Amyotrophic Lateral Sclerosis (ALS): Disease Mechanisms

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Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS), known in the United States as Lou Gehrig’s disease, was first described by the famous French scientist and physician Jean-Martin Charcot in 1869. ALS, the most common adult-onset motor neuron disease, refers to a heterogeneous group of neurodegenerative disorders characterized by the selective loss of upper and lower motor neurons (specialized cells that control movement) in the brain and spinal cord resulting in inevitable paralysis and death. The typical age of onset for most forms of ALS is between 50 and 60 years with an average survival of less than 3 years. This insidious disease is characterized by progressive muscle weakness, atrophy, and spasticity, and is traditionally viewed as lacking in cognitive impairment. However, recent literature documents a variety of cognitive deficiencies in a subset of ALS patients that occur subsequent to neuromuscular deficits. In general, the final fatal event is the loss of the motor neurons that innervate the respiratory muscles and diaphragm. The human impact of ALS is enormous, as it significantly affects a patient’s quality of life (the loss of speech and swallowing is inevitable). The life time risk for developing ALS is about 1 in 200. At present, ALS patients suffer knowing there is no cure, and worse – no truly effective treatment exists to slow disease progression.

While 90–95% of all ALS cases lack an apparent genetic linkage, 5–10% are dominantly inherited disease. Of the familial cases, 15–20% are attributed to mutations in the ubiquitously expressed metalloenzyme copper/zinc superoxide dismutase (SOD1) whose endogenous function is to relieve the oxidative stress generated by normal cellular metabolism. At last count, more than 110 different mutations (scattered throughout the 153 possible positions!) have been identified in familial cases of ALS. Regardless of the nature of the mutation, all provoke the age-dependent and selective loss of motor neurons through acquisition of one or more as yet unidentified toxic properties, not through the loss of enzyme activity. The toxic property itself and the biological basis for the selectivity to motor neurons remain unknown. However, the discovery of disease-causing SOD1 mutations has paved the way for the creation of rodent models on which to model the disease, thus providing a tool to tease out the molecular events that trigger and/or modulate the disease and ultimately develop effective therapies. The extensive use of these models in ALS research has yielded six major themes in the study of motor neuron degeneration (Figure 1).

Mechanisms of Motor Neuron Degeneration

Excitotoxicity

Glutamate is an essential molecule which transmits an excitatory signal across a synapse to an awaiting motor neuron. Deregulated or excessive transmission of glutamate can result in a toxic increase in the intracellular calcium concentration within motor neurons. In general, extracellular glutamate is actively cleared from the synaptic space by closely juxtapositioned astrocytes via the activity of a glial-specific glutamate transporter, excitatory amino acid transporter 2 (EAAT2). In the case of ALS patients and rodent models, a focal loss of this particular transporter correlates well with the loss of motor neurons in the anterior horn of the spinal cord and is further corroborated by the expected increased level of glutamate in the fluid which bathes the brain and spinal cord (cerebrospinal fluid). Furthermore, at-risk motor neurons seem to express a reduced amount of calcium-binding proteins, and thus may be less well equipped to handle a wave of glutamate-mediated calcium influx. To date, excitotoxicity remains one of the few mechanistic links between sporadic and SOD1-mediated familial ALS. In fact, the biological basis for the only food and drug administration (FDA) approved ALS treatment, Riluzole (commercially known as Rilutek), is thought to be through the modulation of the glutamate-mediated excitotoxic response. Despite this, Riluzole offers a very limited extension in lifespan (of ~3 months) and a modest delay in the progression of the disease.

Mitochondrial Dysfunction

Mitochondria are the major energy-producing centers within cells and thus largely responsible for the maintenance of cellular metabolism and survival. The motor neuron is an energy-demanding cell – any interference with its mitochondrial energy production is usually detrimental to its survival. The visual observation of abnormal mitochondrial morphology in the motor neurons of ALS patients in early stages of degeneration was the first implication of

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mitochondria in ALS disease. Similar abnormalities, indicative of mitochondrial damage and dysfunction, were soon identified in rodent models of mutant SOD1-mediated ALS. Using both human (sporadic and familial) cases as well as transgenic models, there has been much focus on components involved in energy production, namely the electron transport chain which drives ATP (the primary energy currency in the cell) generation. While deficits in one or more components of this multicomplex system have been reported, mostly in tissues collected from disease end stage, there has been little agreement between groups on which complex is affected. Moreover, it is unclear whether these defects are causative or merely a consequence of the widespread degeneration of the motor neuron itself that is occurring at this late stage.

The proposal that mitochondrial damage is an initiating event in the failure of motor neurons has gained strong support through the analysis of disease in rodents that express ALS-linked SOD1 mutations. A common feature of all mutant SOD1 proteins (an abundant cytosolic protein) is their preferential association with spinal cord mitochondria purified from affected but not unaffected tissues. This was also observed in samples from familial (SOD1) patients. Moreover, in both cases, the normal endogenous SOD1 was largely excluded from the same spinal cord mitochondria. There is also an intriguing temporal correlation between mitochondrial association and disease progression for the mutant SOD1 proteins. This universal mitochondrial association of various mutant SOD1 proteins may be a critical determinant governing the tissue-specific degeneration of motor neurons (the obligate feature of all ALS cases).

Since optimal mitochondrial function (leading to efficient and sufficient energy production) is central to cellular stability/homeostasis, including the

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**Figure 1** Mechanisms contributing to motor neuron degeneration in ALS.

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subsequent proposed mechanisms, understanding how mutant SOD1 proteins impact mitochondrial function is essential and currently of intense interest. Spinal cord mitochondria from presymptomatic mutant SOD1 animals may have a reduced capacity to buffer intracellular calcium fluxes, a concept that fits quite well with the earlier described mechanism of glutamate-mediated excitotoxicity. An alternate proposal awaiting confirmation is a proposed direct interaction between mutant SOD1 proteins and BCL-2, an antiapoptotic protein residing on the mitochondrial surface. This proposed interaction may sequester BCL-2, thereby quenching its antiapoptotic activity by preventing BCL-2’s interaction with proapoptotic proteins. Since mitochondrial abnormalities are widely reported in sporadic ALS, mitochondrial dysfunction or damage may represent a common pathway in both inherited and sporadic disease.

Protein Aggregation

ALS patient samples typically feature very large intracellular, cytoplasmic inclusions within motor neurons and occasionally in astrocytes. These inclusions are easily identifiable with classic immunohistochemical dyes, but their origin and composition is unresolved. In the mutant SOD1 models, these aggregates are intensely immunoreactive for SOD1 itself, as well as neurofilaments (a structural protein of the motor neuron) and ubiquitin (a component of the protein degradation machinery). Some of these aggregates are detergent resistant and contain misfolded SOD1 and protein folding chaperones (including HSP25 and αβ-crystallin) as well as other proteins including covalent adducts of SOD1 itself. These aggregates occur uniquely in the spinal cords of affected animals, correlate well with disease onset, and may actually be present at the mitochondrial surface.

The prevailing hypothesis is that these large aggregates/inclusions are local enrichments of some toxic species which damage motor neurons. The mechanisms which might contribute to this damage include aberrant chemistry by toxic isoforms of SOD1, co-sequestration of other cellular proteins into these large aggregates (leading to loss of their function), depletion of the protein folding machinery, saturation and eventual disruption of the protein degradation machinery (which has an affinity for misfolded proteins), and interference with mitochondrial function due to aggregation at the mitochondrial surface. The implication of the protein folding pathway is consistent with the co-association of a subset of chaperone proteins, including HSP25, HSP70, HSP40, and αβ-crystallin with SOD1 and SOD1-immunoreactive inclusions. Similar significance can be applied to the proposed defect in protein degradation. Specifically, decreased enzymatic activities of the proteasome, the main organelle involved in protein degradation, has been documented in spinal cord homogenates of mutant SOD1 animals. Finally, it remains controversial as to whether these inclusions are truly damaging. The alternate hypothesis is that these inclusions are in fact protective due to the sequestering of a soluble toxic species. This debate is not unique to ALS, recurring in all of the major examples of human neurodegenerative disease.

Neurofilaments and Axonal Transport

Motor neurons are among the largest and most highly asymmetric cells in nature, with meter long axons generating cell volumes that in humans are 5000 times that of a typical cell. These long processes are specified by an ordered array of neurofilaments, the most abundant structural proteins. Neurofilaments are assembled from neurofilament-L (NF-L), neurofilament-M (NF-M), and neurofilament-H (NF-H). Proper assembly of neurofilaments is essential to the establishment of correct axonal diameters. It is indeed only the largest caliber, neurofilament-rich motor neurons that are most at-risk in all ALS patients (both sporadic and familial) as well as mutant SOD1 rodent models. In the normal motor neuron, neurofilaments form a structural framework that determines axonal caliber (which in turn determines the speed of electrical signal conduction). Furthermore, as discussed earlier, accumulations of neurofilament subunits in motor neuron cell bodies is a frequent feature of ALS cases. In addition, there is some genetic evidence which indicates that mutations in the neurofilament genes may increase the risk of developing ALS. Indeed, mutations in NF-L have already been determined to be causative for one form of motor neuropathy, Charcot–Marie–Tooth disease (type II).

The active movement of cellular proteins and organelles along microtubule tracts in axons, from the cell body all the way to the far-established synapse (and back again), is mediated by ATP-consuming motor proteins. Components traveling anterogradely (toward the synapse) are moved by members of the kinesin family. Defects in the rate of anterograde transport of some components, including SOD1 and neurofilaments, have been documented in mutant SOD1 mice well before the onset of disease. Disturbances in the retrograde movement (from the synapse to the cell body) of cellular constituents, mediated by the single known retrograde motor dynein, have also been implicated in ALS models. In mice, the indirect disruption of dynein function uniquely in postmitotic neurons through disruption of an activation complex (named dynactin) results in impaired
retrograde axonal transport and the eventual development of a late-onset, progressive motor neuron disease that is reminiscent of the ALS mutant SOD1 mouse models. Errors of axonal transport can be a primary cause of late onset motor neuron disease, as demonstrated by point mutations in dynein that provoke motor neuron degeneration in rodents. Interestingly, the introduction of these mutations into mutant SOD1 models leads to a delay in disease onset and extension in lifespan. The molecular basis for this observation is not understood, but perhaps may involve an interplay between the retrograde signaling of toxic and/or trophic factors.

Errors in axonal transport are likely contributors to human ALS. High concentrations of mitochondria and vesicles are found at nerve endings (the neuromuscular junctions where the motor neuron innervates the muscle) in ALS patient samples. Similar distal accumulations have also been reported in mutant SOD1 mouse models. However, what remains unclear is whether these distal accumulations of organelles are due to accelerated anterograde or impaired retrograde transport of these structures. Mitochondria are known to be highly dynamic organelles that are actively transported within cells and through both axonal and dendritic processes of neurons. While the delivery of these organelles is in itself an energy-consuming process, the inefficient delivery of these energy-generating organelles is likely to have significant effects on synaptic activity and neurotransmission, among other previously described cellular processes. The significance of accumulated synaptic vesicles at the nerve terminal remains to be determined but seems to suggest possible problems in synaptic transmission and/or vesicle recycling.

Growth Factor Signaling

Motor neurons require an abundance of both positive and negative signaling molecules to establish and maintain their connections. These signals include those produced by cells in close proximity to their cell bodies (astrocytes and microglia) and those intimately associated with their axons (Schwann cells and oligodendrocytes) and terminals (muscles). The contribution of neighboring cell types to motor neuron degeneration has come to the forefront in ALS research. It is now appreciated that even normal motor neurons (devoid of any disease-causing mutations) are damaged by other nonneuronal cells which do express disease-causing mutant SOD1. This apparent transfer of toxicity to healthy motor neurons may be mediated by a lack of positive trophic signaling from these damage-incurring nonneuronal cells. Candidate molecules that have been implicated include ciliary neurotrophic factor (CNTF), glial cell-line derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and insulin-like growth factor-1 (IGF-1). While these neurotrophins have all been shown to protect motor neurons in culture and/or animal models, all have failed to significantly alter disease in patient trials. However, for IGF-1, CNTF, and GDNF, there are key concerns on whether delivery of appropriate levels of these agents to the nervous system was achieved.

An unexpected involvement of vascular endothelial growth factor (VEGF) has recently been discovered in ALS. VEGF is a growth factor classically thought to participate only in the development of new blood vessels (angiogenesis). Expression of the VEGF gene is regulated by a transcription factor that is itself able to sense low oxygen conditions (hypoxia) and thus upregulate VEGF expression, thereby restoring efficient vascular perfusion in the affected area. Most surprisingly, in a mouse model in which VEGF production was reduced (owing to a mutation in one of its promoter elements which provides normal basal levels of VEGF but does not permit its upregulation in response to hypoxia), a proportion of the mice develop a progressive, late-onset motor neuron disease that is neuropathologically similar to that observed in ALS mice. Furthermore, introducing this mutation of VEGF into mutant SOD1 animals exacerbates the disease. While a firm genetic link of VEGF to ALS pathogenesis remains to be established, an isoform of VEGF has been documented to be neurotrophic for motor neurons grown in culture. Furthermore, the delivery of VEGF whether by viral expression or purified protein into the central nervous system (CNS) of mutant SOD1 animals provides a significant delay in the onset of disease pathology and extends survival. While the exact mechanism involved in VEGF-mediated protection of motor neurons remains to be defined, a clinical trial involving the localized and direct delivery of purified VEGF into the CNS of ALS patients is currently underway.

Neuroinflammation/Glial Activation

It is now appreciated that cell types other than motor neurons contribute to disease initiation and progression. Indeed, the activation of surrounding glial cells, both the trophic-providing astrocytes and the injury-sensing microglia, has been widely reported in both rodent models and patient samples. Microglia share the same ancestry as peripheral tissue macrophages and thus are considered to be the resident immune cells of the CNS. Microglia sense injury in the CNS and rapidly become activated, initiating a neuroinflammatory pathway involving the release of
cytotoxic molecules which affects all neighboring cells, including neurons and astrocytes. Microglial activation correlates well with disease and is observed in human ALS and in all rodent models of ALS and thus is strongly implicated in the disease process. The administration of the FDA-approved antibiotic minocycline to mutant SOD1 mice delays disease progression primarily by preventing microglial activation. Furthermore, the removal of toxic mutant SOD1 from microglial cells limits microglial activation and provides a substantial extension in lifespan in mice which are still expressing the mutant product in all other cell types. This substantial extension in lifespan is actually due to a significant slowing of disease progression after onset. Recognition that microglial activation affects the rate of disease progression offers a significant guidepost to the development of an effective therapy for ALS (as well as other neurodegenerative disease in which neuroinflammation is a component). A clinical trial in which minocycline is included in patient treatment plans is currently underway.

Summary

While many different mechanisms for disease initiation and progression in ALS have been proposed, these should not be seen as mutually exclusive. Many or all are likely to be important contributors that are intricately related to one another. While there may be more than one initiating event occurring within the motor neuron (mitochondrial dysfunction, excitotoxicity, protein aggregation, and defective axonal transport), they are likely to converge on one (or at most a few) common final path(s) to motor neuron death. Trophic signaling and neuroinflammation are likely the primary elements mediating disease progression and thus are attractive targets for new therapeutic strategies.

See also: Amyotrophic Lateral Sclerosis (ALS); Axonal Transport and ALS; Axonal Transport and Neurodegenerative Diseases; Excitotoxicity in Neurodegenerative Disease; Inflammation in Neurodegenerative Disease and Injury; Neurofilaments: Organization and Function in Neurons; Oxidative Damage in Neurodegeneration and Injury; Transgenic Models of Neurodegenerative Disease.

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