— **Minireview** —

Molecular Mechanisms of Zinc-mediated Induction and Chromium(VI)-mediated Inhibition of Mouse *Metallothionein-I* Gene Transcription

Tomoki Kimura*

Department of Toxicology, Faculty of Pharmaceutical Sciences, Setsunan University, 45–1 Nagaotoge-cho, Hirakata, Osaka 573–0101, Japan

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The expression of metallothionein (MT), a heavy-metal-binding protein, is induced by heavy metals such as zinc, copper, cadmium, and mercury. This induction of MT maintains zinc homeostasis and defends against toxic heavy metals by sequestering these metals and lowering their concentrations at critical intracellular sites. However, MT cannot bind chromium(VI), a heavy metal that has been known for over 100 years to be a human carcinogen. Chromium(VI) enters cells via the sulfate anion transporter system and is reduced to intermediate oxidation states, such as chromium(V) and chromium(IV), in the process of forming stable chromium(III) forms. Chromium(VI) is known to inhibit *MT* gene transcription, and recently my colleagues and I reported that chromium(VI) inhibits zinc-induced *MT* gene transcription by modifying the transactivation potential of metal response element-binding transcription factor-1 (MTF-1), a zinc-finger transcription factor. Inhibition of *MT* gene transcription may therefore be involved in the carcinogenicity of chromium(VI). In this review, I briefly summarize the molecular mechanisms of heavy metal-induced *MT* gene transcription and discuss the current status of research on chromium(VI) toxicity and chromium(VI)-mediated inhibition of *MT* gene transcription.

Key words —— metal response element-binding transcription factor-1, metallothionein, zinc, chromium(VI), heavy metal

INTRODUCTION

Metallothionein (MT) is a low-molecular-weight metal-binding protein.[1,2] One-third of its amino acid composition is cysteine, and it lacks disulfides, aromatic amino acids, and histidine. In mouse, four *MT* genes, *MT-I* to *-IV*, have been cloned. *MT-I* and *-II* are actively expressed in many cell types in various organs and tissues, as well as in most cultured cells; in contrast, *MT-III* and *-IV* show a very restricted cell-type-specific expression pattern.[3] In this review, we focus on MT-I and II. Transcription of the *MT* gene is increased in response to some heavy metals, such as zinc, cadmium, copper, and mercury. MT has high affinity for these heavy metals and is usually saturated with zinc, an essential metal. It has been suggested to maintain zinc homeostasis.[4,5] Recently, zinc was recognized as an intracellular signaling factor,[6,7] and MT may modulate this zinc signaling. Defense against cadmium and mercury has been considered to be one of the toxicological roles of MT.[8] Transcriptional activation of the *MT* gene by heavy metals is mediated by a *cis*-acting DNA element, the metal response element (MRE).[9–12] MRE-binding transcription factor-1 (MTF-1) is a highly conserved zinc finger transcription factor that regulates the transcription of *MT*. The induction of *MT* gene transcription is attenuated by hexavalent chromium [chromium(VI)],[13,14] a toxic and carcinogenic heavy metal[15,16] that is present as an environmental pollutant. Chromium(VI) attenuates the induction of *MT* gene transcription by inhibiting the MTF-1-mediated transactivation,[14,17] which may contribute to its carcinogenicity. In this review, I briefly summarize the molecular mechanisms of the induction of *MT* transcription by zinc and other

*To whom correspondence should be addressed: Department of Toxicology, Faculty of Pharmaceutical Sciences, Setsunan University, 45–1 Nagaotoge-cho, Hirakata, Osaka, 573–0101 Japan. Tel. & Fax: +81-72-866-3107; E-mail: tomoki@pharm.setsunan.ac.jp

metals and its inhibition by chromium(VI), with a focus on mouse *MT-I* gene transcription.

MECHANISMS OF ZINC-INDUCED MT GENE TRANSCRIPTION

The molecular mechanisms of MT induction by zinc have been well studied.[12] MTF-1 is an essential transcription factor for both basal and zinc-induced *MT* gene transcription because removal of MTF-1 by gene targeting technology silences endogenous MT genes.[18] MTF-1 has a metalloregulatory DNA-binding domain composed of six Cys_2His_2 zinc fingers. The cytosolic labile zinc concentration is buffered at the picomolar to nanomolar range,[19,20] but the zinc fingers in MTF-1 apparently have low zinc binding affinities, in the nanomolar to submicromolar range.[21,22] By contrast, other zinc-requiring, but not zinc-regulated, transcription factors with canonical Cys_2His_2 zinc fingers [*e.g.*, stimulatory protein 1 (Sp1)] have high zinc affinities (in the picomolar to nanomolar range) and would be saturated with zinc under most physiological conditions. A simple metalloregulatory model is that constitutively expressed MTF-1, with its intrinsically low binding affinity for zinc, would bind zinc only under conditions of zinc excess (Fig. 1). Our recent study revealed why the zinc fingers of MTF-1 show moderate zinc affinities.[23] An unusual (non-canonical) peptide linker connecting the two N-terminal zinc fingers in MTF-1 has a potential role in their zinc-sensing function. Replacing the unusual RGEYT linker between zinc fingers 1 and 2 with a canonical TGEKP linker abolishes the zinc-sensing function of MTF-1, resulting in constitutive DNA binding and transcriptional activation of the *MT-I* gene. Zinc may modulate highly specific, linker-mediated zinc finger interactions in MTF-1, thus affecting its zinc- and DNA-binding activities and its binding to the *MT-I* gene promoter. Very recently, an unusual nuclear localization signal (NLS) was found in a region of MTF-1 spanning zinc fingers 1–3,[24] which includes the metalloregulatory DNA-binding domain of MTF-1, but fusion of the NLS to a cytoplasmic marker protein confers constitutive (zinc-independent) nuclear localization to the protein. It therefore seems that the NLS is not involved in the nuclear localization of MTF-1 in response to zinc.

Recently, my laboratory reported that MTF-1 and the histone acetyltransferase p300 form a complex in response to zinc treatment.[25] The acidic domain of MTF-1 was found to be necessary for the complex formation. NMR chemical shift analyses of backbone $^{13}C\alpha$ revealed that zinc does not bind to the acidic domain of MTF-1, so the zinc-dependent MTF-1-p300 interaction could involve zinc binding to p300 and/or an adaptor protein. Although the mechanisms of the zinc-dependent complex formation are not clear, the complex formation is an important step for *MT-I* gene transcription, because downregulation of endogenous p300 expression by siRNA decreases zinc-dependent *MT-I* gene transcription. It seems that the simple model based on the intrinsically low zinc binding affinity of the zinc fingers of MTF-1 cannot fully explain zinc-induced *MT* gene transcription.

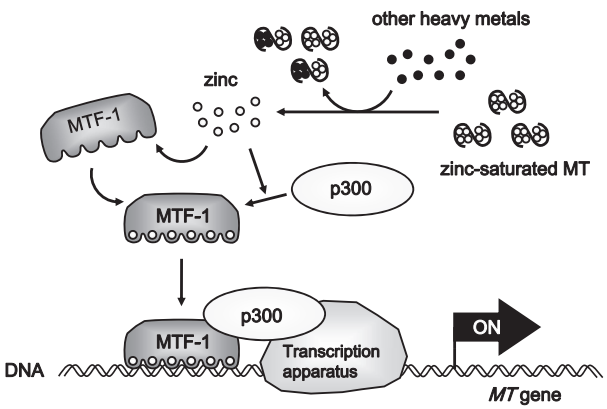

Fig. 1. Proposed Molecular Mechanisms in Mouse *MT-I* Gene Transcription

Heavy metals displace zinc from zinc-saturated MT or other zinc-binding proteins. The zinc binds to MTF-1 and promotes the formation of a complex containing MTF-1 and p300. The MTF-1-p300 complex then binds to DNA and recruits the transcription apparatus, leading to *MT* gene transcription.

MECHANISMS OF HEAVY METAL-INDUCED MT GENE TRANSCRIPTION

Heavy metal-mediated *MT-I* gene transcription is more complicated than zinc-mediated transcription. Zinc can reversibly and directly activate the DNA-binding activity of MTF-1:[26] MTF-1 binds to the MRE in response to zinc, and then induces transcription. In comparison, the DNA binding activity of MTF-1 is not activated by cadmium *in vitro*.[27] Several lines of experiments have shown that zinc, cadmium, and copper activate the DNA-binding activity of MTF-1 *in vivo* in mammalian

cells. The mechanisms of cadmium- and copper-induced MT gene transcription were examined using a cell-free, MTF-1-dependent transcription system.[28] Elevated zinc concentration alone induced transcription in the cell-free transcription system, but induction by cadmium and copper additionally required the presence of zinc-saturated MT, suggesting that these metals cause the release of zinc from MT. We showed using wild-type and MT-I/II-null cells that MTF-1 is activated by the degradation of MT protein.[29] These phenomena can be explained by the preferential binding of cadmium or copper to zinc-saturated MT.

Although cadmium and copper activate MTF-1 by increasing the available zinc concentration, other mechanisms may be also involved in the activation of MTF-1 by heavy metals. One possibility is post-transcriptional modification of MTF-1. Numerous evolutionarily conserved consensus phosphorylation sites in MTF-1 have been reported.[30] Inhibitor studies indicated that multiple kinases and signal transduction cascades, including those mediated by casein kinase II, c-Jun N-terminal kinase, protein kinase C, and tyrosine kinase, are essential for zinc- and cadmium-inducible transcriptional activation.[30–34] Moreover, recombinant MTF-1 acts as a substrate for some kinases *in vitro*. Although the mechanism by which heavy metals such as cadmium, copper, and mercury induce MTF-1-mediated *MT* gene transcription is still not clear, there is no doubt that tolerance to the toxicity of these heavy metals is due to the induction of MT, which sequesters the heavy metals and lowers their concentrations at critical intracellular sites.[8]

OVERVIEW OF CHROMIUM(VI) TOXICITY

Chromium is found in nature primarily as chromite ore with chromium in the trivalent form. This ore is used for manufacturing monochromates, dichromates, chromic acid, and chromium pigments. Although chromium(III) is required in trace amounts for sugar and lipid metabolism in humans, chromium(VI) is toxic and carcinogenic, so that abandoned chromium production sites need environmental cleanup.[15] Long-term inhalation exposure to various chromium(VI) compounds has been shown to result in a high risk of carcinomas of the respiratory organs. Compounds of chromium(VI) have been shown to induce mutations, chromosomal aberrations, DNA damage in the form of single-strand breaks, and DNA-protein and DNA-DNA crosslinks.[35,36] Chromium(VI) enters cells through the sulfate anion transporter[37] and becomes reduced via chromium(V) and chromium(IV) intermediate oxidation states to the stable chromium(III) form. The intermediate forms are thought to be responsible for much of the DNA damage and mutations induced by chromium(VI). MT cannot bind chromium, but by scavenging reactive oxygen species (ROS) through its cysteine residues, MT may act as a protective factor against chromium(VI)-induced DNA lesions, reducing chromium(VI) directly to chromium(III), thereby avoiding the toxic intermediate forms. In fact, downregulation of endogenous MT expression in cultured mammalian cells by preculture in zinc-deficient medium increases malignant transformation caused by chromium(VI) treatment (unpublished data).

INHIBITORY MECHANISMS OF CHROMIUM(VI) ON MT GENE TRANSCRIPTION

Chromium(VI) affects the expression of genes. For example, it upregulates expression of *bcl-2* and *urokinase-type plasminogen receptor*, in the latter case by increasing the stability of the mRNA.[38] Chromium(VI) also downregulates the expression of a diverse set of genes, including *Cyp1a1*,[39] *MT*,[13,14] *phosphoenolpyruvate carboxykinase*,[40,41] and *interleukin-8*.[42] It can also inhibit the activity of nuclear factor-κB (NF-κB) and the activation of polycyclic aromatic hydrocarbon-inducible promoters.[39,42] Chromium(IV) and ROS are key factors in the modification of gene expression. The mechanism by which chromium(VI) affects gene expression has not been thoroughly studied.

We have reported that chromium(VI) inhibits *MT* gene transcription.[17] Chromium(VI) pretreatment blocked the zinc-induced formation of the MTF-1-p300 complex (Fig. 2). Chromatin immunoprecipitation assays revealed that chromium(VI) only modestly reduces recruitment of MTF-1 to the *MT-I* promoter in response to zinc, but drastically reduces the recruitment of RNA polymerase II, thereby inhibiting the transactivation of *MT* in response to zinc. It is interesting to note

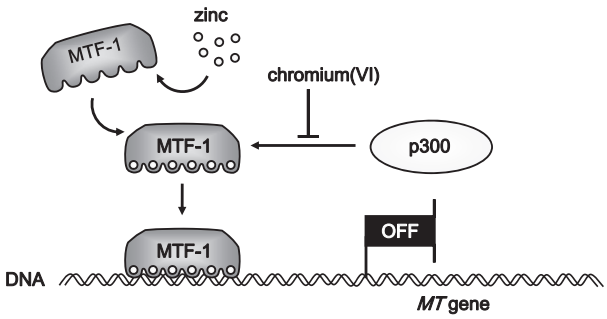

Fig. 2. Proposed Mechanism of Chromium(VI)-mediated Inhibition of *MT* Gene Transcription

Although increases in the intracellular labile zinc concentration lead to increased MTF-1-DNA binding, chromium(VI) prevents the formation of the MTF-1-p300 complex, which in turn prevents the recruitment of the transcription apparatus. Therefore, *MT* is not transcribed.

that chromium(VI) pretreatment also prevents the cytokine-induced interactions between the p65 subunit of NF-κB and the histone acetyltransferase cAMP-response-element-binding protein-binding protein (CBP), a close relative of p300, thus inhibiting induction of the *interleukin-8* gene.[42] Therefore, altering the interactions between histone acetyltransferases and transcription factors may be a common mechanism for chromium(VI) inhibition of gene expression. It has been reported that chromium(VI) can crosslink histone deacetylase-1 (HDAC1) and DNA methyltransferase-1 (DNMT1) complexes to the *Cyp1a1* promoter to inhibit transcriptional responses mediated by the arylhydrocarbon receptor.[43] Inhibitors of HDAC and DNMT can synergistically activate a DNA-methylated and histone-methylated *MT-I* promoter, leading to the formation of an open chromatin structure. However, in MT-expressing cells in general, the chromatin of the *MT-I* promoter has an open structure.[44] Chromium(VI)-induced cross-linking of HDAC1 and DNMT1 complexes is apparently not a mechanism involved in the inhibition of MTF-1 action by chromium(VI). Recently, it was reported that chromium(VI) increases G9a expression.[45] G9a is a histone methyltransferase that specifically methylates histone H3 lysine 9 (H3K9) and downregulates gene expression. The G9a induction in response to chromium(VI) may globally elevate H3K9 dimethylation, resulting in downregulation of some gene transcription. The G9a induction in response to chromium(VI) might modulate zinc-induced *MT* gene transcription. More detailed analyses of chromium(VI)-mediated inhibition of *MT* gene transcription will significantly improve our understanding of chromium(VI) toxicity.

CONCLUSIONS

In this review, I summarized the molecular mechanisms of *MT-I* gene transcription in response to zinc and other heavy metals and inhibition of the transcription by chromium(VI). Much research, including my own, has focused on the cytoprotective role of MT and the mechanism of MT induction. From these studies, MT is thought to be an important intracellular storage site for zinc and possibly other essential trace elements. The induction of MT confers tolerance to heavy metal toxicity by sequestering heavy metals and lowering their concentrations at critical intracellular sites. I suggest here that the carcinogenicity of chromium(VI) is related to its inhibition of MT transcription, which increases DNA lesions and decreases the direct reduction of chromium(VI) to chromium(III); although several mechanisms of the inhibition of *MT-I* gene transcription by chromium(VI) have been investigated, more studies are needed to fully understand this interaction. Recently, zinc was recognized as an intracellular signaling factor. MT and MTF-1 may modulate zinc signaling by regulating intracellular labile zinc concentrations. Understanding the molecular mechanisms of *MT* gene transcription is important not only for understanding the mechanisms of zinc homeostasis and heavy metal toxicity, but also for understanding zinc signaling.

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