Neurotoxicity and ALS: Insights into Pathogenesis

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Contents

Abstract

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder of the motor neurons in the spinal cord, brainstem, and motor cortex. While the mechanisms underlying the development of ALS remain to be fully elucidated, evidence is now emerging to suggest that the pathophysiological mechanisms underlying ALS are multifactorial, reflecting a complex interaction between causal genes and local environment. In particular, dysfunction of metabolic pathways including excessive oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, and defective axonal transport

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systems along with abnormalities of nonneuronal supporting cells may cause critical injury to target proteins and organelles within the motor neuron, thereby leading to neurotoxicity and neurodegeneration in ALS. The clinical effectiveness afforded by anti-glutamatergic agents such as riluzole underscores the importance of glutamate excitotoxicity in the development of neurodegeneration in ALS, with anterior horn cell degeneration mediated by corticomotoneuronal hyperexcitability via an anterograde transsynaptic process. This chapter will review current understanding of the pathophysiological mechanisms underlying the development of ALS, with a particular focus on the role of neurotoxicity in mediating neurodegeneration in ALS.

Keywords
ALS • Glutamate excitotoxicity • Neurodegeneration • Neurotoxicity

1 Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder of motor neurons in the spinal cord, brainstem, and motor cortex (Kiernan et al. 2011). The ALS phenotype is clinically characterized by the presence of upper and lower motor neuron abnormalities (Kiernan et al. 2011; Vucic et al. 2007; Winhammar et al. 2005), whereby lower motor neuron (LMN) features include fasciculations, muscle wasting, and weakness, while upper motor neuron features include weakness, slowness of movement, increased tone, and hyperreflexia. This unique combination of upper and lower motor neuron abnormalities, not encountered in other neurodegenerative diseases, led Charcot to postulate the mechanism of neuronal involvement in ALS pathogenesis (Charcot and Joffroy 1869). Despite Charcot's initial observations, the precise pathophysiological mechanisms, and even the site of disease onset, remain the subject of ongoing debate (Ravits et al. 2007).

Evidence is now emerging to suggest that the pathogenic mechanisms underlying ALS are multifactorial, reflecting interaction between causal genes and local environment (Boillee et al. 2006a; Kiernan et al. 2011; Vucic and Kiernan 2009; Winhammar et al. 2005). These complex pathways, including oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, and defective axonal transport systems (Fig. 1), combined with abnormalities of nonneuronal supporting cells such as the astrocytes, may cause injury of critical target proteins and organelles within the motor neuron, thereby resulting in neurotoxicity and degeneration in ALS (Gonzalez de Aguilar et al. 2007; Gros-Louis et al. 2006; Haidet-Phillips et al. 2011; Neusch et al. 2007; Pasinelli and Brown 2006; Patel and Maragakis 2002; Vucic and Kiernan 2009). As such, this chapter will review current understanding of the pathophysiological mechanisms underlying the development of ALS, with a particular focus on the role of neurotoxicity.

1.1 Glutamate-Mediated Excitotoxicity

Glutamate-mediated excitotoxicity appears to be an important mechanism in ALS pathogenesis (Boillee et al. 2006; Kiernan et al. 2011). Glutamate is the major excitatory neurotransmitter in the central nervous system (Heath and Shaw 2002; Watkins and Evans 1981), synthesized from reductive deamination of alpha-ketoglutarate or from the action of amino acids of aminotransferases (Heath and Shaw 2002). Approximately 20% of the total glutamate pool is stored in presynaptic nerve terminals, and during impulse transmission, glutamate is released from presynaptic neurons through the effects of depolarization, diffusing across the synaptic cleft to activate postsynaptic receptors. Their excitatory signal is terminated upon removal of glutamate from the synaptic cleft by specific glutamate reuptake transporters located on both neurons and astrocytes (Dong et al. 1999; Vandenberg 1998). Within presynaptic astrocytes, glutamate is converted into glutamine by the enzyme glutamine synthetase and then returned to the neuron for resynthesis of glutamate (Laake et al. 1995).

Glutamate receptors are broadly classified into ionotropic or metabotropic receptors (Heath and Shaw 2002). Binding of glutamate to its ionotropic receptors results in a conformational change within the receptor, thereby enabling a passage of Na^+ and Ca^{2+} ions through a central pore. Metabotropic glutamate receptors are linked via G-proteins to second-messenger enzymes, which in turn can regulate a host of cellular activities (Simeone et al. 2004). Based on pharmacological studies, glutamate ionotropic receptors are further classified as (i) *N*-methyl-D-aspartate (NMDA), (ii) α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), and (iii) kainite receptors. This pharmacological classification is supported by cloning studies that have identified six different families of glutamate ionotropic receptors that conform to the original agonist studies (Simeone et al. 2004). *N*-methyl-D-aspartate receptors are permeable to influx of Na^+ and Ca^{2+} and efflux of K^+ (Simeone et al. 2004). An essential feature of NMDA receptors is their voltage-dependent blockade by Mg^{2+} binding within the channel pore, which can be alleviated by depolarization (MacDermott et al. 1986). NMDA receptors are involved in excitatory neurotransmission, which is characterized by a slow rise time and decay. As such, the NMDA receptors are involved in complex physiological processes, such as generation of rhythmic motor activity (Traven et al. 1993), regulation of neuronal migration during embryogenesis (Komuro and Rakic 1993), and memory (Bliss and Collingridge 1993).

The NMDA receptor complex is composed of different subunits derived from 6 genes: NMDAR1 (eight splice variants described), NMDAR2 (A–D), and NMDAR3 (A,B) (Heath and Shaw 2002; Simeone et al. 2004). While the NMDAR1 subunit forms the basic structure of the receptor (Heath and Shaw 2002), the NMDAR2 subunit determines ion channel properties and forms ligand-binding sites (Kutsuwada et al. 1992; Meguro et al. 1992; Michaelis 1998). Functional and pharmacological properties of NMDA receptors are determined through specific combination of NMDAR1 and NMDAR2 subunits (Kutsuwada et al. 1992;

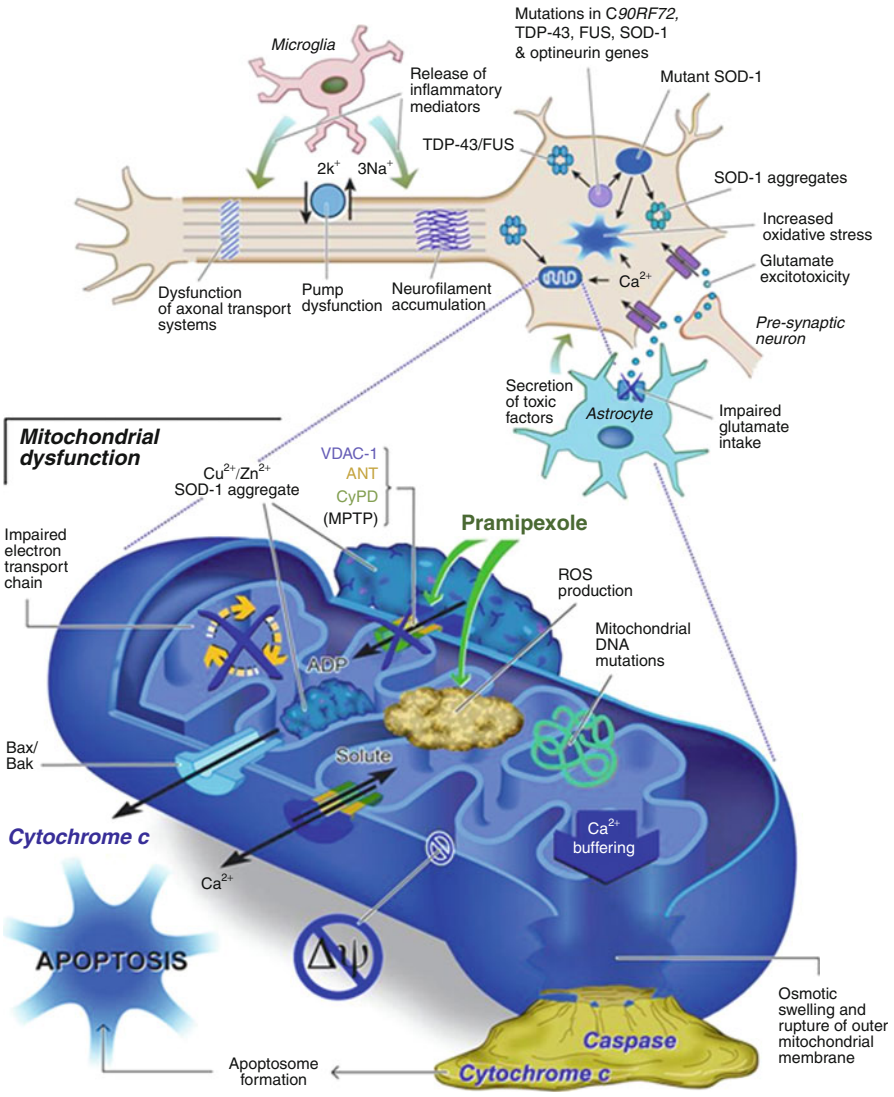

Fig. 1 Multiple interacting pathophysiological mechanisms appear to underlie the development of amyotrophic lateral sclerosis (*ALS*). Glutamate-mediated excitotoxicity is an important mechanism ALS progression and seems to precede the clinical development of ALS. Dysfunction of the excitatory amino acid transporter type 2 (EAAT2), located on astrocytes, in part induces the development of glutamate excitotoxicity via excessive extracellular accumulation of glutamate. In addition, activation of nonneuronal cells (astrocytes and microglia) in ALS results in secretion of proinflammatory cytokines and other cytotoxic factors that ultimately results in further neurotoxicity and degeneration. In conjunction with glutamate-mediated excitotoxicity, other molecular processes induce neurotoxicity via multifactorial mechanisms. Within the neuron, mutations in a host of ALS-related genes, including C9orf72, superoxide dismutase 1 (*SOD1*), TDP-43, and FUS, result in ALS via multiple mechanisms. Specifically, mutations in the SOD-1

Monyer et al. 1992). In addition, there are regional variations in the expression of NMDA receptor subtypes (Ciabarra and Sevarino 1997; Ishii et al. 1993; Kutsuwada et al. 1992; Monyer et al. 1992; Watanabe et al. 1993a, b, 1994a, b), with the NMDAR3B subunit heavily expressed in somatic motoneurons (Chatterton et al. 2002; Nishi et al. 2001).

AMPA receptors mediate a rapid influx of monovalent ions, such as Na^+, K^+, and chloride (Cl^-) but unlike NMDA receptors are impermeable to Ca^{2+} (Heath and Shaw 2002). Four AMPA receptor subtypes have been cloned (*Glu*R1-4) and are composed of three transmembrane domains (M1, M3, M4) and a fourth cytoplasmic hairpin loop (M2), which contributes to the pore-lining region (Dingledine et al. 1999; Simeone et al. 2004). The AMPA receptor exists as a pentameric structure in vivo, which is formed by the arrangement of subunits to create receptor diversity (Heath and Shaw 2002). The GluR2 subunit influences the Ca^{2+} permeability of AMPA receptors, whereby those receptors expressing an immature GluR2 subunit are more permeable to Ca^{2+} ions. Following activation of these AMPA receptors, excessive influx of Ca^{2+} results in neurodegeneration through activation of Ca^{2+}-dependent pathways (Heath and Shaw 2002; Simeone et al. 2004).

1.2 The Role of Glutamate in ALS Pathogenesis

As discussed, glutamate excitotoxicity is mediated by excessive activation of postsynaptic glutamate receptors (Heath and Shaw 2002). In ALS, glutamate excitotoxicity has been postulated to induce anterior horn cell degeneration via a transsynaptic anterograde process mediated by corticomotoneurons (Eisen et al. 1992). Support for such a mechanism has been provided by transcranial magnetic stimulation studies (TMS) which have demonstrated that cortical hyperexcitability, a biomarker of glutamate excitotoxicity, is an early feature in sporadic and familial ALS, linked to motor neuron degeneration (Blair et al. 2010; Caramia et al. 1991; Desiato et al. 2002; Eisen et al. 1993; Prout and Eisen 1994; Vucic and Kiernan 2006, 2009, 2010; Vucic et al. 2008). In addition, longitudinal studies in asymptomatic SOD-1 mutation carriers revealed that cortical hyperexcitability developed prior to the clinical onset of ALS (Vucic et al. 2008), a feature also evident in the G93A SOD-1 mouse model (Browne et al. 2006). Of relevance, loss of γ-aminobutyric acid (GABA) secreting parvalbumin-positive inhibitory interneurons in the motor cortex of ALS patients may further contribute

Fig. 1 (continued) gene result in toxic gain of function of the SOD1 enzyme which affects a host of critical cellular organelles, such as DNA/RNA metabolism. In addition, mitochondrial dysfunction is a feature of ALS, linked to glutamate excitotoxicity and SOD-1 gene mutations, resulting in a reduced production of ATP and calcium-sequestering ability, as well as an increase in free radical formation. Of further relevance, mitochondrial dysfunction may contribute to glutamate excitotoxicity. Ultimately, these multiple pathogenic processes result in critical cell dysfunction and motor neuron degeneration

to the development of cortical hyperexcitability (Nihei et al. 1993), a finding underscored by recent neuroradiological studies reporting a significant reduction of GABA within the motor cortex of ALS patients (Foerster et al. 2012).

Molecular-based studies have provided further evidence for glutamate-mediated excitotoxicity in ALS. Specifically, molecular studies established significant reduction in the expression and function of the astrocytic glutamate transporter (EAAT2), which mediates glutamate reuptake at synapses thereby reducing glutamate excitotoxicity, in the superoxide dismutase-1 (SOD-1) mouse model and the motor cortex and spinal cord of ALS patients (Boillee et al. 2006; Ionov 2007; Rothstein et al. 1993, 1995, Trotti et al. 1999). Of further relevance, dysfunction of the EAAT2 transporter appears to be a preclinical feature in the SOD-1 mouse model (Boston-Howes et al. 2006; Gibb et al. 2007), and an increase in the expression and transporter activity of EAAT2 increases the life span of mutant SOD-1 mice (Rothstein et al. 2005).

At a postsynaptic level, increased expression of AMPA receptors with the unedited GluR2 subunit has been reported in ALS (Kawahara et al. 2004; Kwak and Kawahara 2005; Takuma et al. 1999; Van Damme et al. 2002, 2005). This editing defect appears to be specific for ALS, thereby rendering the motor neurons more permeable to Ca^{2+}, potentially explaining the increased sensitivity of motor neurons to excitotoxicity (Cox et al. 2007; Heath and Shaw 2002). Further support for glutamate excitotoxicity has been provided by the clinical benefit of riluzole in ALS patients (Bensimon et al. 1994; Cheah et al. 2010; Gurney et al. 1996, 1998; Lacomblez et al. 1996). Specifically, riluzole is a glutamate antagonist that exerts effects in the central nervous system by reducing the release of glutamate from presynaptic nerve terminals and enhancing the reuptake of glutamate (Azbill et al. 2000; Cheah et al. 2010; Quinlan 2011; Wang et al. 2004).

For the glutamate hypothesis to be a plausible mechanism of motor neuron degeneration in ALS, it must explain how motor neurons became selectively damaged through overactivity of the glutaminergic system and provide a mechanism by which degeneration occurs. A number of cell-specific molecular features possessed by the motor neurons render them vulnerable to glutamate toxicity in ALS. Motor neurons affected in ALS preferentially express AMPA receptors lacking the functional GluR2 subunit, thereby rendering the motor neurons more permeable to Ca^{2+} (Kawahara et al. 2004; Kwak and Kawahara 2005; Van Damme et al. 2002, 2005). In addition, motor neurons vulnerable to degeneration lack the intracellular expression of proteins parvalbumin and calbindin D28k which are required to buffer intracellular Ca^{2+} (Ince et al. 1993). Of further relevance, increased expression of the inositol 1,4,5-triphosphate receptor 2 (ITPR2) gene was reported in ALS (van Es et al. 2007). The ITPR2 is involved in glutamate-mediated neurotransmission, whereby stimulation of glutamate receptors results in binding of inositol 1,4, 5-triphosphate to ITPR2, which subsequently increases intracellular calcium (Choe and Ehrlich 2006; van Es et al. 2007). Aberrant activity of ITPR2 results in higher intracellular concentration of Ca^{2+} leading ultimately to neurodegeneration (Gutstein and Marks 1997). Of further relevance, motor neurons in ALS, at least in animal

models, appear to be larger, with an increase in distal dendritic branching (Amendola and Durand 2008). Consequently, the input conductance of the motor neurons is increased rendering the motor neuron more vulnerable to electrical and metabolic stresses, in particular those imparted by glutamate excitotoxicity (Quinlan 2011).

Although details of the molecular mechanisms by which glutamate exerts neurotoxicity are still to be fully elucidated, several pathways have been defined. Initially, an influx of Na^+ and Cl^- ions occurs along with water molecules, resulting in acute neuronal swelling that is reversible with removal of agonist (Choi 1987; Shaw and Kuncl 2002; Stys 1998). Subsequently, an influx of Ca^{2+} ions occurs via activation of ionotropic receptors such as the NMDA and Ca^{2+}-permeable AMPA receptors, as well activation of voltage-gated Ca^{2+} channels (Choi 1987; Miller et al. 1989). Ultimately, activation of these ionic pathways results in increased intracellular Ca^{2+} concentration and activation of Ca^{2+}-dependent enzymatic pathways leading to neuronal death (Cox et al. 2007; Meldrum and Garthwaite 1990; Regan et al. 1995; Shaw and Kuncl 2002). Further, glutamate excitotoxicity results in production of free radicals that can further damage the intracellular organelles to thereby cause cell death (Bondy and Lee 1993; Lees 1993; Maher and Davis 1996).

In his original manuscript, Charcot concluded that ALS was a disorder of the brain and that lower motor neuron degeneration resulted from downstream effects (Charcot and Joffroy 1869). This view was not universal, and some of Charcot's contemporaries, such as Gower's, argued that upper and lower motor neuron degeneration were independent. Over the past two decades the site of ALS onset has been revisited to a large extent precipitated by the advent of modern noninvasive technologies such as TMS. Three schools of thought have developed pertaining to the role of the UMN and related pathophysiological processes in ALS: (i) "the *dying forward*" hypothesis; (ii) "the *dying back*" hypothesis, and (iii) "the *independent degeneration*" hypothesis (Fig. 2).

The dying forward hypothesis proposed that ALS was primarily a disorder of the corticomotoneurons, which connect monosynaptically with anterior horn cells (Eisen et al. 1992). Corticomotoneuronal hyperexcitability was postulated to induce anterior horn cell degeneration transsynaptically via an anterograde glutamate-mediated excitotoxic process (Eisen et al. 1992; Vucic and Kiernan 2009). This dying forward hypothesis was based on a number of poignant clinical observations including (i) relative preservation of extraocular and sphincter muscles in ALS, postulated to be due to a paucity of corticomotoneuronal projections onto the motor nuclei innervating these muscles; (ii) absence of an animal model of ALS, ascribed to a lack of direct corticomotoneuronal-anterior horn cell connections (Armand 1982; Lemon and Griffiths 2005); (iii) rarity of pure lower motor neuron forms of ALS, with subclinical upper motor neuron dysfunction invariably detected with TMS studies (Eisen and Shtybel 1990); and (iv) the specificity of dissociated muscle atrophy (Eisen and Kuwabara 2012; Kuwabara et al. 2008; Menon et al. 2011; Wilbourn 2000), termed the split hand phenomenon in ALS remains best explained by a dying forward mechanism (Eisen and Kuwabara 2012; Menon et al. 2011).

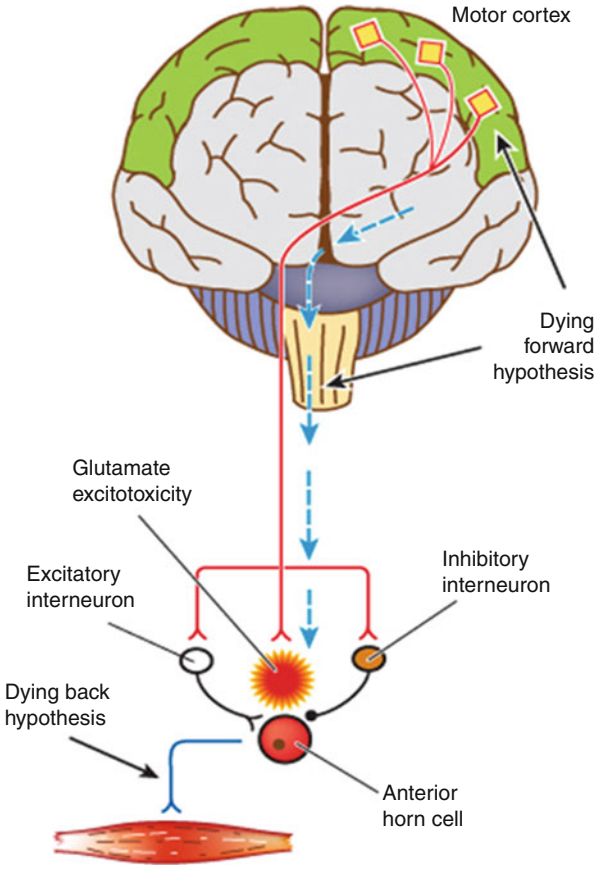

Fig. 2 The dying forward and dying back hypothesis of amyotrophic lateral sclerosis (*ALS*). The "dying forward" hypothesis proposed that ALS was primarily a disorder of the corticomotoneurons (highlighted in *red*), with anterior horn cell degeneration mediated via a transsynaptic anterograde glutamate-mediated excitotoxic process. In contrast, the dying back hypothesis proposed that ALS was primarily a disorder of the lower motor neurons with pathogens retrogradely transported from the neuromuscular junction to the cell body where these pathogens may exert their deleterious effects

Utilizing TMS technology, it is becoming increasingly apparent that cortical hyperexcitability develops as an early feature in sporadic and familial ALS, linked to the process of motor neuron degeneration (Caramia et al. 1991; Desiato et al. 2002; Eisen et al. 1993; Prout and Eisen 1994; Vucic and Kiernan 2006, 2009, 2010; Vucic et al. 2008). Furthermore, longitudinal studies in asymptomatic SOD-1 mutation carriers established that cortical hyperexcitability developed prior to the clinical onset of ALS (Vucic et al. 2008), a feature also reported in the G93A SOD-1 mouse model (Browne et al. 2006). In keeping with a cortical origin of ALS is the now accepted view that ALS and frontotemporal dementia (FTD) represent an overlapping continuum of the same disorder (Lillo and Hodges 2009; Neumann et al. 2006), an observation further underscored by recent genetic discoveries that increased hexanucleotide repeat expansions in the first intron of C9ORF72 gene (9p21) was associated with both ALS and FTD (DeJesus-Hernandez et al. 2011; Renton et al. 2011; Traynor 2012).

The dying back hypothesis proposed that ALS is primarily a disorder of the lower motor neurons, with pathogens retrogradely transported from the

neuromuscular junction to the cell body where they exert their deleterious effects (Chou and Norris 1993). Although some pathological studies have indirectly supported a dying back process (Gould et al. 2006; Pagani et al. 2006; Pun et al. 2006), no pathogens of any type has been identified in relation to ALS. The presence of widespread dysfunction within the frontal cortex, including the primary, supplementary, and prefrontal motor cortices in ALS, remains difficult to reconcile with any dying back process (Miller et al. 2009; Turner et al. 2009; Vucic et al. 2007). In addition, the absence of central pathology in other lower motor neuron disorders such as Kennedy's disease or poliomyelitis provides a further argument against a dying back process (Eisen and Weber 2001; Vucic and Kiernan 2008).

The independent degeneration hypothesis suggested that the upper and lower motor neurons degenerated independently (Gowers 1886–1888). Limited neuropathological studies provided indirect support for independent degeneration, whereby the degeneration of upper and lower motor neurons appeared to be independent (Kiernan and Hudson 1991; Pamphlett et al. 1995). These correlative morphological techniques, however, were significantly confounded by the anatomical and functional complexity of the corticomotoneuronal system (Flament et al. 1993). In particular, there remains considerable variability in the corticomotoneuronal to anterior horn cell ratio, due to synaptic changes, and as such, attempts to correlate upper and lower motor neurons as a "one-off" on autopsy studies are divorced from clinical and in vivo reality (Eisen and Weber 2001).

1.3 Neurotoxicity and the Role of SOD-1 Gene

Mutations in the SOD-1 gene, the first ALS gene reported and mapped to the long arm of chromosome 21 [21 q22.1] (Siddique et al. 1991), was postulated to exert pathogenic effects by resulting in acquisition of aberrant cytotoxic enzyme activity (Andersen 2006a; Dewil et al. 2004; Robberecht 2002). The SOD-1 gene spans 11 kilobases of genomic DNA, comprises five exons and four introns (Levanon et al. 1985), and encodes a highly conserved 153-amino acid long protein, which together with a catalytic copper (Cu) ion and a stabilizing zinc (Zn) ion form a subunit (Andersen 2006; Levanon et al. 1985). A disulfide bridge stabilizes each subunit, and the two identical subunits combine through non-covalent bonds to form the Cu-Zn SOD-1 enzyme. The main function of the SOD-1 enzyme involves free radical scavenging whereby the enzyme catalyses the conversion of the superoxide anion to molecular oxygen and hydrogen peroxide, which in turn is reduced to water by glutathione peroxidase and catalase (Andersen 2006; Fridovich 1986; Shaw and Kuncl 2002). The Cu-Zn SOD enzyme constitutes 0.5–1% of soluble protein in the brain and spinal cord and is located within the cytosol and nucleus and between two mitochondrial membranes (Andersen 2006; Bowling et al. 1995; Pardo et al. 1995).

To date, over 150 different mutations have been reported in the SOD-1 gene, with the majority of being missense mutations, resulting in changes in single amino acids but preserving the SOD-1 protein length. The remaining mutations are either nonsense or deletion mutations that either introduce novel nucleotides or remove existing

nucleotides resulting in alteration of the polypeptide length (Andersen 2006; Dewil et al. 2004; Gros-Louis et al. 2006). An autosomal dominant pattern of inheritance is evident with most mutations, except for the D90A mutation, which may be transmitted in an autosomal recessive manner. Compound heterozygosity has also been reported with SOD1 FALS, whereby two siblings with a slowly progressive ALS phenotype may be carriers of both the D90A and D96N mutations (Hand et al. 2001). The mutations are widely distributed throughout the gene with preponderance for exon 4 and 5 (Andersen 2006; Andersen et al. 2003; Cudkowicz et al. 1997; Radunovic and Leigh 1996). Globally, the most frequent mutation is the substitution of aspartate for alanine (D90A), followed by alanine to valine (A4V) and isoleucine for threonine (I113T) (Andersen 2006; Dewil et al. 2004).

Evidence for a neurotoxic gain of function of the SOD-1 enzyme has been provided by several lines of evidence. Specifically, increased activity of the SOD-1 enzyme was reported in the transgenic SOD-1 mouse models, supporting a toxic gain of function mechanism (Bruijn et al. 1997; Gurney et al. 1994; Ripps et al. 1995; Wong et al. 1995). Of further relevance, SOD-1 knockout mice failed to develop the ALS phenotype, thereby suggesting the importance of SOD-1 enzyme activity in ALS pathogenesis (Reaume et al. 1996). Aberrant biochemical activity of the SOD-1 enzyme has been reported to underlie this toxic gain of function (Andrus et al. 1998; Beckman et al. 1993; Bruijn et al. 1997, 1998, 2004). Specifically, SOD-1 gene mutations reportedly induced structural changes in the SOD-1 enzyme, enabling substrates other than the superoxide anion to gain access to the active center, thereby resulting in increased production of hydroxyl and free radicals (Bogdanov et al. 1998; Liu et al. 1998). In addition, the mutated SOD-1 enzyme also accepts peroxynitrate as a substrate, resulting in nitration of tyrosine residues on critical cellular proteins (Beckman et al. 1993; Beckman and Koppenol 1996; Crow et al. 1997). Ultimately, this aberrant biochemical activity of the SOD-1 enzyme resulted in cell injury and death (Beckman et al. 1993). Importantly, upregulation of protein-tyrosine nitration has been reported in anterior horn cells (Abe et al. 1997; Chou et al. 1996) as well as elevation of 3-nitortyrosine in the spinal cord of ALS patients and transgenic SOD-1 mice (Beal et al. 1997; Ferrante et al. 1997).

Mutations in the SOD-1 gene may also result in improper binding of zinc to the mutated SOD-1 peptide, thereby allowing reduction of SOD-1 bound copper, which in turn results in formation of superoxide anions and cell injury (Estevez et al. 1999). Diminished metal ion binding by the mutated SOD-1 peptide may also release zinc and copper ions, thereby further contributing to neurotoxicity and degeneration (Pasinelli and Brown 2006). Of further relevance, aberrant SOD-1 enzyme activity may result in oxidative stress via upregulation of proinflammatory cytokines (Hensley et al. 2006). Specifically, increased expression of proinflammatory mediators such as nitric oxide, interleukins 1, 6, and 12 has been reported in the SOD-1 mouse model with resultant neurotoxicity to motor neurons in spinal cord preparations (Kim et al. 2006). Importantly, antagonism of these agents with neutralizing antibodies resulted in increased motor neuron survival (Kim et al. 2006).

Alternatively, mutations in the SOD-1 gene may lead to conformational instability and misfolding of the SOD-1 peptide, resulting in formation of toxic

intracellular aggregates. In the transgenic SOD-1 mouse model and human ALS cases, immunoreactive SOD-1 aggregates were reported in motor neurons and glial cells (Bruijn et al. 1998; Jonsson et al. 2006; Zetterstrom et al. 2007). Whether the intracellular aggregates were neurotoxic to motor neurons remains unknown, although a number of possible cytotoxic mechanisms have been proposed, including (i) co-aggregating with vital cellular constitutes, (ii) inhibiting normal proteosome function, and (iii) exerting mechanical or biochemical effects on the cell, such as disruption of axonal transport systems (Bruijn et al. 1997, 1998; Pasinelli and Brown 2006; Williamson and Cleveland 1999).

1.4 Mitochondrial Dysfunction

In conjunction with glutamate excitotoxicity and oxidative stress, there is mounting evidence that mitochondrial dysfunction exerts an important role in the pathophysiology of ALS (Boillee et al. 2006; Chung and Suh 2002; Higgins et al. 2003; Kirkinezos et al. 2005; Lederer et al. 2007; Pasinelli and Brown 2006; Xu et al. 2004). Mitochondria are intracellular organelles whose main function is to generate energy for the cell in the form of ATP. Under conditions of excessive Ca^{2+} load, as may be evident with glutamate excitotoxicity (Dugan and Choi 1994), mitochondrial production of free radicals induces injury of critical neuronal cellular proteins and DNA. In addition, mitochondria remain sensitive to free radical damage at both the protein and DNA level, resulting in further mitochondrial dysfunction (Bowling and Beal 1995). Mitochondrial damage may in turn enhance glutamate excitotoxicity by disrupting the normal resting membrane potential, thereby resulting in a loss of the normal voltage-dependent Mg^{2+}-mediated block of NMDA receptor channels (Heath and Shaw 2002; Shaw and Kuncl 2002).

Mitochondrial degeneration and dysfunction has been reported in ALS patients and in the transgenic SOD-1 mouse model (Higgins et al. 2003; Kong and Xu 1998; Xu et al. 2004). Ultrastructural abnormalities of muscle mitochondria, paracrystalline inclusions, and abnormal cristae have been reported in ALS (Chung and Suh 2002; Comi et al. 1998; Lederer et al. 2007). Dysfunction of mitochondrial enzymes involved in energy generation, such as cytochrome C oxidase and respiratory chain complexes I and IV, as well as downregulation of nuclear genes encoding mitochondrial components within the motor cortex has been reported in ALS (Comi et al. 1998; Fujita et al. 1996; Jung et al. 2002; Kirkinezos et al. 2005; Lederer et al. 2007). Of further relevance, mitochondrial dysfunction including reduction in protein import, impairment in Ca^{2+} sequestering ability, and an exaggerated depolarizing response of the inner mitochondrial membrane to Ca^{2+} stimulation may occur in the presymptomatic stages of ALS (Bilsland et al. 2008; Damiano et al. 2006; Jaiswal et al. 2009; Li et al. 2010; Nguyen et al. 2009). Ultimately, severe damage to the mitochondrial membrane potential, respiration, and electron transfer chain ensues, resulting in reduced ATP synthesis and neurodegeneration (Quinlan 2011).

The transportation and distribution of mitochondria within the neurons appears to be impaired in ALS (Quinlan 2011). Mitochondria are normally highly mobile organelles, evident in both the axons and dendrites (MacAskill et al. 2010). The movement of mitochondria is regulated through Ca^{2+} signalling and synaptic activity (MacAskill et al. 2010). An increase in intracellular Ca^{2+} concentration, as occurs with glutamate excitotoxicity, interrupts the movement of mitochondria within the cell, in particular at the level of the synapse (MacAskill et al. 2009). Abnormalities of mitochondrial distribution and transport have been reported in ALS, with evidence of reduced distribution in the axons and more frequent pauses in mitochondrial movements (Bilsland et al. 2010). Importantly, the slow and fast axonal transport systems, vital for mitochondrial transport, seem to be impaired in ALS and have been linked to glutamate excitotoxicity (Bilsland et al. 2010; De Vos et al. 2007; Quinlan 2011). Ultimately, this interruption in mitochondrial mobility may result in depletion of energy supply in critical neuronal segments, essential for the maintenance of the resting membrane potential and generation of action potentials, with resultant neuronal degeneration.

From a therapeutic perspective, a recent phase II trial of dexpramipexole, a pharmacological agent that enhances mitochondrial function (Cheah and Kiernan 2010), was shown to be effective in slowing ALS disease progression and reducing mortality over a 24-week period (Cudkowicz et al. 2011). Currently, a phase III, multicenter international trial is underway to assess the clinical efficacy of dexpramipexole as add-on therapy to riluzole in ALS (ClinicalTrials.gov-NCT01281189).

2 Non-neuronal Cells and Neurotoxicity in ALS

Dysfunction of nonneuronal cells, astrocytes, and microglia also appears to be important in ALS pathogenesis (Boillee et al. 2006a, b; Haidet-Phillips et al. 2011; Neusch et al. 2007). Molecular studies in the SOD-1 mouse model have established that expression of the mutant SOD-1 protein in either the motor neurons or astrocytes in isolation failed to induce degeneration, thereby suggesting that the pathogenic process in ALS may involve a complex interaction between motor neurons interacting with nonneuronal cells (Gong et al. 2000; Lino et al. 2002; Pramatarova et al. 2001). Underscoring the importance of nonneuronal cells in ALS pathogenesis are findings that motor neuron toxicity appears to be modulated by expression of mutant SOD-1 in nonneuronal cells (Clement et al. 2003). However, mutant SOD-1 expressing nonneuronal cells seem to be more involved in the regulation of disease progression rather than onset of ALS (Beers et al. 2006; Boillee et al. 2006).

Similar to animal models, nonneuronal cells seem also to be an important pathogenic factor in the human disease. Specifically, astrocytes derived from postmortem spinal cord neural progenitor cells (NPCs) of sporadic and familial (SOD1) patients were selectively toxic to motor neurons (Haidet-Phillips et al. 2011). Upregulation of inflammatory genes encompassing chemokines, proinflammatory cytokines and components of the complement cascade, was evident in the neurotoxic astrocytes, further suggesting that neurotoxicity may in

part be mediated by an inflammatory mechanism. In addition, downregulation of mutant and wild-type SOD-1 expression in astrocytes derived from familial and sporadic ALS, respectively, was neuroprotective (Haidet-Phillips et al. 2011). Taken together, these findings suggest that astrocyte-mediated neurotoxicity contributed to neurodegeneration in familial (SOD-1) and sporadic ALS, thereby suggesting that novel cell-based approaches, particularly focusing on nonneuronal supportive cells, may be therapeutic in ALS.

3 Conclusion

Multiple interacting molecular and genetic mechanisms appear to underlie the development of motor neurodegeneration in ALS. Studies in animal models and human ALS have suggested an important role for glutamate neurotoxicity in ALS pathogenesis. Specifically, neurophysiological studies have suggested that cortical hyperexcitability, a biomarker of glutamate neurotoxicity, appears as a primary event in ALS, with motor neuron degeneration mediated via transsynaptic anterograde mechanisms. In conjunction with glutamate excitotoxicity, other molecular processes including mitochondrial dysfunction and abnormalities of axonal transport, together with neurotoxicity of the mutant SOD-1 enzyme, oxidative stress, and dysfunction of nonneuronal supporting cells, such as astrocytes, appear to contribute to ALS pathogenesis. From a therapeutic perspective, further insights into ALS pathogenesis will undoubtedly result in development of novel therapeutic strategies.

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