

REVIEW

Mitochondrial metals as a potential therapeutic target in neurodegeneration

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Transition metals are critical for enzyme function and protein folding, but in excess can mediate neurotoxic oxidative processes. As mitochondria are particularly vulnerable to oxidative damage due to radicals generated during ATP production, mitochondrial biometal homeostasis must therefore be tightly controlled to safely harness the redox potential of metal enzyme cofactors. Dysregulation of metal functions is evident in numerous neurological disorders including Alzheimer's disease, stroke, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and Friedrich's ataxia. This review describes the mitochondrial metal defects in these disorders and highlights novel metal-based therapeutic approaches that target mitochondrial metal homeostasis in neurological disorders.

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Abbreviations

3NP, 3-nitropropionic acid; 6-OHDA, 6-hydroxydopamine; ALS, amyotrophic lateral sclerosis; BBB, blood-brain barrier; Cox, cytochrome C oxidase; DFP, deferiprone; DFX, deferasirox; EC, (–)-epicatechin; ECG, (–)-epicatechin-3-gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; ETC, electron transport chain; FRDA, Friedrich's ataxia; Grx2, glutaredoxin 2; HD, Huntington's disease; IDE, idebenone; IMS, intermembrane space; MnSOD, (SOD2) manganese superoxide dismutase; MPP+, 1-methyl-4-phenylpyridinium; PD, Parkinson's disease; ROS, reactive oxygen species; SN, substantia nigra; SOD1, superoxide dismutase 1; TDP-43, Tar DNA-binding protein

Mitochondrial metal homeostasis

Transition metals are crucial for enzyme activity and correct folding and conformation of proteins. Due to the central role for mitochondria in energy production, which generates reactive oxygen intermediates as by-products, transition metals are required for ATP generation and control of potential oxidative damage in mitochondria. Copper, zinc, iron and manganese are all localized to various compartments in the mitochondria and their regulation is controlled by a diverse range of cofactors, chaperones and ion pumps (Pierrel *et al.*, 2007; Rines and Ardehali, 2013). Mitochondrial control of Cu homeostasis has been well characterized in recent years, although predominantly in yeast rather than mammalian cells (reviewed in Pierrel *et al.*, 2007); however, far less is known of the transport and chaperone processes involved in regulation of other transition metals. This review will

describe the functions of transition metals in the mitochondria and highlight a number of neurological diseases where perturbations to mitochondrial metal homeostasis contribute to pathology. We will discuss the potential of novel therapeutics including metallo-complexes and metal chelators that target mitochondrial dysfunction and/or correct mitochondrial metal disturbances through metal delivery, sequestration or antioxidant properties.

Iron has an essential role in mitochondrial iron-sulfur clusters and haeme-containing proteins, which are crucial for the function of several mitochondrial and cytosolic enzymes including elements of the electron transport chain (ETC) associated with ATP energy production (Atamna *et al.*, 2002; Pierrel *et al.*, 2007). The mechanism of iron import into the intermembrane space (IMS) is unknown; however, mitoferrins pump iron into the matrix (Richardson *et al.*, 2010; Huang *et al.*, 2011).

Cu is an essential element of complex IV (cytochrome c oxidase) in the mitochondria. The Cu is delivered into the IMS from cytosolic pools via Cox17 to additional carriers, Sco1 or Cox11 and subsequently to Cox1 or Cox2 (Kim *et al.*, 2008). Although Cu levels in the mitochondria are relatively low compared with iron levels, Cu exists at least an order of magnitude higher than required for cytochrome c oxidase function, mostly as a low molecular weight soluble ligand, suggesting that there are additional critical functions for Cu in the mitochondria that have yet to be determined.

Manganese has a central role in maintaining antioxidant enzyme function in the mitochondria as the key element of manganese superoxide dismutase (MnSOD also known as SOD2) located in the mitochondrial matrix and has additional enzymatic roles in arginine synthesis (Aschner *et al.*, 2009). MnSOD is essential for removal of superoxide anions released during mitochondrial respiration that are precursors of damaging reactive oxygen species (ROS). In fact, the mitochondria are the primary site for manganese accumulation in the cell and manganese is delivered from the cytosol by a Ca²⁺ uniporter (Gunter and Puskin, 1972; Liccione and Maines, 1988). Interestingly, despite the close similarity between manganese-induced and Parkinson's disease-associated neurotoxicity (see below/see Parkinson's disease section), little is known of manganese handling in the mitochondria. There is also limited information on mitochondrial control of zinc homeostasis. A mitochondrial zinc pool exists in the matrix and is critical for maintenance of zinc-binding metallo-enzymes. A pool can also be mobilized during stress, although the mechanisms are unclear (Sensi *et al.*, 2003). Zinc, along with copper has an essential antioxidant function in Cu/Zn superoxide dismutase 1 (SOD1) that exists in the cytosol and mitochondrial IMS (Field *et al.*, 2003).

Despite the lack of understanding regarding regulation of mitochondrial transition metal pools, particularly in the brain, considerable evidence supports perturbation to these pools in several neurodegenerative disorders (summarized in Table 1). As reviewed below, loss or accumulation of essential transition metal pools in specific compartments of the mitochondria feature strongly in neurodegenerative diseases and may have a critical role in leading to impaired energy production, especially critical for the high energy demands of neurons and/or generation of reactive oxygen species (ROS) and activation of mitochondrial-dependent apoptosis. This review describes the mitochondrial metal defects in amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), stroke, Friedrich's ataxia (FRDA) and Huntington's disease (HD). Mitochondrial metal homeostasis is also altered in AD, but this is reviewed in detail elsewhere (Eckert *et al.*, 2012) and will not be covered in the present review. Importantly, the abnormal metal handling of mitochondria provides a unique set of targets for therapeutic intervention as covered in the final section of this review.

Amyotrophic lateral sclerosis

ALS is a fatal adult-onset neurodegenerative disease in which motor neurons in the spinal cord and brain progressively deteriorate (Bradley, 1995). ALS is characterized by progressive paralysis and wasting of skeletal muscles. The average age of onset is around 50–60 years and patients rarely survive more than 3–5 years after diagnosis, with respiratory failure the most common cause of death (Al-Chalabi *et al.*, 2012). About 5–20% of ALS cases are familial, caused by mutations in SOD1, Tar DNA-binding protein (TDP-43), fused in sarcoma, C9orf72 and multiple other genes, and the remainder of cases are

Table 1

A summary of the cell and animal models, metal distribution and mitochondrial dysfunction in neurological disorders with altered mitochondrial function

Disorder	<i>In vitro/in vivo</i> models	Aberrant metal distribution	Mito protein dysfunction
Amyotrophic lateral sclerosis	Most common models: SOD1 ^{G37R} , SOD1 ^{G93A} transgenic mice	Mitochondrial Fe accumulation	Aberrant mitochondrial localization of SOD1 Deregulation of Fe-S proteins Elevated MnSOD levels
Parkinson's disease	Complex I inhibitors (e.g. rotenone, 6OHDA, paraquat, MPP ⁺ , MPTP) Mn elicits similar symptoms	Mitochondrial Fe accumulation Loss of Cu	Complex I inhibition Inhibitors increase mitochondrial Fe
Stroke	Cerebral artery occlusion ischaemia/reperfusion injury Subarachnoid haemorrhage Rabbit small clot embolic stroke	Possible aberrant Zn redistribution Lysosomal Fe transport to mitochondria	
Friedrich's ataxia	Frataxin knockdown cell models Frataxin knockdown <i>Drosophila</i> , <i>C. elegans</i> models Heterozygous frataxin knockout mice	Mitochondrial Fe accumulation Cytosolic depletion of Fe	Possible impaired Fe-S cluster biogenesis Reduced frataxin expression, overexpression of mitoferrin
Huntington's disease	Complex II inhibitors (e.g. 3NP), CAG140 knock-in mice	Accumulation of Fe, Cu, Zn Possible mito Zn	Complex II/III inhibition Fe-S subunit in complex II

sporadic (Al-Chalabi *et al.*, 2012). Mitochondrial dysfunction is evident in ALS (reviewed in Shi *et al.*, 2010), including mitochondrial abnormalities in the spinal cord of sporadic ALS patients (Sasaki and Iwata, 1996), and impaired mitochondrial function in ALS patients (Bowling *et al.*, 1993) and transgenic mice (Dupuis *et al.*, 2011; Panov *et al.*, 2011).

A portion of the mainly cytosolic Cu/Zn antioxidant protein SOD1 is localized to mitochondria, predominantly in the IMS (Kawamata and Manfredi, 2010), although a portion of mutated SOD1 has been found bound to the mitochondrial outer membrane (Vande Velde *et al.*, 2008). IMS localization of SOD1 is promoted by the copper chaperone for SOD1 (CCS), which delivers copper to SOD1 (Kawamata and Manfredi, 2010). Enhanced delivery of copper to SOD1 by overexpression of CCS increases aggregation of mitochondrial SOD1^{G93A} and induces toxicity in cultured neuronal cells (Cozzolino *et al.*, 2009). Furthermore, increasing copper delivery to mutant SOD1 *in vivo* in double transgenic mice overexpressing SOD1^{G93A} and CCS greatly accelerated the disease course, increased mitochondrial mutant SOD1 content and caused severe mitochondrial pathology (Son *et al.*, 2007). In addition, increasing mitochondrial mutated SOD1 content by genetically introducing an IMS targeting signal to SOD1 causes toxicity in cultured motor neuron cells (Magrane *et al.*, 2009) and, *in vivo*, transgenic mice expressing mitochondrially targeted mutant SOD1^{G93A} develop mitochondrial dysfunction, motor impairment and motor neuron death. However, the abnormalities of mutant SOD1 in mitochondria only partially epitomize the ALS disease phenotype, with no muscle denervation. Hence mitochondrial localization of mutant SOD1 causes many but not all features of ALS, and mutant SOD1 localized to compartments other than mitochondria may contribute to disease (Igoudjil *et al.*, 2011). These studies indicate that increasing the mitochondrial content of ALS-associated mutated forms of SOD1 may contribute to the mitochondrial aberrations evident in ALS.

Perturbations in iron are also evident in ALS. Iron accumulation has been reported in the CNS of both familial and sporadic forms of ALS patients (Oba *et al.*, 1993; Kasarskis *et al.*, 1995) and in mitochondria in the spinal cord of transgenic SOD1^{G37R} ALS model mice (Jeong *et al.*, 2009). Dysregulation of mitochondrial iron proteins including iron-sulfur cluster scaffold protein, mitoferrin and frataxin are evident in *in vivo* and *in vitro* models of ALS (Jeong *et al.*, 2009; Hadzhieva *et al.*, 2013).

A subset of apparently sporadic ALS patients exhibit elevated mitochondrial MnSOD (McEachern *et al.*, 2000). Overexpression of MnSOD rescues neuroblastoma cells in the presence of several different ALS-associated SOD1 mutations (Zimmerman *et al.*, 2007). Selenium exposure induces toxicity, increases MnSOD and induces translocation of SOD1 into mitochondria in neuroblastoma cells (Maraldi *et al.*, 2011). These studies suggest MnSOD responds to ALS-associated mitochondrial stress, but it is unclear whether it helps or contributes to dysfunction.

Stroke

Stroke, or ischaemia, caused by blockage of blood vessels in the brain is a leading cause of adult death worldwide. Blockage

of major blood vessels due to thrombosis or embolism results in deprivation of oxygen and nutrients to local brain tissue. In ischaemia-reperfusion injury, the initial blockage is followed by a potentially more damaging period of re-oxygenation after removal of the blockage. The process results in loss of energy production in neurons and subsequent neuronal cell death and, during reperfusion, this is exacerbated by high levels of oxidative damage from returning oxygen. Although the pathways of neuronal damage are complex, one of the major factors is dysregulated Ca²⁺ and/or zinc influx via glutamate receptors (Sensi and Jeng, 2004), leading to activation of mitochondrial-associated cell death pathways (reviewed in detail in this special issue by Duchen, 2012). In fact, the mitochondria are thought to be the primary target of altered cellular metabolism associated with ischaemia and ischaemia-reperfusion injury (Jordan *et al.*, 2011).

There is currently little understanding of how mitochondrial transition metal homeostasis is altered during stroke. However, it is thought that zinc is released from both mitochondrial and cytosolic stores in response to ischaemia-reperfusion injury, in addition to potential influx via glutamate receptors (Sensi and Jeng, 2004). This leads to generation of ROS and subsequent oxidative damage to neurons. These effects appear to be very potent, occurring at zinc concentrations lower than the concentrations required for Ca²⁺-mediated neurotoxicity (Jiang *et al.*, 2001; Dineley *et al.*, 2005). The importance of abnormal mitochondrial zinc accumulation in ischaemic damage has been supported by studies demonstrating that depletion of zinc reduces mitochondrial cytochrome c release and activation of downstream caspases (Calderone *et al.*, 2004). The role of additional transition metals in mitochondria during ischaemia-reperfusion is even less well understood than zinc. There are currently very few studies on the distribution of copper, iron or manganese pools in mitochondria during ischaemia-reperfusion. Zhang and Lemasters observed that during ischaemia in rat hepatocytes, iron released from lysosomes was transported to mitochondria (Zhang and Lemasters, 2013). However, similar studies in neurons have not been reported. Studies supporting a role for mitochondrial transition metals in the regulation of mitochondrial function during ischaemia have instead come largely from studies on MnSOD. Several reports have described a central role for the mitochondrial manganese-containing MnSOD in neuroprotection against ischaemia-reperfusion injury in gerbils, rats and mice (Wakade *et al.*, 2008; Jung *et al.*, 2009). This protective action is probably related to the essential role of the enzyme in removal of superoxide radicals in mitochondria, and is supported by the therapeutic action of manganese porphyrins as discussed below (Saba *et al.*, 2007). No studies have yet reported changes to mitochondrial copper pools during ischaemia.

Friedrich's ataxia

FRDA is a progressive neurodegenerative disease clinically characterized by progressive ataxia (lack of voluntary muscle coordination), dysarthria (speech impairment), areflexia and progressive motor weakness. Patients also develop hypertrophic cardiomyopathy and diabetes. Symptoms appear in

childhood and progressively worsen through adulthood, commonly fatal in mid-adulthood. Neuropathologically, FDRA involves the degeneration of the dentate nuclei in the cerebellum and the sensory and motor tracts of the spinal cord (Marmolino, 2011).

FDRA is caused by an autosomal recessive inherited GAA repeat expansion in the FXN gene, impairing transcription of the protein frataxin. Frataxin is a small ~210 amino acid mitochondrial protein. In FDRA, frataxin expression is impaired but not absent and is required for survival: full FXN deletion in mice results in embryonic lethality (Cossee *et al.*, 2000).

Although the exact function of frataxin is still being elucidated, extensive evidence links frataxin to iron homeostasis. Genetic models of FRDA are characterized by mitochondrial iron accumulation and cytosolic iron depletion. Loss of frataxin increases the mitochondrial iron importer mitoferrin 2 (Huang *et al.*, 2009), and reintroduction of frataxin to frataxin-deficient yeast cells corrects the iron imbalance between mitochondria and cytosol (Radisky *et al.*, 1999). The FXN gene appears to be regulated by iron, with frataxin expression increased with iron loading and decreased with iron restriction (Sarsero *et al.*, 2003; Li *et al.*, 2008).

Mitochondria are the site of iron-sulfur cluster biogenesis. Loss of frataxin impairs iron-sulfur cluster-dependent proteins, including mitochondrial respiratory chain complexes and aconitase (Rotig *et al.*, 1997; Wilson and Roof, 1997; Anderson *et al.*, 2005; Long *et al.*, 2008). This disruption of iron-sulfur cluster proteins occurs before mitochondrial iron accumulation (Puccio *et al.*, 2001), suggesting that frataxin is involved in iron-sulfur cluster biogenesis (Muhlenhoff *et al.*, 2002). Lack of frataxin is correlated to oxidative stress (Wong *et al.*, 1999), which probably occurs via reactions of excess iron in mitochondria with superoxide.

The above studies provide evidence for a role of frataxin in mitochondrial iron homeostasis. However, these findings were obtained from experimental genetic models. In human FDRA patients, no neurological iron overload has been detected: iron deposits cannot be histologically detected in the affected regions in the CNS (Bradley *et al.*, 2000; Koeppen *et al.*, 2007), and no difference was found in total iron and ferritin between patients and controls in dentate nuclei in cerebellar sections (Koeppen *et al.*, 2007). Nevertheless, cells cultured from FRDA patients and mouse models do show a modest increase in mitochondrial iron (Wong *et al.*, 1999; Puccio *et al.*, 2001; Tan *et al.*, 2001; Calmels *et al.*, 2009), and derangements in mitochondrial iron are evident in cardiac tissue of FRDA patients (Rotig *et al.*, 1997; Bradley *et al.*, 2000).

Parkinson's disease

PD is a progressive adult-onset neurodegenerative disease characterized by resting tremor, bradykinesia and rigidity, as well as cognitive impairment (Litvan *et al.*, 2012). PD is the second most common neurodegenerative disease behind Alzheimer's disease. Pathologically, PD is defined by a selective loss of dopaminergic neurons in the substantia nigra (SN) region of the brain and more widespread aggregation of the protein α -synuclein in Lewy bodies (Spillantini *et al.*,

1997; Goedert, 2001). The incidence of PD is associated with exposure to mitochondrial complex I inhibitors (Tanner *et al.*, 2011), and the inhibition of complex I specifically in the SN (Reichmann *et al.*, 1995; Subramaniam and Chesselet, 2013).

A key pathological hallmark of PD is the accumulation of iron in the SN (Sian-Hulsmann *et al.*, 2011). Concomitant with this characteristic increase in iron is a decrease in copper content (Dexter *et al.*, 1989; 1991; Loeffler *et al.*, 1996; Popescu *et al.*, 2009). Although the elevated iron is most likely glial – iron accumulation is evident in astrocyte mitochondria in aging rat SN (Schipper *et al.*, 1998) – an increase in iron has been detected specifically in dopaminergic neurons in the SN in PD (Oakley *et al.*, 2007). Neuronal mitochondrial iron accumulation can be induced by exposure to complex I inhibitors such as rotenone, paraquat, 1-methyl-4-phenylpyridinium (MPP⁺), and its precursor MPTP, via up-regulation of iron import proteins such as transferrin receptor-2 and mitoferrin-2 (Mastroberardino *et al.*, 2009; Carroll *et al.*, 2011). This accumulation of iron is thought to induce oxidative stress, which contributes to dopaminergic cell death (Mochizuki and Yasuda, 2012). For example, iron induces mitochondrial damage in SK-N-SH dopaminergic cells (Kooncumchoo *et al.*, 2006), whereas genetic or pharmacological iron chelation improves dopaminergic neuron survival in response to MPTP in rats (Kaur *et al.*, 2003).

Although the mechanism of iron accumulation in dopaminergic neurons in PD is not yet known, one hypothesis suggests that iron-sulfur clusters may serve as sensitive mitochondrial iron sensors. When complex I is inhibited, iron-sulfur clusters are oxidized, preventing the binding of iron, and promoting the unchecked influx of iron into mitochondria (Mastroberardino *et al.*, 2009). An alternative possibility is that the characteristic depletion of glutathione evident in PD (Sian *et al.*, 1994; Pearce *et al.*, 1997; Martin and Teismann, 2009) inhibits mitochondrial glutaredoxin 2 (Grx2). As Grx2 is involved in iron-sulfur cluster generation, inhibition of Grx2 leads to impaired iron-sulfur cluster biogenesis and impaired function of iron-sulfur cluster-dependent proteins such as complex I and aconitase (Lee *et al.*, 2009).

Environmental exposure to manganese has been postulated to be a risk factor for PD as accumulation of manganese in the brain causes manganism, a condition that shares many symptoms with PD. However, manganism and PD differ in several neuropathological aspects and could be considered as separate entities (Caudle *et al.*, 2012). Regardless, accumulation of manganese in the brain causes mitochondrial impairment, which contributes to the motor impairments reminiscent of PD (reviewed in Aschner *et al.*, 2009).

The manganese-containing mitochondrial enzyme MnSOD has been studied in relation to PD. MnSOD is up-regulated in genetic models of PD (Andres-Mateos *et al.*, 2007; Xun *et al.*, 2008). In contrast, transgenic mice over-expressing MnSOD are resistant to 6-hydroxydopamine (6-OHDA) toxicity (Callio *et al.*, 2005). Furthermore, dopamine quinones inhibit MnSOD, increasing mitochondrial ROS production (Belluzzi *et al.*, 2012). These studies suggest MnSOD activity is important to prevent oxidative stress in models of PD.

Huntington's disease

HD is an inherited autosomal dominant neurodegenerative disease caused by a CAG expansion in the *huntingtin* gene (Anonymous, 1993). Disease onset and severity is directly correlated to the number of CAG repeats (Andrew *et al.*, 1993). HD causes progressive chorea and cognitive impairment, and is pathologically characterized by the selective loss of striatal neurons and aggregation of the mutant huntingtin protein. While the function of huntingtin is yet to be fully elucidated, deletion is embryonically lethal (Nasir *et al.*, 1995; Zeitlin *et al.*, 1995) and heterozygotes display severe cognitive deficits and neuronal loss in the subthalamic nucleus (Nasir *et al.*, 1995).

HD has long been associated with mitochondrial deficits (reviewed in Oliveira, 2010). Expression of mitochondrial complexes II and III have been found to be reduced in the striatum of HD patients (Brennan *et al.*, 1985; Gu *et al.*, 1996; Benchoua *et al.*, 2006), and transgenic mouse models of HD (Damiano *et al.*, 2013), while inhibitors of complex II such as 3-nitropropionic acid (3NP) recapitulate many features of HD (Beal *et al.*, 1993). Overexpression of the iron-sulfur cluster subunit of complex II is correlated with attenuations of symptoms, suggesting the deficit in the iron-sulfur subunit of complex II may be central to the pathogenesis of HD (Damiano *et al.*, 2013). Further to this, huntingtin appears to be involved in iron regulation: huntingtin-deficient zebrafish display impaired cellular iron uptake (Lumsden *et al.*, 2007) and desferrioxamine treatment increases huntingtin expression in embryonic stem cells (Hilditch-Maguire *et al.*, 2000). Furthermore, iron content is elevated in HD-affected brain regions (Dexter *et al.*, 1991; Bartzokis *et al.*, 1999; Dumas *et al.*, 2012; Dusek *et al.*, 2012; Rosas *et al.*, 2012), and this iron accumulation appears to precede symptom onset (Rosas *et al.*, 2012).

Striatal elevation in copper content has also been reported in HD brains (Dexter *et al.*, 1991), although a more recent study found no change in copper content (Rosas *et al.*, 2012). An elevation in copper may be of importance as copper has been shown to promote huntingtin aggregation (Fox *et al.*, 2007); however, it is currently unclear whether aberrant copper distribution interacts with mitochondria.

An elevation in striatal zinc content has been reported in HD brains (Rosas *et al.*, 2012). Elevated zinc has also been associated with exposure to the complex II inhibitor 3NP (Sheline *et al.*, 2013). Interestingly, inhibiting zinc accumulation attenuates 3NP-induced toxicity (Sheline *et al.*, 2013), suggesting a role for zinc in 3NP toxicity and mitochondrial inhibition in HD.

In summary, a key feature of neurological diseases with impaired mitochondrial functions include a propensity for accumulation of mitochondrial biometals, particularly iron, zinc, copper and manganese, which overwhelm key antioxidant systems and result in oxidative stress-dependent neurotoxicity (Table 1). Therefore, restoration of antioxidant functions through modulation of biometal homeostasis is an attractive therapeutic target for these disorders. This review describes several novel therapeutic approaches to correct biometal homeostasis through metallo-complexes and metal chelating agents, which can be pharmacologically

manipulated to improve brain delivery, mitochondrial targeting and antioxidant functionality.

Therapeutic mitochondrial targeting

Lipophilic cations

Lipophilic cations are ideal mitochondrial targeting agents. Due to lipophilic shielding of the positive charge over the surface of the molecule, these compounds readily cross membranes without requiring specific transporters or energy expenditure. The high membrane potential of mitochondria (~180–200 mV *in vitro*, 130–150 mV *in vivo*) compared with plasma membrane potentials, which are typically in the range of 30–60 mV, results in selective targeting of lipophilic cations to mitochondria (Chen, 1988). To date, the most effective and well-studied metal-based neurotherapeutics belong to two classes of complexes – bis thio semicarbazones (btsc) and pyridyl-porphyrins containing a central metal ligand (for a summary of neuroprotective actions, see Figure 1).

CuII(atism). CuII(atism) is a btsc complex, which has been tested as a neurotherapeutic for several diseases where mitochondrial dysfunction contributes to pathology, including ALS and PD (Soon *et al.*, 2011; Hung *et al.*, 2012). In four PD mouse models, including both genetic and neurotoxin-lesioned models, CuII(atism) was reported to enhance cognitive and motor performance, even when administered after onset of toxin-mediated cell death (Hung *et al.*, 2012). Moreover, CuII(atism) delayed onset of locomotor dysfunction, prevented motor neuron loss and improved survival when administered both to presymptomatic and symptomatic SOD1^{G93A} and SOD1^{G37R} ALS model mice (Soon *et al.*, 2011; McAllum *et al.*, 2013). The mechanism of this therapeutic action is still uncertain but evidence indicates a role for mitochondria in its action.

Therapeutic strategies for diseases of mitochondrial dysfunction may promote indirect benefits to mitochondrial energy metabolism or downstream cellular pathways. Indeed, some controversy exists regarding subcellular trafficking of CuII(atism), reported to localize to cytosolic (Pascu *et al.*, 2010), lysosomal (Price *et al.*, 2011) and microsomal (Obata *et al.*, 2001) compartments. A fluorescent pyrenylated derivative of CuII(atism), CuII(L1) showed punctate staining in M17 neuroblastoma cells with partial colocalization with lysosomal and endoplasmic reticulum, but not mitochondrial organelle markers (Price *et al.*, 2011). However, subcellular targeting of copper complexes may be cell type specific, as CuII(L1) revealed lysosomal localization in HeLa epithelial cells (Lim *et al.*, 2010). Alternatively, it is possible that addition of a pyrene group may affect the cellular trafficking of CuII(atism), as modification of the btsc backbone is known to subtly alter metal delivery properties of different CuII(btsc) complexes (Xiao *et al.*, 2008). Moreover, the localization may be concentration-dependent, as higher concentrations of fluorescently labelled copper compounds can lead to complex aggregation, colloid formation and subsequent localization in lysosomes (Morgan *et al.*, 2011). The identity of the metal ligand also contributes to intracellular targeting,

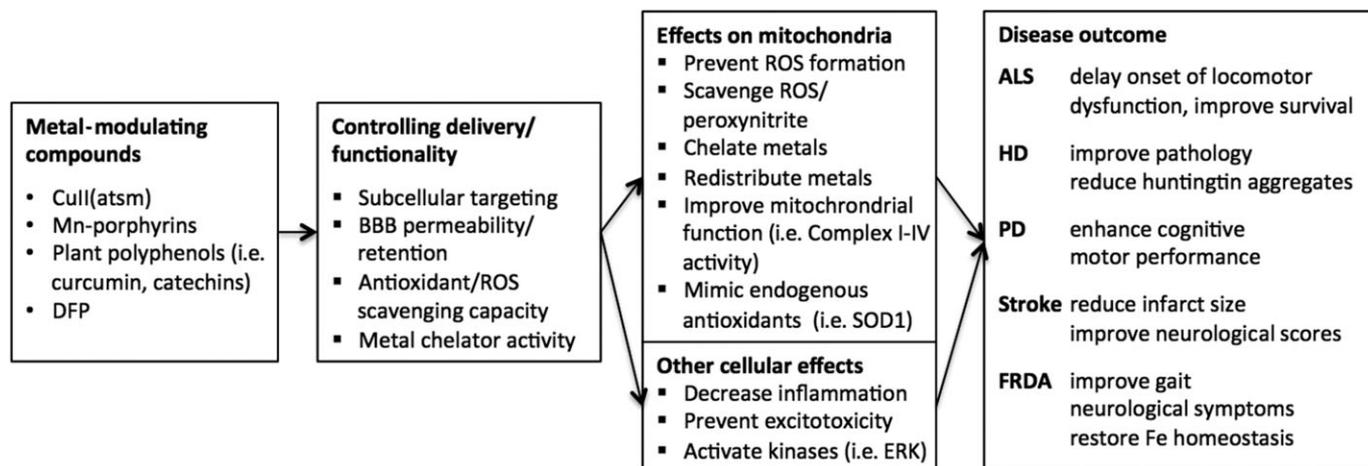


Figure 1

Metal-modulating compounds improve mitochondrial function and disease outcome of neurological disorders. Novel metal-based therapeutics, metal chelators and natural plant-based polyphenolic antioxidants can be chemically modified for subtle control of delivery and functionality. These compounds exert their actions through mitochondrial and other cellular pathways that restore mitochondrial metal homeostasis and lead to therapeutic outcomes in animal models of ALS, PD, HD, stroke and FRDA.

as Zn(btsc) complexes were reported to localize to lysosomes and mitochondria (Pascu *et al.*, 2008). The rapidly increasing resolution of synchrotron X-ray fluorescence microscopy and laser ablation inductively coupled plasma MS elemental imaging is likely to provide crucial insights into the subcellular distribution of unlabeled CuII(btsc) complexes (New, 2013).

Intracellular copper retention of Cu(II) delivered by the btsc coordinate is controlled by cellular reductants, which catalyse reductive transfer of Cu(I) to high-affinity cuproproteins. Structural variations in the btsc backbone, specifically of the R1 and R2 ligands, yields complexes with vastly different cellular copper retention properties, determined by the relative copper-binding affinities of the complex compared with that of cellular reductants. Copper transfer from CuII(atm) to the high copper-affinity proteins, Atx1 and Ctr1 purified from yeast, was detected only in the presence of a strong reductant, dithionite, whereas Apo-Ctr1 alone was capable of irreversible trapping of CuII(gtsc), another btsc compound that delivers copper to all cells (Xiao *et al.*, 2008). *In vivo*, multiple reductants may be responsible for copper retention from CuII(atm). NADH-cytochrome b5 reductase and NADPH-cytochrome P450 reductase were reported to catalyse copper retention in Ehrlich ascites hypoxic brain tumour cells (Obata *et al.*, 2001). Recent work by our group and supported by others implicates impaired mitochondrial function as a prerequisite for copper retention from CuII(atm) (Donnelly *et al.*, 2012; Yoshii *et al.*, 2012). Indeed, dissociation of the coordinate occurred only in hypoxic cells or in mitochondrial xenocybrid cells that encode incompatible ETC components on interspecies mitochondrial and nuclear chromosomes, and hence exhibit overactive glycolytic ATP production and NADH accumulation due to defective ETC function (Donnelly *et al.*, 2012). NADH accumulation in hypoxic cells was also demonstrated to play a

role in ligand dissociation from the complex (Donnelly *et al.*, 2012).

Peroxyntirite has long been known to inhibit components of the mitochondrial ETC (Radi *et al.*, 1994) and induce mitochondrial calcium release (Packer and Murphy, 1994), and selectively induce neuronal mitochondrial dysfunction (Bolanos *et al.*, 1995). A proposed therapeutic mechanism of action of CuII(atm) is the ability to rescue peroxyntirite-dependent toxicity and inhibit nitrate-driven α -synuclein-aggregation (Hung *et al.*, 2012). Moreover, paraquat-induced apoptosis, which involves superoxide generation and mitochondrial cytochrome c and apoptosis inducing factor release (Czerniczyniec *et al.*, 2013), is corrected in SH-SY5Y cells by CuII(atm) treatment (Parker *et al.*, 2012). CuII(atm) also induced phosphorylation of the ERK by a mechanism involving copper release (Donnelly *et al.*, 2012). While it is not known whether the complexes directly target metal delivery to mitochondria in these models, the dramatic effect on mitochondrial function may be attributed to these antioxidant mechanisms, as well as modulation of kinase phosphorylation profiles, leading to reduced neuropathology including TDP-43 aggregate accumulation within stress granules (Parker *et al.*, 2012) and mouse tissues (Soon *et al.*, 2011). CuII(atm) is therefore potentially an ideal self-targeting drug for mitochondrial dysfunction disorders, as this compound specifically depends on mitochondrial impairment for its molecular therapeutic action.

Manganese porphyrins. Considerable attention has been paid in recent years to investigation of the neuroprotective effects of manganese porphyrins in numerous CNS disease models including *in vitro* and *in vivo* models of stroke, spinal cord injury, subarachnoid haemorrhage, cerebral palsy, ALS, PD and oxygen and glucose deprivation (Mackensen *et al.*, 2001; Saba *et al.*, 2007; Sheng *et al.*, 2009; 2011; Wise-Faberowski

et al., 2009; Batinic-Haberle *et al.*, 2011). Manganese has been utilized as the ligand in these complexes, due to its capacity to participate in cellular redox cycling reactions as it exists in numerous oxidation states. To different extents, strongly influenced by the porphyrin backbone, these compounds have been designed to be blood–brain barrier (BBB)-permeable, preferentially accumulate in mitochondria and possess strong antioxidant SOD surrogate activity. Lipophilicity and hence mitochondrial accumulation of the complexes can be enhanced by transfer of pyridyl groups to the meta position (Mackensen *et al.*, 2001), elongation of the alkyl chains (Tovmasyan *et al.*, 2011) or reduction of Mn(III) to Mn(II) (reviewed in Batinic-Haberle *et al.*, 2011). For instance, the higher redox potential of Mn(III) *meso-tetrakis* (N-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP⁵⁺) results in more robust antioxidant activity than its para-isomer MnTE-4-PyP⁵⁺ (Mackensen *et al.*, 2001). Mild toxicity can also be reduced by modulation of the steric properties of the complexes to prevent interactions with DNA.

Given that in ischaemia, superoxide radicals are primarily generated by the mitochondrial ETC (Piantadosi and Zhang, 1996), manganese-pyridyl porphyrins, possessing inherent SOD-like antioxidant and peroxynitrite-scavenging properties (Pasternack *et al.*, 1981) were hypothesized to be ideal therapeutic targets for oxidative mitochondrial dysfunction. To date, the most commonly tested complexes *in vivo* are MnTE-2-PyP⁵⁺ and MnTnHex-2-PyP⁵⁺.

MnTE-2-PyP⁵⁺ significantly reduced neuropathology including cerebral infarct size and substantially enhanced neurological scores in a rat model of ischaemia-reperfusion injury, even when administered 6 h following reperfusion (Mackensen *et al.*, 2001). Moreover, improved mitochondrial function was observed by elevated mitochondrial aconitase activity and reduced oxidative DNA modification in MnTE-2-PyP⁵⁺-treated rats following reperfusion (Mackensen *et al.*, 2001). However, this molecule failed to have an effect in trials in a rabbit cerebral palsy uterus ischaemia model (Yu *et al.*, 2010).

The hexyl substituted, Mn(III) ortho N-n-hexylpyridylporphyrin, (MnTnHex-2-PyP⁵⁺), was reported to be 13 500-fold more lipophilic than its ethyl analogue and thus exhibits stronger mitochondrial accumulation, a greater propensity to be reduced (Keir *et al.*, 2011) and enhanced tissue retention, even at fivefold lower doses (Weitner *et al.*, 2013). This CNS-bioavailable complex was also reported to improve multiple disease indicators in rat stroke models of cerebral artery occlusion, subarachnoid haemorrhage (Sheng *et al.*, 2011) and in a cerebral palsy rabbit uterus ischaemia model (Yu *et al.*, 2010). The substantial benefits afforded by MnTnHex-2-PyP⁵⁺ in a stroke model may be attributed to the high brain retention of the complex, coupled with robust antioxidant activity (Sheng *et al.*, 2011). Similarly, extension of the alkyl chain in MnTnOct-2-PyP⁵⁺ resulted in improved bioavailability compared with the ethyl analogue and three orders of magnitude reduction in the effective dose required to protect rat mixed neuronal and glial cultures from oxygen and glucose starvation (Wise-Faberowski *et al.*, 2009).

The related imidazolium complex, Mn(III) tetrakis[N-N'-diethylimidazolium-2-yl]porphyrin (MnIIITDE-ImP(2)), improved survival, reduced inflammatory, oxidative and nitrosative pathological damage in the SOD1^{G93A} ALS mouse

model (Crow *et al.*, 2005). Another mitochondrial SOD mimic, Mn(III) 1,4,8,11-tetraazacyclodecane (Mn-cyclam), and to a lesser extent its Ni-coordinate, inhibited mitochondrial membrane depolarization and peroxynitrite-mediated cell death in glucose-starved, activated astrocytes (Choi *et al.*, 2003).

Aside from their antioxidant function, investigation of the cellular mechanism of action of this class of compounds has been limited. Manganese porphyrins were reported to inhibit activation of inflammatory transcriptional programmes, including NF- κ B and AP-1 (Sheng *et al.*, 2009; 2011). Recent evidence implicated a pro-oxidant mechanism involving direct oxidation of sulfhydryl groups on p50 NF- κ B, AEP1/Ref-1 or thioredoxin transcription factor subunits. Another mechanism of neuroprotection was proposed whereby metalloporphyrins are able to suppress excitotoxicity and the associated mitochondrial Ca²⁺ influx by preventing postsynaptic NMDA receptor-dependent increases in Ca²⁺ (Tauskela *et al.*, 2005). This activity did not correlate to SOD1 antioxidant properties, indicating that alternative synthesis approaches should also be considered (Tauskela *et al.*, 2005). Modification of the complexes to enhance Ca²⁺/NMDA-mediated neuroprotection could therefore be a useful strategy for disorders, such as ALS, where excitotoxicity significantly contributes to their neuropathology (Ferraiuolo *et al.*, 2011).

Metal chelators

Deferiprone

Another class of therapeutics with metal-dependent antioxidant activity are metal chelators. The BBB-permeable (Fredenburg *et al.*, 1996) iron siderophore, deferiprone (DFP), has been investigated as a therapeutic for FRDA in multiple cell and animal models, as well as two clinical trials. DFP treatment of HEK-293 cells stably transfected with a tetracycline-inducible frataxin shRNA, resulted in restoration of mitochondrial functions including decreased mitochondrial redox potential, ROS production and labile iron pools (Kakhlon *et al.*, 2008). DFP also increased survival and improved motor climbing ability in a frataxin-knockdown *Drosophila* FRDA model (Soriano *et al.*, 2013).

A 6 month clinical trial involving daily DFP administration in young FRDA patients at 20–30 mg·kg⁻¹ improved gait and other neurological signs in all nine patients, and induced a significant progressive reduction in dentate nuclei-localized iron accumulation in eight patients (Boddaert *et al.*, 2007). However, a subsequent 11-month study of combination therapy with DFP and idebenone (IDE), a synthetic analogue of the antioxidant coenzyme Q10, on a cohort of 20 FRDA patients did not show an overall significant improvement in ataxia neurological function scores (Velasco-Sanchez *et al.*, 2011). Nonetheless, as neurological scores are expected to decline with disease progression over the long treatment period (Velasco-Sanchez *et al.*, 2011), it could be inferred that the trial was moderately successful. Moreover, a significant reduction in serum ferritin concomitant with increased transferrin was observed in DFP and IDE co-treated patients (Velasco-Sanchez *et al.*, 2011), suggesting that restoration of iron homeostasis could be the mechanism involved.

However, care must be taken with administration of non-selective iron chelators, as these can often have toxic consequences. For example, deferasirox (DFX) is highly toxic in people without iron overload (Singh *et al.*, 1995), and oral administration of dipyriddy is toxic to rats (Groce and Kimbrough, 1982).

Interestingly, the iron affinity of the chelators, DFP, DFX and alicylaldehyde isonicotinoyl hydrazone, was inversely correlated with their ability to improve mitochondrial function in frataxin knockdown cells (Kakhlon *et al.*, 2008). It has been hypothesized that the reason DFP is more effective than other iron chelators (Kakhlon *et al.*, 2008) is its ability to act as a frataxin mimetic ionophore, with functions both in chelation of excess iron and redistribution to iron-deficient subcellular sites (Goncalves *et al.*, 2008). Consistent with this, DFP shuttled iron from iron donor to iron acceptor probes in multiple cellular compartments, including extracellular media, nuclei, mitochondria and endosomes (Sohn *et al.*, 2008). This indicates that DFP is capable of iron mobilization from accumulated stores to sites of low iron bioavailability and therefore acts as a membrane permeable multidirectional iron transporter. Indeed, DFP increased soluble mitochondrial iron and prevented aggregation of insoluble Fe³⁺ in a frataxin-knockdown *Drosophila* model (Soriano *et al.*, 2013). However, caution must be exercised with the use of DFP, as excessive depletion of labile mitochondrial iron pools may promote adverse effects including loss of mitochondrial aconitase activity and growth inhibition, as reported with high dose treatments in control and FRDA patient fibroblasts (Goncalves *et al.*, 2008).

Natural plant-based polyphenols

The neuroprotective role of various natural plant-based polyphenolic compounds has been examined in numerous cell and animal models of neurodegeneration. These include baicalin (from skullcap flowers), tocotrienol (from palm oil), epigallocatechin gallate (EGCG; from green tea), rosmarinic acid (from rosemary), tannic acid (from various plant sources), nordihydroguaiaretic acid (from the creosote bush), curcumin (from turmeric), myricetin (from numerous sources including berries, nuts, fruit, vegetables) and kaempferol (from various sources including tea, berries, fruit and vegetables). These compounds are potent metal chelators and free radical scavengers and have been reported to restore multiple parameters of mitochondrial function including mitochondrial lipid peroxidation, membrane potential, mitochondrial swelling and cytochrome c release (Kamat and Devasagayam, 1995; Mandel *et al.*, 2005; Ono and Yamada, 2006; Cheng *et al.*, 2008; He *et al.*, 2009; Mu *et al.*, 2009; Caruana *et al.*, 2011). This review will focus on two of these: the green tea catechin, EGCG and curcumin.

Green tea catechins

Green tea, which has long been recognized for numerous health benefits, contains a number of polyphenolic flavonoids called catechins, including EGCG, epigallocatechin (EGC), (-)-epicatechin (EC) and (-)-epicatechin-3-gallate (ECG), in order of abundance. These natural compounds are strong antioxidants and have been reported to chelate iron with a similar efficacy as DFP (for a detailed review, see

Mandel *et al.*, 2005). Green tea consumption has been linked to increased cognitive performance and is reported to significantly lower the risk of developing PD (Kuriyama *et al.*, 2006; Hu *et al.*, 2007). ECGG is implicated in hippocampal neuroprotection in a gerbil model of ischaemia/reperfusion injury by carotid artery occlusion (Lee *et al.*, 2000), is shown to prevent 6-OHDA-induced neurotoxicity *in vitro* (Levites *et al.*, 2002) and dopaminergic neuron loss in the SN in the MPP⁺-induced mouse model of PD (Levites *et al.*, 2001). Moreover, EGCG can both attenuate fibrillar α -synuclein aggregation and induce dissociation of formed fibrils (Ono and Yamada, 2006). Similar to the other antioxidant metal-modulating agents described above, catechins exhibit a biphasic action, where lower concentrations mediate neuroprotection and higher doses induce apoptosis, suggestive of promising therapeutic potential for both neurodegeneration and cancer (Weinreb *et al.*, 2003).

Of the green tea polyphenols, EGCG displays the most robust neuroprotective activity, and hence is the best studied of the catechins (Nie *et al.*, 2002). The molecular mechanism of action of EGCG involves direct antioxidant activity through free radical scavenging (Salah *et al.*, 1995; Nanjo *et al.*, 1996), as well as through potent induction of endogenous antioxidant systems such as SOD1 and catalase (Levites *et al.*, 2001). In a study examining the free radical scavenging capacity of EGCG and closely related molecules, it was determined that the ortho-trihydroxyl group on the aromatic ring and the galloyl substituent are primarily responsible for the potent redox properties of EGCG (Nanjo *et al.*, 1996). In the mitochondria, EGCG inhibited 6-OHDA-mediated mitochondrial membrane depolarization and prevented impaired mitochondrial complex I-V function following a hypoxic insult in rats (Sutherland *et al.*, 2005). Moreover, EGCG preferentially targets the mitochondria and displays specific neuroprotective activity to mitochondrial toxic stimuli, but not other apoptosis-inducing agents in rat cerebellar neurons (Schroeder *et al.*, 2009).

In addition to direct effects on mitochondrial function, EGCG activates neuroprotective cellular signalling pathways including the antioxidant response through nrf2-dependent transcription of haeme oxygenase-1 (Romeo *et al.*, 2009). Moreover, in neuronal SH-SY5Y cells, EGCG stimulated the PKC phospho cascades, and attenuated the expression of the pro-apoptotic genes activated by 6-OHDA (Levites *et al.*, 2002). As described for other metal-modulating compounds, EGCG has also been reported to induce ERK activation (Levites *et al.*, 2002; Schroeter *et al.*, 2007), which may contribute to neuroprotection.

Curcumin

Curcumin, a natural plant polyphenol derived from the spice, turmeric, has been shown to exert potent anti-cancer activity. Curcumin is able to bind metal ions and act as a metal ionophore (Dairam *et al.*, 2008; Garcia *et al.*, 2012). Curcumin was demonstrated to exert pleiotropic cellular effects, which include antioxidant activity and direct detoxification of ROS and peroxynitrite (Iwunze and McEwan, 2004). Hence, the neuroprotective efficacy of curcumin has been assessed in various diseases of mitochondrial metal dysfunction including AD, PD and stroke models. For a detailed review on mitochondrial dysfunction and curcumin

treatment in AD models see Eckert *et al.* (2012). Although curcumin is able to cross the BBB, its rapid elimination from the brain has resulted in several drug design approaches to improve bioavailability. Recently, modification of natural compounds such as curcumin by combining individual neuroprotective functionalities into multi-target complexes based on two scaffolds is gaining increasing support. Diester coupling of amino acid moieties has been used as a delivery platform to improve bioavailability, as amino acid transport systems facilitate prodrug influx and intracellular esterase-mediated reactions liberate functional curcumin molecules (Dubey *et al.*, 2008). Naturally, curcumin derivatives display altered metal-binding kinetics, largely dependent on the nature of the aromatic ring substituents (Ferrari *et al.*, 2013).

Knowledge regarding the cellular localization of curcumin is limited. One study demonstrated that curcumin accumulates primarily in the membrane with some nuclear localization (Kunwar *et al.*, 2008), but more research is required to determine the intracellular targeting of curcumin and its derivatives in brain cells. Curcumin is a potent iron chelator (Jiao *et al.*, 2006; 2009) and induces up-regulation of iron regulatory proteins and the transferrin receptor (Jiao *et al.*, 2006), indicative of systemic iron deficiency. Curcumin has been shown to bind Fe²⁺ ions, which prevents iron-mediated hydroxyl radical production (Dairam *et al.*, 2008). Curcumin also strongly binds and stabilizes Fe³⁺ (Dairam *et al.*, 2008; Garcia *et al.*, 2012), thereby inhibiting iron redox cycling.

Curcumin and its derivatives have been tested in numerous cell and animal models of PD. Treatment of N27 dopaminergic neurons with a glutamic acid substituted curcumin derivative-induced neuroprotection involving increased GSH synthesis and a reduction in oxidative stress, lipid peroxidation and H₂O₂ production (Harish *et al.*, 2010). N27 neurons were also protected from MPP⁺-induced cytotoxicity by Di-glutamoyl curcumin (Mythri *et al.*, 2011). Furthermore, curcumin improved survival of dopaminergic neurons in the SN in MPTP-lesioned C57BL/6 mice via JNK-dependent inhibition of mitochondrial swelling and cytochrome c release (Pan *et al.*, 2012). Curcumin-fed rats that had been 6-OHDA-lesioned displayed elevated striatal dopamine levels and had a decreased number of iron-positive cells in the SN, suggesting that curcumin-mediated iron chelation may be responsible for protection of dopaminergic neurons from Fenton chemistry-dependent neurodegeneration (Du *et al.*, 2012). Curcumin has also been reported to reduce huntingtin accumulation and improve rearing behaviour in HD CAG140 knock-in mice (Hickey *et al.*, 2012).

Similar to CuII(atsm), the cellular mechanism of action of curcumin involves activation of kinase signalling pathways. Pharmacological inhibition of the Akt, but not MAPK, pathways decreased curcumin-stimulated Nrf2 activation and Nrf2-dependent gene expression in primary rat cultured cortical neurons (Wu *et al.*, 2013). Moreover, blocking Akt prevented curcumin-mediated reduction in infarct size and oxidative stress in a rat transient middle cerebral artery occlusion stroke model (Wu *et al.*, 2013). Nrf2 activation was also reported to contribute to curcumin-mediated amelioration of mitochondrial dysfunction in spinal cord astrocytes treated with H₂O₂ (Jiang *et al.*, 2011).

As mentioned earlier, limitations with bioavailability of curcumin have been overcome by the development of mul-

tifunctional drug candidates including CNB-001, which combines the neuroprotective actions of curcumin and cyclohexyl bisphenol A, and was detected in the mouse brain 6 days after a single oral dose (Liu *et al.*, 2008). CNB-001, but not curcumin, was reported to protect PC12 cells from excitotoxicity and glucose starvation, despite a 20-fold lower antioxidant activity (Liu *et al.*, 2008). CNB-001 also restored ATP levels and protected HT22 cells from glutamate toxicity (Lapchak *et al.*, 2011). The *in vivo* action of CNB-001 in a rabbit small clot embolic stroke model was also associated with induction of the neuroprotective BDNF, PI3K, Akt and calcium-calmodulin-dependent kinase signalling pathways (Lapchak *et al.*, 2011).

Curcumin is widely reported to reduce cellular ROS production (Wang *et al.*, 2010; Jiang *et al.*, 2011; Liu *et al.*, 2011) and prevent toxicity associated with pathological protein aggregation, including both the PD-associated α -synuclein and ALS-associated TDP-43 aggregates (Wang *et al.*, 2010; Lu *et al.*, 2012). Moreover, curcumin and its derivatives exhibit numerous protective effects on mitochondria. Curcumin improved multiple indicators of mitochondrial function, including cytochrome c release, mitochondrial membrane depolarization and caspase activation in a PC12 neuroblastoma cell model of A53T α -synuclein toxicity (Liu *et al.*, 2011). Mitochondrial dysfunction was also attenuated in a model of aluminium-induced cytotoxicity, as demonstrated by enhanced activity of the mitochondrial NADH dehydrogenase, succinic dehydrogenase and cytochrome c oxidase complexes (Sood *et al.*, 2011). Addition of various substituents on the aromatic rings appears to yield protection against mitochondrial dysfunction. Indeed, mitochondrial swelling, membrane potential and NADH dehydrogenase activity of mice isolated brain mitochondria were substantially improved by a glutamoyl diester of curcumin, as compared with curcumin alone (Mythri *et al.*, 2011). Similarly, a dimethoxy curcumin derivative also attenuated TDP-43-induced loss of mitochondrial membrane potential and mitochondrial NADH dehydrogenase activity (Lu *et al.*, 2012).

Conclusion

The therapeutic approaches presented above demonstrate immense promise in treating neurological diseases of mitochondrial dysfunction by restoration of aberrant mitochondrial metal homeostasis. The common theme from the complexes presented in this review is that the protective action on mitochondrial function is mediated through antioxidant mechanisms that prevent toxic build-up of redox active metals in the vicinity of ETC components (Figure 1). Derivatives of these prototype complexes can therefore be engineered to improve specific molecular properties such as BBB and organelle targeting, antioxidant function and metal binding and release properties. *In vitro* disease models with high throughput screening capabilities are required to examine various functionalities of derivative and multi-scaffold molecules for targeted design of novel multifunctional metal-based therapeutics for mitochondrial dysfunction. Together with an improved understanding of the fundamental molecular mechanism(s) of action of the

complexes, these strategies will lead to improved outcomes in animal and clinical trials.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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