal shrunken and indistinct nucleoli. Besides, the number of Purkinje cell decreased significantly in 70 mg/kg group (Fig. 1B) ($P < 0.001$). These results suggested that Cd induced cerebellar injury. As shown in Fig. 1C, dose-dependent

cerebellar injury was observed as determined by through PCoA analysis.

Effects of Cd on Homeostasis of Trace Elements

There are 5 trace elements in chicken's cerebellum by ICP-MS. As shown in Fig. 2A, B, the content of Cd and Cu was increased significantly, especially in 70 mg/kg group (Fig. 2A, B) ($P < 0.05$, $P < 0.01$, $P < 0.001$). As shown in Fig. 2C–E, the content of Se, Fe, and Zn was decreased (Fig. 2C–E) ($P < 0.05$, $P < 0.01$, $P < 0.001$). As shown in Fig. 2F, heatmap showed the content of 5 trace elements in cerebellum. As shown in Fig. 2G, the correlation analysis showed that Cd positively correlated with Cu. In contrast, Cd had a negative correlation with Se, Zn, and Fe. Among these elements, Cd had a negative correlation with Se (Fig. 2G) ($P < 0.05$).

Effect of Cd on Oxidative Stress in Chicken's Cerebellum

The markers of oxidative stress results showed that the oxidation products H_2O_2 and MDA were increased significantly, especially in 70 mg/kg group (Fig. 3A, B) ($P < 0.05$, $P < 0.01$, and $P < 0.001$). On the other hand, T-SOD, GSH-PX, CAT, and T-AOC level were decreased (Fig. 3C–F) ($P < 0.01$ and $P < 0.001$). Above all, these results suggested that Cd caused oxidative stress in cerebellum.

Effect of Cd on Selenoprotein Transcriptome

The expressions of selenoprotein biosynthesis-related factors and selenoprotein transcriptome were tested by qRT-PCR and western blot. The results showed that the expression of selenoprotein biosynthesis-related factors and selenoprotein transcriptome decreased significantly (Fig. 4B–G, L, M) ($P < 0.05$, $P < 0.01$ and $P < 0.001$) along with the content of Se decreased. In addition, we demonstrated that at the protein level, the protein expression of SepSecS and Gpx4 was consistent with mRNA expression (Fig. 4I, J) ($P < 0.01$ and $P < 0.001$). Furthermore, heatmap showed that the mRNA expression of selenoprotein biosynthesis-related factors and selenoprotein transcriptome under Cd exposure in Fig. 4H, K. Above all, these results suggested that Cd inhibited selenoprotein transcriptome in the cerebellum.

Effect of Cd on the MTF1-Mediated Metal Response

The result of MTF1-mediated metal response-related genes shows that Cd-induced MTF1, MT1, MT2, and DMT1 increased significantly in low-dose (35 mg/kg) Cd group, whereas high-dose Cd group was considerably decreased

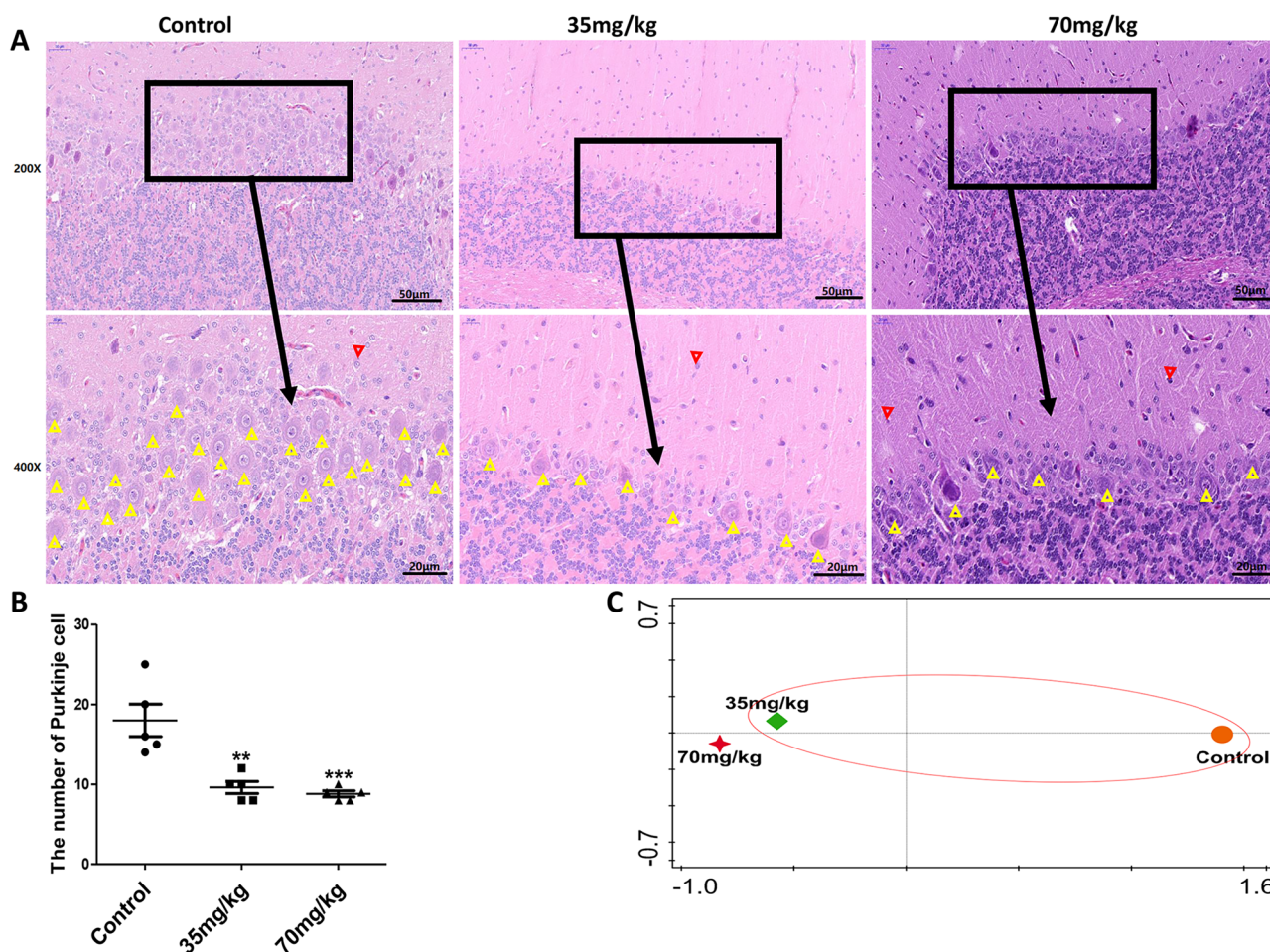


Fig. 1 Effect of Cd on the cerebellum histopathological changes. **A** The histopathological morphology in control, 35 mg/kg, and 70 mg/kg groups (H&E, 200 \times , 400 \times). **B** The number of Purkinje cells in control, 35 mg/kg, and 70 mg/kg groups. **C** The PCoA analysis in

control, 35 mg/kg, and 70 mg/kg groups. Notes: yellow triangle: Purkinje cells. Values are expressed as the mean \pm SD. Significant differences between Cd-treated groups and control groups are denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

when compared with the control group (Fig. 5B, D–F) ($P < 0.05$, $P < 0.01$ and $P < 0.001$).

It is worth noting that the tendency expression of MTF1 protein is consistent with gene expression. Concretely, in the low-dose Cd group, it is increased significantly, whereas high Cd group was considerably decreased when compared with the control group (Fig. 5C) ($P < 0.01$).

Effect of Cd on Zn, Fe, and Cu Transporters

The result showed that zinc exporters including ZNT3, ZNT5, and ZNT10 mRNA expression were decreased significantly, especially in 70 mg/kg group (Fig. 6A–C) ($P < 0.01$ and $P < 0.001$), while zinc importers including ZIP8 and ZIP10 expressions were significantly increased in 35 mg/kg group when compared with the control group (Fig. 6D, E) ($P < 0.001$). However, the expression of the zinc importers

was decreased significantly in the 70 mg/kg group when compared with the control group (Fig. 6D, E) ($P < 0.05$). As for Fe and Cu transporters, the result showed that Cd exposure significantly increased the level of iron importer TF and copper exporter ATP7B expression in the 35 mg/kg group when compared with the control group (Fig. 6F, H) ($P < 0.001$), while their expressions were significantly decreased in 70 mg/kg group (Fig. 6F, H) ($P < 0.05$, $P < 0.001$).

However, the expression of iron exporter FPN1 and copper importer CTR1 was considerably decreased, especially in 70 mg/kg group (Fig. 6G, I) ($P < 0.01$ and $P < 0.001$). Furthermore, the result of correlation analysis between MTF1 and Zn-, Fe-, and Cu-related transporters showed that MTF1 had a positive correlation with Zn-, Fe-, and Cu-related transporters. Among them, MTF1 had a significant positive correlation with MT1 (Fig. 6J) ($P < 0.001$), ZIP8, and ATP7B (Fig. 6J) ($P < 0.05$).

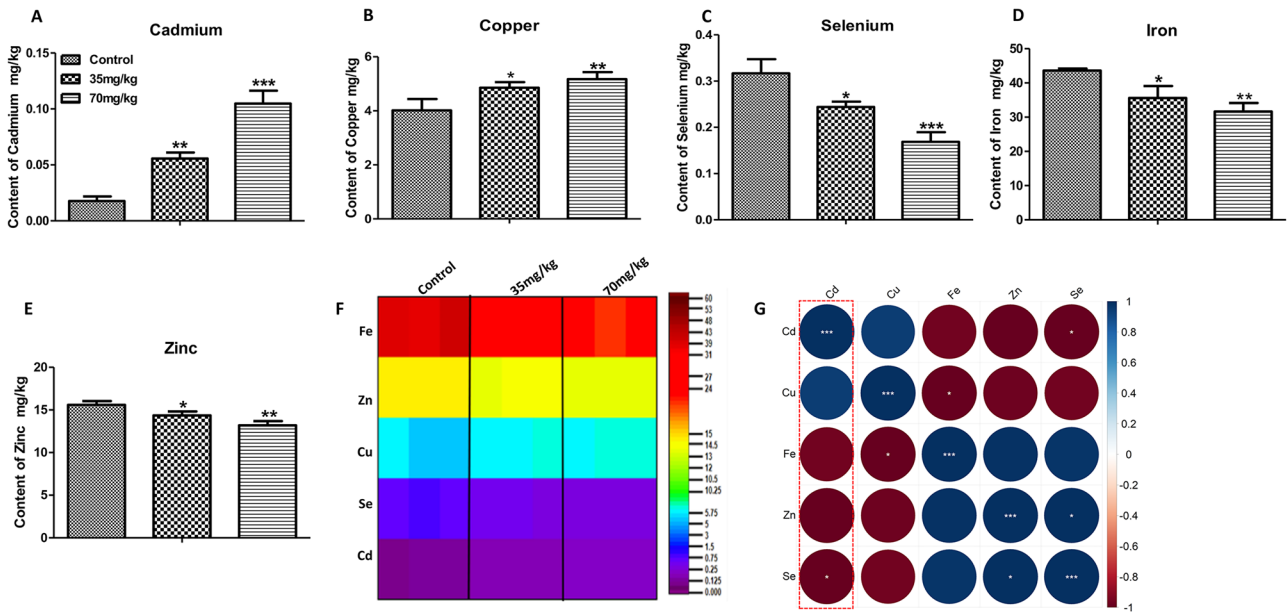


Fig. 2 The analysis of 5 trace elements in chicken’s cerebellum. **A** The content of Cadmium in chicken’s cerebellum. **B** The content of copper in chicken’s cerebellum. **C** The content of Selenium in chicken’s cerebellum. **D** The content of Iron in chicken’s cerebellum. **E** The content of Zinc in chicken’s cerebellum. **F** The content of Fe, Zn, Cu, Se, and Cd in chicken’s cerebellum. **G** Correlation analysis between Cd and Fe, Zn, Cu, and Se. From –1 (red) to +1 (blue) rela-

tive to correlation analysis for 5 trace elements in chicken’s cerebellum with red indicating negative correlation, blue indicating positive correlation, and white indicating no correlation. Values are expressed as the mean ±SD. Significant differences between Cd-treated groups and control groups are denoted as follows: **P*<0.05, ***P*<0.01, ****P*<0.001

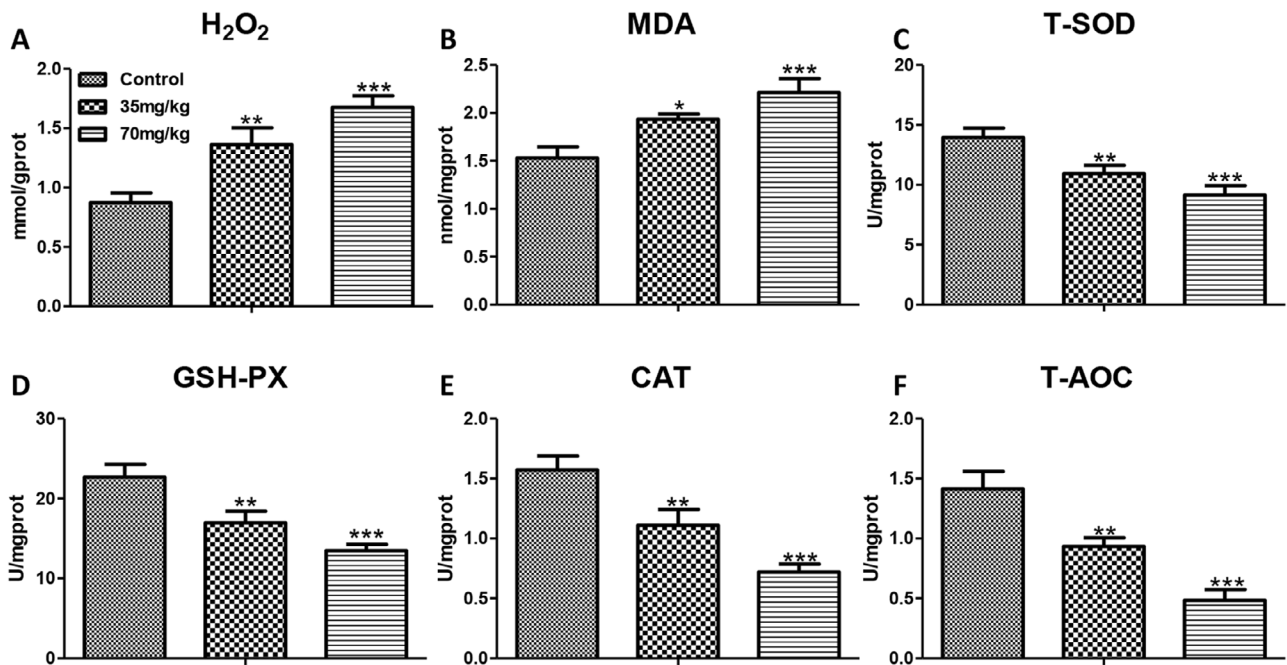


Fig. 3 Effect of Cd on oxidative stress indices. **A** H₂O₂ content. **B** MDA content. **C** T-SOD activity. **D** GSH-PX activity. **E** CAT activity. **F** T-AOC level. Values are expressed as the mean ±SD. Signifi-

cant differences between Cd-treated groups and control groups are denoted as follows: **P*<0.05, ***P*<0.01, ****P*<0.001

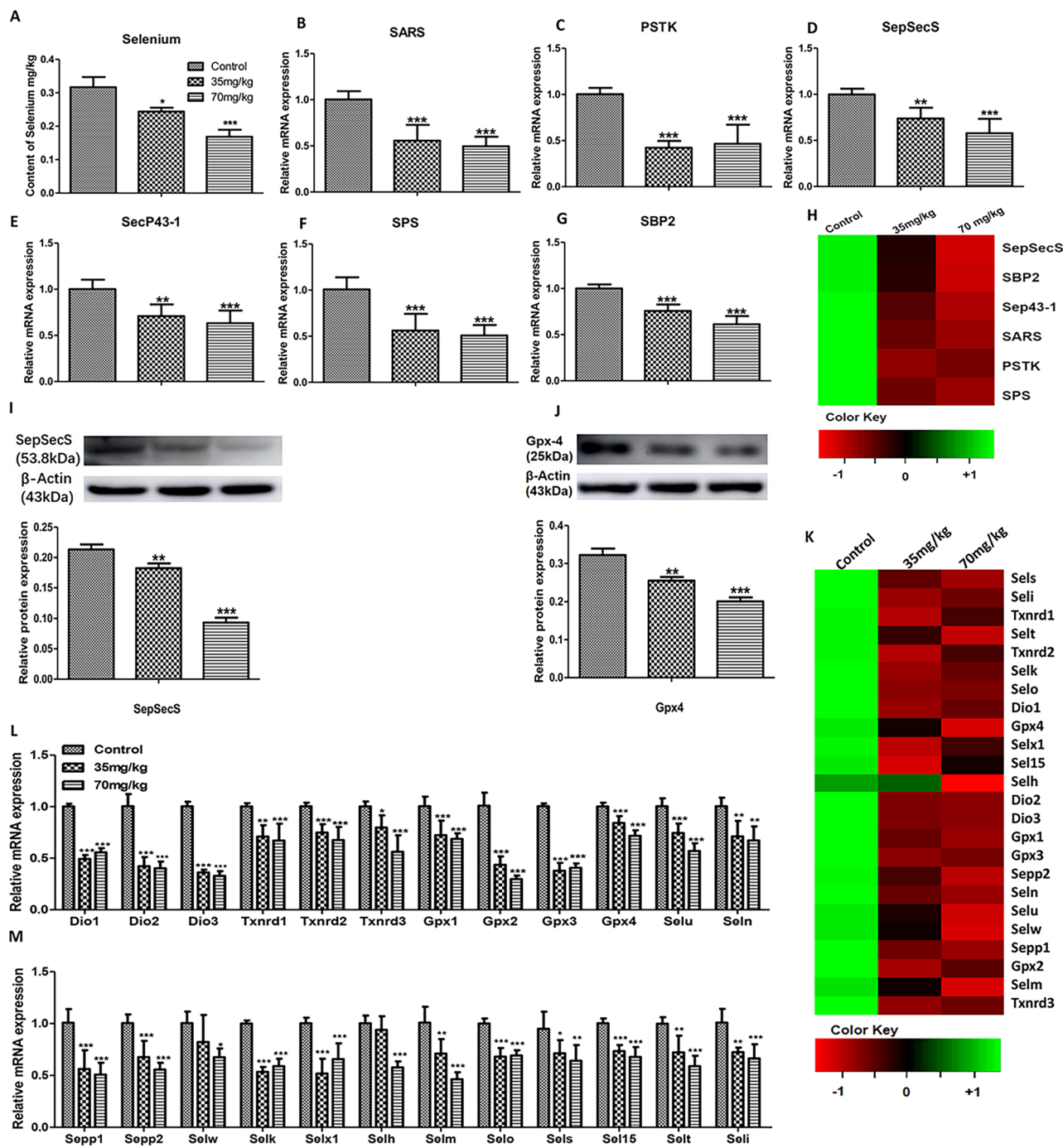


Fig. 4 Effect of Cd-induced selenoprotein biosynthetic related factors and selenoprotein expression. **A** The content of selenium in the cerebellum. **B** Relative mRNA expression of SARS. **C** Relative mRNA expression of PSTK. **D** Relative mRNA expression of SepSecS. **E** Relative mRNA expression of SecP43-1. **F** Relative mRNA expression of SPS. **G** Relative mRNA expression of SBP2. **H** The heatmap of 6 selenoprotein biosynthetic related factors. **I** The relative protein expression of SepSecS. **J** The relative protein expression of Gpx4. **L, M** The 24 selenoproteins mRNA expression level. **K** The

heatmap of 24 selenoproteins mRNA expression. From -1 (red) to +1 (green) relative to values for chicken’s cerebellum in the Con group. The color scale represented the relative mRNA levels, with green indicating up-regulated genes, red indicating down-regulated genes, and black indicating unchanged genes. Values are expressed as the mean±SD. Significant differences between Cd-treated groups and control groups are denoted as follows: **P*<0.05, ***P*<0.01, ****P*<0.001

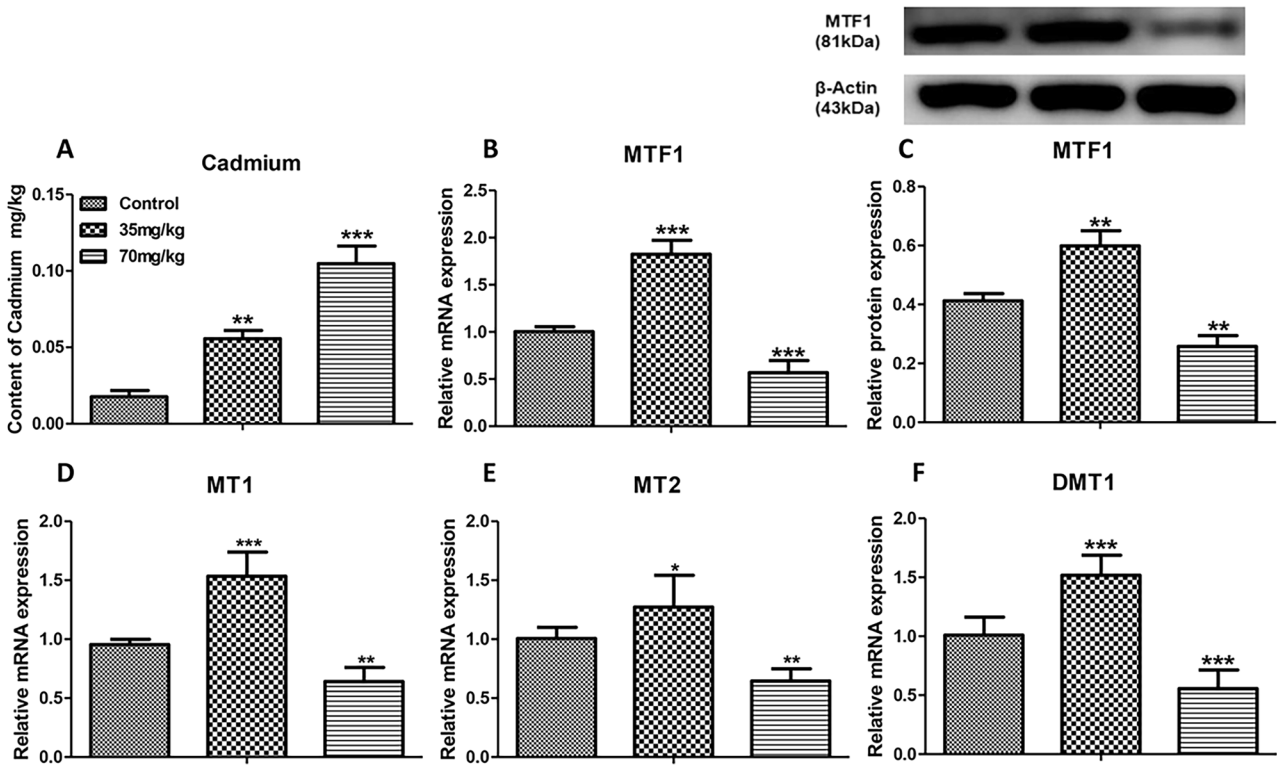


Fig. 5 Effect of Cd on MTF1-mediated metal response. **A** The content of Cd in the cerebellum. **B** Relative mRNA expression of MTF1. **C** Relative Protein expression of MTF1. **D** Relative mRNA expression of MT1. **E** Relative mRNA expression of MT2. **F** Relative

mRNA expression of DMT1. Values are expressed as the mean \pm SD. Significant differences between Cd-treated groups and control groups are denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

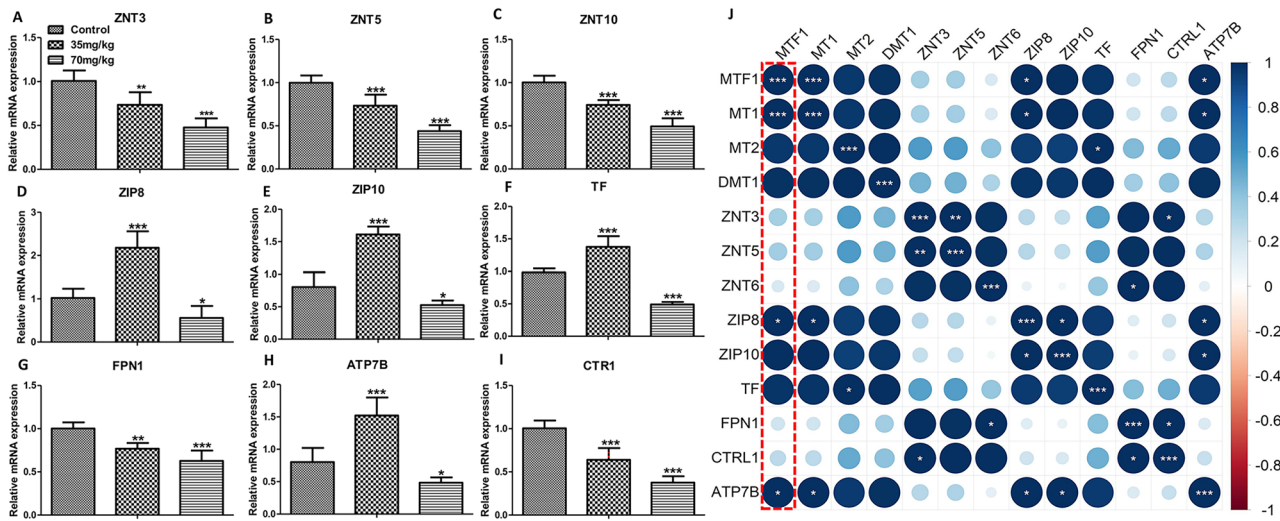


Fig. 6 Effect of Cd on Zn, Fe and Cu related transporters. **A** Relative mRNA expression of ZNT3. **B** Relative mRNA expression of ZNT5. **C** Relative mRNA expression of ZNT10. **D** Relative mRNA expression of ZIP8. **E** Relative mRNA expression of ZIP10. **F** Relative mRNA expression of TF. **G** Relative mRNA expression of FPN1. **H** Relative mRNA expression of ATP7B. **I** Relative mRNA expression of CTR1. **J** Correlation analysis of MTF1-mediated metal response.

From -1 (red) to $+1$ (blue) relative to correlation analysis of MTF1-mediated metal response-related genes in chicken’s cerebellum with red indicating negative correlation, blue indicating positive correlation, and white indicating no correlation. Values are expressed as the mean \pm SD. Significant differences between Cd-treated groups and control groups are denoted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Discussion

Cd is an environmental contaminant, which threatens the health of human beings and wild animals (Berglund and Nyholm 2011; Zhu et al. 2018). It can pass the blood–brain barrier (BBB) and enter the brain, causing neurotoxic effects (Mendez-Armenta et al. 2001). Besides, a recent study has shown that Cd exposure can cause neuronal death (Wei et al. 2015). Notably, Cd-induced neurotoxicity includes cell death, receptor dysfunction, and behavioral changes (Wang et al. 2014). Former researches have proposed that Cd exposure decreased cerebellar cell viability and caused degeneration in cortical neurons in the rat brain (Varmazyari et al. 2020). Thus, these studies suggested that Cd through destructing the normal histological structure induced neuro-organ injury. The result of this study found that Cd induced neuronal shrunken, darkly stained pyknotic neurons; the number of Purkinje cell decreased, and dendrites of neurons were observed, which indicate that Cd caused injury in cerebellar neurons.

Cerebellum is one of the major nervous organs that maintain coordination in animals. Notably, previous study indicated that higher Cd accumulation was found in cerebellum when compared with cerebellum in peacock blennies (Naija et al. 2017). Furthermore, it has been reported that with the dose of Cd increased, the content of Cd and Pb was also increased, while the contents of Se, Cr, and Fe were decreased in chicken's pectorals (Qu et al. 2020). Cd not only can interfere the capability of cells to manage transition metals and disturb the homeostatic (Ammendola et al. 2014), but also disrupt the homeostasis of trace elements that resulted in oxidative damage in chicken's kidney (Ge et al. 2019). It is worth noting that Cd exposure significantly increased the content of Cd in the chicken's brain (Zhang et al. 2016). Consistent with previous study, in this study, we had detected 5 elements by ICP-MS in chicken's cerebellum; the results found that the content of Cd and Cu was enhanced in cerebellum, while Cd treatment significantly reduced the contents of Se, Zn, and Fe in the 35mg/kg group; this situation gets more serious in the 70mg/kg group. These findings suggested that Cd disordered metal homeostasis in chicken cerebellum.

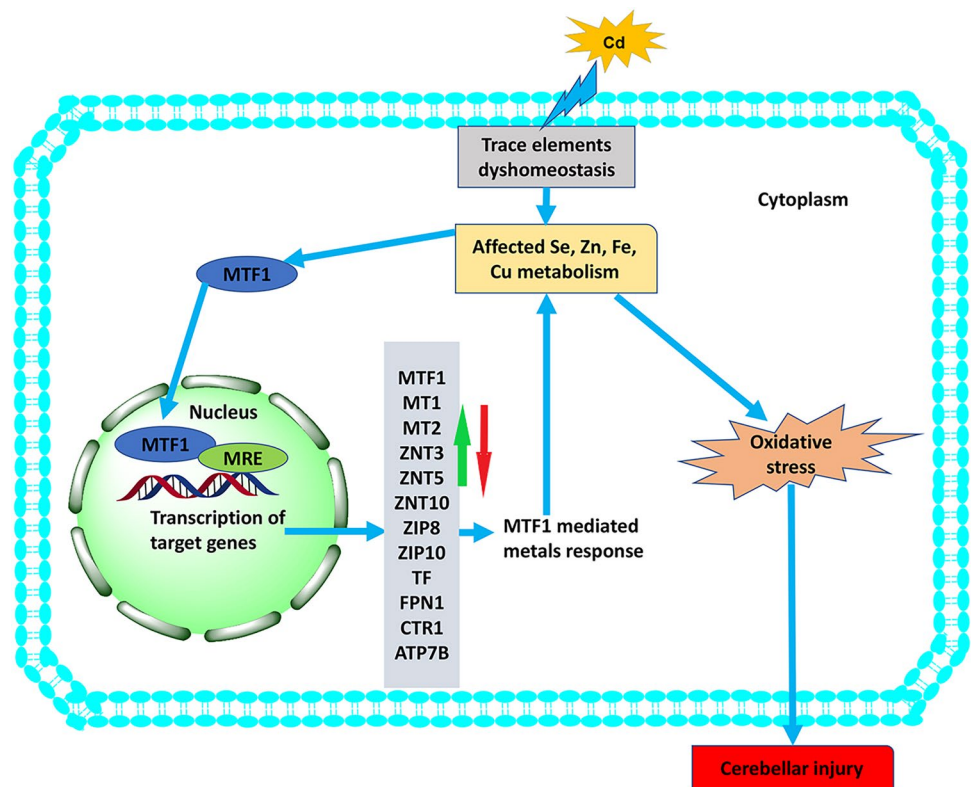
Se is a wholesome trace element that plays a critical role in biological antioxidative metabolism process (Xiong et al. 2020). Besides, Se is an integral part of some enzymes including glutathione peroxidases, deiodinases, and thioredoxin reductases (Zoidis et al. 2018). It could prevent oxidative injury of toxins (Zhang et al. 2020a). Se not only has positive effects of animal, such as resistance to oxidative damage and reduction of chronic inflammatory (Dehkordi et al. 2017), but also have a strong function that against Cd triggered oxidative stress. Notably,

previous study also documented that Cd exposure depleted the content of Se in chicken's kidney (Ge et al. 2019). Consistent with previous research, this study found that chronic Cd treatment obviously decreased Se content and inhibited selenoprotein transcriptome and increased H₂O₂ and MDA contents, while antioxidative stress indexes decreased in chicken's cerebellum. These results suggest that Cd caused oxidative stress in chicken's cerebellum.

MTF1 is a transcription factor that not only regulates metal transporters including Zn, Fe, and Cu (Cuillel et al. 2014; Fu et al. 2017b; Nemmiche and Guiraud 2016), but also can induce the expression of MTs. MTs are a family of stress proteins that are involved in the process of detoxification and anti-oxidation (Sheng et al. 2015). Besides, MTs are known to protect cells against oxidative stress, especially providing protection against Cd toxicity (Shen et al. 2019). Furthermore, MTs play an essential role in protection against Cd toxicity in snails (Dvorak et al. 2019). Former researches have proposed that MTs participate in the homeostasis of Zn, Fe, and Cu and thus protecting metal toxicity and oxidative damage (Chen et al. 2011; Menezo et al. 2011). It is worth noting that the expressions of MT and MTF1 proteins were firstly found in the cattle group which had low Cd concentration in cattle kidney and liver tissues (Buranasinsup et al. 2011). Besides, MT1 and MT2 expressions were induced in liver and lung cells even upon low-dose Cd exposure (Shen et al. 2019). It has been reported that MTF1 is a molecular target of Cd-induced cerebral toxicity (Talukder et al. 2021). Thus, MTF1 and MTs are involved in Cd toxicity. In keeping with previous study, this study showed that Cd activated the MTF1 expression along with the increase of MTs in low-dose Cd exposure, whereas high-dose Cd inhibited MTs and MTF1, which suggest that MTF1 can be activated by low-dose Cd, which induced MT expression.

Increasing evidence suggests that changes in the activity of specific Zn transporters might play a role in regulating the general Zn homeostasis (Cai et al. 2018). Notably, zinc transporters are crucial for the MTF1-mediated metal response (Grzywacz et al. 2015; Muraina et al. 2020). The main function of the ZNT family is to promote the transfer of Zn from cytoplasm into the extracellular, as well as the compartmentalization of zinc in various organelles to reduce the content of cytoplasmic zinc for detoxification (Palmiter and Findley 1995). Besides, the function of ZIP family is to promote the transfer of Zn from extracellular into the cytoplasm that maintain the homeostasis of zinc (Troche et al. 2015). It has been reported that Cd accumulation within the cell induced Zn starvation, which may be due to Cd compete with Zn for the metal binding site (Ammendola et al. 2014). Notably, previous study also documented that Cd-induced alterations of Zn homeostasis in rat hippocampal neurons, lead to the hippocampal neurons

Fig. 7 Schematic diagram illustrating the proposed mechanism on the MTF1-mediated metal response in Cd-induced cerebellum injury in chicken



injury, and Zn supplementation able to partial protection against Cd neurotoxicity (Ben Mimouna et al. 2019). Consistent with previous study, in this study, the result showed that Cd exposure led to reduction in Zn level, and low-dose Cd activated the MTF1 protein expression level along with the increase of MTs and ZIP expression, which suggests that MTF1-mediated metal response was activated by low-dose Cd, which tries to adjust Zn homeostasis.

Fe metabolism disorder plays a key role in neurodegeneration (Bi et al. 2020). Transferrin (TF) and ferroportin 1 (FPN1) are the MTF1-regulated proteins, which are involved in Fe homeostasis (Fu et al. 2017a). Besides, it has been reported that DMT1 was involved in neuronal cell Fe homeostasis (Aral et al. 2020). The proper homeostasis of Fe is managed by Fe importer and exporter, which prevent Fe overload or Fe starvation in cells. FPN1 at the basal membrane exports Fe into the circulation; TF carries Fe to various tissues and cells (Tsuji 2020). Previous study reported that MTF1 and TF have strong positive correlation, suggesting that these genes are involved in transportation of iron in fish liver (Fu et al. 2017b). Cu is essential for the central nervous system (Zhang et al. 2020b). Cu uptake transporter CTR1 and efflux transporters ATP7B were involved in Cu homeostasis. The function of ATP7B is to transport excess Cu in the cytoplasm, and the function of CTR1 is to transport Cu from extracellular transport to the cytoplasm when Cu deficiency in the cytoplasm (Kitada et al. 2008). What is noteworthy is that MTF1

can upregulate ATP7B expression and eliminate Cu through serum ceruloplasmin and bile (Hardyman et al. 2016). However, Cd exposure induced disordered iron homeostasis on HEL cells (Wang et al. 2020) and causes a marked depletion of the content of Fe in rat's liver and kidney (Whanger 1979). Besides, Cd exposure disturbed Cu and manganese homeostasis, and with the dose of Cd increased, Cu deposition on hepatopancreas was observed (Nica et al. 2019), suggesting that Cd exposure disrupts Cu homeostasis. In line with previous study, this study showed that Cd exposure led to reduction in Fe level and enhanced the content of Cu. In addition, MTF1-mediated metal response was activated by low-dose Cd, which means that MTF1-mediated metal response tries to adjust Fe and Cu homeostasis. However, it was inhibited under the high-dose Cd exposure. Above all, these results suggest that Cd exposure disrupted homeostasis via inhibiting MTF1-mediated metal response to further induce cerebellar injury.

Conclusion

This study revealed that cerebellum is the target organ that Cd accumulated in Cd exposure that inhibited selenoprotein transcriptome, caused oxidative stress, disturbed metals homeostasis, and modulated MTF1 and its target metal-responsive genes, which affected redox homeostasis that was

involved in the mechanism of cerebellar injury. In conclusion, Cd through disturbing MTF1-mediated metal response induced cerebellar injury (Fig. 7).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12640-022-00474-x>.

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Declarations

Conflict of Interest The authors declare no competing interests.

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