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Positive and negative regulators of the metallothionein gene (Review)

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Abstract. Metallothioneins (MTs) are metal-binding proteins involved in diverse processes, including metal homeostasis and detoxification, the oxidative stress response and cell proliferation. Aberrant expression and silencing of these genes are important in a number of diseases. Several positive regulators of *MT* genes, including metal-responsive element-binding transcription factor (MTF)-1 and upstream stimulatory factor (USF)-1, have been identified and mechanisms of induction have been well described. However, the negative regulators of *MT* genes remain to be elucidated. Previous studies from the group of the present review have revealed that the hematopoietic master transcription factor, PU.1, directly represses the expression levels of *MT* genes through its epigenetic activities, and upregulation of *MT* results in the potent inhibition of myeloid differentiation. The present review focuses on PU.1 and several other negative regulators of this gene, including PZ120, DNA methyltransferase 3a with Mbd3 and Brg1 complex, CCAAT enhancer binding protein α and Ku protein, and describes the suppression of the *MT* genes through these transcription factors.

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1. Introduction

The metallothioneins (MTs) are a group of low molecular weight, cysteine-rich intracellular proteins, which are involved in maintaining intracellular metal homeostasis by binding metals, including zinc and copper. There are 10 functional isoforms of MTs, which are divided into four classes, designated MT-1 to -4, on the basis of small differences in protein sequence, expression and characteristics (1,2). They maintain transition metal ion homeostasis and redox balance, serve as anti-oxidants and protect against DNA damage and apoptosis (3). Reduced expression of *MT* has been observed in liver (4), colon (5) and prostate (6) cancer. It was suggested that during the transformation of normal colorectal tissue to adenomatous polyps and adenocarcinoma, a progressive decrease in the expression of *MT* occurs (7,8). The role of *MT* in these types of cancer remains to be elucidated, however, considering its anti-oxidant activity and its protective potential against DNA damage, this reduction may increase susceptibility to toxin-induced damage. Indeed, an *MT* knockout in mice has been reported to induce a higher rate of induced carcinogenesis (9). Conversely, aberrant overexpression of *MT* has been observed in various types of human cancer, including breast cancer, gallbladder cancer, melanoma and lymphoma (10-13). It has been suggested that the overexpression of *MT* may protect cells from free radical-induced DNA damage and lipid peroxidation (14). Overexpression of *MT* has been demonstrated to be important in drug resistance, since nuclear expression of *MT* protects DNA in ovarian cancer cells from the toxic effect of treatment with cisplatin (15). This indicates that aberrant under/over-expression of *MT* are important in various types of cancer.

A study revealed that the hematopoietic master transcription factor, PU.1, directly suppresses the *MT-1A* and *MT-1G* promoter through DNA methylation and histone deacetylase (HDAC) activity (16). Additionally, it was revealed that *MT-1A* is suppressed, while the expression of PU.1 is induced, during 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced monocytic differentiation of THP-1 cells (17). Notably, the suppression of *MT-1s* by PU.1 is required for the proper differentiation of myeloid cells.

Although there are several reviews regarding the regulation of the *MT* gene (18-20), reviews regarding the suppressive regulation of *MT* genes are relatively scarce. Therefore, this

review summarized the regulation of *MT* genes and particularly focused on PU.1 and other suppressive regulators of the *MT* genes.

2. Positive regulators of *MT* genes

The basal activity of *MT* is regulated by several general transcription factors, including the TFIID complex comprising TATA-binding protein (TBP), TBP associated factors and Sp1 (18-20). In addition, *MT* can be activated by a variety of stimuli, including metal ions, cytokines and growth factors (1). Several inducible expression regulators of the *MT* genes have been identified, including metal-responsive element (MRE)-binding transcription factor (MTF)-1 (21,22), upstream stimulatory factor (USF)-1 (23) and nuclear factor (NF)1 (24). Since a number of reviews summarize the details of the positive regulation of *MT* genes (18-20), the present review describes the above essential factors.

The *MTF-1* gene is a central regulator of the metal-inducible expression levels of *MT-1* and *MT-2*. In addition to zinc, other heavy metals (e.g. cadmium), hypoxia, oxidative stress, stress hormones (glucocorticoids), nitric oxide and high temperature induce the transcriptional activity of MTF-1 (25-28). Andrews *et al* (23) reported that MTF-1 is essential for the upregulation of the gene expression of *MT-1* in visceral endoderm cells and that optimal expression is dependent upon the interactions of the basic helix-loop-helix transcription factor, USF -1, with an E-box-1 containing sequence at -223 bp in the *MT-1* promoter (23).

NF1 is a protein expressed ubiquitously in higher eukaryotes, and distinct highly conserved genes encode four isoforms of the NF1 protein (NF1-A, NF1-B, NF1-C and NF1-X) (29-31). NF1 binding sites were identified in various *MT* promoters, with the exception of *MT*-IB (19). LaRochelle *et al* (24) previously demonstrated that NF1 binds to the mouse *MT*-1 promoter *in vivo* and this binding is zinc inducible and MTF-1 dependent. It was revealed by transient transfection assays into HepG2 cells, that NF1 activates the mouse *MT-1* promoter. The authors demonstrated that NF1 and MTF-1 synergistically activate the mouse *MT-1* gene in response to metal ions (24). However, Majumder *et al* (32,33) previously demonstrated that NF1 isoforms inhibit the activity of the *MT-1* promoter in HepG2 cells. This is contradictory to the earlier study (24), however, this result may be due to the experimental condition in which Majumder *et al* have used extremely high expression levels of the NF1 vector, ~30- to 1000-fold more vector compared with the earlier study (24). LaRochelle *et al* demonstrated that the expression levels of the transcriptionally active mutant of NF1 reduced the zinc-induced *MT-1* promoter by up to 50%, in a dose-dependent manner and may also indicate that NF1 is a positive regulator of the gene expression of *MT-1* (24).

3. Negative regulators of *MT* genes

To date, several factors are reported to regulate the suppression of *MT* genes, including PZ120 (34), DNA methyltransferase (Dnmt) 3a with Mbd3 and Brg1 complex (35), C/EBP α (36), Ku protein (37) and PU.1 (16,17).

Tang *et al* (34), reported the cloning of a novel zinc finger protein with a molecular mass of 120 kDa (PZ120), through Southwestern cloning, which interacts specifically with the human gene transcription initiation site of *MT-2A*. PZ120 is a ubiquitously expressed protein and possesses a conserved poxvirus and zinc finger (POZ) motif, which is a structure existing in several transcriptional repressors. This protein has been revealed to repress the transcription of the *MT-2A* promoter (34).

Datta *et al* (35) purified DNA methyltransferase (Dnmt) 3a from mouse lymphosarcoma cells and revealed that Dnmt 3a-associated polypeptides identified the methyl CpG binding protein, Mbd3, histone deacetylase 1 and components of the Brg1 complex (35). A chromatin immunoprecipitation assay reveled that Dnmt 3a, Mbd3 and Brg1 are associated with a transcriptionally silent methylated *MT-1* promoter in the mouse lymphosarcoma cells. The authors further clarified that the catalytic activity of Dnmt3a was not important for the repression of the *MT-1* gene; however, ATP-dependent chromatin remodeling of Brg1 was (35). It was also revealed that methylated and unmethylated *MT-1* promoters are differentially regulated by several methyl CpG binding proteins, including methyl CpG binding protein (MeCP) 2 and Mbd1, 2 and 4 (38).

CCAAT enhancer binding protein (C/EBP) is important in the terminal differentiation of cells, particularly in myeloid cells and adipose cells (39). Yin *et al* (36) demonstrated that forced expression of C/EBPα decreased the expression levels of the *MT* isoforms 1A, B, F and H, and 2A and 3 in prostate cancer cells, and that this suppression is mediated through its promoter activity. Furthermore, it was revealed that the forced expression of C/EBPα led to an increased cytotoxicity of zinc in prostate cancer cells (36). However, in human hepatocellular carcinoma cells, the inactivation of C/EBPα through the activation of phosphatidylinositol 3-kinase led to the downregulation of the expression of *MT* (4). Therefore, the role of C/EBPα in the gene regulation of *MT* may differ among tissues.

It was previously reported that the large subunit (p80) of the Ku protein contained repressor activity for the *MT*-1 promoter (37). Additionally, it was revealed that this repression is due to the hypermethylation of a CpG island in the *MT-1* promoter (40).

Rodent and human *MT* genes contain CpG islands in their promoter (19,41). It was first reported in 1981 that DNA methylation controls the inducibility of the mouse *MT-1* gene (42). Since then, >100 studies have been published demonstrating that the *MT* promoter is regulated by DNA methylation in its promoter region. Arriaga *et al* (43) demonstrated from the analysis of colorectal cancer, that the mRNA expression levels of five isoforms (*MT-1G*, *1E*, *1F*, *1H* and *1M*) were lost during the transition from normal mucosa to tumor, whereas *MT-1X* and *MT-2* were less downregulated and their expression was correlated with overall protein positivity. It was also demonstrated that hypermethylation of the *MT-1G* gene occurred in cell lines and in 29% of tumor samples. Faller et al (44) analyzed specimens from patients with melanoma and demonstrated that in 1/17 (6%) of the benign naevi, 16/43 (37%) primary tumors and 6/13 (46%) of metastases exhibited *MT-1E* gene methylation. Peng *et al* (45) revealed

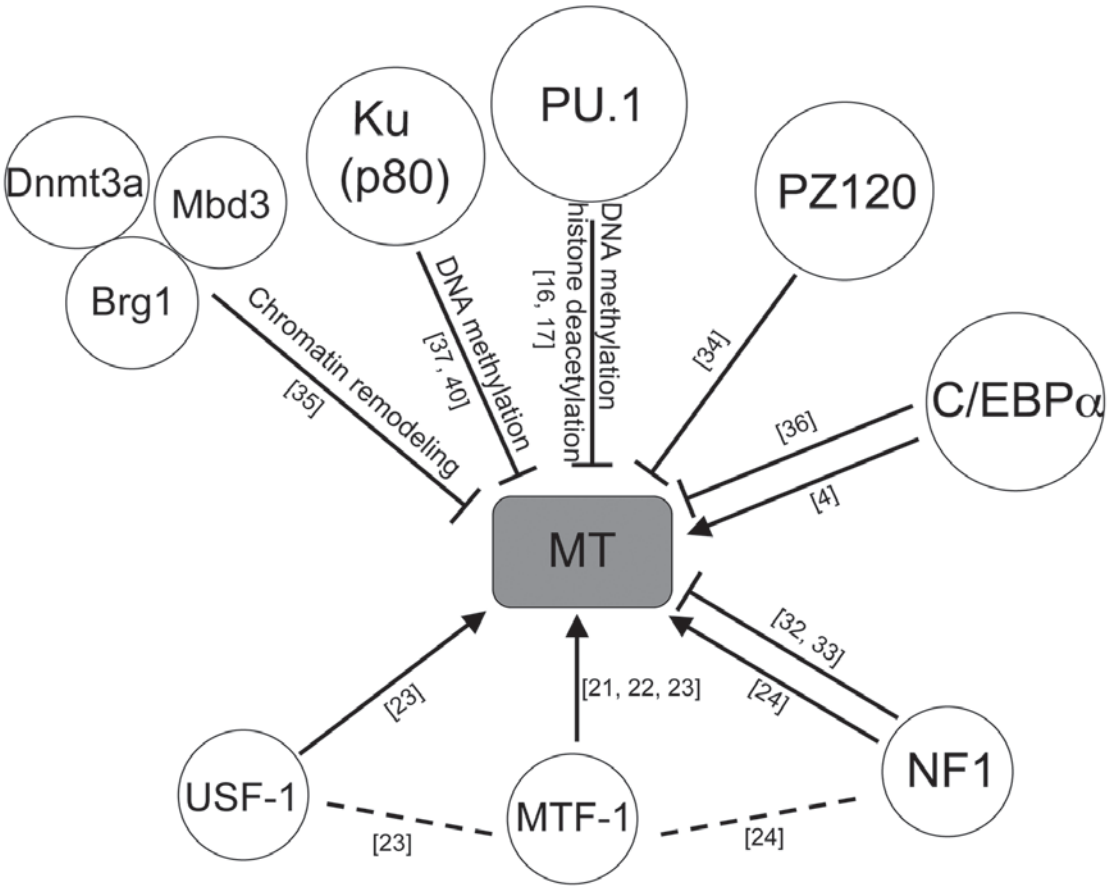

Figure 1. Schematic presentation of the negative and positive regulators of *MT*. Dotted line indicates a possible interaction. The indicated numbers in brackets are the references cited in this review. *MT*, metallothionein; Dnmt3a, DNA methyltransferase 3a; Mbd3, methyl-CpG-binding domain protein 3; PZ120, zinc finger protein with a molecular weight of 120 kDa; C/EBPα, CCAAT-enhancer-binding protein α; NF1, nuclear factor 1; MTF-1, metal regulatory transcription factor 1; USF-1, upstream transcription factor 1.

using quantitative pyrosequencing, unique DNA methylation profiles in the *MT*-3 promoter region in esophageal adenocarcinomas (EACs). This previous study concluded that EACs are characterized by frequent epigenetic silencing of the *MT*-3 gene. In addition, in colon cancer, not only DNA methylation (41,43), but the loss of heterozygosity (5) is also important in the downregulation of the *MT* genes (*MT*-1F, *MT*-1G, *MT*-1X and *MT*-2A).

4. PU.1-a master hematopoietic transcription factor previously identified as a novel negative regulator of *MT-1s*

A previous study revealed that *MT-1s* genes are epigenetically suppressed by the activity of PU.1 (16). PU.1 is a hematopoietic master transcription factor, predominantly expressed in immature myeloid cells and B cells, and downregulation of this factor is important in various hematological malignancies (46,47). To identify downstream target genes of PU.1, the authors generated cell lines expressing reduced levels of PU.1 by stable transfection of PU.1 short inhibitory RNAs into K562 human myeloid leukemia cells (K562PU.1KD cells) and PU.1-overexpressing K562 cells (K562PU.1OE cells). Dual microarray analyses were performed using these cell lines. Notably, the expression levels of all the functional *MT* isoforms expressed in humans (*MT-1A*, *-B*, *-E*, *-F*, *-G*, *-H* and *-X* and *MT*-2) were increased by varying degrees in the K562PU.1KD cells. Furthermore, there were negative correlations between the mRNA expression of PU.1 and the mRNA expression of the *MT-1s* in 43 primary specimens from patients with acute myeloid leukemia (AML). Additionally, it was revealed that PU.1 directly binds and epigenetically suppresses the *MT-1s* promoter, in concert with MeCP2, through the suppression of the enzymatic activities of HDAC and Dnmt. The proportion of the methylated CpG sites is tightly associated with the expression levels in *MT-1s* promoters (16). Next, the authors examined whether the expression levels of PU.1 and *MT-1A* are indeed correlated with each other, and whether the expression of *MT-1A* is regulated by PU.1 during TPA-induced THP-1 monocyte differentiation. As a result, it was revealed that the expression of *MT-1s* is suppressed during monocytic differentiation in the THP-1 cells (17). Chromatin immunoprecipitation analysis demonstrated that PU.1 and MeCP2 bind to the same region in the *MT-1A* promoter, and the binding of these proteins to this promoter was increased during differentiation. Consistently, the proportion of methylated CpG sites was markedly increased during differentiation (17). These results suggest that *MT-1s* are repressed through the epigenetic activity of PU.1 in hematopoietic cells.

5. Conclusion

The positive and negative regulators described in this review are summarized in Fig. 1. The consequences of these *MT* gene regulations have been reported to be through the normal physiological aspects to disease, including inflammation, aging and malignancies (1,3,48,49). It was recently demonstrated that the overexpression of *MT-1G* potently inhibited the retinoic acid induced myeloid differentiation of NB4 acute promyelocytic leukemia cells (50). This is consistent with the literature, suggesting that the downregulation of PU.1 is the cause of AML (46) and results in the overexpression of *MT*, leading to the inhibition of differentiation, which is important in leukemogenesis.

*MT*s are multifunctional proteins and exhibit different biological behavior in different tissues. Therefore, further clarifying the underlying mechanisms and the roles of *MT*, may lead to an improved understanding of the biology of normal physiology and malignancies from another aspects.

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