Toxicity of Nickel Compounds Mediated by HTZ1, Histone Variant H2A.Z, in Saccharomyces cerevisiae

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Nickel compounds have toxic and carcinogenic effects. Several cellular targets have been identified and the toxicity is thought to be mediated by genetic and epigenetic factors. Gene expression from chromatin is regulated by posttranslational histone modifications, ATP-dependent chromatin remodeling, and the incorporation of histone variants. Nickel compounds decrease acetylation levels of all four histones and increase ubiquitylation of H2A and H2B and dimethylation of H3 lysine 9. Less attention has been focused on histone variants in nickel toxicity. Here we demonstrate that a null mutation of H2A.Z (HTZ1 in Saccharomyces cerevisiae), a variant of H2A, decreases the sensitivity to soluble nickel compounds. In addition, we show that a mutation in the acetylatable residues in Htz1p does not alter the sensitivity to nickel compounds. Furthermore, sensitivity to nickel compounds of the null mutant of SWR1 encoding the catalytic subunit of the ATP-dependent chromatin remodeling complex that specifically loads Htz1p into chromatin, was identical to that of the htz1 mutant. Taken together, these results reveal that the incorporation into chromatin, but not acetylation, of Htz1p is important to the toxicity of nickel compounds.

Key words histone variant; epigenetics; nickel compound; genetics

Both water soluble and insoluble nickel compounds penetrate the cytoplasm and nucleus, and exhibit toxic and carcinogenic activities in humans and animals.1–3) Exposure to nickel compounds, insoluble nickel sulfide and soluble nickel sulfate, resulted in gene deletions and point mutations in a Chinese hamster ovary cell line.4) In rat kidney, nickel sulfide was incorporated or replaced at specific locations.5–7) H2A.Z (Saccharomyces cerevisiae), one of the H2A variants and is conserved from yeast to humans.6) H2A.Z and nickel toxicity. In this study, we observed the effects of HTZ1’s disruption on the sensitivity to nickel compounds in yeast. Our findings revealed that the nickel toxicity appeared with the incorporation of Htz1p into chromatin mediated by the chromatin remodeling factor SWR1, but not acetylation of Htz1p.

MATERIALS AND METHODS

Yeast Strains and Manipulations To generate the htz1Δ (YS532), Pgal1-HTZ1 (YS533), and swr1Δ (YS544) strains, deletion of HTZ1, introduction of the GAL1 promoter into the HTZ1 locus, and deletion of SWR1, respectively, were done in strain W303a by polymerase chain reaction (PCR) targeting using the his5+ cassette as a selecting marker. Standard yeast manipulations were performed as described.8–11) For spotting analyses, cells were harvested, resuspended at 1×10⁷ cells/ml, and subjected to 10-fold serial dilutions, and 2μl of each dilution was spotted per plate. The plates were incubated at 30 °C for 3 d. Sensitivity to methyl methanesulfonate (MMS) (Sigma-Aldrich, St. Louis, MO, U.S.A.) (0.1%) or bleomycin (BLM) (Sigma-Aldrich) (10 mU/ml) was assayed on YPD (yeast extract/peptone/dextrose) or YPG (yeast extract/peptone/galactose). Nickel chloride or nickel sulfate (0.2 mM or 0.4 mM) was added to synthetic complete medium with dextrose or galactose. When cells were transformed with plasmids, synthetic complete medium lacking uracil was used. Typical results from two or three experiments are shown. Cell growth image on the plates was taken by FAS-III (Toyobo, Osaka, Japan) and was quantified by the NIH Image program (version 1.62).

Plasmid Construction Plasmids expressing Htz1p regulated by the endogenous promoter were generated by PCR amplification of the HTZ1-coding sequences along with 1-kb upstream and downstream regions from yeast genomic DNA. The amplified product was subcloned into the SacI/KpnI site

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of CEN and 2µ based plasmids, pRS416 and pRS426, respectively. All fragments generated by PCR were verified by sequencing. Mutations of acetylation sites of HTZ1 were introduced using the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, U.S.A.) following the manufacturer's instructions, and confirmed by sequencing.

Preparation of Cell Lysate and Western Blot Analysis
Cells were grown to mid-log phase, harvested by centrifugation, and suspended in 1× sodium dodecyl sulfate (SDS) loading buffer [50 mM Tris–HCl (pH 6.8), 100 mM dithiothreitol, 2% SDS, 0.01% bromophenol blue, 10% glycerol]. Samples were boiled for 8 min, loaded onto a 15% SDS–polyacrylamide gel, transferred to a nitrocellulose membrane, and detected with the ECL Western blotting analysis detection system (Amersham Biosciences, Piscataway, NJ, U.S.A.). Anti-Htz1p antibody was obtained from Abcam, Cambridge, U.K.

DNA Localization
Cells were grown to mid-log phase in liquid synthetic complete medium with dextrose, harvested, fixed with paraformaldehyde, stained with 4',6'-diamino-2-phenylindole (DAPI) (22 μg/ml), and directly visualized under a fluorescence microscope (Olympus, Tokyo, Japan; BX51).

RESULTS AND DISCUSSION
Effect of HTZ1's Disruption on Sensitivity to DNA Damaging Reagents
Gene expression is regulated by several factors including DNA-binding transcription factors, histone modification enzymes, chromatin remodeling factors and histone variants. HTZ1, one of the histone H2A variants in Saccharomyces cerevisiae, contributes to both transcriptional activation and repression. To investigate the sensitivity of the null mutant of HTZ1 to genotoxins and epimutagens, we prepared a HTZ1 deletion strain (htz1Δ) and a strain in which Htz1p expression is regulated by the GAL1 promoter (PGAL1-HTZ1). With this strain, the expression of Htz1p is repressed in dextrose medium, but induced in medium supplied with galactose. Cell lysates from the wild-type, deletion, and GAL1 promoter-introduced strains grown in dextrose and galactose medium were used for the examination of the Htz1p expression by Western blotting (Fig. 1A). Htz1p was detected in the wild-type strain but not the deletion strain. In the PGAL1-HTZ1 strain, overexpressed Htz1p was observed in the galactose-containing medium, but no Htz1p was detected in the dextrose-containing medium. Equivalent amounts of protein in each lane were visualized by Coomassie brilliant blue staining.

Previous reports have shown that the htz1Δ strain is sensitive to MMS, a genotoxic reagent.24) To examine whether

Fig. 1. Effect of HTZ1’s Disruption on the Sensitivity to DNA Damaging Reagents and Nickel Compounds
(A) Cell lysates were prepared from the wild-type (lanes 1, 4), the HTZ1 deletion (lanes 2, 5), and GAL1 promoter-introduced (lanes 3, 6) strains grown in dextrose (lanes 1 to 3) and galactose (lanes 4 to 6)-containing medium and probed with anti-Htz1p antibody. Equal protein loading was shown by Coomassie staining. (B) Effects of disruption and over-expression of HTZ1 on sensitivity to mutagens (MMS and BLM). Serial dilutions of wild-type and mutant strains were spotted and grown on YPD (left) or YPG (right) in the absence (−) or presence of MMS (0.1%) and BLM (10 μM/ml) (upper). Quantification of cell growth image on the plates was also shown (lower). (C) Sensitivity to nickel ion in several strains indicated at the left of the panel. Cells were grown on a synthetic complete medium with dextrose (left) or galactose (right) containing 0.2 mM of nickel compounds.
HTZ1 is involved in the sensitivity to some genotoxic reagents, serial dilutions of wild-type and mutant strains were spotted and grown on the plates with or without genotoxic reagents, and cell growth image on the plates was quantified by the NIH Image program (Fig. 1B). We confirmed the previous result and also showed that the deletion strain is sensitive to bleomycin, which produces superoxide and hydroxide-free radicals and induces DNA strand breaks. Such sensitivity was also observed in the PGAL1-HTZ1 strain grown in the dextrose (i.e., HTZ1 repressed condition). On the other hand, the sensitivity to MMS and bleomycin of the PGAL1-HTZ1 strain grown in the galactose medium (i.e., HTZ1 overexpressed condition) was indistinguishable from that of the wild-type strain. These observations indicate that the HTZ1 deletion strain is sensitive to genotoxic reagents. Furthermore, the expression level of Htz1p in the wild-type strain is enough to gain the sensitivity and excess amounts of Htz1p do not affect it.

Effect of HTZ1’s Disruption on the Sensitivity to Nickel Ions Next we observed the sensitivity of the HTZ1 deletion strain to nickel compounds (nickel chloride and nickel sulfate) (Fig. 1C). Though the growth of the wild-type strain was inhibited by nickel compounds, the htz1Δ strain was resistant to these compounds. In the PGAL1-HTZ1 strain, the cell growth was not significantly inhibited by nickel compounds in the dextrose-containing medium (i.e., HTZ1 repressed condition). On the other hand, inhibition of cell growth detected in the galactose-containing medium (i.e., HTZ1 overexpressed condition) was indistinguishable from that of the wild-type strain. The sensitivity of PGAL1-HTZ1 to nickel compounds in dextrose medium is more similar to that of the HTZ1 deletion strain than that of the wild-type one, while the sensitivity of PGAL1-HTZ1 in galactose medium is more similar to that of the wild-type strain than that of the HTZ1 deletion one. These results suggest the sensitivity to the nickel compounds to be dependent on HTZ1 and excess amounts of Htz1p do not increase the sensitivity.

Effect of Mutations in Modifiable Lysine Residues of HTZ1 on the Sensitivity to Nickel Ions Several histone modifications have been reported and multiple residues on each of the four core histones and histone variants have been identified as potential modification targets. From these findings, the histone code hypothesis, which postulates that these modifications regulate gene function dependent on high order chromatin organization, has been proposed. Previous reports showed that Htz1p has four acetylatable lysine residues (K3, K8, K10, K14) in its amino terminal region. One of them, K14 but not K3, K8, or K10, is acetylated by NuA4 histone acetyltransferase complex and htz1-K14R mutant displays sensitivity to the microtubule destabilizing agent methyl-1-(butylcarbamoyl)-2-benz-imidazol-carbamate (benomy1). Thus, mutation in one of acetylatable lysine residues affects the sensitivity to chemicals.

To test the effect of the mutation of the acetylatable lysine residue on the sensitivity to the nickel compounds, we constructed wild-type and mutated Htz1p expression plasmids containing the native HTZ1 promoter on either CEN or 2μ-based plasmids and 10-fold serial dilutions of each strain including plasmids were spotted onto the indicated medium (Fig. 2). In the mutants, four lysine residues were altered individually to arginine (R) residue. The wild-type strain containing the empty vector was sensitive to nickel chloride. In contrast, the HTZ1 deletion strain including the empty vector was resistant to nickel chloride, consistent with Fig. 1C. The sensitivity of the wild-type strain to nickel chloride is the same as that of the htz1Δ strain including the wild-type Htz1p expression plasmid. Further, cells possessing all of the four mutant Htz1p expression plasmids had the same influence as the strain including the wild-type plasmid on the sensitivity to nickel chloride. The same results were obtained when 2μ plasmids were used. Though we cannot rule out the possibility that a combination of acetylation of several lysine residues affects the sensitivity to nickel compounds, our results suggest that the acetylation of lysine residues on Htz1p may not be concerned with the sensitivity to nickel chloride.

Sensitivity to Nickel Ions in swr1 and htz1 Mutants Since the acetylation of Htz1p did not affect the sensitivity, we focused on the incorporation of Htz1p into chromatin. Htz1p is specifically loaded onto chromatin by an ATP-dependent chromatin remodeling complex, SWR-C, the catalytic subunit of which is Swr1p. To determine whether the incorporation of Htz1p into chromatin is required for the sensitivity to nickel compounds, we prepared a SWR1 null mutant. The disruption of SWR1 did not affect the level of Htz1p or cell growth (Fig. 3). The disruption of SWR1 decreased the sensitivity to nickel compounds and the sensitivity of the swr1 mutant was the same as that of the htz1 mutant (Fig. 3B). This indicates that the toxicity of nickel compounds is mediated by the incorporation of Htz1p into chromatin.

Effect of the Nickel Ion Treatment on DNA Localization in Wild-Type Strain and htz1 and swr1 Mutants Nickel compounds induce specific morphologic chromosomal changes including decondensation, formation of sister chromatid exchange, etc. in cultured mammalian cells. We examined whether nickel chloride affects the localization of DNA in yeast (Fig. 4). DNA in the wild-type strain was stained with DAPI after growing in the absence or presence of nickel chloride. A few diffuse spots appeared in the presence of nickel chloride in the wild-type strain, though a sin-
A

WT
Htz1Δ

CBB

1 2 3

B

WT
htz1Δ

swr1Δ

NiCl₂

(-)

NiSO₄

Fig. 3. Effect of SWR1's Disruption on the Sensitivity to Nickel Compounds

(A) Cell lysates were prepared from the wild-type (lane 1), the HTZ1 deletion (lane 2), and the SWR1 deletion (lane 3) strains and probed with anti-Htz1p antibody. Equal protein loading was shown by Coomassie staining. (B) Effects of disruption of HTZ1 and SWR1 on sensitivity to 0.2 mM nickel compounds. Serial dilutions of wild-type and mutant strains were spotted and grown onto plates containing 0.2 mM NiCl₂ and 0.2 mM NiSO₄.

Fig. 4. Effect of the Nickel Chloride Treatment on DNA Localization in Wild-Type Strain and htz1 and swr1 Mutants

The wild-type strain and htz1 and swr1 mutants were exposed to 0.2 mM nickel compounds for 25 h and the localization of DNA in cells was identified by DAPI staining. Typical results from two experiments are shown.

Single white circle was detected in the absence of nickel chloride.

Though the histone variant Htz1p is one of the chromatin components, depletion of HTZ1 did not affect the localization of DNA in the control medium. In the htz1 mutant, the DAPI staining profile in the presence of nickel chloride was indistinguishable from the profile in its absence. These phenomena were also observed in the swr1 mutant. The same events were observed in the medium including nickel sulfate (data not shown). These results suggest the diffusion of DNA induced by nickel compounds to be mediated by the Swr1p-dependent incorporation of Htz1p into chromatin.

We presented here that the depletion of HTZ1 increased the sensitivity to genotoxins, while it decreased the sensitivity to nickel compounds, which alter the epigenetic state of chromatin, including acetylation, methylation and ubiquitylation of histones. Generally, genetic and epigenetic mutations affect each other. For example, global DNA hypomethylation has been linked to chromosomal instability. On the other hand, the rearrangement of genes coding for histone acetyltransferases in chromosome translocations induces aberrant histone modifications and leads to acute myeloid leukemia and/or benign uterine leiomyomata. Several mutations found in genes encoding histone acetyltransferases also perturb histone acetylation status and are linked with various diseases. Though status of histone modifications and the mechanism for the diffusion of DNA localization induced by nickel compounds under our experimental conditions have not been clarified, the diffusion of DNA generated by the treatment of nickel compounds was dependent on the Swr1p-mediated incorporation of Htz1p into chromatin. The diffusion of DNA is generally concerned with the genomic instability including gene amplification, microsatellite instability and chromosomal aberrations. Genetic and epigenetic mechanisms of nickel toxicity have been proposed. Our findings suggest the incorporation of histone variants into chromatin mediated by a chromatin remodeling factor to also be one significant factor in the toxicity of nickel.

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