

The “metal transcription factor” MTF-1: biological facts and medical implications

Peter Lichtlen, Walter Schaffner

Institute of Molecular Biology, University of Zurich, Zürich, Switzerland

Summary

Metallothioneins (MTs) are a class of small, cysteine-rich proteins that have an important function in heavy metal metabolism and detoxification and in the management of various forms of cell stress. Several lines of evidence suggest a role for metallothioneins in therapy resistance of malignant tumours, regulation of blood pressure and protection against some neurological diseases. Basal and heavy metal-induced expression of the stress-inducible metallothionein-I and -II genes and some other stress-regulated genes depends on the zinc-finger transcription factor MTF-1. MTF-1 acts as a cellular stress-sensor protein and,

besides its crucial role in metallothionein expression, is essential for liver development since mice null mutant for MTF-1 die *in utero* due to hepatocyte degeneration. Under pathological conditions, MTF-1 seems to be involved in clinically important processes such as tumour angiogenesis and drug resistance. It thus seems generally advisable to monitor MTF-1 activity in stress-related processes including aging and carcinogenesis.

Key words: cadmium toxicity; cell stress; oxidative stress; transcription control

Introduction

Living organisms constantly need to cope with harmful environmental conditions such as heavy metal load, UV irradiation and oxidative stress. On the other hand, trace amounts of several heavy metals, notably the transition heavy metals zinc and copper, are essential for life. Zinc is crucial for the proper functioning of a large number of proteins including zinc-containing enzymes, transcription factors of the so-called zinc-finger family or other regulatory nuclear proteins containing single or multiple zinc ions as integral components. Zinc deficiency can lead to severe growth retardation, immune deficiency, impaired hair growth and fertility problems due to reduced sperm production [1–3], while a genetic defect in zinc uptake results in *acrodermatitis enteropathica* [4, 5]. In mammals bioavailability of zinc is controlled by a well-balanced sys-

tem. Metallothioneins (MTs), a group of small, cysteine-rich proteins particularly abundant in the kidney and liver are, due to their ability to bind heavy metals (reviewed in [6]), centrally involved in the homeostatic regulation of zinc concentrations and in detoxification of non-essential heavy metals. Under physiological conditions MTs primarily bind zinc but at the same time have a particularly high affinity for potentially toxic heavy metals [6]. For example, cadmium taken up in food is stored in a form tightly bound to metallothioneins, where it remains with a half-life of approximately 15 years [7]. Under heavy metal load, MT expression is strongly induced at the level of transcription [8]. This induction is mediated by the transcription factor MTF-1, an essential protein for liver development and cell stress response [9–11].

Glossary

Transcription factor: Protein which is necessary to start or control gene expression by recruiting the transcription apparatus (RNA-polymerase and associated proteins) to gene promoters.

Zinc finger: Protein domain (typically for DNA-binding) with a zinc ion as a structural component.

Promoter: DNA sequence to which the transcription apparatus binds in order to start transcription.

MRE: “Metal-responsive element”, a specific DNA-sequence motif to which MTF-1 binds and thereby activates expression of an associated gene.

Activation domain: Protein domain of transcription factors required to recruit the transcription apparatus.

Transgene: Gene that is artificially introduced and stably integrated into the genome and transmitted to subsequent generations.

Functions of metallothionein under physiological and pathological conditions

The mouse has four genes encoding distinct metallothioneins, designated MT-I to MT-IV (reviewed in [12]), but only the expression of MT-I and MT-II is ubiquitous and stress-inducible. The human genome encodes the same four types of metallothioneins, the stress-inducible forms having been multiplied by several gene duplication events [12]. Associations have been established between MTs and a number of diseases [13]. Several lines of evidence indicate that MTs play a role in carcinogenesis and tumour cell drug resistance [14, 15]. Although the role of metallothioneins in cancer remains controversial, it is clear that at least in some malignant tumours a high expression of metallothionein genes is correlated with increased metastatic potency, resistance to therapy and a poor prognosis [16–20]. Metallothionein-I and -II

have also been shown to be neuroprotective proteins in animal models of brain trauma, familial amyotrophic lateral sclerosis (ALS) and multiple sclerosis [21–24].

Somewhat unexpectedly, mouse strains with targeted deletion of both the MT-I and MT-II genes do not exhibit an altered phenotype under normal laboratory conditions [25, 26]. Nevertheless, these mice show increased sensitivity to cadmium intoxication, a dramatically reduced myotonic reflex of mesenteric arteries and, following kainic acid-induced seizures, a higher number of convulsions as well as a prolonged convulsion time and increased hippocampal neurodegeneration [26–28]. The latter phenotype is also observed on deletion of the brain-specific metallothionein-III gene [29].

MTF-1 directs metallothionein expression

Expression of the metallothionein-I and -II genes is induced, at the transcriptional level, by heavy metal load in particular [8]. The promoter regions of metallothionein genes contain so-called metal-responsive elements (MRE's; core consensus sequence TGCRCNC) which are responsible for induction by heavy metals [30, 31]. In 1988 the factor binding to the MRE promoter sequences was described as MTF-1 (metal-responsive element-binding transcription factor-1 or, for short, metal transcription factor-1). It required elevated zinc concentrations for optimal DNA binding [32]. Subsequently, MTF-1 was cloned and characterised [9]. MTF-1 is a ubiquitously expressed zinc finger protein that is essential for basal and

heavy metal-induced expression of metallothioneins [10]. Therefore, MTF-1 is the key regulator of metallothionein expression and recent data suggest that it is also involved in the response to oxidative and hypoxic stress, as might be expected for a stress-sensor molecule [11, 33].

Human MTF-1 is a 753 amino acid protein and its encoding gene has been mapped to the short arm of chromosome 1 (1p33) [34]. The N-terminal part of the protein is followed by six zinc fingers of the Cys₂-His₂ type that harbour the DNA-binding activity and sense the level of zinc [10]. In the C-terminus, MTF-1 contains three activation domains, each with distinct properties in transcriptional activation [35].

MTF-1 is activated under various stress conditions

When cells are treated with heavy metals MTF-1 is activated, binds to MRE's and induces transcription of target genes, notably metallothioneins [8, 9, 36]. In resting cells, most MTF-1 localises to the cytoplasm whence it is translocated from the cytoplasm to the nucleus under several different stress situations [37, 38]. The finding that MTF-1 requires an elevated concentration of zinc for strong binding to DNA suggests that MTF-1 is activated by allosteric regulation of DNA binding via binding of metals to the transcription factor itself [9, 10, 32]. Although other heavy metals readily induce metallothionein gene transcription in cultured cells, none of them can substitute for zinc in a cell-free DNA binding reaction of MTF-1 [10]. The most likely scenario is replacement of zinc by these other heavy metals in cellular and/or extracellular zinc-storage proteins, leading to concomitant activation of MTF-1 by the released zinc.

In addition, MTF-1 can be phosphorylated upon metal induction, as a result of the activation of a complex kinase signalling transduction pathway which includes protein kinase C (PKC), phosphoinositol-3 kinase (PI3K), c-Jun N-terminal kinase (JNK) and a tyrosine-specific kinase [39]. This suggests that metal ions such as cadmium could also activate MTF-1 by stimulating kinases.

However, the response of MTF-1 is not confined to heavy metals but is also activated by treatment of cells with hydrogen peroxide [40]. In line with this, MTF-1 has been reported to be greatly overexpressed in a radioresistant cervical carcinoma cell line relative to its expression in a radiosensitive cell line of the same origin [41]. These findings imply a role for MTF-1 in the regulation of genes involved in the cellular response to oxidative stress and suggest that MTF-1 might play a crucial role in therapy resistance of certain tumours.

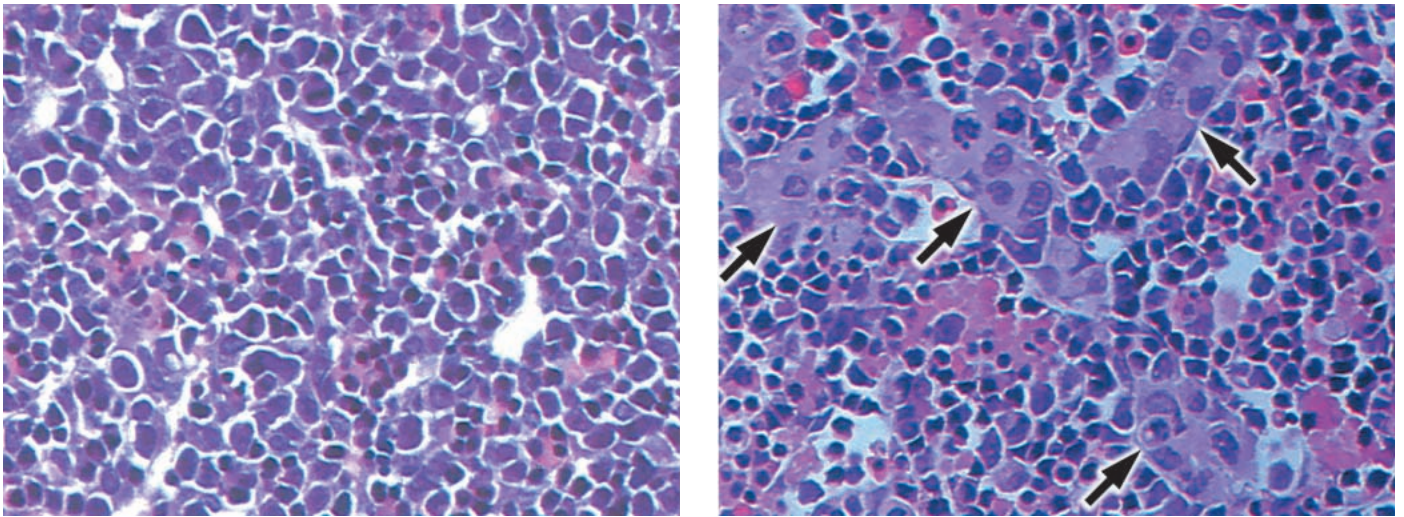
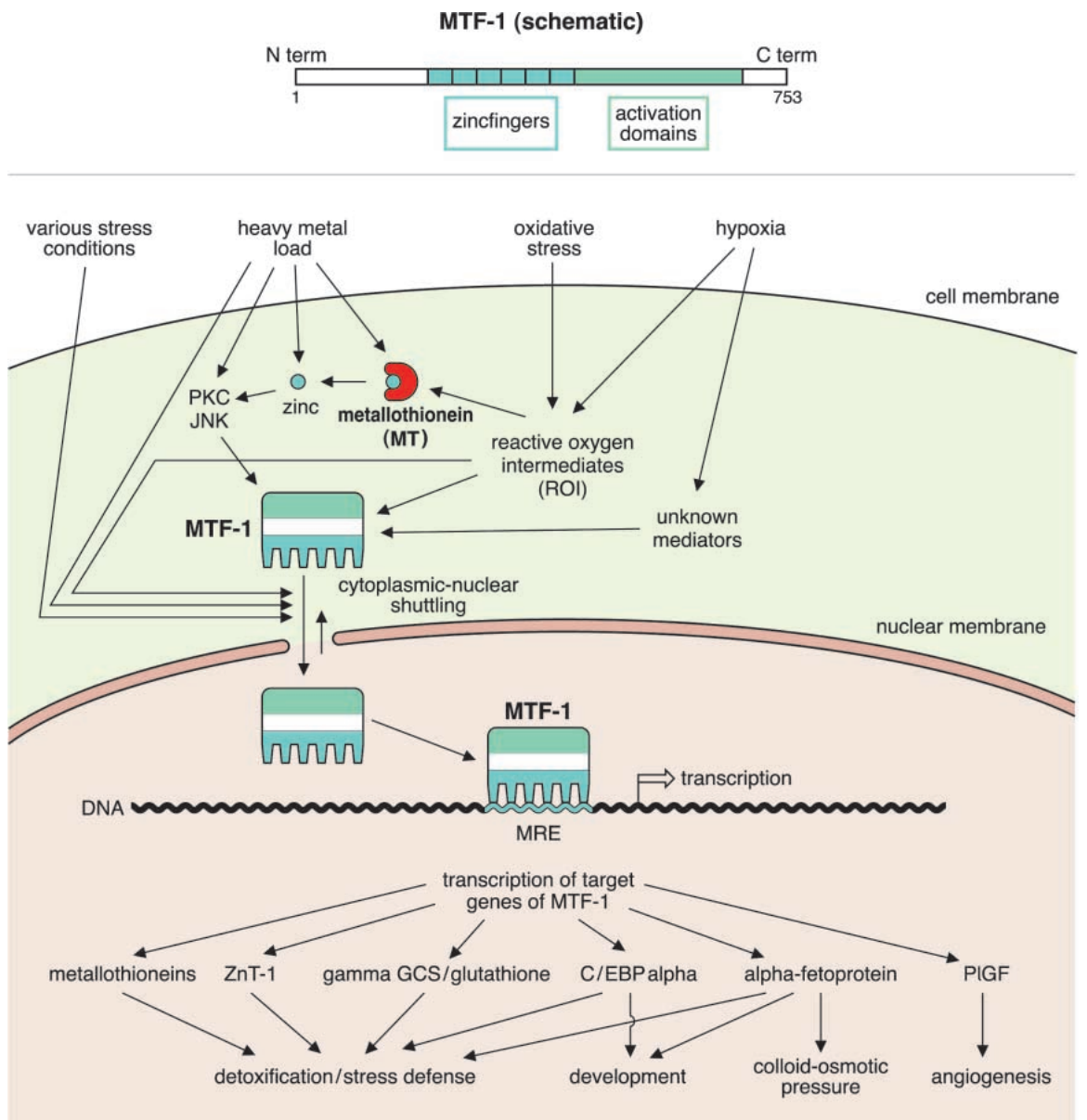


Figure 1

Histology of wild-type (A) and MTF-1 knockout (B) embryos showing the disrupted liver phenotype in MTF-1^{-/-} embryos. Haematoxylin and eosin-stained sagittal liver sections of embryos at stage E13.5. MTF-1^{-/-} (B) embryonic livers show enlarged, congested sinusoids and dissociation of the epithelial compartment. The remaining hepatocytes are clustered to form contiguous cell patches (arrows).

Figure 2

Schematic view of human MTF-1 protein (top). Overview of known and inferred activation pathways and downstream functions of the metal transcription factor MTF-1 (below). See text for details.



MTF-1 is essential for liver development

To investigate the physiological function of MTF-1 *in vivo*, we generated MTF-1 null mutant mice by targeted gene disruption and found this knockout to be lethal. A closer investigation revealed that MTF-1 knockout embryos die *in utero* around embryonic day (E) 14, due to degeneration of the embryonic hepatocytes [11] (see fig. 1). Interestingly, loss of other proteins involved in the management of cell stress results in similar phenotypes. This raises the question whether the embryonic liver at this stage may be particularly susceptible to a lack of stress-response regulators. For example, the phenotypes of mice null mutant for c-jun and SEK-1 strongly resemble that of MTF-1 knockout mice, though they are not identical [42, 43]. Among the latter, SEK-1/MKK4 is a kinase which has been shown to participate *in vitro* in stress-activated cascades that terminate with the p38 and SAPK/JNK kinases, which in turn result in phosphorylation and activation of transcription factors such as c-jun [44]. C. Séguin and colleagues have shown that JNK is also involved in phosphorylation of MTF-1 [39]. Taken together, activation of MTF-1 may overlap with activation of c-jun and thereby result in transcriptional activation of some common target genes needed for correct hepato-

cyte differentiation. Furthermore, the fact that MTF-1 levels in c-jun knockout fibroblasts are not changed (and vice versa [11]) speaks in favour of a parallel rather than epistatic relationship between these two essential transcription factors.

The lethal embryonic phenotype of MTF-1 knockout mice initially prevented investigations at stages later than E14. Nevertheless, fibroblasts taken into culture and neural tissue grafted into wild-type recipient mice survived [11, 45]. For a more thorough analysis, we generated so-called conditional knockout mice in order to study the role of MTF-1 in the development and functioning of tissues other than the liver. These studies are based on generation of transgenic mouse strains in which MTF-1 expression can be eliminated at a chosen time point or in a chosen tissue, using the so-called cre/lox system [46]. Indeed, preliminary data using such mice indicate that MTF-1 can be eliminated in adulthood, but that these mice are more susceptible to heavy metal stress. Hence MTF-1 probably plays a dual role in being essential for liver development and thereafter required to cope with cell stress (Ying Wang, P.L., W.S., unpublished results).

MTF-1 target genes and medical implications

The observation that the double knockout of metallothionein-I and -II genes was viable, while the knockout of the control protein MTF-1 was lethal, suggested that MTF-1 activates other important genes besides those encoding metallothioneins (fig. 2). Gamma glutamyl-cysteine synthetase heavy chain (γ GCS_{hc}), a key enzyme for glutathione synthesis, was the first additional putative candidate gene identified [11]. Nevertheless, we recently found glutathione levels in E12.5 embryonic MTF-1 knockout livers to be at least as high as in wild-type littermates, questioning the contribution of γ GCS_{hc} for the MTF-1 knockout phenotype [47]. In the same study we presented C/EBP α and α -foetoprotein (AFP) as likely *in vivo* target genes of MTF-1. Both genes provide new clues to the molecular pathogenesis of the knockout phenotype: C/EBP α , another transcription factor, is required to maintain the differentiated, non-proliferating state of hepatocytes [48]. In addition, it plays a role in cellular energy metabolism and, interestingly, is also involved in the cellular stress response, as indicated by its induction during acute phase response [49, 50]. AFP is expressed during embryogenesis, and its expression is turned off after birth [51]. It is clinically important as a tumour marker for primary hepatocellular carcinoma and for prenatal diagnosis of *spina bifida*. Physiologically it is responsible for maintenance of

embryonic colloid-osmotic pressure, and hence its down-regulation in MTF-1 knockout embryos could explain the late stages of the knockout phenotype, which are marked by generalised oedema [11]. In addition, AFP acts as a scavenger for heavy metals and reactive oxygen intermediates (ROI) [51]. The zinc transporter ZnT-1 was shown to be yet another *in vivo* target gene of MTF-1 [52]. It is expressed in the liver and also plays an important role in zinc metabolism in the brain [53].

In sum, MTF-1 is a crucial transcriptional regulator for basal expression of at least three important genes (MT-I, MT-II, ZnT-1) involved in zinc metabolism. The toxicity of zinc for neurons [53] would warrant studies with neural cell-specific conditional MTF-1 knockout mice. Such studies might also cast more light on the recent findings that the gene encoding the prion protein Prp, whose scrapie form causes Creutzfeldt-Jacob disease, contains MRE's in the promoter region (Ying Wang and W.S., unpublished results) and its gene product is a copper-binding protein [54].

Solid tumours usually grow under hypoxic conditions and must induce angiogenesis in order to grow. Of particular interest in this context is the recent finding that under hypoxic conditions expression of placenta growth factor (PlGF), encoding a member of the VEGF family of angiogenic factors, is induced in an MTF-1-dependent man-

ner in fibroblasts [33]. Furthermore, preliminary results of xenograft studies suggest that MTF-1 deficiency retards the formation of ras-transformed fibrosarcomas, resulting in significantly reduced tumour masses when compared to MTF-1-expressing tumours at two weeks after injection of the cells into nude mice (Brian Murphy, P.L., W.S., unpublished data).

As is typical of higher eukaryotes, different forms of stress response produce overlapping patterns of gene activity. Thus, it would not be surprising to find new roles for MTF-1 in a variety of stress conditions other than those evoked by heavy metal, hypoxia, xenobiotic components and reactive oxygen intermediates. The downside of this ability of cells to cope with various forms of stress, however, might be a propensity to malignant

growth and therapy resistance of tumours, as a result of MTF-1 boosting expression of metallothioneins and other cytoprotective proteins. Given the multiple facets of MTF-1 activity, it certainly seems advisable to monitor changes in MTF-1 expression and activity in studies involving cell stress, aging and cancer.

We thank Fritz Ochsenbein for preparation of the figures.

Correspondence:

*Professor Dr. W. Schaffner
Institute of Molecular Biology
University of Zurich
Winterthurerstrasse 190
CH-8057 Zurich*

References

- MacDonald RS. The role of zinc in growth and cell proliferation. *J Nutr* 2000;130(5S Suppl):1500S-8S.
- Prasad AS. Zinc and immunity. *Mol Cell. Biochem* 1998;188:63-9.
- Hamdi SA, Nassif OI, Ardawi MS. Effect of marginal or severe dietary zinc deficiency on testicular development and functions of the rat. *Arch Androl* 1997;38:243-53.
- Prasad AS. Zinc:an overview. *Nutrition* 1995;11(1 Suppl):93-9.
- Muga SJ, Grider A. Partial characterization of a human zinc-deficiency syndrome by differential display. *Biol Trace Elem Res* 1999;68:1-12.
- Kägi JH. Evolution, structure and chemical activity of class I metallothioneins:an overview. In: Suzuki KT, Imura N, Kimura M, eds. *Metallothionein III*. Basel/Switzerland: Birkhäuser; 1993.
- Nordberg M, Nordberg GF. On the role of metallothionein in cadmium induced renal toxicity. *EXS* 1987;52:669-75.
- Durnam DM, Palmiter RD. Transcriptional regulation of the mouse metallothionein-I gene by heavy metals. *J Biol Chem* 1981;256:5712-6.
- Radtke F, Heuchel R, Georgiev O, Hergersberg M, Gariglio M, Dembic Z, Schaffner W. Cloned transcription factor MTF-1 activates the mouse metallothionein-I promoter. *EMBO J* 1993;12:1355-62.
- Heuchel R, Radtke F, Georgiev O, Stark G, Aguet M, Schaffner W. The transcription factor MTF-1 is essential for basal and heavy metal-induced metallothionein gene expression. *EMBO J* 1994;13:2870-5.
- Günes C, Heuchel R, Georgiev O, Müller KH, Lichtlen P, Schaffner W, et al. Embryonic lethality and liver degeneration in mice lacking the metal-responsive transcriptional activator MTF-1. *EMBO J* 1998;17:2846-54.
- Heuchel R, Radtke F, Schaffner W. Transcriptional regulation by heavy metals, exemplified at the metallothionein genes. In: Baeuerle PA ed.; *Inducible Gene Expression*. Birkhäuser, Boston, MA, Vol I: 206-240.
- Simpkins CO. Metallothionein in human disease. *Cell Mol Biol* 2000;46:465-88.
- Cherian MG, Huang PC, Klaassen CD, Liu YP, Longfellow DG, Waalkes MP. National Cancer Institute workshop on the possible roles of metallothionein in carcinogenesis. *Cancer Res* 1993;53:922-5.
- Cherian MG, Howell SB, Imura N. Role of metallothionein in carcinogenesis. *Toxicol Appl Pharmacol* 1994;126:1-5.
- Jasani B, Schmid KW. Significance of metallothionein overexpression in human tumors. *Histopathology* 1997;31:211-4.
- Moussa M, Kloth D, Peers G, Cherian MG, Frei JV, Chin JL. Metallothionein expression in prostatic carcinoma: correlation with Gleason grade, pathologic stage, DNA content and serum level of prostate-specific antigen. *Clin Invest Med* 1997;20:371-80.
- Janssen AML, van Duijn W, Oostendorp-van de Ruit MM, Kruidenier L, Bosman CB, Griffioen G, et al. Metallothionein in human gastrointestinal cancer. *J Pathol* 2000;192:293-300.
- Jin R, Bay BH, Chow VT, Tan PH. Metallothionein 1F mRNA expression correlates with histological grade in breast carcinoma. *Breast Cancer Res & Treatment* 2001;66:265-72.
- Hishikawa Y, Kohno H, Ueda S, Kimoto T, Kumar Dhar D, Kubota H, et al. Expression of metallothionein in colorectal cancers and synchronous liver metastases. *Oncology* 2001;61:162-7.
- Penkowa M, Giralt M, Thomsen PS, Carrasco J, Hidalgo J. Zinc or copper deficiency-induced impaired inflammatory response to brain trauma may be caused by the concomitant metallothionein changes. *J Neurotrauma* 2001;18:447-63.
- Nagano S, Satoh M, Sumi H, Fujimura H, Tohyama C, Yanagihara, et al. Reduction of metallothioneins promotes the disease expression of familial amyotrophic lateral sclerosis mice in a dose-dependent manner. *Eur J Neurosci* 2001;13:1363-70.
- Penkowa M, Hidalgo J. Metallothionein treatment reduces proinflammatory cytokines IL-6 and TNF-alpha and apoptotic cell death during experimental autoimmune encephalomyelitis (EAE). *Exp Neurol* 2001;170:1-14.
- Espejo C, Carrasco J, Hidalgo J, Penkowa M, Garcia A, Saez-Torres I, et al. Differential expression of metallothioneins in the CNS of mice with experimental autoimmune encephalomyelitis. *Neurosci* 2001;105:1055-65.
- Michalska AE, Choo KHA. Targeting and germ-line transmission of a null mutation at the metallothionein I and II loci in mouse. *Proc Natl Acad Sci USA* 1993;90:8088-92.
- Masters BA, Kelly EJ, Quaife CF, Brinster RL, Palmiter RD. Targeted disruption of metallothionein I and II genes increases sensitivity to cadmium. *Proc Natl Acad Sci USA* 1994;91:584-8.
- Pearce LL, Gandle RE, Han W, Wasserloos K, Stitt M, Kanai AJ, et al. Role of metallothionein in nitric oxide signaling as revealed by a green fluorescent fusion protein. *Proc Natl Acad Sci USA* 2000;97:477-82.
- Carrasco J, Penkowa M, Hadberg H, Molinero A, Hidalgo J. Enhanced seizures and hippocampal neurodegeneration following kainic acid-induced seizures in metallothionein-I and -II-deficient mice. *Eur J Neurosci* 2000;12:2311-22.
- Erickson JC, Hollopeter G, Thomas SA, Froelick GJ, Palmiter RD. Disruption of the metallothionein-III gene in mice:analysis of brain zinc, behaviour, and neuron vulnerability to metals, aging and seizures. *J Neurosci* 1997;17:1271-1281.
- Stuart GW, Searle PF, Palmiter RD. Identification of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences. *Nature* 1985;317:828-31.
- Serfling E, Lubbe A, Dorsch-Hasler K, Schaffner W. Metal-dependent SV40 viruses containing inducible enhancers from the upstream region of metallothionein genes. *EMBO J* 1985;4:3851-9.
- Westin G, Schaffner W. A zinc-responsive factor interacts with a metal-regulated enhancer element (MRE) of the mouse metallothionein-I gene. *EMBO J* 1988;7:3763-3770.
- Green CJ, Lichtlen P, Huynh NT, Yanovsky M, Schaffner W, Murphy BJ, et al. Placenta growth factor gene expression is induced by hypoxia in fibroblasts:a central role for metal transcription factor MTF-1. *Cancer Res* 2001;61:2696-703.

- 34 Brugnera E, Georgiev O, Radtke F, Heuchel R, Baker E, Schaffner W, et al. Cloning, chromosomal mapping and characterization of the human metal-regulatory transcription factor MTF-1. *Nucleic Acids Res* 1994;22:3167-73.
- 35 Radtke F, Georgiev O, Müller HP, Brugnera E, Schaffner W. Functional domains of the heavy metal-responsive transcription factor MTF-1. *Nucleic Acids Res* 1995;23:2277-86.
- 36 Aufder Maur A, Belser T, Wang Y, Günes C, Lichtlen P, Schaffner W, et al. Characterization of the mouse gene for the heavy metal-responsive transcription factor MTF-1. *Cell Stress & Chaperones* 2000;5:196-206.
- 37 Smirnova IV, Bittel DC, Ravindra R, Jiang H, Andrews GK. Zinc and cadmium can promote rapid nuclear translocation of metal response element-binding transcription factor-1. *J Biol Chem* 2000;275:9377-84.
- 38 Saydam N, Georgiev O, Nakano MY, Greber UF, Schaffner W. Nucleo-cytoplasmic trafficking of metal-responsive transcription factor MTF-1 is regulated by diverse stress signals. *J Biol Chem* 2001;276:25487-95.
- 39 LaRochelle O, Gagné V, Charron J, Soh JW, Séguin C. Phosphorylation is involved in the activation of metal-regulatory transcription factor 1 in response to metal ions. *J Biol Chem* 2001;276:41879-88.
- 40 Dalton TP, Li Q, Bittel D, Liang L, Andrews GK. Oxidative stress activates metal-responsive transcription factor-1 binding activity. Occupancy in vivo of metal response elements in the metallothionein-I gene promoter. *J Biol Chem* 1996;271:26233-41.
- 41 Archary MP, Jaggernauth W, Gross E, Alfieri A, Klinger HP, Vikram B. Cell lines from the same cervical carcinoma but with different radiosensitivities exhibit different cDNA microarray patterns of gene expression. *Cytogenetics & Cell Genetics* 2000;91:39-43.
- 42 Hilberg F, Aguzzi A, Howells N, Wagner EF. c-Jun is essential for normal mouse development and hepatogenesis. *Nature* 1993;365:179-181.
- 43 Ganiatsas S, Kwee L, Fujiwara Y, Perkins A, Ikeda T, Labow MA. SEK1 deficiency reveals mitogen-activated protein kinase cascade crossregulation and leads to abnormal hepatogenesis. *Proc Natl Acad Sci USA* 1998;95:6881-6886.
- 44 Sanchez I, Hughes RT, Mayer BJ, Yee K, Woodgett JR, Avruch J. Role of SAPK/ERK kinase-1 in the stress-activated pathway regulating transcription factor c-jun. *Nature* 1994;372:794-8.
- 45 Lichtlen P, Georgiev O, Schaffner W, Aguzzi A, Brandner S. The heavy metal-responsive transcription factor-1 (MTF-1) is not required for neural differentiation. *Biol Chem* 1999;380:711-5.
- 46 Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. *Science* 1995;269:1427-9.
- 47 Lichtlen P, Wang Y, Belser T, Georgiev O, Certa U, Schaffner W, et al. Target gene search for the metal-responsive transcription factor MTF-1. *Nucleic Acids Res* 2001;29:1514-23.
- 48 Timchenko NA, Harris TE, Wilde M, Bilyeu TA, Burgess-Beusse BL, Darlington GJ, et al. CCAAT/enhancer binding protein alpha regulates p21 protein and hepatocyte proliferation in newborn mice. *Mol Cell Biol* 1997;17:7353-61.
- 49 Wang ND, Finegold MJ, Bradley A, Ou CN, Abdelsayed SV, Darlington GJ, et al. Impaired energy homeostasis in C/EBP alpha knockout mice. *Science* 1995;269:1108-12.
- 50 Burgess-Beusse BL, Darlington GJ. C/EBP alpha is critical for the neonatal acute-phase response to inflammation. *Mol Cell Biol* 1998;18:7269-77.
- 51 Mizejewski GJ. Alpha-fetoprotein as a biologic response modifier: relevance to domain and subdomain structure. *Proc Soc Exp Biol Med* 1997;215:333-62.
- 52 Langmade SJ, Ravindra R, Daniels PJ, Andrews GK. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT-1 gene. *J Biol Chem* 2000;275:34803-9.
- 53 Cuajungco MP, Lees GJ. Zinc metabolism in the brain: relevance to human neurodegenerative disorders. *Neurobiol Dis* 1997;4:137-69.
- 54 Brown DR. Copper and prion disease. *Brain Res Bulletin* 2001;55:165-73.

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website <http://www.smw.ch> (direct link from each SMW record in PubMed)
- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

Editorial Board

Prof. Jean-Michel Dayer, Geneva
 Prof. Peter Gehr, Berne
 Prof. André P. Perruchoud, Basel
 Prof. Andreas Schaffner, Zurich
 (Editor in chief)
 Prof. Werner Straub, Berne
 Prof. Ludwig von Segesser, Lausanne

International Advisory Committee

Prof. K. E. Juhani Airaksinen, Turku, Finland
 Prof. Anthony Bayes de Luna, Barcelona, Spain
 Prof. Hubert E. Blum, Freiburg, Germany
 Prof. Walter E. Haefeli, Heidelberg, Germany
 Prof. Nino Kuenzli, Los Angeles, USA
 Prof. René Lutter, Amsterdam,
 The Netherlands
 Prof. Claude Martin, Marseille, France
 Prof. Josef Patsch, Innsbruck, Austria
 Prof. Luigi Tavazzi, Pavia, Italy

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:

http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
 SMW Editorial Secretariat
 Farnsburgerstrasse 8
 CH-4132 Muttenz

Manuscripts: submission@smw.ch
 Letters to the editor: letters@smw.ch
 Editorial Board: red@smw.ch
 Internet: <http://www.smw.ch>