Role of transition metals in the pathogenesis of amyotrophic lateral sclerosis

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Abstract

ALS (amyotrophic lateral sclerosis) is a devastating progressive neurodegenerative disorder resulting in selective degeneration of motor neurons in brain and spinal cord and muscle atrophy. In approx. 2% of all cases, the disease is caused by a mutation in the Cu,Zn-superoxide dismutase (SOD1) gene. The transition metals zinc and copper regulate SOD1 protein stability and activity, and disbalance of the homoestasis of these metals has therefore been implicated in the pathogenesis of ALS. Recent data strengthen the hypothesis that these transition metals are excellent potential targets to develop an effective therapy for ALS.

Introduction

With an incidence of 1–2 per 100000 individuals per year, ALS (amyotrophic lateral sclerosis) (OMIM #105400), also referred to as Lou Gehrig’s disease, is one of the most common motor neurodegenerative disorders. This progressive disease is characterized by selective degeneration of both upper and lower motor neurons in the brain, brainstem and spinal cord, resulting in paralysis due to muscle weakness and atrophy. In a small percentage of cases, dementia is observed. The majority of patients die within 3–5 years of symptom onset as a consequence of respiratory failure. Only 10% of all ALS cases are inherited (familial ALS (fALS)), whereas the vast majority is sporadic (sALS) [1]. Despite common pathological features [2], sALS generally develops later than fALS (average age of onset: 56 years compared with 46 years respectively) [3], and juvenile cases have also been described [1].

Although several genes, most notably SOD1 (Cu,Zn-superoxide dismutase), have been implicated in the aetiology of ALS and have provided initial insight into disease development, the mechanisms of selective motor neuron degeneration are still unclear. A variety of pathophysiological processes have been proposed to play a role, which include oxidative stress, glutamate-mediated excitotoxicity, protein aggregation and transition-metal-induced toxicity (Figure 1) (reviewed in [1]).

Transition metals such as copper are essential trace elements that function as catalytic cofactors in cuproenzymes such as SOD1. On the other hand, copper can be highly toxic due to production of ROS (reactive oxygen species). Therefore cellular copper concentrations are kept in tight balance by proteins mediating copper uptake, distribution, storage and excretion (reviewed in [4]). Cellular copper excretion is promoted by the copper-transporting P-type ATPases ATP7A and ATP7B. Mutations in the genes encoding these proteins result in Menkes disease (copper deficiency) and Wilson’s disease (copper overload) respectively. Both disorders are potentially lethal and manifest with distinct neurological features, further illustrating necessity and toxicity of copper in the CNS (central nervous system). Cellular copper excretion also potentially involves COMMD1 [copper metabolism (Murr1) domain containing 1], as a deletion of this gene results in the development of a copper overload disorder in dogs [5]. Free intracellular copper is detoxified primarily by MT (metallothionein) proteins, and a special function in intracellular copper sequestration is provided by copper chaperones, which specifically present the metal to different cuproenzymes. In this context, copper incorporation into SOD1 is mediated by CCS (copper chaperone for SOD1) [6].

The transition metal zinc is an essential and potentially toxic trace element required for proper conformation and activity of proteins involved in cellular growth, differentiation and metabolism. Deficiency of this metal can result in immunosuppression, night blindness, delayed wound healing, cardiac failure and growth retardation. In the CNS, zinc is located primarily in synaptic vesicles of nerve terminals that surround the cell bodies of spinal motor neurons [7]. Zinc homoeostasis is maintained by regulating the expression and activity of zinc transporters and buffering proteins such as MTs (reviewed in [8]).

In the present review, we discuss the role of transition metals in the pathogenesis of ALS, with a particular focus on copper and zinc.
Genetics of ALS

Mutations in SOD1 are carried by 20% of fALS patients [9]. Currently, more than 130 fALS-associated mutations in SOD1 have been identified [10]. Most of these are associated with a recessive pattern of inheritance. The recessive mutations are usually related to juvenile onset. The vast majority comprises point mutations, distributed over all five exons of the gene [10]. Interestingly, marked variations in disease onset, severity and rate of decline have been observed in patients with the same distinct mutations. This implies that other genetic variations and/or environmental influences are involved as potential disease modifiers and/or contributing factors for ALS. Indeed, smoking, decreased physical activity and diet have been shown, in combination with a mutation in SOD1, to provoke disease onset [3,11]. Besides SOD1, mutations in a number of other genes are associated with fALS. These genes are involved in processes such as hypoxia and oxidative stress, maintenance of cytoskeletal structure and intracellular transport, neurotransmission, RNA metabolism and motor neuron survival (Table 1). Genetic linkage studies, candidate-gene approaches and genome-wide association studies have identified common polymorphisms in yet other genes associated with an increased risk of developing sALS. Interestingly, these genes are involved in the same processes as fALS-associated genes (Table 1). The relative contributions of these and other, yet to be determined, gene variants must be determined in additional in vitro and ex vivo studies.

Function and structure of SOD1

The cytoplasmic protein SOD1, comprising approx. 1% of soluble proteins expressed in brain and spinal cord [12], functions as an antioxidant enzyme by catalysing the disproportionation of superoxide radicals (O₂•⁻) to hydrogen peroxide (H₂O₂). As a stable homodimeric enzyme, each subunit contains one zinc (Zn²⁺) and one copper (Cu²⁺) ion. Zinc binding to the subunits is necessary for structural properties of the protein [13]. Normally, zinc is bound by SOD1 in a tetrahedral geometry by ligands from His71, His80, Asp83 and His63 [14]. Zinc ions are indirectly crucial for SOD1 activity by changing local structure and binding affinities of copper-binding regions as well as stabilizing the global structure of the dimeric enzyme [13]. The presence of a copper ion at the active site of the protein is essential for its enzymatic activity [13]. In an oxidized form, copper ions are bound by SOD1 in a five-co-ordinate geometry by ligands from His46, His48, His120, His63 and a water molecule [14]. fALS-associated SOD1 mutations at sites of metal co-ordination and at the dimer interface result in loss of protein stability and function. In addition, the majority of these mutant SOD1 (mSOD1) proteins are exceptionally prone to aggregate [15].

Pathogenesis of ALS

The enormous achievements in the genetic analysis of ALS have identified a complex interplay between oxidative injury, protein aggregation, glutaminergic excitotoxicity,
<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Disease</th>
<th>Inheritance</th>
<th>Onset</th>
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<td>fALS1</td>
<td>Dominant and Recessive</td>
<td>Adult and juvenile</td>
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<td>TRPM2</td>
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<td>fALS (?)</td>
<td>Recessive (?)</td>
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<td>Adult</td>
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<td>Adult</td>
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<td>–</td>
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<td>Adult</td>
<td>[69]</td>
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<td>Apolipoprotein E (ε-4)</td>
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<td>–</td>
<td>–</td>
<td>[71]</td>
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<td>–</td>
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neuroinflammation, impaired axonal transport and mitochondrial function and/or dysfunction of critical proteins responsible for the selective degeneration of motor neurons in ALS (Figure 1).

**Toxic gain-of-function of mSOD1**

fALS-associated mutations in SOD1 do not necessarily result in a loss of its enzymatic activity. Indeed, mice lacking the enzyme do not develop motor neuron disease, whereas transgenic mice expressing fALS-associated human mSOD1 develop paralysis [16]. It is therefore generally believed that a toxic gain-of-function of mSOD1 is responsible for the pathological phenotype seen in fALS [17]. Most fALS-associated mSOD1s do not display diminished, but rather enhanced, catalytic activity. Instead of solely using O$_2^{•−}$ radicals as substrate, mSOD1 will modify other substrates such as nitric oxide (NO), to increase the cellular level of toxic radicals [e.g. H$_2$O$_2$ and peroxynitrite (ONOO$^−$)] and to induce neurodegeneration (Figure 1). These studies raised the question of whether reduced copper incorporation in mSOD1 would be beneficial. A deletion of Ccs leads to a significant reduction in the amount of copper-loaded mSOD1, but Ccs$^{+/−}$ mice expressing different fALS-associated mutations in SOD1 (G93A, G85R and G37R) do not display modified disease onset and progression compared with mSOD1/Ccs$^{+/+}$ mice [18]. In line with these observations, disruption of the active site of the SOD1 protein in mice by mutating the four histidine residues involved in copper orientation in SOD1 (mSOD1$^{H46R/H48Q/H65G/H120G}$) resulted in an induction of motor neuron disease in a similar manner as to other murine models of fALS such as mSOD1$^{G93A}$ and mSOD1$^{G37R}$ [19]. Since recent data indicate that copper incorporation into SOD1 is not absolutely dependent on CCS, copper could still be relevant in ALS pathogenesis associated with mSOD1.

In contrast with copper binding, almost all mSOD1 proteins demonstrate a decreased affinity for zinc ions. These proteins have enhanced their copper binding as copper is also bound to zinc-binding sites in mSOD1 [20]. Owing to this altered copper co-ordination, zinc-deficient mSOD1s change their enzymatic activity from antioxidant to pro-oxidant by the formation of ONOO$^−$, resulting in induction of motor neuron apoptosis [21].

**Oxidative stress**

In spinal cords of both sALS and fALS patients, increased levels of typical oxidation products (e.g. lipid peroxidation and protein glycoxidation) and of downstream protein biomarkers, such as haem oxygenase 1, have been identified [22,23]. The CNS is particularly sensitive to oxidative stress [17], but oxidative stress in muscle also contributes to ALS pathogenesis [24]. Administration of antioxidant molecules has proven beneficial in sALS patients, in a mouse model expressing a fALS-associated mutation in human SOD1 (mSOD1$^{G93A}$) [25,26] and in some other neurodegenerative disorders, such as AD (Alzheimer’s disease) and PD (Parkinson’s disease), indicating that oxidative stress is a pathologic factor involved in multiple neurodegenerative disorders.

**Protein aggregation and impairment of axonal transport**

A hallmark of ALS is the formation of protein aggregates in the motor neurons of the spinal cord. These aggregates contain several different proteins, including neurofilament proteins and ubiquitin and, in the case of SOD1-associated fALS, also mSOD1 proteins. An impairment of the UPS (ubiquitin–proteasome system) may underlie this aggregate formation. Accumulation of ubiquitin-positive deposits in the spinal cord and brain have been reported in fALS mSOD1$^{G93A}$ model mice, but this was not associated with a reduced proteasomal activity as measured in vitro [27,28]. Alternatively, protein aggregates may be toxic to motor neurons by entrapping proteins critical for viability, such as Bel-2, thus inducing apoptosis [29], or by interfering with intracellular transport [30,31] (Figure 1).

**Excitotoxicity**

The glutamate-induced release of Ca$^{2+}$ ions into the cell by the NMDA (N-methyl-D-aspartate) receptors and the AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors is increased in sALS patients due to a reduced uptake of glutamate from the synaptic cleft by the glutamate-specific EAAT2 (excitatory amino acid transporter 2) [32,33]. In this way, cell damage and neuronal death are induced. The increasing intracellular calcium levels induce Zn$^{2+}$ release in the synaptic cleft, thereby enhancing activation of AMPAR (AMPA receptor) (Figure 1) (reviewed in [34]). In contrast with zinc, copper seems to protect against NMDAR (NMDA receptor)-provoked excitotoxicity. Activation of NMDAR induces ATP7A-mediated Cu$^{2+}$ release in the synaptic cleft, which inhibits synaptic activity and decreases the intracellular Ca$^{2+}$ concentrations [35].

The relevance of excitotoxic processes as critical mediators of ALS pathogenesis is underscored by a moderate prolongation of patient survival induced by the only drug currently approved for ALS treatment, riluzole [36]. However, it should be noted that other anti-glutamatergic drugs had no significant clinical benefit. Furthermore, loss of EAAT2 is also involved in the pathogenesis of other neurodegenerative disorders such as AD [37].

**Transition metals and ALS**

**Copper and ALS**

Copper is of particular relevance to the development and function of the CNS because a variety of cuproenzymes operating in the CNS are dependent on copper to become active (e.g. dopamine β-hydroxylase). Copper has been implicated in the pathogenesis of several neurodegenerative disorders, including AD, prion encephalopathies and ALS [38,39]. Increased copper concentrations have been reported in erythrocytes of SOD1-associated fALS patients [40,41], and altered levels of the copper-responsive MT-1 protein were found in the spinal cords of mSOD1$^{G93A}$ mice [42]. Several studies have therefore investigated the
effect of copper chelators on disease onset and progression. Interestingly, decreased spinal cord copper concentrations, due to administration of copper chelators to transgenic mice expressing mSOD1<sup>G93A</sup>, result in prolongation of their lifespan compared with untreated mSOD1<sup>G93A</sup> mice [43]. Further in vitro studies have determined that copper chelation diminishes the cytotoxic effects of exogenous mSOD1 protein in lymphoblasts of fALS patients [44].

To determine the contribution of copper to disease progression, mSod1<sup>G86R</sup> transgenic mice were crossed with female carriers of the X-chromosome-linked mottled/brindled (Mobr) mutation [43]. The Mobr mice are an excellent model for the human copper deficiency disorder Menkes disease because of a mutation in Atp7a. In these double-mutant mice, decreased copper levels in the CNS, secondary to reduced intestinal copper uptake, caused a delayed onset of ALS. Despite the fact that the mean survival of these mice was extended compared with single mSod1<sup>G86R</sup> mice, the death rate was unaffected. This implies that a decrease in copper levels seems to be beneficial for disease onset and progression in ALS, but cannot prevent the complete neurodegenerative phenotype and associated mortality.

**Zinc and ALS**

In contrast with the hypothesis that zinc overload is involved in the pathogenesis of ALS [45], no differences in distribution of free zinc or significant changes in free zinc levels have been identified in spinal cords of mSOD1<sup>G93A</sup> mice and in blood of sALS patients compared with non-diseased controls [7]. Paradoxically, zinc deficiency induces acceleration of disease progression in mSOD1<sup>G93A</sup> transgenic mice, which can be abrogated by supplementation of the metal [7]. Additional studies are necessary to clarify the exact role of zinc, but it appears most beneficial for ALS patients to maintain normal dietary zinc intake.

**Other transition metals and ALS**

An early indication for a role of Fe<sup>2+</sup> in the pathogenesis of ALS was provided by elevated iron levels in some regions of the brain of sALS patients. In addition, the expression of the iron storage protein ferritin was induced at late stages of the disease in mSOD1<sup>G93A</sup> transgenic mice, indicating high Fe<sup>2+</sup> concentrations [1]. Iron chelators have beneficial effects on ALS progression as they promote, among others, the transcription of VEGF (vascular endothelial growth factor), one of the genes implicated in sALS [46] (Table 1). VEGF expression is positively correlated with neuroprotection in transgenic mice expressing mSOD1<sup>G93A</sup> [47] and the phenotype of Vegf-knockout mice largely resembles that of murine ALS models [46].

Large inconsistencies exist between different studies regarding the role of iron and other metals such as lead, cadmium, mercury and selenium in ALS pathogenesis. Defining a correct and reproducible read-out for measurement of metal concentrations in blood and tissues will help to compare the different studies.

**Concluding remarks**

Despite decades of intense research, the precise mechanisms of selective neurodegeneration of motor neurons in the multifactorial disease ALS remain largely elusive. Identification of other genetic causes of ALS is ongoing, but these studies require larger populations to allow solid conclusions. Copper and zinc play important roles in the pathogenesis of ALS since these metals are essential for normal SOD1 function. In addition, although to a different extent, they are involved in a variety of toxic processes associated with ALS pathogenesis. For both metals, chelation therapies had beneficial effects in murine and cellular models of the disease. We consider proteins that regulate cellular copper levels, such as MTs, ATP7A, ATP7B and COMMD1, as promising potential therapeutic targets for ALS. By decreasing cellular copper concentrations, ROS production and subsequently oxidative stress will be reduced, leading to a less toxic environment for motor neurons.

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**References**


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