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Review

Transgenic mouse models of amyotrophic lateral sclerosis

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Abstract

The discovery of missense mutations in the gene coding for the Cu/Zn superoxide dismutase 1 (SOD1) in subsets of familial cases was rapidly followed by the generation of transgenic mice expressing various forms of SOD1 mutants. The mice overexpressing high levels of mutant SOD1 mRNAs do develop motor neuron disease but unraveling the mechanisms of pathogenesis has been very challenging. Studies with mouse lines suggest that the toxicity of mutant SOD1 is unrelated to copper-mediated catalysis but rather to propensity of a subfraction of mutant SOD1 proteins to form misfolded protein species and aggregates. However, the mechanism of toxicity of SOD1 mutants remains to be elucidated. Involvement of cytoskeletal components in ALS pathogenesis is supported by several mouse models of motor neuron disease with neurofilament abnormalities and with genetic defects in microtubule-based transport. Here, we describe how transgenic mouse models have been used for understanding pathogenic pathways of motor neuron disease and for pre-clinical drug testing.

Keywords: Sclerosis; Mutant SOD1; Toxicity

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurological disorder that is characterized by the selective loss of motor neurons leading to progressive weakness, muscle atrophy with eventual paralysis and death within 5 years of clinical onset. Approximately 10% of ALS cases are familial, the remainder ALS cases being diagnosed as sporadic (90%). The discovery a decade ago of missense mutations in the gene coding for the Cu/Zn superoxide dismutase 1 (SOD1) in subsets of familial cases directed most ALS research to elucidating the mechanism of SOD1-mediated disease. Unraveling the mechanisms of toxicity of SOD1 mutants has been surprisingly difficult. Nonetheless, many neuronal death pathways have been revealed through studies with transgenic mice expressing SOD1 mutants. Another key question in understanding ALS is to what extent cytoskeletal abnormalities such as intermediate filament (IFs) accumulations, a hallmark of the disease, actively participate in the neurodegenerative mechanism. Again, transgenic mouse approaches have been used to clarify the role of IF proteins in motor neuron death but with complex results. In addition, there is growing evidence that genetic defects in components of the microtubule-based transport might be implicated in degeneration of motor neurons. Here, we will review the mouse studies that contributed toward understanding the pathogenic pathways of motor neuron disease and the testing of therapeutic approaches. A list of various mouse models with motor dysfunction is shown in Table 1.

2. Mice expressing ALS-linked SOD1 mutants

2.1. Toxicity unrelated to copper-mediated catalysis

To date, 114 different mutations have been discovered in the SOD1 gene that account for ~20% familial ALS cases [1,2]. SOD1 is an abundant and ubiquitously expressed protein. Because of its normal function in catalyzing the conversion of superoxide anions to hydrogen peroxide, it was first thought that the toxicity of different SOD1 mutants could result from decreased free-radicals scavenging activity. However, different SOD1 mutants showed a remarkable degree of variation with respect to enzymatic activity. Mice expressing mutants $SOD1^{G93A}$ (glycine substituted to alanine at position 93) or $SOD1^{G37R}$ developed motor neuron disease despite elevation in SOD1 activity levels [3]. Moreover, SOD1 knockout mice did not develop motor neuron disease [4]. The conclusion from these combined results

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Table 1
Mouse models with motor neuron dysfunction

Mouse	Pathological changes	References
Protein misfolding		
Mutant SOD1	Loss of 70% spinal motor neurons IF and SOD1 aggregates	[5–7, 30,31]
Synuclein mutant	Perikaryal inclusions and spheroid-like inclusions of synuclein in motor neurons Inclusions also abundant in raphe nuclei and the pons	[119]
Intermediate filament disorganization		
hNF-H overexpressor	Perikaryal accumulations and axonal atrophy Altered conductivity but no neuronal loss	[60,61]
mutant NF-L	Perikaryal and axonal NF accumulations with trapped organelles in motor neuron and sensory neurons No degeneration of sensory neurons but massive degeneration of spinal motor neurons	[64]
NF-L−/−	Perikaryal accumulation of NF-M and NF-H Developmental loss of 20% motor neurons Altered nerve conductivity Mild sensorimotor dysfunction	[50,56,57]
Peripherin overexpressor	Age-dependent IF aggregates in perikarya and axons 40% loss of spinal motor neurons	[66,67,69]
Defects in microtubule-based transport		
Dynamitin overexpressor	Abnormal gaits and decrease in strength Axonal IF swellings 25% loss of motor axons at 16 months	[77]
KIF1B heterozygous	Staggering gait after 1 year of age	[73]
Knockout	Progressive muscle weakness	
KIF5A knockout	NF accumulations especially in sensory neurons Degeneration of sensory axons but not of motor axons after 5 months	[75]
Dynein mutations heterozygous	Progressive motor dysfunction Loss of 4 to 70% of motor neurons at 16 months	[78]
pmn mouse	Axonal swellings and early-onset motor neuron degeneration	[80,81]
Short tau overexpressor	Spheroidal inclusions containing tau, NFs and tubulin 20% loss of motor axons at 12 months	[86]
P25 overexpressor	Abnormal tau phosphorylation and axonopathy	[120]
Others		
VEGF ΔHRE	Late-onset motor dysfunction Loss of 40% motor axons at 7 months	[86]
Als2 knockout	Late-onset degeneration of cerebellar Purkinje cells	[97,98]

was that the mutations in SOD1 provoke a gain of new toxic properties. Unlike transgenic mice overexpressing the wild-type SOD1, the mice bearing the SOD1^{G93A}, SOD1^{G37R} or SOD1^{G85R} mutants developed a motor neuron disease with many pathological changes reminiscent of human ALS [5–7].

Both the nature of the toxicity of SOD1 mutants and the reason for the susceptibility of motor neurons are not fully understood. Initially, studies have focused on aberrant copper-mediated catalysis as potential source of toxicity. One hypothesis was that SOD1 mutations enhanced the ability of the enzyme to use hydrogen peroxide as substrate to generate toxic hydroxyl radicals that can damage cellular targets including DNA, protein and lipid membranes [8]. Another hypothesis was that the misfolding of SOD1 induced by mutations would allow the access of abnormal substrates such as peroxynitrite to the catalytic site leading to the nitration of tyrosine residues [9]. However, neither the peroxidase activity nor peroxynitrite hypotheses were supported by transgenic mouse studies. The absence of endogenous SOD1 or the addition of wild-type SOD1 did not affect disease progression in mice expressing mutant SOD1^{G85R} [10]. Moreover, the gene knockout for the copper chaperone for SOD1 (CCS) that delivers copper to SOD1 catalytic site had no effect on disease progression in mutant SOD1 transgenic mice [11]. Finally, transgenic mice overexpressing a mutant form of SOD1 lacking two of the four histidines residues coordinating the binding of the Cu at the catalytic site still developed motor neurodegeneration despite a marked reduction in SOD1 activity [12]. Thus, the combined studies with genetically altered mice indicate that SOD1 mutants cause motor neuron disease through the gain of a new function that is independent of the enzymatic activity involving the copper catalytic site.

2.2. Complex pathways to motor neuron degeneration

The most prevailing view is that the toxicity of SOD1 mutants is related to the propensity of mutant SOD1 to form noxious misfolded protein species and aggregates [10–14]. However, the toxicity of these protein aggregates is still poorly understood. Deleterious effects could result from the co-sequestering of essential cellular components and from overwhelming the capacity of the protein folding chaperones [15,16] and/or of ubiquitin proteosome pathway to degrade important cellular regulatory factors [17]. In cultured neurons, increasing levels of Hsp70 decreased formation of SOD1 mutant aggregates and toxicity [16]. However, increasing Hsp70 levels by ~10-fold did not affect disease pathology in mice expressing SOD1 mutants [18].

Studies on transgenic mice expressing various SOD1 mutants suggest that the motor neuron death pathway is complex and that it involves multiple cascades of events including oxidative damage, excitotoxicity, alterations in calcium homeostasis, caspase activation, changes in levels of Bcl-2, mitochondrial defects [19,20] and Fas transduction [21]. Analysis of mice expressing mutant SOD1^{G37R} also revealed a deregulation of Cdk5 and Cdk4 activities in the spinal cord [22,23], which are Cdks with possible involvement in apoptosis and neurodegeneration ([24,25]. However, recent studies do not support a role for Cdk5 in pathogenesis caused by SOD1 mutants. First, the knockout of p35 gene did not affect disease onset and progression in the SOD1^{G93A} mice [26]. Second, the elimination by gene targeting of the neurofilament NF-M and NF-H tail domains containing the Cdk5 phosphorylations sites conferred protection in SOD1^{G37R} mice, rather than deleterious effects anticipated from a loss of phosphorylation sink for Cdk5 activity [27]. One possible explanation for the benefit of removing the neurofilament tail domains may be an enhancement of anterograde transport [27] as defects in axonal transport have

been detected at early stages of disease in mice expressing mutant SOD1 [28,29].

Many transgenic mice have been generated in which ALS-linked SOD1 mutants of different biochemical properties were expressed. Based on mRNA expression levels established by Jonsson et al. [30], the rate of synthesis of mutant SOD1 in the widely used mouse strain SOD1^{G93A} with survival of approximately 130 days corresponds to 40 times the normal synthesis rate of mouse SOD1. For many other transgenic strains (G85R, D90A, G93Adl and G127X) with later onset disease, the synthesis rates correspond to approximately 20-fold the synthesis rate of endogenous SOD1 (Table 2). Thus, very high levels of mutant SOD1 mRNAs seem required for development of ALS-like phenotypes within the short life span of mice. Moreover, the life span of the ALS mice is inversely proportional to gene dosage. For example, in the SOD1^{G127X}, the survival times in hemizygous mice was twice as long as in mice homozygous for the transgene [30]. Yet, the steady-state levels of mutant SOD1 proteins in spinal cords may differ widely between transgenic mouse strains. The level of human SOD1 protein in the young G93A strain is of 17-fold higher than normal mouse SOD1 level, whereas the young G85R and G127X mice exhibit levels that are only 90% and 45% of the mouse SOD1 level [30]. Transgenic mice expressing the mutant SOD1^{L126Z} with mRNA levels comparable to other ALS mouse models also exhibited very low levels of mutant SOD1 proteins [31]. Such widely different steady state protein levels must reflect different stabilities and degradation of the various human SOD1 mutants. It is noteworthy that despite low human SOD1 protein levels in the young G85R, G127X and L126Z mice, their life span remains similar to the G37R or G93A mice that express similar human SOD1 mRNA levels but with much higher steady state protein levels at early stages. Nonetheless, the mutant SOD1 mice (G85R, G127X and L126Z) with low-steady state protein levels showed similar amounts of detergent-insoluble aggregates in the spinal cord at end-stage of disease [7,30,31]. The combined studies suggest that the motor neuron disease may be caused by long-term exposure to noxious misfolded mutant SOD1 species with propensity to aggregate. However, the exact mechanism of toxicity of the misfolded SOD1 species remains unknown.

2.3. Involvement of non-neuronal cell types

Pathological analysis of SOD1^{G93A} mice carried out at various ages at neuromuscular junctions, ventral root and spinal cord revealed that motor neuron pathology begins at the distal axon and progresses in a “dying back” pattern [32]. Thus, the SOD1^{G93A} mice show end plate denervation a long time before ventral root axons loss and motor neuron loss. The failure to detect mutant SOD1^{L126Z} proteins in distal nerve fibers of transgenic mice suggest that direct damage to axons by mutant SOD1 is not a requirement for such axonal dying back mechanism [31].

In addition to intrinsic motor neuron death pathways, there is now compelling evidence that non-neuronal cells might contribute to pathogenic process in mice expressing SOD1 mutants. In transgenic mice or rats expressing mutant SOD1, there is a reduction in levels of astroglial glutamate transporter EAAT2 that may provoke a glutamate-induced excitotoxicity [7,33]. Excess glutamate can cause neuronal death via abnormal activation of glutamate receptors, allowing Ca^{2+} entry into the cell and altering cytosolic free Ca^{2+} homeostasis. Moreover, microglial activation may be involved in the neurodegenerative process. Robust NF-κB activity and expression of proinflammatory cytokines and chemokines were detected by in situ hybridization within spinal cord in SOD1^{G37R} mice [34]. The chronic induction of innate immunity by intraperitonial injection of lipopolysaccharides (LPS) exacerbated disease by about 3 weeks in SOD1^{G37R} mice [35], suggesting that inflammation may contribute to neurodegenerative processes. Conversely, an attenuation of neuroinflammation by minocycline or COX-2 inhibitors extended the longevity of ALS mice [36,37].

To unravel what cell types produce the deleterious effects leading to motor neuron death, transgenic mice expressing SOD1 mutants under astrocyte- or neuronal-specific gene promoters have been generated. Expression of the SOD1^{G86R} mutation under the GFAP promoter produced astrocytosis but no motor neuron disease [38]. Recently, transgenic mice expressing a SOD1^{G37R} cDNA under the prion gene promoter were reported to develop motor neuron disease [39]. This demonstrates that expression of mutant SOD1 in the neuromuscular unit is sufficient to cause disease and that expression of mutant SOD1 in microglia is not a requirement to trigger disease. Surprisingly, neuron-specific expression of SOD1 mutants with NF-L or Thy1 gene promoters in mice did not induce motor neuron disease [40,41]. However, the possibility remained that the level of transgene expression during aging was below the threshold necessary to provoke disease. This concern has subsequently been addressed by the generation of chimeric mice comprised of mixtures of normal and SOD1 mutant expressing cells [42]. These chimeric mouse studies with SOD1 mutants have demonstrated that neurodegeneration is delayed or eliminated when motor neurons expressing mutant SOD1 are surrounded by healthy wild-type cells. Moreover, these studies show evidence of damage to wild-type motor neurons by surrounding cells expressing mutant SOD1. Such results emphasize the importance of a motor neuron milieu, but the mechanism by which the toxicity of mutant SOD1 may be transferred from one cell to another is still unclear.

2.4. Chromogranin-mediated secretion of SOD1 mutants

A search for proteins that interact with mutant SOD1 led to the recent discovery that chromogranins are interacting with mutant forms of SOD1, but not wild-type SOD1 [43]. The chromogranins, namely chromogranin-A (CgA) and chromogranin-B (CgB), are soluble, acidic glycophosphoproteins and are major constituents of secretory large dense-core vesicles (LDCV) in neurons and endocrine cells. The studies show that CgA and CgB, which are abundant proteins in motor neurons and interneurons, may act as chaperone-like proteins to promote secretion of misfolded SOD1 mutants. Moreover,

the results suggest that extracellular mutant SOD1 can induce microgliosis and motor neuron death. Such pathogenic mechanism in ALS based on toxicity of secreted SOD1 mutant would be consistent with findings that the disease is not strictly autonomous to motor neurons and that toxicity is transferable from one cell to another.

3. Mice with disorganized IFs

Neurofilament and peripherin proteins are two types of IFs detected in the majority of axonal inclusion bodies, called spheroids, in motor neurons of ALS patients [44,45]. Multiple factors can potentially cause the accumulation of IF proteins including deregulation of IF protein synthesis, proteolysis, defective axonal transport, abnormal phosphorylation, and other protein modifications. Evidence for neurofilament involvement in disease came from the discovery of codon deletions or insertion in the KSP phosphorylation domain of the neurofilament NF-H gene in a small number of sporadic ALS patients (~1% cases) [46] and from the report of mutations in the rod domain of the NF-L gene in cases of Charcot–Marie–Tooth disease type 2 [47,48]. Recently, a peripherin frameshift mutation has also been reported in a case of ALS [49].

3.1. IF gene knockout mice

In the past decade, genetic manipulation in mice has been used to address the role of neurofilament and peripherin proteins in neuronal function and disease. Mice knockout for any of the neuronal IF proteins, i.e., neurofilament proteins, peripherin or α-internexin, do not develop gross developmental defects or motor neuron disease [50–55]. Yet, IF deficiencies are not completely innocuous. The reduction in caliber of myelinated axons lacking NF-L was accompanied by 50% reduction in conduction velocity [56], a feature that would be very deleterious for large animal species. The NF-L null mice exhibited mild sensorimotor dysfunction and spatial deficits, but without overt signs of paresis [57]. Moreover, altered cytochrome oxidase activity in numerous hindbrain regions has been detected in the NF-L null mice [58]. Significant loss of motor axons has also been observed in the NF-L null mice [50] and in double NF-M/NF-H knockout mice [53,55].

In peripherin knockout mice, the number and caliber of myelinated motor and sensory axons in the L5 roots remained unchanged but there was a substantial reduction (~ 34%) in the number of L5 unmyelinated sensory axons, demonstrating a requirement of peripherin for the proper development of a subset of sensory neurons [59].

3.2. Neurofilament overexpressors

The overexpression in mice of any of the three wild-type neurofilament subunits alone can provoke the accumulation of neurofilaments in neuronal cell bodies [60,61]. For example, high-level expression of human NF-H proteins cause large perikaryal neurofilament accumulations. The sequestration of neurofilaments in cell body resulted in atrophy of motor axons and altered axonal conductances but without motor neuron death even in two-year old mice [63]. Surprisingly, overexpressing NF-L in NF-H transgenic mice reduced the perikaryal swellings and rescued the motor neuron dysfunction illustrating again the importance of subunit stoichiometry for proper neurofilament assembly and transport [62]. The proof that neurofilament abnormalities can induce neuronal death came from the expression of an assembly-disrupting NF-L transgene having a Leucine to Proline substitution near the end of the conserved rod domain [64]. Mice expressing this NF-L mutant at only 50% of the endogenous NF-L level exhibited within 4 weeks after birth a massive loss of motor neurons. However, the exact mechanism of toxicity of mutant NF-L is not yet fully understood. Increasing the levels of bcl-2 did not protect the large motor neurons from the toxicity of mutant NF-L [65].

3.3. Peripherin abnormalities

The sustained overexpression of wild-type peripherin in mice caused the selective loss of motor neurons during aging [66,67]. The onset of neuronal death was precipitated by the absence of NF-L, as revealed by cross-breeding of peripherin transgenic mice with NF-L knockout mice. This context is reminiscent of the findings in ALS in which there is a reduction of NF-L mRNA levels in affected motor neurons [68]. In addition, it induced formation of perikaryal and axonal IF inclusions resembling spheroids in motor neurons of human ALS. The toxicity of peripherin overexpression in mice may be related in part to the axonal localization of IF aggregates. This is supported by the rescue of peripherin-mediated disease in mice by the overexpression of NF-H transgene [69]. A reasonable explanation is that perikaryal sequestering of excess peripherin protein reduces the formation of deleterious axonal IF accumulations. Other mechanisms may also contribute to the toxicity of peripherin overexpression. In vitro studies have shown that dorsal root ganglion (DRG) neurons from peripherin transgenic embryos die when grown in a proinflammatory CNS culture environment rich in activated microglia [70], suggesting that peripherin aggregates might predispose neurons to deleterious effects of a proinflammatory environment. To investigate the role of peripherin in disease caused by SOD1 mutations, $SOD1^{G37R}$ mice that lack peripherin or that overexpress peripherin have been generated [71]. The excess or absence of peripherin did not affect the onset and progression of motor neuron disease in mutant SOD1 mice. Thus, it can be concluded that peripherin is not a key contributor of motor neuron degeneration associated with toxicity of mutant SOD1. Nevertheless, because mutations in SOD1 are responsible for only ~2% of all ALS cases, it remains possible that peripherin might contribute to motor neuron loss in ALS of other etiologies. Further support for the peripherin involvement in disease came from the findings of toxic peripherin splicing variant [72] and from the discovery of a frameshift mutation in the peripherin gene of a human ALS case [49]. This mutation in exon 1 predicts the production of a truncated protein of 76 amino acids of the peripherin head domain. Because this peripherin species lacks

coiled-coil sequences to enable self-assembly into IF structure, it is surprising that transfection experiments with the frameshift mutant *PRPH*^{228delC} in SW13 cells caused disruption of neurofilament network assembly. Future studies with transgenic mice bearing this PRPH frameshift mutant might provide new insights into the molecular mechanisms of motor neuron disease.

4. Mice with defects of microtubule-based transport

Axonal transport is essential to neurons because of the extreme polarity and size of these cells. In humans, spinal motor neurons may have axons of more than 1 meter in length. Most proteins must be synthesized in cell bodies and transported to nerve terminals through axonal transport. Various molecular motors, which are multi-subunit ATPases members of the kinesin family and dynein, move cargos along microtubules in the anterograde and retrograde directions, respectively. Impairment of axonal transport has recently emerged as a common factor in several neurodegenerative disorders. Mutations that disrupt either kinesin or the dynein complex cause impairment of axonal transport, blockade of membranous cargoes and axonal degeneration.

4.1. Kinesins

The creation of mice heterozygotes for disruption of the kinesin KIF1B gene provided the proof that defects in axonal transport can provoke neurodegeneration [73]. These mice showed defect in transporting synaptic vesicle precursors and they suffer from progressive muscle weakness similar to human neuropathies. This discovery subsequently led to the identification of a loss-of-function mutation in the motor domain of the KIF1B gene in patients with Charcot–Marie–Tooth disease type 2A [73]. In addition, missense mutations in the conventional KIF5A are responsible for a hereditary form of spastic paraplegia [74] and disruption of KIF5A gene in mice was reported to cause neurofilament transport impairment [75].

Table 2
Different steady-state protein levels in mice expressing various mutant SOD1 transgenes

Mouse strain	hSOD1 mRNA levels relative to mouse SOD1	Spinal cord protein levels relative to mouse SOD1 in young mice	Life span	References
In vivo stable SOD1 mutants				
G93A	40	17	124 days	[30]
G93Adl	20	8	253 days	[30]
D93A	20	20	407 days	[30]
G37R line 29	–	5	365 days	[6]
G37R line 42	–	12	154 days	[6]
In vivo unstable SOD1 mutants				
G85R	17	0.90	345 days	[7,30]
G127x	25	0.45	250 days	[30]
L126Z	high	low	210 days	[31]

4.2. Dynein

Dynein is a molecular motor involved in retrograde axonal transport of organelles along microtubules. Dynein activity requires association with dynactin, a multiprotein complex that activates the motor function of dynein and participates in cargo attachment [76]. The overexpression of the p50 subunit of dynactin, dynamitin, disrupts the dynein/dynactin complex thereby inhibits motor activity. Transgenic mice overexpressing dynamitin developed a late-onset and progressive motor neuron disease resembling ALS with neurofilamentous swellings in motor axons [77]. Other mouse mutants, called *legs at odd angles* (*Loa*) and *cramping 1* (*Cra1*) that arose by mutagenesis with N-ethyl-N-nitrosourea, were found to carry missense mutations the dynein heavy chain 1 gene [78]. The *Loa* and *Cra1* mice bearing heterozygous dynein mutations develop progressive motor neuron due to impairment in retrograde transport. The notion that impairment of retrograde axonal transport may play causative role in pathogenesis is further supported by the discovery of missense mutations in the dynactin/p150^{glued} cause a lower motor neuron disease in humans [79].

4.3. The pmn mouse

Mice that are homozygous for the *pmn* mutation develop a progressive caudio-cranial degeneration of their motor axons from the age of 2 weeks and die four to 6 weeks after birth. This autosomal recessive disease was discovered by spontaneous mutation in mice. Evidence for the importance of the axonal transport machinery in motor neuron disease came from the identification the gene mutation responsible for the *pmn* in the mouse. Two groups identified the *pmn* mutation as a Trp to Gly substitution at the last residue of the tubulin-specific chaperone (Tbce) protein [80,81]. The Tbce is essential for proper tubulin assembly and for the maintenance of microtubules in motor axons. This suggests that altered function of tubulin cofactors might be implicated in human motor neuron diseases.

4.4. Tau

Tau is a microtubule-associated protein involved in stabilization of microtubules. There are six tau isoforms that are derived from a single gene via alternative splicing of the primary gene transcript. Abnormalities of tau in human disease are known as tauopathies that include Alzheimer's disease (AD), frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP) and amyotrophic lateral sclerosis/parkinsonism–dementia complex of Guam [82]. Transgenic mice overexpressing the shortest tau isoform developed axonal degeneration of spinal neurons and motor weakness [83]. These tau transgenic mice are characterized by the presence of filamentous aggregates of hyperphosphorylated tau, not only in cortical and brain stem neurons, but also in spinal neurons [84]. The inclusions contain 10- to 20-nm tau-positive straight filaments. Gliosis has been detected in the spinal cord with degeneration of axons in ventral

roots. Neurofilaments are also associated with these aggregates, demonstrating that abnormalities in tau protein can directly affect neurofilaments. Breeding experiments with tau transgenic mice and NF-L knockout mice revealed an alleviation of tau-mediated disease by reducing the neurofilament content [85].

5. Mice with targeted disruption of hypoxia response element

Vascular endothelial growth factor is a cytokine crucial for angiogenesis. Targeted disruption in mice ($VEGF^{\delta/\delta}$) of the hypoxia response element sequence in the vascular endothelial growth factor gene resulted in severe motor deficits at 5–7 months of age with pathological changes resembling ALS such as neurofilament accumulations in the spinal and brainstem motor neurons [86]. The mechanism of disease is still unclear. It has been suggested that chronic vascular insufficiency and possibly a lack of VEGF neuroprotection might result in motor neuron degeneration. The $SOD1^{G93A}$ mice crossed with $VEGF^{\delta/\delta}$ mice die earlier because of more severe motor neuron degeneration [87]. Some studies found an association of that some human haplotypes in the VEGF upstream promoter sequence with risk of ALS [87,88] and there is evidence for a VEGF deregulation in response to hypoxia in patients with ALS [89].

6. The wobbler

The wobbler mouse in another model of motor neuron disease which has been extensively investigated [90]. The wobbler mice originated from a spontaneous mutation that is transmitted by an autosomal recessive gene *wr* mapping to chromosome 11. However, the exact genetic defect has not yet been identified. The symptoms of wobbler mice can be recognized early in the postnatal period. The disease is associated with the degeneration and loss of spinal motor neurons. Pathology of cortical motor neurons has also been reported [90]. The wobbler phenotype is characterized by perikaryal vacuolar degeneration and swelling of motor neurons, astrogliosis and microglia activation. There is evidence of dysfunctional mitochondrial respiration in the wobbler with decreased activity of complex IV in a manner similar to what has been reported in the spinal cord of patients with sporadic ALS [91]. Ubiquitin and hyperphosphorylated NF-H immunoreactivities have also been detected in cortical neurons of affected animals [92]. Moreover, increased expression of neurofilament NF-M protein has been observed in affected motor neuron in the wobbler mice [92]. To what extent the NF-M overexpression contributes to pathogenesis remains unknown. In future, it would be of interest to use the NF-M knockout mice to address this question.

7. Mice knockout for Als2

Deletion mutations were discovered in coding exons of a new gene mapping to chromosome 2q33, *ALS2* coding for Alsin, from patients with an autosomal recessive form of juvenile ALS (JALS), primary lateral sclerosis and infantile-onset ascending hereditary spastic paralysis (IAHSP) [93–96]. The ALS2 gene is ubiquitously expressed. It encodes a protein having guanine nucleotide exchange factor (GEF) homology domains which are known to activate small guanosine triphosphatase (GTPase) belonging to the Ras superfamily. The RCC1-like, DH/PH and VPS9 domains are GEF for small GTPase Ran (Ras-related nuclear), Rho (Ras-homologous member) and Rab5 (Ras-related in brain 5), respectively.

Als2 knockout mice have been reported recently by two groups [97,98]. These studies demonstrate that absence of Als2 does not produce a severe phenotype in mice. However, the studies by Cai et al. [97] showed that the Als2 null mice develop age-dependent deficits in motor coordination and primary motor cultured motor neurons lacking Als2 were more susceptible to oxidative stress. Whereas Cai et al. detected no neuropathological changes in their Als2 null mice, Hadano et al. [98] showed that Als2-null mice develop an age-dependent and slow progressive loss of cerebellar Purkinje Cells, a reduction in ventral motor axons during aging, astrogliosis and evidence of deficits in endosome trafficking. Our laboratory also generated a mouse knockout for *Als2* using a targeting vector by replacing exon 2 and part of exon 3 with a 1.1 kb Neo cassette (Kriz J, Millecamps S, Urushitani M, Zhu Q and Julien JP, unpublished results). In agreement with the two other groups, our Als2 knockout mice developed only mild motor dysfunction as determined by rotarod test. Nonetheless, electron microscopy (EM) provided evidence of pathological changes. In 18-month-old Als2 null mice, EM revealed the presence of degenerating axons in corticospinal tracts (Fig. 1). So, it is anticipated that the

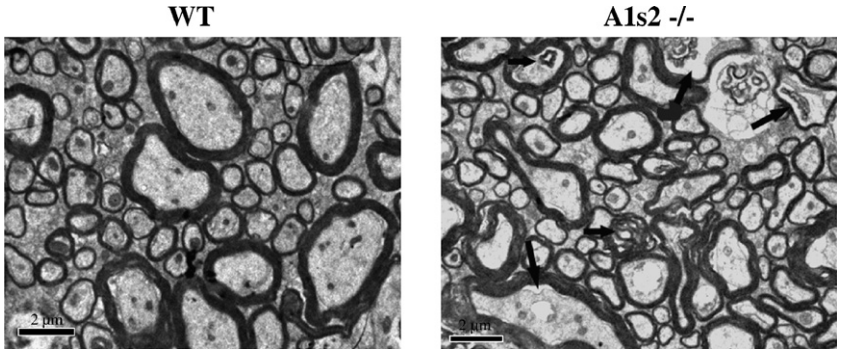

Fig. 1. Electron microscopy reveals degenerating axons (arrows) in corticospinal tract of Als2 null mice (Als2−/−) as compared to a normal mouse (WT) at 18 months of age.

Alsin knockout mice derived from different laboratories might be useful to investigate some aspects of Als2 functions in endosomal trafficking and the long-term vulnerability of large neuronal subsets to degeneration.

8. Testing therapeutic strategies in mice expressing mutant SOD1

Presently, there is no effective pharmacological treatment for ALS. Transgenic animal models that exhibit many of the pathological changes in human ALS provide useful tools for drug testing. Table 3 lists some of the genetic manipulations and drugs tested in mice expressing SOD1 mutants. Many of the pharmacological approaches tested so far have produced only modest beneficial effects. Vitamin E, gabapentin and salicylate had no effect on survival of SOD1^{G93A} mice [99–101]. Riluzole, a glutamate antagonist and the only drug currently approved for ALS treatment, extended the life span of SOD1^{G93A} mice by 10–15 days without affecting disease onset. More neuroprotection was provided in SOD1^{G93A} mice by the intra-cerebroventricular administration of N-Benzyloxycarbonyl–Val–Ala–Asp–fluoromethylketone (zVAD-fmk), a broad caspase inhibitor [102]. Celecoxib treatment significantly delayed the onset of weakness and weight loss and prolonged survival by 25% [37]. Celecoxib is an inhibitor of cycloxygenase 2 (COX-2), an enzyme that plays a role in inflammatory processes and in production of prostaglandins that can stimulate the release of glutamate from astrocytes.

Table 3
Therapeutic interventions in animals expressing mutant SOD1

	ALS mouse	Increased life span	References
Pharmacologicgal Treatments			
Creatine (diet)	SOD1^{G93A}	20 days	[115]
Riluzole (diet)	SOD1^{G93A}	10–15 days	[100]
Gabapentin (diet) SOD1^{G93A}		no effect	[99]
Vitamin E (diet) SOD1^{G93A}		no effect	[99]
Lysine Acetyl-salicylate (diet)	SOD1^{G93A}	no effect	[130]
Minocycline (diet)	SOD1^{G37R}	21–35 days	[36,103,104]
	SOD1^{G93A}	11–21 days	
Cocktail of Minocycline-riluzole-nimodipine	SOD1^{G37R}	42 days	[105]
Ceftriaxone	SOD1^{G93A}	10 days	[106]
Ginseng	SOD1 ^{G93A}	7 days	[121]
zVAD-fmk (i.c.v.)	SOD1^{G93A}	27 days	[102]
SOD1 injection	SOD1^{G93A}	24 days	[122]
AOL 10150	SOD1^{G93A}	20 days	[107]
Transgene overexpression			
Human NF-H	SOD1^{G37R}	2–4 months	[125]
Bcl-2	SOD1^{G93A}	30–35 days	[126]
Dominant inhibitor of caspase-1	SOD1^{G93A}	27 days	[127]
Gene knockout			
NF-L−/−	SOD1^{G85R}	6 weeks	[22,128]
	SOD1^{G37R}	10–15 weeks	
Tailess NFH and NFM	SOD1^{G37R}	8 weeks	[27]
Peripherin −/−	SOD1^{G37R}	No change	[71]
nNOS−/−	SOD1^{G93A}	No change	[129]
Il-1beta −/−	SOD1^{G37R}	No Change	[34]
CCS−/−	SOD1^{G85R}	No change	[11]
	SOD1^{G37R}		
	SOD1^{G93A}		
Viral gene therapy			
IGF1	SOD1^{G93A}	37 days	[110]
GDNF	SOD1^{G93A}	25 days	[123]
VEGF	SOD1^{G93A}	40 days	[111]
Cardiotrophin	SOD1^{G93A}	27 days	[124]
RNAi	SOD1^{G93A}	100 days	[112–114]

Minocycline, a second-generation tetracycline with anti-inflammatory properties, has been shown to increase survival in at least three independent laboratories, using two different mutant SOD1 mouse lines, and using various modes of drug delivery [36,103,104]. Minocycline may confer neuroprotection by multiple pathways as it can reduce microglial activation, caspase-1, caspase-3, iNOS, p38 MAP kinase and mitochondrial cytochrome *c* release. Moreover, the addition of riluzole and nimodipine further enhanced the effect of minocycline on survival [105]. Minocycline is a clinically well-tolerated drug. There is an on-going human trial to test the effectiveness of minocycline in ALS. Another antibiotic, the beta-lactam antibiotic ceftriaxone, can confer certain protection in mouse model of ALS presumably through elevation of expression of glutamate transporter EAAT2 (also called GLT-1) [106]. An elevation in EAAT2 would attenuate glutamate neurotoxicity. A clinical trial is also in progress to test the efficacy of ceftriaxone in human ALS. Another compound that can catalytically decompose oxidants as peroxynitrite, the manganese porphyrin AEOL 10150, was found to extend the survival of SOD1G93A mice by approximately 20 days when administered intra-peritoneally or subcutaneously at symptom onset [107].

Apoptotic pathways might represent targets for disease intervention in ALS. Thus, many neurotrophic factors such as IGF-1, GDNF, CNTF and VEGF might confer protection to motor neurons. The failure of neurotrophic factors in human trials so far may be due in part to the limited delivery of the proteins to the target neurons. Recently, the intracerebroventricular delivery of recombinant VEGF was found to delay disease onset and to extend survival in a SOD1^{G93A} rat model [108]. Another approach for treatment of motor neuron disease that may be considered for future would involve the delivery of viral vectors to mediate expression of either growth factors such GDNF, IGF-1 and VEGF [109–111]. Another strategy for treatment of familial ALS cases would be to use viral vectors encoding RNAi molecules to target SOD1 mRNA for degradation [112–114]. Remarkably, SOD1 silencing with lentiviruses almost doubled the longevity of mice expressing SOD1^{G93A} mutant [112].

Yet, mice are not humans and sometimes therapeutic approaches that confer benefits with mouse models might fail in a human trial. A good example is creatine, a compound believed to improve mitochondrial function. Creatine administered in drinking water was found to extend the longevity of mice expressing mutant SOD1 [115,116]. However, a clinical trial of creatine was found to be ineffective in human ALS [117]. So, the results in mouse models should be interpreted with caution. Many factors must be considered including pharmacokinetics, as well as routes and timing of drug administration. There is also the question of validity of the mutant SOD1 mice as models of gene defects that account for only 2% of ALS cases.

9. Future directions

Despite important effort devoted in the past decade toward elucidating the mechanism of disease caused by SOD1 mutations, the neurodegeneration mechanism is still not fully understood. The SOD1 mutants cause disease through acquisition of toxicity. Yet, it is not resolved how SOD1 mutants can trigger through protein misfolding some death pathways selectivity in neuronal subsets. There is evidence that pathogenic SOD1 mutants can form pore-like oligomeric structures [131]. Whether mutant SOD1 aggregates can permeabilize membranes through formation of ion channels is an intriguing possibility that merits to be considered.

In light of the emerging evidence for a crucial role of axonal transport in motor neuron disease, there is a need to clarify the cytoskeletal changes associated with human ALS. The abnormal IF accumulations, a hallmark of ALS, emerged as intrinsic factors that may affect the disease either negatively or positively. Yet, the mechanisms underlying the formation and neurotoxicity of IF accumulations are not fully understood. Disorganization of IF network could result from a variety of primary causes including neurofilament gene mutations, deregulation of IF gene expression, post-translational modifications and axonal transport defects. Motor neuron loss has been observed in some transgenic mouse models exhibiting an axonal localization of IF swellings supporting the view of "axonal strangulation" disease model by which IF swellings can block axonal transport [2,3]. Other toxic mechanisms may also be involved. For instance, in vitro culture studies suggest that peripherin aggregates can predispose neurons to apoptotic death induced by a proinflammatory CNS environment [70]. Thus, the presence of IF aggregates may sometimes lower the threshold to neuronal death in a toxic environment.

A key role of axonal transport in pathogenesis of motor neuron disease was recently supported by the inhibition of molecular motors in transgenic mice. Therefore, studies on the neuronal cytoskeleton and molecular motors of the microtubule-based transport might offer promising research avenues to understand the selective vulnerability of motor neurons to disease.

Although intracellular dysfunction such as axonal transport defects may cause motor neuron degeneration, there is evidence that the disease is not strictly cell autonomous in mutant SOD1 models. Chimeric mouse studies suggested that non-neuronal cells are involved in the disease process but the molecular mechanisms in various cell types that contribute to motor neuron death remain to be elucidated [42]. Recent findings demonstrated that chromogranins can interact selectively with mutant forms of SOD1 to promote secretion of these molecules [43]. Extracellular mutant SOD1 and chromogranin A can trigger microglial activation and death of motor neurons in culture. If extracellular SOD1 mutant contributes to pathogenesis, there is a rationale to develop approaches for clearance of extracellular SOD1 mutant. Moreover, in mutant SOD1 mice, there is an enhanced signal for chromogranin A in activated astrocytes. It has been suggested that a potential implication of enhanced chromogranin levels is that the N-terminal peptides of chromogranin A can act on blood vessels as antiangiogenic agents [118]. Perhaps, this action would be counteracted with VEGF treatment. Whether alteration in the vasculature of spinal cord contributes to ALS pathogenesis is another question that remains to be explored. In future, it will be of interest to generate mutant SOD1 mice in context of deficiency or excess of chromogranin expression.

Another question is whether results of drug testing in ALS mouse models are predictive of human outcomes. Is the widely used $SOD1^{G93A}$ mouse strain a valid pre-clinical model for drug testing? The synthesis rate of human SOD1 mutant in this mouse corresponds to 40 times the synthesis of endogenous mouse SOD1. It has been proposed that extreme levels of mutant SOD1 proteins in such mouse models can produce artifacts such as vacuoles, which may not be relevant to human ALS pathogenesis [30]. Perhaps, it would be more appropriate to consider testing of therapeutic approaches and potential drugs in experimental models with late onset of disease that mimic better the human situation, i.e., with lower gene copy number encoding the mutant SOD1 proteins. For example, overexpression of a human NF-H transgene extended the life span by several months in mice from line 29 overexpressing $SOD1^{G37R}$ by 5-fold but these same the hNF-H transgene had little effect in mice of line 42 overexpressing SOD^{G37R} by 12-fold [22].

In view of the complexity of the disease, a combination of different therapies acting in synergy will probably be needed for effective ALS treatment. In a near future, new strategies might include a search for agents that can prevent the abnormal aggregation of proteins, mutant SOD1 or IF proteins. As more associated genes are discovered, new therapeutic approaches could potentially be derived. Gene therapy approaches involving the use of recombinant viruses offer a promising strategy for the delivery of genes to enhance motor neuron survival or to silence specific deleterious genes such as mutant SOD1. The next few years should also provide some perspective on the potential of neural stem cells to replace or to repair damaged neurons. Finally, a better understanding of ALS pathogenesis through the use of animal models and the development of efficient therapeutics will require the discovery of new genes and biomarkers associated with the disease, especially for sporadic ALS cases.

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