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The "Dying-Back" Phenomenon of Motor Neurons in ALS

Michal Dadon-Nachum · Eldad Melamed · Daniel Offen

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Abstract Amyotrophic lateral sclerosis (ALS) is a lethal disease, characterized by progressive death of motor neurons with unknown etiology. Evidence from animal models indicates that neuronal dysfunction precedes the clinical phase of the disease. However, in parallel extensive nerve sprouting and synaptic remodeling as part of a compensatory reinnervation processes and possibly also of motor neurons pathology was demonstrated. Therefore, the weakness in muscle groups will not be clinically apparent until a large proportion of motor units are lost. This motor unit loss and associated muscle function which precedes the death of motor neurons may resemble the "die-back" phenomena. Studies indicated that in the early stages the nerve terminals and motor neuron junctions are partially degraded while the cell bodies in the spinal cord are mostly intact. Treatments to rescue motor neurons according to "dying-forward" model of motor neuron pathology in ALS have shown only limited success in $SOD1^{G93A}$ transgenic mice as well as in humans. If cell body degeneration is late compared with axonal degeneration, early intervention could potentially prevent loss of motor neurons. Therefore, it should be considered, according to the dying back hypothesis, to focus on motor neurons terminals in order to delay or prevent the progressive degradation.

Keywords Amyotrophic lateral sclerosis · Motor neuron · Dying back

M. Dadon-Nachum · E. Melamed · D. Offen (✉)
The Neuroscience Laboratory, Felsenstein Medical Research Center, Rabin Medical Center,
Petah Tikva 49100, Israel
e-mail: danioffen@gmail.co

M. Dadon-Nachum · E. Melamed · D. Offen
Sackler School of Medicine, Tel-Aviv University,
Tel Aviv, Israel

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, was first described in 1869 by a French neurologist, Jean Martin Charcot. The disease is characterized in a progressive degeneration of upper and lower motor neurons. The death of the upper motor neurons, found in the motor cortex in the brain, leads to spasticity, hyperexcitability of reflexes and the appearance of pathological reflexes, such as the Babinski sign. The death of the lower motor neurons, which are found in the brain stem and in the spinal cord, leads to weakness and atrophy of the muscles followed by progressive paralysis (Mohajeri et al. 1999; Acsadi et al. 2002; Bruijn et al. 2004; Séverine et al. 2006; Aguilar et al. 2007; Lev et al. 2009; Offen et al. 2009).

ALS has a worldwide prevalence of 1–2 per 100,000, and death occurs within 3–5 years of the onset of the disease, due to respiratory failure. ALS occurs mainly in adults (45–60 years of age), and most cases are sporadic, although 5% to 10% of ALS cases are inherited in an autosomal dominant pattern of which about 20% are caused by a mutation in the Cu/Zn superoxide dismutase (SOD1) gene on chromosome 21. The etiology of sporadic ALS is unknown, although it is generally believed that sporadic and familial ALS may share pathological mechanisms (Mohajeri et al. 1999).

The pathophysiology of the disease includes a reduced secretion of neurotrophic factors, protein aggregations, malfunctioning of the mitochondria, rupture in the axonal passage, destruction in the calcium metabolism, changes in the skeletal proteins, high levels of glutamate and oxidative damage (Bruijn et al. 2004; Ilieva et al. 2007; Lev et al. 2009). Preventing or slowing down motor neuron degeneration and death in ALS are critical goals of future therapies, as are means of enhancing axonal regeneration (Fig. 1).

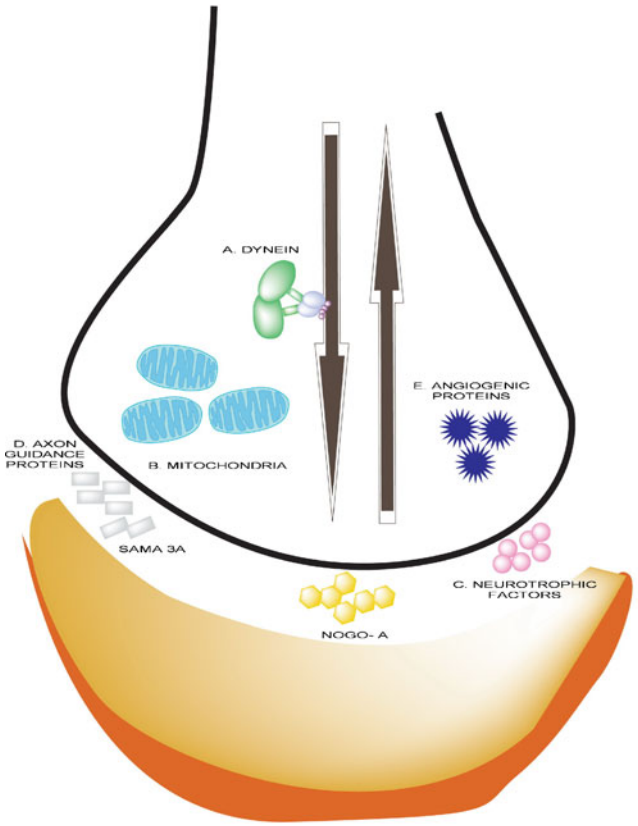

Fig. 1 Possible mechanisms of the dying-back phenomenon. **a** Disruption to the dynein activity leads to impaired retrograde transport. This disruption might be results of several factors such as aggregation of the mutant SOD1 which interfere with the dynein–dynactin complex or overexpression of dynamitin subunit of dynactin can interrupt dynein activity. **b** Enlarged mitochondria at the NMJ reasons are dysfunctional and as consequence synapses are lost. **c** Reduced growth factors signaling can cause to end plate abnormalities. **d** Overexpression of axon guidance proteins such as Sema-3A and Nogo-A can cause inappropriate repelling of the motor axon from the NMJ. **e** Mutations in angiogenic proteins inhibit neurite outgrowth and negatively affect motor neuron survival

In recent years, accumulating data on genetic causes of ALS is emerging. This genetic information has helped develop animal models for the study of ALS, enabling the elucidation of the pathogenetic mechanisms operating in the disease. The most commonly used rodent model of ALS exploits the G93A mutation, in which the amino acid glycine is exchanged against alanine at position 93 of human SOD1 ($SOD1^{G93A}$ transgenic mice). These mice imitate much of the human ALS phenotype as they lose motor neurons, develop progressive paralysis and die at 4–5 months of age (Mohajeri et al. 1999; Gruzman et al. 2007; Turner and Talbot 2008; Lev et al. 2009).

The identification of the initial mechanisms leading to the progressive loss of motor neurons in ALS is crucial, especially in reference to the estimated numbers and sizes of motor units, for several reasons. Firstly, there is growing evidence indicating that weakness in a particular muscle group may not be clinically apparent until a large proportion of motor units are lost. Therefore, it may be important to monitor changes in the motor unit population prior to the onset of weakness. This kind of data will provide valuable insights into possible early detection. Secondly, this information might be crucial for early treatment and to the understanding of the mechanisms of new drugs treating the disease (Felice 1997; Durand et al. 2006).

The functioning and plasticity of the peripheral neural system depends on the specific properties of its components. Distinct subtypes of neurons and their particular functions have been best characterized in motor pools, the groups of motor neurons that innervate one defined skeletal muscle. Each muscle unit (one muscle and its motor pool) consists of a defined set of functionally distinct motor units (one motor neuron and the muscle fibers it innervates). In diseases that target motor neurons, extensive nerve sprouting and synaptic remodeling occurs as part of compensatory reinnervation processes and possibly also of motor neuron pathology. Evidence from animal models indicates that, in these diseases, neuronal dysfunction precedes the clinical phase of the disease, but the mechanisms underlying disease progression still need to be defined (Frey et al. 2000).

In this review, we will try to describe and discuss the "dying-back" hypothesis in ALS. According to the dying-back theory, pathological changes in motor axons and nerve terminals appear to precede motor neuron degeneration and clinical symptoms. Moreover, it raises the possibility that disease process my start distally at the nerve terminal or at the neuromuscular junction (NMJ) and progress towards the cell body.

Presymptomatic Detection of Pathological Changes in Motor Neurons Terminals

"Dying back" or slowly evolving distal to proximal axonal degeneration is a common pattern seen in a wide variety of degenerative and toxic conditions of the central and peripheral nervous system (Fischer et al. 2003). Many animal and human studies have dealt with the problematic issue of pathological observations without correlation with disease stages. This phenomenon leads to limited information regarding the mechanisms of disease progression. Several animal models of motor neuron disease have been characterized as "dying-back" neuropathies. In the ALS and the progressive motor neuropathy mouse models, morphological analysis of the NMJ show synaptic weakening and "dying back" prior to the manifestation of clinical symptoms (Frey et al. 2000).

Parkhouse et al. (2008) showed that diminished retrograde uptake and transport precedes the onset of debility in

SOD1^{G93A} transgenic mice. Fischer et al. (2003) examined the cell body, axon and NMJ at multiple time points to illustrate the spatiotemporal progression of motor neuron pathology in the SOD1^{G93A} transgenic mouse. They related their findings to the clinical onset of weakness in these animals. Although signs of clinical disease were recognized at about 80 days, quantitative analysis demonstrated denervation at the NMJ by day 47, followed by severe loss of motor axons from the ventral root between days 47 and 80, and loss of α motor neuron cell bodies from the lumbar spinal cord after day 80. This pattern implies that motor neuron disease in SOD1^{G93A} transgenic mouse is actually a "dying-back" motor neuropathy where distal axon degeneration occurs early during the disease, before neuronal degeneration, and onset of symptoms (Fischer et al. 2003). They also found that the longest and largest nerve fibers with the highest metabolic demand seemed to be the most susceptible to dying back. Thus, it has been suggested that distal axonal degeneration represents a size dependent "undernourishment" of the most distal region of the axon. At the same study, neuropathological analysis of a 58-year-old ALS patient who died unexpectedly, demonstrated grouped atrophy and fiber-type grouping in skeletal muscles from the legs and thorax. Ventral roots at all levels showed postmortem autolytic changes but little axonal degeneration, and all levels of the spinal cord appeared normal. Astrocytic and microglial activations were not prominent at any level, while the corticospinal tract degeneration was not noted, and motor cortex appeared normal. This case, though anecdotal and non-quantified, showed a similar pattern of disease with prominent evidence of motor neuron degeneration only in muscle (Fischer et al. 2003).

Hegedus et al. (2007) used EMG in order to record whole muscle and motor unit isometric contractile forces from both fast and slow twitch muscles in the SOD1^{G93A} mutant mouse. They explored the time course of loss of functional motor units and whether a differentiation in the loss of function of fast versus slow twitch muscles occurs. They found a significant decline in both the whole muscle contractile force and the number of functional motor units from fast but not from slow twitch muscle, 50 days before the onset of clinical symptoms. The loss of motor units from the fast twitch hind limb muscled was initially quick and then reached a plateau in the symptomatic phase of the disease. Their results supported immunohistochemical evidence indicating that denervation proceeds in a muscle specific manner, with faster muscle fibers denervating before slower ones.

Felice et al. (1997) compared the number of motor unit estimates between different functional tests in a longitudinal study of 21 ALS patients over a 12-month period. Their results showed early changes in the estimated numbers and sizes of motor units as recorded in a single muscle group, long before onset of clinical symptoms. They hypnotized that a possible explanation for this result may relate to the sequential pathological changes occurring in motor units. Moreover it was postulated that motor neuron end plates are lost prior to the onset of weakness in ALS. Initially, the reinnervation of orphaned muscle fibers via the collateral sprouts of remaining axons is fully compensatory and therefore there is no change in muscle strength. At some critical point for each muscle group, the compensatory process of reinnervation is unable to compensate for denervation and muscle weakness becomes apparent (Felice. 1997; Hayworth and Gonzales-Lima 2009).

The evaluation of disease progression and treatment efficacy in ALS patients and in animal models of the disease has traditionally relied on forced exercise tasks. However, this battery of tests has proven insensitive to detecting motor deficits prior to the onset of overt clinical symptoms, despite evidence that has demonstrated early motor system defects in presymptomatic SOD1^{G93A} transgenic mice. Hence, it is extremely important to focus on parameters in the NMJ region, where first signs of the disease can be observed at its earliest stages.

Mechanisms Underlying the "Dying-Back" Phenomenon

There are many possible explanations for the "dying-back" pattern seen in the in SOD1^{G93A} transgenic mouse and ALS patients. Over the years, several possibilities for the delayed clinical phenotype and especially for the "dying-back" hypothesis have been suggested. Some of the main theories which have been tested will hereby be described.

One of the earliest explanations suggested for the phenomena was investigated by Rotestein et al. (1993). In this work, the authors argued that the dying-back pattern might be due to a sublethal insult to the cell body that results in undernourishment of the distal axon. Accumulation of insoluble complexes of mutant SOD1 protein or chronic glutamate toxicity could be responsible for insufficient maintenance of the distal axon, leading to early denervation at the NMJ, while the cell body remains structurally intact.

Later, this theory was expanded and further studied, and Zhang et al. (1997) described an early accumulation of abnormal neurofilaments in SOD1^{G93A} transgenic mice, leading to the slowing down of the anterograde and retrograde axonal transport. Since one of the major functions of the neurofilaments is to provide mechanical support, disruption of the neurofilament network might play an early role in the degeneration of motor neurons (Zhang et al. 1997; Williamson and Cleveland. 1999).

Recent evidence regarding the critical role of retrograde axonal transport in the maintenance of motor neurons has emerged from the study by LeMonte et al. (2002). Transgenic mice over expressing the dynamitin subunit of dynactin, a protein that regulates dynein activity, developed late-onset, progressive motor neuron disease due to the inhibition of retrograde transport.

The dynactin–protein complex is required for dynein mediated retrograde axonal transport of vesicles and organelles along the microtubule system. It provides the link between the specific cargo, the microtubule, and cytoplasmic dynein during vesicle transport. (Shaw 2005). Therefore, both in mice and humans, axonal abnormalities that inhibit retrograde transport confer selective damage to motor neurons, possibly by preventing the delivery of target-derived neurotrophic factors back to the cell body (Fischer et al. 2004).

A different approach to the dying-back hypothesis has focused on the functional subtypes of motor units. Muscle fibers express characteristic type-specific muscle protein isoforms, each having its unique functional role in muscle activity. Thus, motor units represent functionally distinct subunits of the peripheral motor system. Frey et al. (2000) reported that synapse types that fail to exhibit terminal sprouting are selectively vulnerable, whereas synapses undergoing robust paralysis-induced sprouting are selectively resistant. They proposed a dying-back model in which the clinical features of motor neuron diseases are determined by the sensitivity of peripheral neuromuscular synapses to neuronal dysfunction. These synapses on fibers which are mostly plastic are particularity resistant, while those on fibers which are less competent to sprout are the most vulnerable. Differential sprouting in the target region may ensure that, under conditions of partial dysfunction, essential muscle functions associated with the maintenance of posture and motor coordination are conserved at the expense of muscle force (Frey et al. 2000).

Several other studies have discussed the selective loss of functional motor units as an explanation for the pattern of disease seen in ALS patients and animal models. The main idea is that the death of the neuronal motor axon, which starts from the NMJ, may be linked to the characteristics that distinguish motor neurons innervating muscle fibers expressing a slow type isoform from those expressing fast type isoforms. According to the Henneman size principle, motor neurons that innervate fast motor units are those with the largest innervation ratios, soma sizes and axon calibers (Henneman and Mendell 1981; Gordon et al. 2004). Smaller motor neurons, which are recruited first under physiological conditions, innervate the smallest and slowest motor units. It has been shown that axons, in $SOD1^{G93A}$ transgenic mice and in human patients, are susceptible in a caliber-specific order, with the largest caliber axons being the most susceptible to loss, (Feinberg et al. 1999; Bendotti et al. 2001; Fischer et al. 2003).

Hegedus et al. (2007) provided further supporting evidence that motor unit type, and the associated differences in innervation ratio, axon caliber, and soma size appear to have a role in the specific vulnerability of motor neurons. Hence, larger soma size and axon diameter appear to be important determinants of differential motor neuron susceptibility and their results corroborate the hypothesis that implicates soma size and metabolic demands in the preferential susceptibility of motor neurons.

Pun et al. (2006) provided insights into the mechanisms of selective vulnerability in ALS. Selective axonal vulnerability might involve the selective primary damage to subtypes of axons or failure by vulnerable axons to compensate for disease related burdens. Their results provide evidence that in $SOD1^{G93A}$ transgenic mice, fast firing axons in the hind limb are affected selectively, synchronously and early on, leading to an abrupt loss of their peripheral synapses. In contrast to phasic motor neurons, axons of slow firing motor neurons are particularly resistant to disease: they compensate efficiently and maintain expanded motor units up to the final phase of disease. However, delayed regeneration after nerve crush and a slowly progressing reduction in innervation of this subtype suggest that these motor neuron axons are also vulnerable to disease, albeit to a much smaller extent. According to their results, it can be concluded that the onset of the clinical phase coincides with the weakening and loss of fast firing innervations, and the progressive weakening of slow motor neuron axons determines clinical progression during the end phase of disease. This study identifies physiological subtypes of related neurons and axons differing in their vulnerability to disease, suggesting that differences among these neurons are critically important to disease progression. The selective vulnerability of fast firing and fast fatigue resistant motor neuron axons is consistent with clinical observations in ALS patients that slow motor units tend to be spared and expand in disease. Fast firing motor neuron axons have the largest diameter and innervate the greatest numbers of muscle fibers among α motor neurons. These large sizes might result in higher demands on axonal transport and higher vulnerability to its dysfunction (Pun et al. 2006).

Another explanation of the dying-back pattern has been raised by Bendotti et al. (2001). In this study, enlarged mitochondria were observed in the distal part of the motor axons of the phrenic nerve and in the large axons of the sciatic nerve of $SOD1^{G93A}$ transgenic mice at the presymptomatic stage of the disease. Supporting data comes from a study in which mitochondrial enlargement in the motor nerve terminals of sporadic ALS patients in the early stages, was observed (Siklos et al. 1996).

A recent study checked biochemical changes in the hind limb muscle of young presymptomatic $SOD1^{G93A}$ transgenic mice. In this work, it was demonstrated that cdk5 activity was reduced at the early stages of the disease. Cdk5 is a proline-directed serine/threonine kinase that is involved in numerous cellular functions in the central nervous system. It is also implicated in various aspects of muscle function such as acetylcholine receptor clustering and myogenesis. Cdk5 activity is also upregulated in the muscle following denervation. These results suggest the presence of altered muscle function in very young, presymptomatic $SOD1^{G93A}$ mice (Park and Vincent 2008).

A different view has been offered by Pasterkamp and Giger (2009) who hypothesized that aberrant function or expression of axon guidance proteins contributes to the early pathological changes in motor neuron connectivity of ALS. Axon guidance proteins influence axonal transport and synaptic function. A direct relationship between axon guidance proteins and ALS has recently been reported for Sema3A. An increase of Sema3A in a specific subset of muscle fibers known as fast fatigable muscle fibers was found in $SOD1^{G93A}$ transgenic mouse versus wild type mouse. The precise role of Sema3A in the pathogenesis of ALS is unclear but its increased expression might lead to the repulsion of motor axons away from the NMJ, eventually resulting in axonal denervation and motor neuron degeneration. When Sema3A is secreted by non-neuronal cell aggregates, it acts as a chemorepellent for motor axons. Sema3A secreted by terminal Schwann cells may adhere to basal lamina associated molecules at the NMJ and restrict the growth permissive properties of the synapse microenvironment. De Winter et al. (2006) observed higher expression of Sema3A in Schwann cells' terminals in presymptomatic versus symptomatic $SOD1^{G93A}$ mice. Thus, Sema3A can act directly on motor nerve terminals during presymptomatic stages and induce the retraction of these terminals from the NMJ before the motor neuron death occurs.

One more axon guidance protein which is associated with ALS is Nogo-A. Nogo-A is predominantly expressed in embryonic and early postnatal skeletal muscles. In adults, however, the expression levels of Nogo-A decreases. It was found that Nogo-A expression is upregulated in the skeletal muscle of both ALS patients and $SOD1^{G93A}$ mice at the early stages of the disease, before the onset of clinical symptoms. This upregulation correlates with the severity of the disease and is most robust in slow-twitching fibers, when almost all Nogo-A-immunoreactive fibers are atrophic (Jokic et al. 2005).

Recently, ALS patients have been found to have mutations in the code of the Angiogenin gene (ANG). ANG has a variety of functions including neovascularization and axon guidance; it regulates neurite extension and path finding, while mutations, such as those found in ALS patients, cause ANG to inhibit neurite outgrowth and negatively affect motor neuron survival. At present, the molecular mechanisms underlying the degenerative effect of mutant ANG are unknown. It was hypothesized that axonal sprouting and regeneration are processes ongoing throughout life and may be of extra importance to motor neurons due to their high level of activity and long axonal projections, making them more prone to axonal damage. Impaired axonal regeneration or sprouting due to mutant Angiongenin may therefore lead to a gradual loss of motor axons and consequently, motor neurons (Schmidt et al. 2009).

Vascular endothelial growth factor (VEGF), another angiogenic protein, was also suggested to contribute to the pathogenesis of ALS (Lambrechts and Carmeliet 2006). Oosthuyse et al. (2001) linked VEGF to ALS, when a motor neuron disease phenotype developed in mice that were genetically deficient for the hypoxia response elements of the VEGF promoter. Since this observation, mounting evidence has indicated a direct role for VEGF as a neuroprotective factor, independent of its effect on angiogenesis that attenuates the phenotype of $SOD1^{G93A}$ mice. The precise role of VEGF in the pathogenesis of ALS is poorly understood. However, results from a recent study demonstrate that mutant SOD1 protein might be involved in destabilizing VEGF mRNA, thereby reducing VEGF expression (Lu et al. 2007).

Accumulating data from new studies highlight novel theories and examine various directions to explain the dying-back hypothesis. Many possible mechanisms have been suggested in an attempt to improve the time of detection and in its wake, the onset of treatment. These highly significant investigations pave the way for revealing the different factors underlying the causes of the disease.

Muscle-Targeted Treatments

The potential role of muscle in ALS is highlighted by the therapeutic benefit of muscle-targeted treatments. The main therapeutic strategy for ALS directed to the dying-back hypothesis is the muscle expression of neurotrophic factors. Neurotrophic factors are small, naturally occurring polypeptides that support the development and survival of neurons, and therefore have been considered in the past few years as candidates for therapy options for different neurodegenerative diseases, including ALS (Mohajeri et al. 1999; Acsadi et al. 2002).

In previous studies, the brain-derived neurotrophic factor was shown to prevent the loss of motor units and to contribute to the maintenance of muscle mass when administered to the hind limb muscles of mice after

peripheral nerve injury (Fryer et al. 2000; Hu and Kalb 2003; Mousavi et al. 2004; Ozdinler and Macklis 2006). Glial-derived neurotrophic factor (GDNF) and insulin growth factor 1 (IGF-1) are two of the most potent survival factors known for motor neurons. Several studies have shown that GDNF and IGF-1 can prevent motor neuron degeneration in mice and rats after axotomy, as well as programmed cell death of motor neurons during development (Mohajeri et al. 1999; Fryer et al. 2000; Acsadi et al. 2002; Ozdinler and Macklis 2006; Sakowski et al. 2009). In $SOD1^{G93A}$ transgenic mice, over expression of GDNF and/or IGF-1 in muscles resulted in hyperinnervation of the muscles by motor neurons (Mohajeri et al. 1999; Fryer et al. 2000; Musarò et al. 2001; Acsadi et al. 2002; Kaspar et al. 2003; Rabinovsky et al. 2003; Dobrowolny et al. 2005; Ozdinler and Macklis 2006; Li et al. 2007; Sakowski et al. 2009). Moreover, motor neurons are able to bind, internalize, and retrogradely transport GDNF and IGF-1 from muscle in a receptor-dependent manner (Mohajeri et al. 1999; Fryer et al. 2000; Acsadi et al. 2002; Ozdinler and Macklis 2006; Sakowski et al. 2009).

In addition, GDNF is important for neuron branching at the NMJ and for modulating synaptic plasticity (Crone and Lee 2002). The enhanced expression of GDNF in muscle of $SOD1^{G93A}$ transgenic mice delays disease onset, improves locomotor performance, and increases lifespan. This, in contrast to an increase in centrally derived GDNF produced by astrocytes, which elicits only a transient improvement of locomotor performance (Li et al. 2007). In addition, survival of motor neurons is improved when GDNF levels in muscle of $SOD1^{G93A}$ transgenic mice are increased. The number of large myelinated axons at the end-stage of the disease is also increased as compared with normal $SOD1^{G93A}$ transgenic mice. This effect was not found in the distal segment, which is in agreement with the dying-back hypothesis. Finally, this study shows that augmented expression of GDNF in muscle of $SOD1^{G93A}$ transgenic mice reduces the number of completely denervated NMJs (Li et al. 2007).

In another study investigating the regenerating properties of GDNF, the latter was administered to the peripheral nervous system or muscle tissue to act as a focal attractant to guide new motor axons distally (Deshpande et al. 2006). This study showed the formation of new NMJs resulting in functionally active motor units and the restoration of motor function in a paralysis model in adult rats.

VEGF is another factor that contributes to the pathogenesis of ALS. Increased expression of VEGF in motor neurons of $SOD1^{G93A}$ transgenic mice increases their survival, with one study also reporting increased lifespan and enhanced motor performance (Azzouz et al. 2004; Wang et al. 2007). Also intracerebroventricular administration of VEGF in a rat model of ALS dramatically increases motor neuron survival, while an intraperitoneal injection of VEGF leads to preservation of NMJs (Storkebaum et al. 2005; Zheng et al. 2007).

Pun et al. (2006) suggested that different subtypes of axons show distinct progression of cillary neurotrophic factor (CNTF) sensitive axonal deficits during disease. In their study, they detected disease related reduction in CNTF stimulus in fast firing axons. They noticed that CNTF alleviates disease. According to their findings, protection by CNTF specifically involved a boosting of adaptive responses in disease affected axons.

Treatments to rescue motor neurons according to a "dying forward" model of motor neuron pathology in ALS have shown only limited success in $SOD1^{G93A}$ transgenic mice as well as in humans. By separating the motor neuron from its target muscle, axonal degeneration may be the more important contributor to the progressive deterioration of motor function in ALS, therefore representing a very important therapeutic target. Furthermore, if cell body degeneration is late compared with axonal degeneration, early intervention could potentially prevent the loss of motor neurons (Sagot et al. 1997; Coleman and Perry 2002).

Summary

Pathological observations associated with ALS are largely with no reference to the stage of the disease and thus provide only limited information regarding the mechanism of disease progression.

Due to the difficulties in studying human cases, particularly because of the near impossibility of obtaining pathological specimens at the early stages of the disease, transgenic mice expressing mutated SOD1 have provided an opportunity to investigate the early and presymptomatic stages of the disease (Kong and Xu 1998). Accumulating data indicate that, while a significant loss of motor neurons is not observed until approximately 90 days of age in $SOD1^{G93A}$ mice, denervation of NMJs is detected as early as 47 days of age (Fischer et al. 2003).

ALS is a complex disease and cannot be explained by the occurrence of a single event or the disturbance of a single gene or protein. The disease is likely to be the result of a multistep process, ultimately leading to motor neuron degeneration and clinical symptoms. It emerges that subtle changes at the NMJ may elicit subtle changes in motor neuron connectivity which may then render the system more vulnerable to subsequent genetic or environmental insults (Schmidt et al. 2009).

Clinically detectable muscle deficits are present at the time of earliest pathological dysfunction in the $SOD1^{G93A}$ transgenic mouse model of ALS. Motor unit loss and associated

muscle function precedes motor neuron death in accordance with the "die-back" hypothesis. Moreover, the time course of presymptomatic preferential motor unit loss supports the available anatomical evidence that has suggested that motor neurons innervating the slower muscle fibers are more resilient in the SOD1^{G93A} transgenic mice. In addition, it was shown that the largest motor neurons with the biggest axon calibers are the most vulnerable to cell death, implicating size as a contributing factor for selective vulnerability. These large motor neurons also have the greatest innervation ratios.

Different mechanisms have been suggested for the "dying-back" pattern leading to the progressive loss of motor neurons in ALS. One of the theories is that an accumulation of insoluble complexes could be responsible for insufficient maintenance of the distal axon. As a result, axonal abnormalities that inhibit retrograde transport confer selective damage to motor neurons, possibly by preventing the delivery of target-derived neurotrophic factors back to the cell body. Another explanation might be an aberrant function or expression of axon guidance proteins that contributes to the early pathological changes in motor neuron connectivity of ALS. Irregular axon guidance protein expression influences axonal transport and synaptic function and finally leads to motor neuronal loss.

Treatment according to the dying-back hypothesis—a muscle-targeted therapy may delay motor impairment, extending the life span of SOD1^{G93A} mice and hopefully of ALS patients.

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