Preliminary Investigation of MAPK Regulation of Downstream Effector Proteins Under Cadmium Stress (Postprint)

Authors: Chang Na, Zhang Lei, Huang Jinhai, Zhang Lilin

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Abstract

Cadmium, as a non-essential metal, poses a significant threat to human health. Studies have shown that cadmium accumulation leads to increased -amyloid protein[1] and formation of Lewy bodies[2], thereby causing neurodegenerative diseases such as Alzheimer' s disease and Parkinson' s disease. From the perspective of pathogenic mechanisms, cadmium induces cell death by disrupting the homeostasis of intracellular signaling pathways including Ca2+, NO, ROS, and MAP kinase[3]. Current research on MAPK primarily focuses on the responses of MAPKs themselves and their upstream components under cadmium stress, while studies on the regulatory mechanisms of MAPK activation on downstream effector proteins and the types of effector proteins are relatively limited.

Previous work in our laboratory found that deletion of three core components (HOG1, SLT2, and KSS1) of the mitogen-activated protein kinase signaling pathway in yeast cells exhibited sensitivity to cadmium stress[4], and conducted in-depth research on two of these pathways—the Cell Wall Integrity (CWI) pathway and the High Osmolarity Glycerol (HOG) pathway. In this study, the Saccharomyces cerevisiae wild-type strain BY4741 and its HOG1 and SLT2 gene deletion mutants in the same background were used as research subjects. HiSeq2500 was utilized to detect gene expression levels under 50 M CdCl2 stress, and the DESeq differential expression analysis software was employed for differential expression analysis between sample groups. Genes involved in cadmium stress response and regulated by Hog1p and Slt2p were selected for functional analysis. Ten differentially expressed genes were randomly selected for RT-qPCR validation of the RNA-seq results.

Based on the RNA-Seq data, we set the screening criteria for differentially expressed genes as FC>1.5 and FDR<0.05, and used GO, COG, and KEGG databases for functional annotation analysis of the differentially expressed genes.

The results showed that under cadmium stress, hog1Δ had 103 differentially expressed genes compared with BY4741. GO annotation functions were mainly concentrated in antioxidant activity, molecular transport, metabolism, signal transduction, and stress response, which was consistent with the KEGG analysis results revealing two major response pathways—the MAPK transduction pathway and amino acid biosynthesis metabolic pathway. COG analysis results further demonstrated that the functions of differentially expressed genes were concentrated in metabolism and transport processes. Through Venn diagram analysis of differentially expressed genes, 26 genes positively regulated by Hog1p were identified, which may be involved in Cd2+ transport; while 5 genes including TEC1 and SFG1 were negatively regulated by Hog1p. Under cadmium stress, slt2Δ had 9 differentially expressed genes compared with the BY4741 strain, and analysis by all three databases confirmed that functions were concentrated in cell cycle regulation and protein synthesis/processing. Currently, 8 genes related to ion transport and antioxidant stress functions, including NCW2, PRM5, YCT1, etc., have been selected from the gene pools regulated by the two deletion mutants. Double gene deletion strains were constructed and subjected to serial dilution phenotype analysis. It has been demonstrated that hog1yct1 can partially rescue the cadmium-sensitive phenotype caused by HOG1 deletion. Based on the phenotypic results, YCT1 is hypothesized to be located downstream of MAPKs-Hog1p, and corresponding cadmium atomic absorption experiments also support this conclusion. YCT1 is a high-affinity transporter for cysteine[5], and there is currently no evidence linking it to Hog1p. Genetic and physicochemical validation experiments for their interaction are currently underway.

Full Text

A Preliminary Study on MAPK Regulation of Downstream Effector Proteins Under Cadmium Stress

Na Chang, Lei Zhang, Jinhai Huang, Lilin Zhang*

School of Life Sciences, Tianjin University, No. 92 Weijin Road, Tianjin 300072, China

*Corresponding author. E-mail: lyliazhang@163.com

Cadmium, as a non-essential heavy metal, poses significant threats to human health. Studies have demonstrated that cadmium accumulation leads to increased -amyloid protein [1] and formation of Lewy bodies [2], thereby contributing to neurodegenerative diseases such as Alzheimer' s and Parkinson' s. Mechanistically, cadmium disrupts cellular homeostasis of signaling pathways including Ca2+, NO, ROS, and MAP kinase, resulting in cell death [3]. Current MAPK research primarily focuses on the responses of MAPKs themselves and their upstream components under cadmium stress, whereas studies on how MAPK activation regulates downstream effector proteins and the identity of these effectors remain limited.

Our previous work revealed that deletion of three core components (HOG1, SLT2, and KSS1) of the mitogen-activated protein kinase signaling pathway confers cadmium sensitivity in yeast cells [4]. We performed in-depth investigations on two pathways—the Cell Wall Integrity (CWI) pathway and the High Osmolarity Glycerol (HOG) pathway. In this study, we employed the Saccharomyces cerevisiae wild-type strain BY4741 and its HOG1 and SLT2 deletion mutants in the same genetic background. Using HiSeq2500, we measured gene expression levels under 50 M CdCl2 stress and applied DESeq software for differential expression analysis between sample groups. Genes involved in cadmium stress response and regulated by Hog1p and Slt2p were selected for functional analysis. Ten differentially expressed genes were randomly chosen for RT-qPCR validation of RNA-seq results.

Based on RNA-Seq data, we established differential expression gene screening criteria of FC>1.5 and FDR<0.05. Functional annotation and analysis were performed using GO, COG, and KEGG databases. Under cadmium stress, hog1Δ exhibited 103 differentially expressed genes compared to BY4741. GO annotation revealed functions primarily concentrated in antioxidant activity, molecular transport, metabolism, signal transduction, and stress response. KEGG analysis identified two major response pathways—the MAPK signaling pathway and amino acid biosynthesis metabolism—consistent with GO results. COG analysis further confirmed that differentially expressed genes were functionally enriched in metabolism and transport processes. Venn diagram analysis identified 26 genes positively regulated by Hog1p that may participate in Cd2+ transport, while five genes including TEC1 and SFG1 were negatively regulated by Hog1p.

Under cadmium stress, slt2Δ displayed nine differentially expressed genes compared to BY4741. Analysis across all three databases demonstrated functional enrichment in cell cycle regulation and protein synthesis/processing. We have currently selected eight genes related to ion transport and antioxidant stress functions from the gene pools regulated by both deletion mutants, including NCW2, PRM5, and YCT1. Double deletion strains were constructed for each gene, and serial dilution phenotype analysis was performed. We demonstrated that hog1yct1 partially rescues the cadmium-sensitive phenotype caused by HOG1 deletion. Phenotypic results suggest YCT1 functions downstream of MAPKs-Hog1p, with cadmium atomic absorption experiments supporting this conclusion. YCT1 is a high-affinity cysteine transporter [5], though no prior evidence has linked it to Hog1p. Genetic and biochemical validation experiments for their interaction are currently underway.

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Author Biography

Na Chang, female, born in April 1993, holds a bachelor' s degree and is currently a master' s student at the School of Life Sciences, Tianjin University. Contact phone: 13820273325 Email: 13820273325@163.com

Note: Figure translations are in progress. See original paper for figures.