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Zinc protects against cadmium-induced toxicity by regulating oxidative stress, ions homeostasis and protein synthesis

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HIGHLIGHTS

- Global transcriptome profiles induced by Cd or Cd plus Zn were obtained.
- Zn efficiently reversed the expression of Cd-induced differentially expressed genes.
- Supplementary Zn during Cd exposure lowered Cd-induced oxygen stress.
- Zn prevented the disruption of Fe- and Zn-ion homeostasis induced by Cd.
- Zn inhibited the synthesis of S-rich proteins and antioxidant enzymes.

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ABSTRACT

The widespread environmental toxin cadmium (Cd) is associated with numerous human diseases. The essential trace element zinc (Zn) strongly counteracts Cd-induced toxicity; however, the mechanism is incompletely understood. Here, we conducted RNA sequencing and bioinformatics analyses to determine the global gene expression profiles of yeast cells exposed to Cd or Cd plus Zn. We identified 912 Cd-induced and 627 Cd plus Zn-induced differentially expressed genes (DEGs). Adding Zn during Cd exposure efficiently reversed the expression of 92.1% of Cd-induced DEGs; that of 48.7% was entirely reversed. Gene Ontology, Cluster of Orthologous Group and KEGG Ontology analyses revealed that the response of yeasts to Cd or Cd plus Zn was mainly involved in metal-specific oxidative stress; energy production and conversion; ion homeostasis and ribosome biogenesis and translation. Exposure of yeasts to Cd plus Zn protected them from oxidative stress by efficiently inhibiting the expression of genes associated with Cd-triggered oxidative stress and preventing the disruption of Fe- and Zn-ion homeostasis and reduced glutathione and partially restored mitochondrial membrane potential. Moreover, Zn reduced the intracellular level of Cd to prevent the replacement by Cd of elements required for antioxidant enzyme activity and to protect protein sulphydryl groups against oxidation by free radicals. Further, Zn inhibited the synthesis alterations of Cd-induced ribosomal proteins, S-containing amino acids, S-rich proteins and antioxidant enzymes. Conversely, the investigation results of our study on the yeast model revealed that the Cd-treated protective effects of Zn on Cd-induced toxicity might be partially protective.

1. Introduction

Cadmium (Cd) is a heavy metal that is extensively used in the manufacture of alloys, pigments, electroplates and batteries and is a widespread environmental toxicant. Therefore, Cd and its compounds are classified as type 1 carcinogens by the International Agency for Research on Cancer (Beyersmann and Hartwig, 2008). Non-occupational exposure to Cd mainly originates from diet and smoking, with an estimated individual worldwide dietary Cd intake ranging from 10 to 40 μg/day in unpolluted areas to several hundred micrograms in Cd to polluted regions (Jomova and Valko, 2011). Cd is very toxic at relative low concentrations and accumulates gradually during a person's life, and its biological half life range is 10–30 years; therefore, relatively low levels of Cd intake are a risk to health (Nawrot et al., 2010). Targets of Cd toxicity

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include the lungs, liver, kidney and bone as well as the cardiovascular and immune systems (Jomova and Valko, 2011; Hartwig, 2010; Wang and Du, 2013). Cd-associated diseases include osteoporosis, nephropathy, cancer, diabetes and cardiovascular and neurodegenerative diseases (Jomova and Valko, 2011; Hartwig, 2010; Wang and Du, 2013; Khlifi and Hamza-Chaffai, 2010; Jiang et al., 2007).

Growing interest focuses on developing effective measures to prevent the toxic effects of Cd and to develop therapeutic interventions. Unfortunately, no effective therapy for Cd poisoning is available (Sandbichler and Hockner, 2016). Therefore, there are numerous, compelling reasons to identify natural treatments for Cd-induced toxicity. For example, certain vitamins, trace elements, natural compounds and phytochemicals efficiently prevent or mitigate the toxic effects of Cd on the liver, kidneys and the skeletal and circulatory systems (Sandbichler and Hockner, 2016; Babaknejad et al., 2016; Messaoudi et al., 2009; Bernhoft, 2013; Brzóska et al., 2015). The trace metal zinc (Zn) is required for diverse biological activities such as cell proliferation, reproduction, immune function, antioxidative and anti-inflammatory defence and cell death (Marchan et al., 2012; Stefanidou et al., 2006). Zn strongly reverses Cd-induced toxicity in a variety of model organisms (Sandbichler and Hockner, 2016; Babaknejad et al., 2016; Messaoudi et al., 2009; Bernhoft, 2013; Chouchene et al., 2016; Chemek et al., 2016; McCarty, 2012; Rogalska et al., 2011). However, the mechanism of these protective effects needs to be explored in future studies.

The mechanisms of metal toxicity and detoxification are conserved in eukaryotic organisms (Wysocki and Tamas, 2010; Bleackley and Macgillivray, 2011). The budding yeast *Saccharomyces cerevisiae* serves as a model for studying the mechanisms of biological responses to toxic metals, including Cd. Genome-wide changes in gene and protein expression underlying Cd-induced stress were analysed using microarrays (Momose and Iwahashi, 2001; Hosiner et al., 2014; Jin et al., 2008), RNA sequencing (RNA-Seq) (Huang et al., 2016) and proteomics techniques (Fauchon et al., 2002; Vido et al., 2001). However, little information is available about the effects of the genome-wide changes underlying the interactions between Cd and Zn.

Here we used RNA-Seq to analyse the expression of redox balance-related genes in yeasts exposed to Cd (Huang et al., 2016) and to investigate the genes involved in the protective effect of Zn on Cd-induced toxicity in yeasts. Our results provide a framework for further studies as well as clues that enhance our understanding of the molecular mechanisms of Zn that protect against Cd-induced toxicity associated with the regulation of oxidative stress, ion homeostasis and protein synthesis.

2. Materials and methods

2.1. Strain, media and growth conditions

The *S. cerevisiae* wild-type strain CICC 31094 is closely related to the widely used *S. cerevisiae* strain S288c (Huang et al., 2016). Yeasts were grown in 500-ml Erlenmeyer flasks containing 100 ml of yeast extract peptone dextrose (YPD) medium (2% peptone, 1% yeast extract and 2% glucose) at 30 °C and were shaken at 200 rpm overnight (approximately 16 h). The cultures were then refreshed with medium to adjust the cultures to an optical density (OD) at 600 nm (OD_{600}) = 0.2 and then grown to early exponential phase (OD_{600} = 1.0). The cultures were supplemented with different concentrations of $CdSO_4$ or $ZnSO_4$ or not supplemented with either (untreated control) and then incubated for 2 h. Cell growth was measured by counting the number of colony-forming units (CFUs) that formed on agar plates. The relative growth rate is expressed as the ratio of the initial CFUs divided by the number of CFUs of the control or cells treated with either or both metals. The data are presented as the average ratio of three independent cultures.

2.2. RNA isolation, cDNA library construction, RNA-Seq and bioinformatic analyses of transcriptome data

Yeasts were grown in YPD medium at 30 °C to an OD_{600} = 1.0, and the cultures were then divided into four aliquots. One culture was allowed to continue to grow under the same conditions, the second contained 320 μM $CdSO_4$, the third contained 320 μM $CdSO_4$ and 320 μM $ZnSO_4$ and the fourth contained 320 μM $ZnSO_4$. All cultures were then incubated at 30 °C for 2 h before harvest using centrifugation at 6000 × *g* for 10 min. The methods for RNA isolation, cDNA library construction, RNA-Seq and the analyses of the transcriptome data were performed as previously described (Huang et al., 2016). Total RNA was extracted and purified using the Ribopure Yeast kit and Micro Poly(A) purist Kit according to the manufacturer's instructions (Ambion, Austin, TX). For each RNA-Seq library, two individual cultures of yeast cells were taken and pooled for sequencing on Illumina HiSeq™ 2000 (Illumina Inc., USA). The RNA-Seq library was prepared and sequenced according to the manufacturer's instructions (www.illumina.com). The construction of the libraries and RNA-Seq were performed by the Biomarker Biotechnology Corporation (Beijing, China).

2.3. Measurement of intracellular Cd and Zn concentrations

Cells were grown under the same conditions described above to prepare samples for RNA-Seq. The pellets were washed twice with distilled-deionised water and dried at 70 °C to a constant weight. The dried pellet was digested with HNO_3 and HCl (1:3) at room temperature and incubated overnight. The concentrations of Cd and Zn in acid-digested fluids were determined using an atomic absorption spectrophotometer (AA-800, PerkinElmer). The amounts of Cd and Zn in the pellets were determined using a standard curve. Each experiment was repeated three times, and the results are presented as averages.

2.4. Flow cytometry analysis of reactive oxygen species and mitochondrial membrane potential

Cells were grown under the same conditions described above for RNA-Seq samples. *Reactive oxygen species* (ROS) and mitochondrial membrane potential (MMP) were assessed using flow cytometry (Accuri C6, BD Biosciences) as previously described (Huang et al., 2016). To measure MMP, the cells (5×10^6 cells/ml) were incubated with 40 nM 3,3-dihexyloacarbocynine iodide ($DiOC_6(3)$; Sigma-Aldrich) for 30 min at 30 °C in the dark. To measure ROS, sample cells were incubated with 5 μg/ml dihydroethidium (Sigma-Aldrich) for 30 min at 30 °C in the dark.

2.5. Measurement of reduced glutathione and oxidised glutathione

Reduced glutathione (GSH) and oxidised glutathione (GSSG) concentrations were determined using the recycling method as previously described (Huang et al., 2016; Israr et al., 2006). Briefly, cells (0.5 g wet) were harvested and homogenised in the cold in 3.0 ml of 5% sulphosalicylic acid. The homogenate was centrifuged, and a 0.5-ml aliquot was added to a microfuge tube, followed by the addition of 0.5 ml of reaction buffer (0.1 M phosphate buffer [pH 7.0], 0.5 mM ethylene diamine tetraacetic acid (EDTA) and 50 μl of 3 mM 5′ dithio-bis-(2-nitrobenzoic acid)). After 5 min, the GSH concentration was measured according to the absorbance of the reaction mixture at 412 nm. NADPH (100 μl, 0.4 mM) and 2 μl of