Neuroscience Letters 440 (2008) 289–293

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Cadmium specifically induces MKP-1 expression via the glutathione depletion-mediated p38 MAPK activation in C6 glioma cells

Sang-Mi Kim[a], Jong-Gu Park[a], Won-Ki Baek[a], Min-Ho Suh[a], Hyung Lee[b], Sun Kyun Yoo[c], Kyung-Hwan Jung[d], Seong-Il Suh[a,*], Byeong-Churl Jang[a,**]

[a] Chronic Disease Research Center, Keimyung University School of Medicine, 194 Dongsan-dong, Jung-gu, Daegu, Republic of Korea
[b] Department of Neurology, Keimyung University School of Medicine, 194 Dongsan-dong, Jung-gu, Daegu, Republic of Korea
[c] Department of Oriental Medicine and Food Biotechnology, Joongbu University, Kumsan-gun, Chungnam, Republic of Korea
[d] Division of Food & Biotechnology, Chungju, National University, Chungju, Chungbuk, Republic of Korea

ARTICLE INFO

Article history:
Received 6 December 2007
Received in revised form 28 April 2008
Accepted 20 May 2008

Keywords:
Cadmium
MKP-1
NAC
Glutathione
p38 MAPK
C6 cells

ABSTRACT

Cadmium is a toxic heavy metal and an environmental pollutant. Mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) is a negative regulator of the family of MAPK. In this study, we investigated the effect of heavy metals on MKP-1 expression in C6 rat glioma cells. Cadmium treatment induced MKP-1 at both protein and mRNA levels while cobalt or manganese treatment did not, suggesting the specificity. Cadmium treatment also depleted intracellular GSH and activated p38 MAPK, JNKs, and AKT. Profoundly, pretreatment with thiol-containing compounds NAC or GSH, but not vitamin E, blocked GSH depletion, 38 MAPK activation and MKP-1 expression by cadmium. Moreover, pharmacological inhibition of p38 MAPK by SB203580 suppressed the cadmium-induced MKP-1. Collectively, these results demonstrate that cadmium specifically induces MKP-1 by transcriptional up-regulation in C6 cells in a mechanism associated with the glutathione depletion-dependent p38 MAPK activation.

Cadmium is a toxic heavy metal and an environmental pollutant. It can accumulate in the body, mostly kidney, liver, lung, and brain [9]. Acute cadmium exposure leads to lung damage [1] while chronic exposure causes renal toxicity [25]. In vitro cadmium induces tumor cell death via glutathione depletion, generation of reactive oxygen species (ROS), Bcl-2 suppression, or caspase activation [30,29,11,14]. Recent data also demonstrate that cadmium treatment elevates inflammatory COX-2 expression and PGE_2 release [20,18], suggesting a link between the cadmium-mediated cytotoxicity and inflammation.

Mitogen-activated protein kinase (MAPK) phosphatase-1 (MPK-1) is a member of the family of dual specificity phosphatases. MKP-1 functions to inactivate the member of MAPKs, including extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinase (JNKs) and p38 MAPK, by dephosphorylation [5,13,6]. MKP-1 is an immediate early response gene and its expression is rapidly induced in cells after treatment with cellular stress and inflammatory or mitogenic stimuli [24,28,23]. However, the underlying mechanism of MKP-1 induction remains unclear. While MKP-1 expression by interleukin-1β occurs in the p38 MAPK-dependent in HeLa cells [16], JNK-dependent MKP-1 expression is shown in anisomycin or UV-treated fibroblasts [2]. A role for ERKs in MKP-1 expression by serum in fibroblasts is also reported [4]. However, MKP-1 expression by calcium and serum in fibroblasts, respectively, is independent of p38 MAPK or JNKs and ERKs [23]. It suggests that MAPKs differentially regulate MKP-1 expression with cells or agonists specificity. Notably, cadmium treatment leads to activation of various signaling proteins, including MAPKs, protein kinase C, and the PI3K downstream effector AKT [18,7,15]. However, cadmium treatment also diminishes phosphorylation of AKT in H9c2 rat heart cells [19]. Little is known about the regulation of MKP-1 expression by cadmium.

In this study, we investigated the effect of cadmium on MKP-1 expression in C6 rat glioma cells.

DMEM, penicillin, and streptomycin were from GIBCO-BRL. FBS was from Hyclone. Anti-rabbit or mouse secondary horseradish peroxidase antibodies and ECL Western detection reagents were from Amersham Biosciences. Bradford reagent was from Biorad. Antibodies against phospho-ERKs (p-ERKs), ERKs, p-JNKs, JNKs, p-p38 MAPK, p38 MAPK, p-AKT, or AKT were from Cell Signaling Technology. Antibodies against MKP-1 and IκB-α were from Santa Cruz Biotechnology. PD98059 (PD), SB203580 (SB), and LY294002 (LY) were purchased from Biomol. SP600125 (SP)

* Corresponding author. Fax: +82 53 255 1398.
** Corresponding author. Tel.: +82 53 250 7032; fax: +82 53 250 8005.
E-mail addresses: seong@dsmc.or.kr (S.-I. Suh), jangbc12@kmu.ac.kr (B.-C. Jang).

0304-3940/$ – see front matter © 2008 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2008.05.064

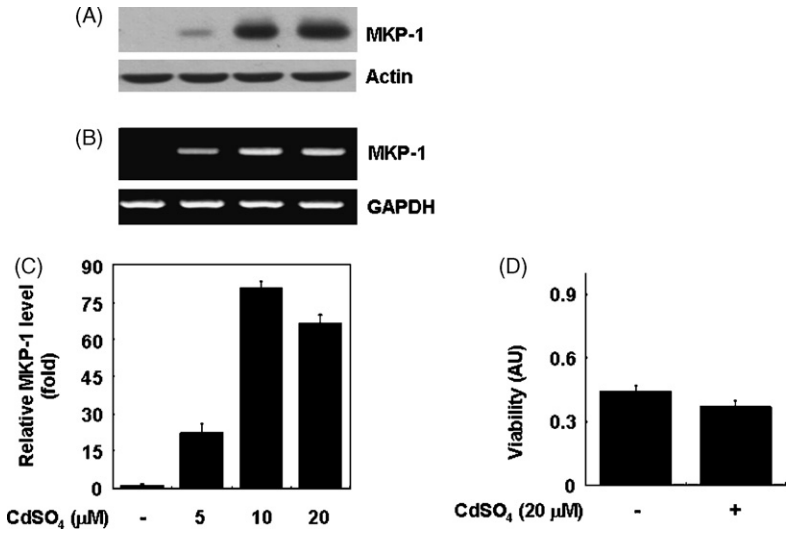

Fig. 1. Induction of MKP-1 protein and mRNA by cadmium in C6 cells. C6 cells were treated with the indicated concentrations of cadmium ($CdSO_4$) for 8 h. Whole cell lysates and total RNA were prepared and analyzed for MKP-1 or actin protein by Western blot (A) and for MKP-1 or GAPDH mRNA by analyses of RT-PCR (B) or real-time PCR (C). (D) C6 cells were treated with $CdSO_4$ for 8 h, and cell viability was measured by a MTS assay. Data are mean ± S.E. of three independent experiments.

and proteinase inhibitor cocktail (100×) were from Calbiochem. Reagents for RT-PCR were from PerkinElmer. Other reagents were from Sigma.

C6 cells were grown in DMEM supplemented with 10% FBS and 1% antibiotics at 37 °C at 95% air and 5% CO_2. Cells were washed with phosphate-buffered saline and exposed to cell lysis buffer (50 mM Tris–Cl (pH 7.4), 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 0.25% sodium deoxycholate, 1% Triton X-100, 1% Nonidet P-40, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 1 mM NaF, proteinase inhibitor cocktail (1×)). Cells were harvested and centrifuged at 12,000 rpm. The supernatant was saved and protein concentrations were determined with Bradford reagent.

C6 cells were grown in 96-well plates (10,000 cells/well). Cells were treated without or with cadmium for 8 h, at which point they were incubated with MTS (20 μl/well). The absorbance was then measured at 595 nm using a microplate reader.

Proteins (70 μg) were separated by SDS-PAGE (10%) and transferred onto nitrocellulose membranes (Millipore). The membranes were washed with TBS (10 mM Tris, 150 mM NaCl) supplemented with 0.05% Tween 20 (TBST) followed by blocking with TBST containing 5% non-fat dried milk. The membranes were incubated with antibodies specific for MKP-1, actin, p-ERKs, p-p38 MAPK, p-JNKs, p-AKT, or IκB-α, and exposed to secondary antibodies coupled to horseradish peroxidase. Immunoreactivities were then detected by ECL reagents. Subsequently, the membrane used for phosphoproteins was stripped and reprobed for total protein of ERKs, JNKs, p38 MAPK, or AKT with respective antibody. The membrane used for MKP-1 was stripped and reprobed with an anti-actin antibody.

Three micrograms of total RNA were reverse transcribed using a random hexadeoxynucleotide primer and reverse transcriptase to synthesize cDNA in 40 μl volume. One microlitre out of cDNA was amplified by PCR with the following primers: MKP-1 sense, 5′-CAG TGG AGA TCC TGT CCT TC TG-3′; MKP-1 anti-sense, 5′-TTG CAG CAG CTG GCC GAT GAA GC-3′; GAPDH sense, 5′-GGTGAAGGTCGGTGTGAACG-3′; GAPDH anti-sense, 5′-GGTAGGAACACGGAAGGCCA-3′. The PCR conditions applied were: MKP-1, 25 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s; GAPDH, 27 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s. Real-time PCR amplification was performed on the LightCycler using the 'LightCycler FastStart DNA master SYBR Green 1' kit (Roche Diagnostics) as we previously reported [12] with slight modifications.

Intracellular GSH content was measured using a GSH Assay Kit (Calbiochem). Briefly, 1×10^7 cells were sonicated in 5% metaphosphoric acid. The homogenate was centrifuged at $3000 \times g$ for 10 min, and its supernatant was used for GSH measurement according to the manufacturer's instruction.

We initially determined the effect of cadmium on MKP-1 expression in C6 cells. Treatment with cadmium (5, 10 and 20 μM) for 8 h induced MKP-1 protein and mRNA expressions (Fig. 1A–C). Control actin or GAPDH was not affected by cadmium. Results of MTS analysis showed no significant cytotoxicity in C6 cells by cadmium treatment at 20 μM for 8 h (Fig. 1D) in which high MKP-1 was induced. However, treatment with cadmium at 20 μM for 24 h caused a significant decrease in the cell viability (data not shown). In subsequent kinetic studies, cadmium treatment induced a time-dependent expression of MKP-1 protein and mRNA in which maximal MKP-1 protein and mRNA was induced after 8 and 4 h, respectively (Fig. 2A).

In this study, the exposure of cadmium into C6 cells also increased phosphorylation of ERKs, JNKs, p38 MAPK, and AKT in a time-dependent manner (Fig. 2B). Total protein of ERKs, JNKs, p38 MAPK, or AKT was not affected by cadmium. Considering the previous reports [15,19], AKT seems to be positively or negatively regulated by cadmium in different cells in a concentration-related manner. MKP-1 is best known for its specificity toward ERK [6], but has a substrate preference for p38 MAPK and JNKs [8]. The present findings showing decreased ERKs and AKT phosphorylation but sustained p38 MAPK and JNKs phosphorylation in the cadmium-treated C6 cells for 8 h (Fig. 2B) in which high MKP-1 is induced (Fig. 2A) may suggest that the cadmium-induced MKP-1 in C6 cells may selectively dephosphorylate ERKs and AKT via a negative feedback. Treatment with peroxovanadium, another heavy metal, induces MKP-1, which specifically inactivates JNKs in PC12 cells [21]. Thus, MKP-1 induced by different heavy metals may have different substrate specificity against MAPKs, depending on cell types. Cadmium is an oxidative stressor [10]. NF-κB is a redox-sensitive transcription factor and its activation is associated with proteolytic degradation of IκB-α [3]. In C6 cells, IκB-α was not degraded by cadmium (Fig. 2B), indicating no NF-κB activation by cadmium in C6 cells.

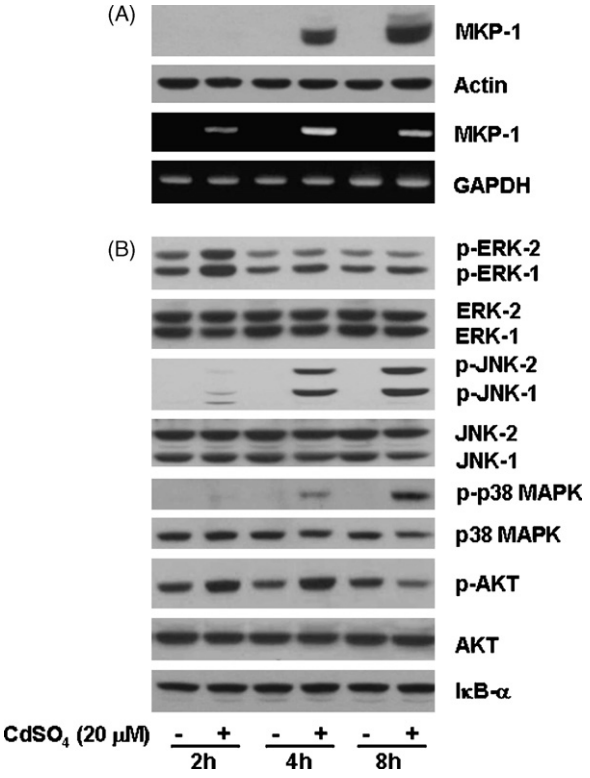

Fig. 2. Effect of cadmium on MAPKs, AKT, or IκB-α in C6 cells. (A) C6 cells were treated with $CdSO_4$ for the indicated times. Whole cell lysates and total RNA were prepared and subjected to immunoblot analysis and RT-PCR, respectively. (B) C6 cells were treated with $CdSO_4$ for the indicated times. Whole cell lysates were prepared and subjected to immunoblot analysis with respective antibody.

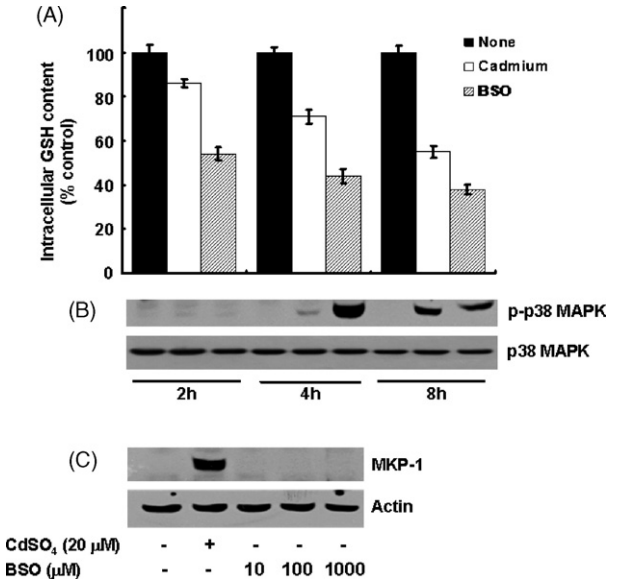

Fig. 3. Effect of cadmium or BSO on intracellular GSH, p38 MAPK activation and/or MKP-1 expression in C6 cells. (A and B) C6 cells were treated with $CdSO_4$ (20 μM) or BSO (1 mM), a GSH depletor for the indicated times. At each time, intracellular GSH level in the $CdSO_4$- or BSO-treated C6 cells was determined by a GSH assay (A). Data are mean ± S.E. of three independent experiments. Whole cell lysates were prepared and analyzed by Western blot (B). (C) C6 cells were treated with the indicated does of $CdSO_4$ or BSO for 8 h. Whole cell lysates were prepared and subjected to Western blot.

In this study, the exposure of cadmium into C6 cells decreased intracellular GSH in a time-dependent manner, as assessed by the effect of BSO, a GSH depletor (Fig. 3A). Notably, as induced by cadmium, BSO treatment also activated p38 MAPK (Fig. 3B), supporting that GSH depletion leads to p38 MAPK activation [17]. However, while cadmium induced MKP-1, BSO at the concentrations used did not (Fig. 3C), suggesting the specific ability of cadmium to induce MKP-1 expression in C6 cells.

In this study, pretreatment with SB (a p38 MAPK inhibitor) specifically blocked MKP-1 protein and mRNA expressions by cadmium (Fig. 4A–C), suggesting a role for p38 MAPK in the MKP-1 transcriptional up-regulation by cadmium in C6 cells. The MKP-1 promoter contains multiple *cis*-acting elements, including Sp1, Sp3, AP1, AP2, E-box, and two CRE elements [22]. Considering that two CREs within the MKP-1 promoter may bind CRE-interacting proteins including ATF-2 [22], and, that p38 MAPK regulates ATF-2 activity [26], p38 MAPK may influence the cadmium-mediated MKP-1 transcription via ATF2 in C6 cells.

In this study, pretreatment with NAC (a GSH precursor) or GSH (a reducing agent), but not vitamin E (VE, a ROS scavenger) that prevented GSH depletion by cadmium (Fig. 5C) blocked the cadmium-induced MKP-1 protein (Fig. 5A) and mRNA (Fig. 5B) expressions and p38 MAPK activation (Fig. 5D) in C6 cells. This suggests that in C6 cells the cadmium-induced MKP-1 is due to a depletion of glutathione rather than a production of ROS and the cadmium-induced GSH depletion precedes p38 MAPK phosphorylation. To our knowledge, it is the first reporting a network between cadmium exposure, glutathione depletion, p38 MAPK activation and MKP-1 expression. It is previously shown that single addition of NAC at 0.1 or 1 mM to culture medium of C6 cells for 24 h does not influence intracellular GSH [27]. Supporting it, single addition of NAC (20 mM) or GSH (20 mM) to culture medium of C6 cells for 8 h did not modulate intracellular GSH, as assessed by the effect of BSO (data not shown). No effect by extracellular GSH on intracellular GSH level in C6 cells may be explained by that GSH is easily oxidized in cell culture medium [10]. The underlying mechanism of how NAC or GSH, when pretreated, elicits its inhibitory effect on the cadmium-mediated GSH depletion in C6 cells remains unclear at present.

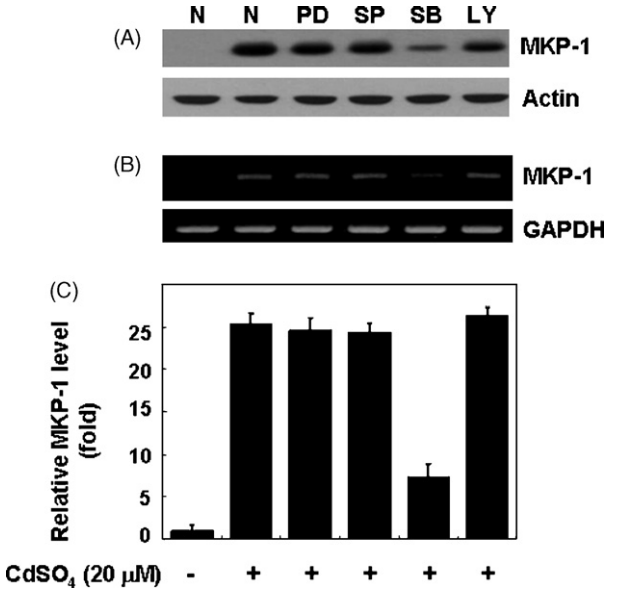

Fig. 4. A role for p38 MAPK in the cadmium-induced MKP-1 expression in C6 cells. C6 cells were pretreated for 1 h with vehicle (N), PD (50 μM), SP (25 μM), SB (25 μM) or LY (25 μM) and then exposed to $CdSO_4$ for additional 8 h. Whole cell lysates and total RNA were prepared and analyzed by Western blot (A) and by RT-PCR (B) or real-time PCR (C), respectively.

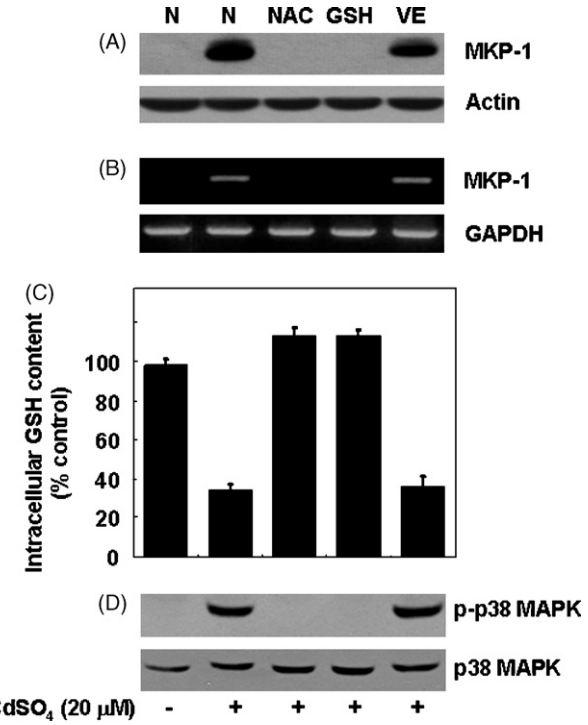

Fig. 5. Involvement of GSH depletion in the cadmium-induced MKP-1 expression and p38 MAPK activation in C6 cells. (A–D) C6 cells were pretreated for 1 h with vehicle (N), NAC (20 mM), GSH (20 mM), or vitamin E (VE, 100 μM) and then exposed to $CdSO_4$ for additional 8 h. Whole cell lysates and total RNA were prepared and analyzed by Western blot (A) and RT-PCR (B), respectively. (C) Measurement of intracellular GSH level in the conditioned cells by a GSH assay. Data are mean ± S.E. of three independent experiments. (D) Measurement of the level of p-p38 MAPK or p38 MAPK in the conditioned cells by Western blot.

We next evaluated the effect of other heavy metals on MKP-1 expression in C6 cells. Compared with cadmium, neither cobalt nor manganese induced MKP-1 (Fig. 6), suggesting a high degree of specificity for cadmium to induce MKP-1 in C6 cells. However, given that the exposure of cobalt (100 μM) into PC12 cells induces MKP-1 [24], MKP-1 up-regulation by heavy metals may be the concentrations and/or cell types-dependent.

Although speculative, the cadmium-induced MKP-1 seen in C6 cells may regulate cell apoptosis. Dephosphorylation of ERKs and AKT (survival proteins) in conjunction with glutathione depletion and sustained activation of p38 MAPK and JNKs (apoptosis-related proteins) may lead to long-term apoptosis of C6 cells by cadmium poisoning. In conclusion, we have demonstrated that cadmium specifically induces MKP-1 expression in C6 cells in a mechanism associated with GSH depletion and the subsequent activation of p38 MAPK.

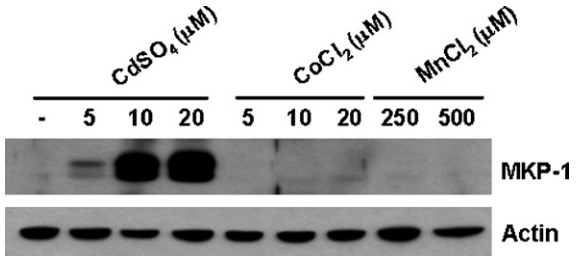

Fig. 6. Effects of other heavy metals on MKP-1 expression in C6 cells. C6 cells was exposed to $CdSO_4$, $CoCl_2$, or $MnCl_2$ with the indicated concentrations for 8 h. Whole cell lysates were prepared and analyzed for Western blot.

Acknowledgements

The authors gratefully acknowledge Dr. David Bishop-Bailey (Queen Mary University of London, United Kingdom) for critical reviews of the manuscript and Ms. Hwa Hong for excellent technical assistants. This work was supported by Grant number R13-2002-028-02001-0 from Basic Research Program of the Korea Science and Engineering Foundation (KOSEF) and by KOSEF grant funded by the Korea government (MOST) (R01-2006-000-10323-0).

References

[1] M.H. Bhattacharyya, A.K. Wilson, S.S. Rajan, M. Jonah, Biochemical pathways in cadmium toxicity, in: R.K. Zalups, J. Koropatnicks (Eds.), Molecular Biology and Toxicology of Metals, Taylor and Francis, London, UK, 2000, pp. 34–74.
[2] D. Bokemeyer, A. Sorokin, M. Yan, N.G. Ahn, D.J. Templeton, M.J. Dunn, Induction of mitogen-activated protein kinase phosphatase 1 by the stress-activated protein kinase signaling pathway but not by extracellular signal-regulated kinase in fibroblasts, J. Biol. Chem. 271 (1996) 639–642.
[3] A. Bowie, L.A. O'Neill, Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries, Biochem. Pharmacol. 59 (2000) 13–23.
[4] J.M. Brondello, A. Brunet, J. Pouysségur, F.R. McKenzie, The dual specificity mitogen-activated protein kinase phosphatase-1 and -2 are induced by the p42/p44MAPK cascade, J. Biol. Chem. 272 (1997) 1368–1376.
[5] M. Camps, A. Nichols, S. Arkinstall, Dual specificity phosphatases: a gene family for control of MAP kinase function, FASEB J. 14 (2000) 6–16.
[6] Y. Chu, P.A. Solski, R. Khosravi-Far, C.J. Der, K. Kelly, The mitogen-activated protein kinase phosphatases PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo toward the ERK2 sevenmaker mutation, J. Biol. Chem. 271 (1996) 6497–6501.
[7] S.M. Chuang, I.C. Wang, J.L. Yang, Roles of JNK, p38 and ERK mitogen-activated protein kinases in the growth inhibition and apoptosis induced by cadmium, Carcinogenesis 21 (2000) 1423–1432.
[8] C.C. Franklin, A.S. Kraft, Conditional expression of the mitogen-activated protein kinase (MAPK) phosphatase MKP-1 preferentially inhibits p38 MAPK and stress-activated protein kinase in U937 cells, J. Biol. Chem. 272 (1997) 16917–16923.
[9] L. Gerhardsson, V. Englyst, N.G. Lundstrom, S. Sandberg, G. Nordberg, Cadmium, copper and zinc in tissues of deceased copper smelter workers, J. Trace Elem. Med. Biol. 16 (2002) 261–266.
[10] D. Han, C.K. Sen, S. Roy, M.S. Kobayashi, H.J. Tritschler, L. Packer, Protection against glutamate-induced cytotoxicity in C6 glial cells by thiol antioxidants, Am. J. Physiol. 272 (1997) R1771–R1778.
[11] J.Y. Im, S.G. Paik, P.L. Han, Cadmium-induced astroglial death proceeds via glutathione depletion, J. Neurosci. Res. 83 (2006) 301–308.
[12] B.C. Jang, K.J. Lim, M.H. Suh, J.G. Park, S.I. Suh, Dexamethasone suppresses interleukin-1beta-induced human beta-defensin 2 mRNA expression: involvement of p38 MAPK, JNK, MKP-1, and NF-kappaB transcriptional factor in A549 cells, FEMS Immunol. Med. Microbiol. 51 (2007) 171–184.
[13] S.M. Keyse, Protein phosphatases and the regulation of mitogen-activated protein kinase signaling, Curr. Opin. Cell. Biol. 12 (2000) 186–192.
[14] M.S. Kim, B.J. Kim, H.N. Woo, K.W. Kim, K.B. Kim, I.K. Kim, Y.K. Jung, Cadmium induces caspase-mediated cell death: suppression by Bcl-2, Toxicology 145 (2000) 27–37.
[15] H. Konishi, H. Matsuzaki, M. Tanaka, Y. Takemura, S. Kuroda, Y. Ono, U. Kikkawa, Activation of protein kinase B (Akt/RAC-protein kinase) by cellular stress and its association with heat shock protein Hsp27, FEBS Lett. 410 (1997) 493–498.
[16] M. Lasa, S.M. Abraham, C. Boucheron, J. Saklatvala, A.R. Clark, Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38, Mol. Cell. Biol. 22 (2002) 7802–7811.
[17] J.H. Limon-Pacheco, N.A. Hernandez, M.L. Fanjul-Moles, M.E. Gonsebatt, Glutathione depletion activates mitogen-activated protein kinase (MAPK) pathways that display organ-specific responses and brain protection in mice, Free Radic. Biol. Med. 43 (2007) 1335–1347.
[18] T. Miyahara, T. Katoh, M. Watanabe, Y. Mikami, S. Uchida, M. Hosoe, T. Sakuma, N. Nemoto, K. Takayama, T. Komurasaki, Involvement of mitogen-activated protein kinases and protein kinase C in cadmium-induced prostaglandin E2 production in primary mouse osteoblastic cells, Toxicology 200 (2004) 159–167.
[19] H. Murata, Y. Ihara, H. Nakamura, J. Yodoi, K. Sumikawa, T. Kondo, Glutaredoxin exerts an antiapoptotic effect by regulating the redox state of Akt, J. Biol. Chem. 278 (2003) 50226–50233.
[20] P. Rockwell, J. Martinez, L. Papa, E. Gomes, Redox regulates COX-2 upregulation and cell death in the neuronal response to cadmium, Cell Signal. 16 (2004) 343–353.
[21] L. Rumora, A. Shaver, T. Zanic-Grubisic, D. Maysinger, Differential regulation of JNK activation and MKP-1 expression by peroxovanadium complexes, Neurochem. Int. 38 (2001) 341–347.

[22] S. Ryser, A. Massiha, I. Piuz, W. Schlegel, Stimulated initiation of mitogen-activated protein kinase phosphatase-1 (MKP-1) gene transcription involves the synergistic action of multiple *cis*-acting elements in the proximal promoter, Biochem. J. 378 (2004) 473–484.
[23] J.C. Scimeca, M.J. Servant, J.O. Dyer, S. Meloche, Essential role of calcium in the regulation of MAP kinase phosphatase-1 expression, Oncogene 15 (1997) 717–725.
[24] K.A. Seta, R. Kim, H.W. Kim, D.E. Millhorn, D. Beitner-Johnson, Hypoxia-induced regulation of MAPK phosphatase-1 as identified by subtractive suppression hybridization and cDNA microarray analysis, J. Biol. Chem. 276 (2001) 44405–44412.
[25] Z.A. Shaikh, T.T. Vu, K. Zaman, Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants, Toxicol. Appl. Pharmacol. 154 (1999) 256–263.
[26] H. Song, S.H. Ki, S.G. Kim, A. Moon, Activating transcription factor 2 mediates matrix metalloproteinase-2 transcriptional activation induced by p38 in breast epithelial cells, Cancer Res. 66 (2006) 10487–10496.
[27] V.C. Stewart, R. Stone, M.E. Gegg, M.A. Sharpe, R.D. Hurst, J.B. Clark, S.J. Heales, Preservation of extracellular glutathione by an astrocyte derived factor with properties comparable to extracellular superoxide dismutase, J. Neurochem. 83 (2002) 891–984.
[28] M.L. Toh, Y. Yang, M. Leech, L. Santos, E.F. Morand, Expression of mitogen-activated protein kinase phosphatase 1, a negative regulator of the mitogen-activated protein kinases, in rheumatoid arthritis: up-regulation by interleukin-1beta and glucocorticoids, Arthritis Rheum. 50 (2004) 3118–3128.
[29] W. Wätjen, D. Beyersmann, Cadmium-induced apoptosis in C6 glioma cells: influence of oxidative stress, Biometals 17 (2004) 65–78.
[30] W. Wätjen, M. Cox, M. Biagioli, D. Beyersmann, Cadmium-induced apoptosis in C6 glioma cells: mediation by caspase 9-activation, Biometals 15 (2002) 15–25.