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Review

Localization, Regulation, and Function of Metallothionein-III/ Growth Inhibitory Factor in the Brain

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The metallothionein (MT) family is a class of low molecular, intracellular, and cysteine-rich proteins with a high affinity for metals. Although the first of these proteins was discovered nearly 40 years ago, their functional significance remains obscure. Four major isoforms (MT-I, MT-II, MT-III, and MT-IV) have been identified in mammals. MT-I and MT-II are ubiquitously expressed in various organs including the brain, while expression of MT-III and MT-IV is restricted in specific organs. MT-III was detected predominantly in the brain, and characterized as a central nervous system-specific isomer. The role of MTs in the central nervous system has become an intense focus of scientific research. An isomer of MTs, MT-III, of particular interest, was originally discovered as a growth inhibitory factor, and has been found to be markedly reduced in the brain of patients with Alzheimer's disease and several other neurodegenerative diseases. MT-III fulfills unique biological roles in homeostasis of the central nervous system and in the etiology of neuropathological disorders.

Key words: neuroprotectin, metal transport, localization, gene expression, neurodegenerative disease

Metallothioneins (MTs), which are low molecular, intracellular, and cysteine-containing proteins with a high affinity for metals [1-4], were first discovered by Margoshes and Vallee in 1957 [5]. Previous studies on the structure and biological functions of MT have identified 4 major isoforms (MT-I, MT-II, MT-III, and MT-IV) in mammals. MT-I and MT-II are extensively expressed in all mammalian tissues [6]. In the central nervous system, MT-I and MT-II are conspicuously absent from neuronal cell populations, but abundant in astroglias [7, 8]. MT-IV is expressed in squamous epithelial cells [9] but appears to be absent from the brain. MT-III, originally discovered as a growth inhibitory factor (GIF), is a brain-specific isomer. MT-III resembles other members of the MT family: it consists of 68 amino acids containing 20 cysteine residues in conserved positions, and binds to zinc (Zn) and copper (Cu) [10, 11]. Uchida *et al.* reported that MT-III protein is normally secreted from cortical astrocytes, and that it is markedly depleted in the brain of patients with Alzheimer's disease (AD) [10]. Many reports have suggested that MTs in the brain might be involved in the regulation of brain metal homeostasis and neural protective function against toxic metals [13-15]. We speculated that the expression mechanism and function of MT-III are different from those of MT-I and MT-II isomers.

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Although MT-I and MT-II are directly inducible in astrocytes by cadmium (Cd), Zn, or dexamethazone (Dex), MT-III mRNA is not directly inducible [12]. MT-III expression may be regulated by factors other than classical MT inducers (Cd, Zn *etc.*), and this difference in inducibility is useful for identifying these factors. Further, MT-III was found to exert growth inhibitory activity on neurons cultured with AD brain extract [10]. This function is very distinctive, given that other MTs exhibit no growth inhibitory activity. Based on these findings, a previous report postulated that loss of MT-III might result in elevated neurotrophic activity, which induces abortive sprouting exhaustion and eventual neuronal death. Uchida *et al.* showed that MT-III is down regulated in several other neurodegenerative diseases in addition to AD [16]. These findings indicate that reductions in MT-III may correlate with neuronal loss and/or neuroprotection, and that the exact roles of MT-III should be elucidated. In this review, we summarize the structure, localization and function of MT-III, and investigate the regulation of MT-III expression in various neurodegenerative statuses.

Structure of MT-III

MT-III is a low molecular weight, heat-stable, cysteine-rich, and acidic protein that possesses a high affinity for metal. Three Zn and 4 Cu atoms are bound to human MT-III [10]. The numbers of the metals in MT-III are species-specific. Human, mouse, and rat MT-III consist of 68, 68, and 66 amino acids residues, respectively [10, 11, 17–19] (Fig. 1). MT isoforms are structurally identical to the same number of cysteine residues [2, 11]. The position of all 20 constituent cysteine residues is completely conserved, though they differ in their total charge due to differences in certain amino acids other than cysteine [14] (Fig. 1). Human MT-III contains 7 additional amino acids: a single threonine residue inserted at the N-terminal region and 6 residues, consisting of glutamic acid and alanine, inserted at the C-terminal region [10]. The additional glutamic acids make MT-III acidic, in contrast to MT-I and MT-II [10]. The growth inhibitory activity on the survival of rat cultured cortical neurons in extract of AD brains is a specific biological activity of MT-III (*i.e.*, not of MT-I or MT-II) [10]. The sequence of 5–23 in MT-III contains 2 prolines (at positions 7 and 9) is essential for this activity [20, 21], and the activity is dependent not only on the sequence but also on the conformation [22]. The gene of the human MT-III localizes on chromosome 16, which location is the same as that in MT-I and MT-II [11]. Human, mouse, and rat MT-III genes have been isolated and sequenced [17–19, 23]. These genes show high homology to MT-encoding genes in the 5' flanking region. Metal responsive elements (MREs) characteristic of MT promoters were identified within this region, though the MREs of MT-III were unresponsive to Zn [23]. CTG triplet repeat elements, which were identified within the mouse and rat MT-III promoter region, suppressed gene expression [24], but such repeat has not been identified in the human MT-III gene [19]. Future study on these inactive MREs and this silencing region might reveal the mechanism of regulation of MT-III expression.

Cell-Specific Distribution of MT-III

Many studies have shown the localization of 3 isoforms of MTs (MT-I, MT-II, and MT-III) in the mammalian brain. There are some regional differences in localization among specific isoforms. All MT mRNAs were highly expressed in the olfactory bulb, though MT-III mRNA was higher in the hippocampus and lower in the cerebellum as compared with expression of MT-I and MT-II [25]. *In situ* hybridization revealed that the constitutive expression of MT-I mRNA was predominantly localized in glial cells of the Purkinjie cell layer of the cerebellum [25]. MT-I and MT-II are abundant in glial

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                          10        20        30        40        50        60
mouse MT-III  MDPETCPCPTGGSCTCSDKCKCKGCKCTNCKKSCCSCCPAGCEKCAKDCVCKGEEGAKAEAEKCSCCQ
rat   MT-III  MDPETCPCPTGGSCTCSDKCKCKGCKCTNCKKSCCSCCPAECEKCAKDCVCKGEEGAKAE KCSCCQ
human MT-III  MDPETCPCPSGGSCTCADSCKCEGCKCTSCKKSCCSCCPAECEKCAKDCVCKGGEAAEAEAEKCSCCQ
      MT-Ia   MDPN CSCATGGSCTCTGSCKCKECKCNSCKKSCCSCCPMSCAKCAQGCICKGA      SEKCSCCA
      MT-II   MDPN CSCAAGDSCTCAGSCKCKECKCTSCKKSCCSCCPVGCAKCAQGCICKGA      SDKCSCCA
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Fig. 1 Comparison of the amino acid sequences of mouse MT-III [19], rat MT-III [18], human MT-III [10], human MT-Ia [71], and human MT-II [72]. Cystein residues appear in boldface in all sequences.

cells (both protoplasmic and fibrous astrocytes) but not in neurons, with the exception of neurons in the CA3 region of the hippocampus [7].

Several studies have reported on the cell-specific distribution of MT-III protein and MT-III mRNA. However, results have not yet reached a consensus on this matter. Uchida *et al.* reported immunohistochemical findings that showed that MT-III expressed in astrocytes in the gray matter of the normal human brain [10]. Anezaki *et al.* reported that astrocytes were immunostained more strongly than neurons in the cerebral cortex of the normal rat brain [26]. Electron microscopic immunohistochemistry revealed a high level of MT-III immunoreactivity throughout the soma and the fine processes in astrocytes. Neurons also exhibited MT-III immunoreactivity, but the activity was restricted to a subset of the neuronal population. In contrast to the pattern of astrocytes, activity in the neurons was localized predominantly in the processes including axons and dendrites [27]. Recently, an immunohistochemical study using phagimid-antibody specific for rat MT-III demonstrated that MT-III in the normal brain was localized abundantly in neuronal cell bodies in CA1–3 regions and the dentate gyrus of the hippocampus, the cerebral cortex, the olfactory bulb, and Purkinjie cells in the cerebellum [28]. On the other hand, MT-III mRNA expression has been reported in neurons by *in situ* hybridization using an antisense probe of MT-III [7]. MT-III mRNA was predominantly expressed in neurons of regions with high concentrations of vesicular Zn, such as the hippocampus, piriform cortex, and amygdala [7]. β-Galactosidase activity in transgenic mice using 11.5 kb of the mouse 5' flanking region of MT-III fused to the *E. coli lac Z* gene was localized to neurons containing Zn in synaptic terminal vesicles [7]. Further, transgenic mice with the human MT-III gene and 20 kb of the MT-III 5' flanking region were found to express high levels of MT-III and its mRNA as well as Zn, but no other metals [29]. This interesting distribution of MT-III in zinc-containing neurons led to the recent hypothesis that MT-III might serve as a neuromodulator in addition to performing functions common to MT isoforms. Further, in an *in vitro* system, Kobayashi *et al.* reported that MT-III mRNA expression was detected in primary cultured astrocytes, but not in neurons, microglia, and fibroblasts prepared from rat brain [18]. Both primary cultured neurons and astrocytes from neonatal CF-1 mice expressed MT-III mRNA [30, 31]. Thus, there remains some disagreement among findings on the cell-specific distribution of MT-III protein and MT-III mRNA. Further investigation is needed to clarify the storage and turnover of MT-III in neurons and astrocytes.

Several studies have reported that MT-III expressed in several organs other than the brain. The MT-III expression was detected in human kidney, and MT-III protein was localized strongly in the cytoplasm of distal tubules [32, 33]. Moffatt *et al.* showed that MT-III mRNA was expressed in the testis, prostate, epididymis, tongue, ovary, uterus, stomach, heart, and seminal vesicles of the rat [34]. However, the MT-III mRNA levels in these organs were extremely lower than the level in brain. In testis, epididymis, prostate, and tongue, the levels were 22% that of the brain level, and levels were lower still in the other organs. *In situ* hybridization analyses on human testis showed that MT-III mRNA localized nonuniformly in Leyding cells and the seminiferous tubule [34]. In addition, Garrett *et al.* reported that selected epithelial and stromal cells of the normal human prostate were shown to have low levels of MT-III expression [35]. MT-III tissue-specific expression in several peripheral organs may be broader than previously reported.

MT-III Expression in Developmental and Aged Animals

Kobayashi *et al.* reported that the level of MT-III mRNA in the rat brain increased rapidly from Day 10 to Day 17 after birth and reached a plateau at Week 4, and showed no decrease in the aged rat [18]. In the other reports, brain MT-III mRNA in rat and mouse were increased throughout the developmental period, reaching adult levels by approximately Week 3 [7, 34, 36]. On the other hand, in the mouse brain, the level of MT-I mRNA increased rapidly on Day 16 after birth and reached a maximum level at Week 3, but decreased in the adult mouse [7]. In other reports, MT-I mRNA levels in mouse liver were very high and increased until Day 12 after birth, then began a gradual decrease to adult levels in 36 day old mice, whereas MT-I mRNA levels in the mouse brain were uniformly low from birth to Week 6 [34]. The mRNA accumulation curves during development for these MTs were different. Kojima *et al.* reported that in aged dog brains MT-I and MT-II protein localized in astrocytes in the cerebral cortex and around the blood vessels, where severe age-related morphological

changes were detectable [37]. MT-III and its mRNA were demonstrated in neurons in the Zn-rich regions such as the hippocampus and parahippocampus regardless of the intensity of the age-related changes. We observed age-dependent increases in MT-III-immunopositive cell number in the cerebral cortex, piriform cortex, amygdaloid nucleus, and hypothalamus of aged rat. In addition, lipopolysaccharide (LPS)-induced marked elevation of MT-III was seen in these brain regions of young-adult rats, but not in aged rats, suggesting that inducibility of brain MT-III against inflammatory stress is reduced with aging (Asanuma *et al.*, unpublished observation in reports for Grants-in-Aid for Comprehensive Research on Aging and Health from the Japanese Ministry of Health and Welfare).

Regulation of MT-III Expression by Classical MT Inducers and Other Chemicals

MT-I mRNA is induced by heavy metals, glucocorticoids, LPS, cytokines, oxidative agents, and other stress factors in brain [12, 38-40]. Zheng *et al.* reported that MT-I mRNA appeared to be enhanced in mice given MT-I inducers (LPS, Zn, Cd, Dex, ethanol (EtOH), and kainic acid (KA)), whereas Cd, Dex, EtOH, and KA down-regulated brain MT-III mRNA levels by approximately 30% [12]. In addition, KA, Dex, or LPS treatment reduced the MT-III mRNA expression in the hippocampus, whereas in the cortical region KA, Cd, or EtOH treatment enhanced it. Chemical-induced alterations in MT-III mRNA occur ununiformly throughout the brain. In murine-cultured astrocytes, Cd, Zn, Hg, and Dex increased MT-I and MT-II mRNA expression, whereas MT-III mRNA expression was unaffected [30]. In murine neuron culture, however, Zn, Cd, Hg, and Dex decreased MT-III mRNA expression by 30-60% [31]. Thus, these *in vitro* studies indicated that MT-I and MT-II were directly inducible in astrocytes, while the MT-III transcriptional regulation might be different from that of MT-I and MT-II.

Uchida reported that in cultured astrocytes, MT-III was highly expressed in confluent astrocytes, but the expression was down regulated in low density growing astrocytes [41]. Further, she reported that epidermal growth factor (EGF) increased MT-III protein and mRNA, and interleukin (IL)-1β decreased MT-III mRNA but not MT-III protein [41]. Several studies have reported very weak expression of MT-III mRNA in transformed glial cell lines. Masters *et al.* detected MT-III mRNA by solution hybridization in mouse astrocytes but not in transformed human astrocyte cell lines [7]. In a previous report, we found very low levels of MT-III mRNA expression in a glial cell line [42]. Based on the findings in these reports, we suppose that MT-III expression is regulated by some neuronal factor *in vivo*, and it is necessary to identify this neuronal factor.

We reported previously that the expression of MT-III mRNA in a mouse glial cell line (VR-2g) is elevated following administration of 4-methylcatechol and dopamine (DA) which increase the level of nerve growth factor (NGF) in mouse astroglial cells. However, our previous studies showed that the induction of MT-III mRNA by these drugs occurred more slowly and achieved lower expression than that of NGF mRNA, indicating that MT-III expression is induced in a manner different from that of NGF mRNA [42, 43]. We investigated the mechanism of MT-III mRNA induction by DA to examine the effects of DA agonists and antagonists [43]. Based on the finding that DA agonists and antagonists did not regulate the MT-III mRNA expression, it is assumed that DA receptors did not participate in the induction by DA [43]. Further, 6-hydroxydopamine (6-OHDA) increased the level of MT-III mRNA. Neurotoxin 6-OHDA is thought to produce nigrostriatal dopaminergic lesions through the production of reactive oxigen species. DA-induced MT-III mRNA expression was abrogated by a group of antioxidants (glutathione, vitamin E, and ascorbic acid) known to scavenge reactive oxygen species [43]. These results suggest that drugs capable of generating reactive oxygen species may induce MT-III mRNA expression.

We examined the expression of MT-III mRNA in the basal ganglia of 6-OHDA-lesioned hemi-parkinsonian rats and its regulation by levodopa, which may be a source of free radical formation [44, 45]. Levodopa treatment significantly increased expression of MT-III mRNA in both non-lesioned and lesioned substantia nigra, and in non-lesioned striatum [44]. In the lesioned side of the striatum, levodopa tended to increase the level of MT-III mRNA. The lack of response of MT-III mRNA expression to levodopa in the lesioned striatum was probably due to the decreased number of cells expressing MT-III mRNA: the basal level of MT-III mRNA was significantly lower than that of the non-lesioned striatum [44].

Altered MT-III Expression Following Acute Experimental Brain Injuries

Hozumi *et al.* investigated changes in MT-III protein and MT-III mRNA in rat brains after stab wounds [27, 46, 47]. The level of MT-III protein and MT-III mRNA began to increase 4 days after the operation, reached a maximum at 14–21 days, and remained increased up to Day 28. Yuguchi *et al.* reported that the strong MT-III mRNA expression was detected surrounding the injury site on Day 4 and in the perifocal site at 2 to 3 weeks after ablation with a razor blade [48]. Gliosis is a common pathological response in the central nervous system following injury of any kind. It is characterized by a large number of reactive astrocytes, which increase the level of glial fibrillary acidic protein (GFAP). The stab wound in rats is the most common and best-defined model of gliosis. GFAP is the major protein of intermediate filaments in astrocytes and is a specific marker for astrocytes. The level of GFAP began to increase on Day 1, reached a maximum of 200% at 5–7 days, and declined significantly by 21 days after stab wound [49, 50]. The level of MT-III increased more slowly, and remained elevated for a longer period than that of GFAP [49, 50], although both proteins are expressed in reactive astrocytes [47, 49]. Hozumi, based on these differential patterns of MT-III and GFAP, suggested that MT-III might play an important role in brain tissue repair following brain injury, and might also offer new insights into the mechanism of gliosis investigated mainly from the viewpoint of GFAP [51].

Several studies have reported changes of MT-III protein following left middle cerebral artery occlusion in the rat. Inuzuka *et al.* reported that the MT-III level declined to 56% of the sham-operated control value at 7 days after operation [52]. Thereafter, it increased, and returned to the normal level by 21–28 days. Yuguchi *et al.* examined the change of MT-III mRNA by *in situ* hybridization and Northern blot analysis [53, 54]. On the first day, MT-III mRNA expression tended to decrease in the hemisphere ipsilateral to the injury. On the third and fourth day, the expression increased diffusely in the hemisphere of the affected side, including the subcortical area. Two weeks after ischemia, the MT-III mRNA expression increased again but only in the peri-infarcted area [54]. Yanagitani *et al.* showed that MT-III mRNA and protein levels were up-regulated in the cerebrum just after ischemic stress, and MT-III protein increased in neurons in injured brain after ischemia in neuronal cell bodies in the CA1–3 regions of the hippocampus, dentate gyrus, cerebral cortex, olfactory bulb, and Purkinjie cells in the cerebellum [28]. These increases in MT-III expression after brain injuries may be related to astroglial reaction, and may serve to protect neurons against toxicity caused by oxygen radicals.

MT-III Expression in AD and Other Neurodegenerative Diseases

MT-I and MT-II were highly expressed in astrocytes and microcapillaries in the AD brain [55]. Table 1 summarizes the altered MT-III expression in AD and other neurodegenerative diseases. Uchida *et al.* reported that MT-III-positive astrocytes were greatly reduced in the AD cortex [10]. However, other reports showed that neither MT-III mRNA nor MT-III protein levels

Table I Metallothionein-III expression in neurodegenerative diseases

Disease	MT-III expression (altered cells)	References
Alzheimer's disease	↓ (reactive astrocytes in gray matter)	10
〃	→	55, 56
〃	↑	57
Meningitis	↑ (reactive astrocytes in ceredral cortex)	16
Creutzfeldt-Jakob disease	↑ (reactive astrocytes in ceredral cortex)	16
Old cerebral infarcts	↑ (reactive astrocytes)	16
Multiple-system atrophy	↓ (reactive astrocytes in lesioned areas)	16
Parkinson's disease	↓ (reactive astrocytes in lesioned areas)	16
Progressive supranuclear palsy	↓ (reactive astrocytes in lesioned areas)	16
Amyotrophic lateral sclerosis	↓ (reactive astrocytes in lesioned areas)	16
Down syndrome	↓ (reactive astrocytes around senile plaques)	59

were significantly decreased in all cases of AD [56, 57]. Carrasco *et al.* reported that MT-III protein levels of patients with AD were increased when compared with similarly aged control brains [58]. Uchida *et al.* examined MT-III expression in other neurodegenerative diseases [16]. MT-III was increased in reactive astrocytes in the cerebral cortex in cases of meningitis and Creutzfeldt-Jakob disease or in reactive astrocytes surrounding old cerebral infarcts. MT-III was reduced in the subset of reactive astrocytes in lesioned areas of degenerative diseases such as multiple-system atrophy, Parkinson's disease (PD), progressive supranuclear palsy, and amyotrophic lateral sclerosis (ALS) [16]. In elderly Down syndrome brains with AD-type dementia, MT-III was decreased around senile plaques [59]. These reductions of MT-III expression around senile plaques may be correlated with neuronal loss or degeneration, and may lead to sprouting responses, which may be involved in the formation of senile plaques.

To examine the involvement of MT-III in parkinsonism, we analyzed the expression of MT-III mRNA in the striatum and the substantia nigra of hemi-parkinsonian rats using 6-OHDA [44]. The level of MT-III mRNA was significantly decreased in the striatum and increased slightly in the substantia nigra of 6-OHDA-lesioned side [44]. The levodopa treatments showed enhanced expression of MT-III mRNA in the substantia nigra of the 6-OHDA-lesioned side, whereas no significant effect was observed in the lesioned striatum by the treatment [44]. These results suggested that the levels of radical scavengers and reducing agents, including MT-III, are decreased in the brain of PD, and that levodopa administration fails to induce MT-III mRNA expression in the parkinsonian brain, thereby accelerating the progression of PD.

MT-III Gene-deficient Mice

Erickson *et al.* generated mice lacking the MT-III gene by means of targeted gene disruption [60]. Brain chemical, morphological, and behavior studies were performed using MT-III gene-deficient (MT-III−/−) mice under normal conditions, after metal exposure, KA treatment, and in the aged [60]. MT-III−/− mice showed a decreased concentration of Zn in several brain regions including the hippocampus, but the pool of histochemically reactive Zn was unaffected, as determined by both TSQ histofluorescence and Timm's staining [60]. These results suggest that MT-III is not critical for the regulation of this pool of Zn under steady-state conditions. MT-III−/− mice showed normal spatial learning in the Morris water maze [60]. Further, both normal and MT-III−/− mice had great difficulty learning the maze after 2 years, though the age-related increase in GFAP expression was enhanced in the gene-deficient mouse brain [59]. Astrocytes cultured from normal and MT-III−/− mice showed no difference in sensitivity to either Zn or Cd toxicity [60]. MT-III−/− mice were more sensitive to seizures induced by KA, and subsequently exhibited greater neuron injury in the CA3 field of the hippocampus, where large amounts of Zn would be released from mossy fibers in the dentate gyrus during seizure [60]. On the other hand, MT-III transgenic mice, in which the level of MT-III was elevated, were more resistant to seizure-induced neuronal damage in the CA3 field [60].

Functions of MT-III in the Brain

There are some regional differences in localization between MT-I and MT-III in the brain [7, 10, 25-28]. In addition, although the 5'promoter region of MT-III was highly homologous to that of MT-I [17-19, 23], MT-III expression is not regulated by inducers of MT-I [12, 30, 31]. Penkowa recently reported that MT-III expression was not altered in the brain of MT-I and MT-II gene-deficient (MT-I + II−/−) mice [61]. The regulation and cellular distribution specific to MT -III are important to an understanding of the function of MT-III in the brain. Although the functional significance of MT-III expression remains somewhat speculative, the main functions of MT-III are believed to be as follows.

The most apparent and distinctive function of MT-III is growth inhibitory activity on neurons on culturing with AD brain extract [10, 21, 56]. Constitutive MT-III expression by transformation with the MT-III gene inhibited the growth of baby hamster kidney cells when they were deprived of Zn [62]. Either MT-I or MT-II did not mimic this inhibitory activity. The expression of proinflammatory cytokines IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) was high in MT-I + II−/− mice, whereas the expression of growth factors was low [61]. These findings suggest that MT-I and MT-II have major regulatory functions in the brain's inflammatory response to injury, especially in the angiogenesis process [61]. On the other hand, induction of MT-III by cytokines remains unclear [30, 41]. Uchida *et al.* suggested

that MT-III is synthesized in resting astrocytes and is a quiescent state-specific protein [16, 41]. They also reported that MT-III expression was increased by EGF [41], which stimulates biological responses, astrocytic proliferation and differentiation, and neuronal differentiation [63-65]. In addition, EGF induces secretion of apolioprotein E (ApoE) [66]. Uchida suggested that both ApoE and MT-III might play a role in the repair of neuronal damage: ApoE functions in lipid transport to neurons, and MT-III functions in inhibition of aberrant neurite sprouting [41].

The colocalization of MT-III and Zn in neurons suggests several possible unique functions of MT-III. Masters *et al.* suggested that MT-III participate in the utilization of Zn as a neuromodulator [7]. MT-III might play a role in protecting neurons from Zn toxicity, or it might facilitate transport of Zn. MT-III is not required in the protection against exogenous Zn or Cd, because MT-III$-/-$ mice were not more sensitive than normal mice to Zn or Cd toxicity [60]. On the other hand, MT-III$-/-$ mice were more sensitive to seizures induced by KA [60]. During seizures, Zn, along with glutamate, is released from synaptic vesicles, and both would activate KA and AMPA receptors on postsynaptic neurons in a synergistic manner. After release, Zn is probably recovered by specific uptake mechanisms then recycled into synaptic vesicles. Aschner *et al.* suggested that MT-III might play a role in this recycling process [14].

MT-I and MT-II are known to scavenge free radicals and protect cells against oxidative stress [67, 68]. MT-III has been known to scavenge hydroxyl radicals *in vitro* more efficiently than MT-I or MT-II [69, 70]. Several studies reported that MT-III was up-regulated in reactive astrocytes in the acute phase following brain injury [28, 46-54]. Based on previous findings, we propose that the expression of MT-III may be induced by reactive oxygen species, and MT-III may protect against the oxidative stress. However, at a later stage of neurodegenerative diseases, neuronal damage may disrupt normal neuroglial interaction, and, consequently, MT-III synthesis may be reduced in reactive astrocytes [16, 51]. Given that MT-III eliminates the hydroxyl radicals and protects against neuronal death [70], marked decrease in MT-III in reactive astrocytes may lead to aggravation of cell damage in neurodegenerative diseases.

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