

## A Different Role of Metallothionein-3 (Mt3) in Oxidative Stress and Neurodegeneration of Brain

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### Introduction

Brain injury comes from various pathological conditions. Among those, acute brain injuries such as trauma and ischemia are mainly caused in the oxidative stress of brain cells [1-3]. Labile zinc accumulation in the brain significantly contributes to oxidative brain injury [4-8]. On the other hand, neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), Parkinson's (PD), Alzheimer's (AD), and Huntington's (HD) are caused by progressive loss of structure or function of neurons. Different neurodegenerative disorders show many parallels including atypical protein assemblies as well as induced cell death [9,10]. Unlike the case of oxidative injury, zinc deficiency in the brain aggravates chronic neurodegeneration [11], suggesting that zinc dyshomeostasis plays a key role in the brain diseases.

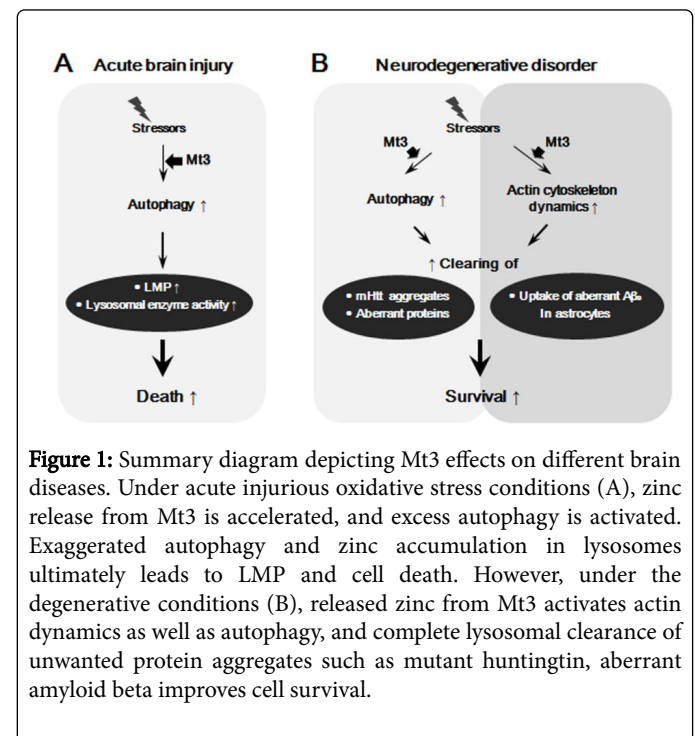
Together with ubiquitin-proteasome pathway, autophagy-lysosome pathway has an important role in clearing of troublesome proteins and organelles [10]. Especially, zinc in lysosomes has a crucial role in autophagy pathway [11-13]. Metallothionein-3 (Mt3) is a zinc-binding protein enriched in the central nervous system (CNS) [14,15] and its deficiency also has a crucial role in the autophagy as well as amyloid beta (A $\beta$ ) endocytosis in the brain, thereby finally leading to AD as well as oxidative brain injury [11]. Here, we explain in detail how Mt3 is involved in two different brain injuries.

### The Role of Mt3 in Oxidative Brain Injury

Zinc plays a major role in neuronal and glial oxidative injuries [5,16,17]. In peroxynitrite- and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-induced cell death, increases in free zinc levels induced p38 kinase activation and apoptosis [12,18,19]. Of many zinc sources, Mt3 is one of the major regulators of cellular zinc in the brain because this protein contains metal-cystein content in it and zinc has a high affinity for this protein. Therefore, Mt3 is able to accept or release zinc in response to changes in oxidative status [20,21] (Figure 1).

Except for its function as a zinc buffer, Mt3 may have more complex effects in the brain. The biological functions of Mt3 in the oxidative brain injuries may be from increased lysosomal enzyme activity and autophagy [8,12]. Various oxidative stressors initiate lysosomal membrane permeabilization (LMP), and thus a large amount of lysosomal enzymes containing proteases cathepsins are secreted from lysosomal lumen into cytosols. The secreted enzymes further activate caspases, finally leading to apoptotic cell death [8]. Besides, autophagic cell death mechanism has been recently proposed, as certain forms of cell death are attenuated by inhibition of autophagy [3]. A growing body of evidence shows that autophagic death contributes in acute brain injury [22,23]. A study with cultured model of oxidative cell

injury revealed that Mt3 plays a key role in astrocytic cell death [12]. That is, *Mt3*-null mice presented altered mobility of lysosomal membrane protein 1 and reduced activity of lysosomal enzymes [12]. Abnormal function of lysosomes contributes to autophagy defect because lysosomes are the endpoint organelle in the autophagy pathway [24]. For this reason, *Mt3*-null condition may serve the beneficial effect on oxidative injury because the desensitization of lysosomal rupture and defect of autophagosome-lysosome fusion sequentially protect cells from apoptotic and autophagic cell death [8,12,16].



**Figure 1:** Summary diagram depicting Mt3 effects on different brain diseases. Under acute injurious oxidative stress conditions (A), zinc release from Mt3 is accelerated, and excess autophagy is activated. Exaggerated autophagy and zinc accumulation in lysosomes ultimately leads to LMP and cell death. However, under the degenerative conditions (B), released zinc from Mt3 activates actin dynamics as well as autophagy, and complete lysosomal clearance of unwanted protein aggregates such as mutant huntingtin, aberrant amyloid beta improves cell survival.

### The Role of Mt3 in Neurodegenerative Disorders

Like two sides of the same coin, *Mt3*-null condition indicates a different effect depending on the type of encephalopathy. For instance, contrary to its positive effect in oxidative brain injury, knock-out of *Mt3* negatively influences on neurodegenerative disease [25,26]. Degenerative brains show a decrease in the function of lysosome and autophagy, thus highly accumulating autophagosomes in diseased brains [27]. Unwanted proteins in cells disturb cell to cell communication in brain. Autophagy largely contributes to recycling of cellular proteins by clearing of unnecessary proteins and it partly

serves smooth synaptic transmission in the brain. Mt3 level is downregulated in the AD brain [25,28] and *Mt3*-null astrocytes indicated low level of zinc in lysosome and autophagy defect [12]. Moreover, decrease in lysosomal zinc level contributes to mutant huntingtin (mHtt) aggregates in GFP-tagged mHtt polyglutamine (polyQ) expansion 74-transfected astrocytes [13], suggesting that cellular zinc plays a key role in degenerative brains.

A $\beta$  is the main component of amyloid plaques and the most damaging form of A $\beta$  may be oligomeric A $\beta$ s rather than the plaques themselves [29]. For this reason, misfolded oligomeric A $\beta$ s in extracellular space should be cleared by microglia and astrocytes because they block cell to cell signaling at synapses [30,31]. Thus, A $\beta$ s clearance has a key role in the pathologies of AD. Uptake of oligomeric A $\beta$ s in cultured astrocytes occurs mainly in a clathrin-dependent manner with a help of actin cytoskeleton, indicating that the role of actin is pretty important for endocytosis [11]. It has been recently found that disruption of actin cytoskeleton blocks A $\beta$ s endocytosis and the absence of *Mt3* resulted in a defect in actin polymerization [32], thereby A $\beta$ s uptake in *Mt3*-null astrocytes noticeably decreased [11]. Therefore, AD and PD are accelerated by *Mt3*-null condition but etiological mechanisms are different.

## Conclusion and Discussion

Deficiency in *Mt3* may lead to two different changes in the brain, which are lysosomal biogenesis and cytoskeleton dynamics. Specifically, in the oxidative brain injury, knock-out of *Mt3* may protect cells from oxidative damage because of desensitized lysosomal biogenesis and autophagy process. However, in the case of degenerative brain, dysfunction of lysosomes and actin cytoskeleton in *Mt3*-null astrocytes may contribute to accumulation of damaged proteins and toxic A $\beta$ s proteins. Except the function of Mt3 in lysosomes and cytoskeleton, metal-ion homeostasis by Mt3 may also play an important role in neurodegenerative diseases [33]. In PD, Cu(II) removal from the  $\alpha$ -synuclein ( $\alpha$ -Syn)-Cu(II) complex by thiolate ligands of Mt3 efficiently prevents  $\alpha$ -Syn and dopamine oxidation,  $\alpha$ -Syn oligomerization, and ROS formation [34].

Apart from the features of Mt3 described herein, Mt3 has other roles in the human diseases. Mt3 induction may serve as anti-inflammatory, anti-apoptotic agent and provides protection in cell therapy because zinc from Mt3 augments transcriptional regulation of genes involved in growth, cell proliferation, and differentiation [35]. Even though the role of Mt3 has not been clearly clarified yet and reported effects of Mt3 are not consistent, Mt3 may be closely associated with various cancers. In the bladder [36], breast [37,38], and prostate cancers [39], Mt3 expression was highly elevated and this alteration acted as a poor prognostic indicator. On the contrary, Mt3 level was downregulated in gastric carcinoma [40] and esophageal squamous cell carcinoma (EACs) [41]. In particular, it has been found that DNA methylation at -127 to -8 CpG sites of the promoter of *xis* essential for Mt3 mRNA expression. In addition, as significant hypermethylation at different sites within the promoter of Mt3 has been observed in EACs [42], aberrant patterns of DNA methylation may directly lead to the severe human disorders. As both the DNA methyltransferases (DNMTs) and some histone methyltransferases (HMTs) play a role in the establishment and maintenance of DNA methylation in mammals [43,44], inhibitors of these enzymes might serve as the novel therapeutic strategies for the Mt3 associated human diseases. Taken together, control of Mt3 function in human may offer hope for the

therapeutic advances that could ameliorate many disease simultaneously.

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