Animal Models of Motor and Sensory Neuron Disease

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Motor and Sensory Tracts

Movements of many parts of the body, from the lips and the eyelids to the hands and toes, have their origin in the brain in a specialized region called the motor cortex (Figure 1). Upper motor neurons receive information there that they transmit to spinal motor neurons, often through an intermediate interneuron. Firing of the lower motor neuron triggers muscle contraction. Upper motor neuron degeneration induces brisk tendon reflexes, spasticity, and hyperreflexia, whereas denervation of the muscle upon lower motor neuron degeneration or loss leads to muscle weakness and loss of tone.

The sensory system works in the opposite direction with information flowing to the brain from the periphery. Receptors, primarily located at the surface of the skin, provide initial sensory input (touch, pressure, vibration perception, pain, and temperature) to the axons of the sensory neurons which carry these signals into the spinal cord. The information is then transferred to the neurons located in the sensory cortex, where the information is decoded by the brain.

Gene Deletion (‘Knockout’) and Transgenic Mouse Models

Two main principles are used to reproduce inherited human pathologies in the mouse (Figure 2). When the human disease is recessive, a situation usually caused by the absence of one particular protein, a genetic mimic in mice can be constructed by disrupting the corresponding gene in the mouse genome (Figure 2[a]). Upper motor neurons receive information there that they transmit to spinal motor neurons, often through an intermediate interneuron. Firing of the lower motor neuron triggers muscle contraction. Upper motor neuron degeneration induces brisk tendon reflexes, spasticity, and hyperreflexia, whereas denervation of the muscle upon lower motor neuron degeneration or loss leads to muscle weakness and loss of tone.

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Neurodegeneration in Human

Motor Disease

Amyotrophic lateral sclerosis  ALS, more familiarly known in the United States as Lou Gehrig’s disease, is the most prominent adult motor neuron disease, with a typical age of disease onset of 50–60 years. Most (90%) incidences of ALS are referred to as sporadic because there is no family history of disease and hence no evidence for a major genetic component. Disease involves the selective alteration and death of both the upper and the lower motor neurons, resulting in spasticity, hyperreflexia, and progressive weakness of skeletal muscles, atrophy, and death due to respiratory muscle paralysis within 1–5 years after onset.

For the incidences (~10%) of ALS with a genetic origin, determination of the gene(s) responsible has been significantly challenged by the wide heterogeneity of the disease, genetically and clinically. Many genetic loci have been associated with different forms of motor neuron degeneration. Most prominently, mutations in the gene encoding for Cu/Zn superoxide dismutase (SOD1), an enzyme used to detoxify an aberrant oxygen species, have been shown to account for 20% of the familial cases of ALS. Dominantly inherited, analysis of enzymatic activity of mutated SOD1 in ALS patients together with genetic manipulations in mice have clearly revealed that SOD1-mediated toxicity is not due to a reduction in activity of this enzyme but, rather, due to a gain of a new toxic property or properties. Accordingly, transgenic rodent models have been generated by inserting into their genome a human SOD1 gene carrying different ALS-causing mutations. Many models have been created to dissect the disease-associated toxic mechanism(s), the most extensively used being transgenic for the
amino acid substitution mutations G93A (glycine substituted to alanine at position 93), G37R (glycine to arginine at position 37), and G85R (glycine to arginine at position 85). Although none of these completely recapitulate all features of the human disease, they all develop progressive motor neuron degeneration with slightly different symptoms and disease progressions that are characteristic for each mutation, with onset and survival determined by the level of mutant expression. All of these mice develop motor neuron degeneration, limb weakness associated with neurofilament misaccumulation, impaired axonal transport, and axonal swelling.

Mouse and rat models for ALS have been extensively used to test proposals for a wide range of pathological mechanisms. These include impairment of the chaperone/degradation machinery, dysfunction of mitochondria, oxidative stress, alteration of cytoskeletal architecture and axonal transport, glutamate-mediated excitotoxicity, aberrant growth factor signaling, microglial cell involvement, and inflammation. Another aspect particularly important to the identification of therapeutic targets is whether other cell types contribute to motor neuron degeneration. Motor neurons require support provided by several cell types in the central nervous system which also express the mutant SOD1. Use of a transgene that can be eliminated selectively from motor neurons has shown that mutant damage in motor neurons initiates disease onset and an early phase of disease progression. A similar approach has proven that mutant damage within microglial cells, the immune cells of the central nervous system, has no effect on disease onset but accelerates disease progression.

Additional forms of human ALS-like motor neuron disease, all associated with motor neuron degeneration and death, have been designated ALS2–ALS8. Significant divergence in the age of onset and clinical presentation has led to disagreement as to whether these should be classified as forms of ALS. This is
1. Introduction of modified gene into ES cells

2. Injection of targeted ES cells into embryos

3. Implantation of embryos into a foster mother

Chimeric mice (mixture of normal cells and gene targeted cells)

4. Breed to a normal mouse

Mice with the disrupted ('Knockout') or replaced gene ('Knock in') (all cells carry the modified gene)

Gene insertion: ('transgenic' mouse)

1. Injection of a specific gene into a fertilized egg

Transgenic mice (all cells carry the transgene)

Gene modification: replacement ('knock in' mouse) or disruption ('knockout' mouse)

Figure 2 Continued
especially so for ALS2, an infantile and juvenile-onset form of motor neuron disease which is caused by loss of an apparent guanine exchange factor enzyme, termed alsin, that can activate one or more enzymes from the family of small GTPases known as G-proteins. Proposed G-protein partners of alsin include Rab5 and Rac1. Alsin has been shown to modulate processes such as cytoskeleton organization and transport of membrane cargoes. Recessive AL2 motor neuron disease has been reproduced in mice by deleting the corresponding gene (Figure 2(a)). This first revealed that the absence of alsin in the mouse does not produce a juvenile disease that has as severe a phenotype as found in human. The lack of perfect concordance between the human and mouse genetic defect has been proven to be true for many other diseases. Importantly, lower motor neurons (those that directly trigger contraction of skeletal muscles) are almost completely spared in mice deleted of the ALS2 gene, which do not develop muscle weakness. Analysis of voluntary running, however, revealed that these mice move slowly, a well-recognized clinical sign of an upper motor neuron defect. Additionally, the upper motor neurons degenerate. Thus, the animal model reinforces that the pathology and the phenotype of loss of ALS2 provoke an upper motor neuron disease quite distinct from ALS, resembling instead hereditary spastic paralysis.

ALS4 is a dominantly inherited juvenile form of ALS characterized by distal muscle weakness and atrophy before 25 years of age. Rare in humans, this disease is caused by mutation in the senataxin (SETX) gene. The function of the senataxin protein is not known, but its sequence suggests DNA/RNA helicase activity, potentially acting in DNA repair pathways and RNA processing. Senataxin mutations are also causative of a recessive disease termed AOA2 (ataxia–oculomotor apraxia 2); symptoms include balance difficulties and defects in coordination of movements (ataxia) associated with an alteration of eye movement (oculomotor apraxia).

Axonal transport has been shown to play an essential role in neuronal survival. Two families of motor proteins, the kinesins and dynein, which power transport of cargoes along the microtubules away from the nerve cell body (anterograde) or returning components from the nerve ending to the cell body (retrograde), respectively, have been shown to cause or contribute to motor neuron disease in humans. This has been replicated in mice. In humans, mutation in the subunit p150 of the dynactin complex (which interacts with the dynein complex) has been identified to be causative of an unusual form of lower motor neuron disease in early adulthood, characterized by vocal fold paralysis, progressive weakness, and muscle atrophy. Although a mouse model of the p150 dynactin mutation has not been reported, it is widely established that alteration of the dynein–dynactin complex causes motor deficits. Indeed, overexpression of dynamin (also called p50 of the dynactin complex) has been shown in mice to disrupt dynein–dynactin interaction leading to late-onset slowly progressive motor neuron degeneration, characterized by muscle weakness, trembling, abnormal gait, and deficits in strength and endurance. Progressive alterations of muscle function and motor coordination have been linked in mice to distinct mutations in the dynactin gene. The corresponding mutants, Loa (Legs at odd angles) and CraI (cramping 1), develop motor deficits, confirming that alteration in the dynein motor complex and its presumed effects on moving components through axons are of unusual importance for motor axons. A completely unexpected and unexplained finding is that both dynavin mutations delay disease progression and increase the life span of mice that develop early onset motor neuron disease caused by expression of an ALS-causing SOD1 mutant. Understanding what is causing the motor deficits in the Loa and CraI mice will certainly help us to understand what leads to this amelioration of disease in SOD1 mutant mice and might help to identify new therapeutic approaches for ALS.

Figure 2  Mouse models for human neurodegenerative disease. Strategies for gene modification or replacement. (a) Gene modification. In this approach, the gene of interest is modified in vitro and then introduced into (brown) embryonic stem (ES) cells. Following recombination in the ES cells, the modified gene replaces the endogenous gene on one of the two chromosomes. The corresponding targeted ES cells are then injected into mouse embryos at the blastocyst stage and are implanted into a foster mother. Those females produce progeny mice that are mixtures of normal cells and modified cells. Mice carrying the modified gene in all cells (also called ‘knockout mice’ in the case of a gene disruption and ‘knock in’ when the gene is replaced) are obtained by standard breeding with normal mice. (Box 1) To selectively disrupt a gene in a specific tissue, the gene region to be deleted is flanked by two lox sites, which are small sequences recognized and cut by an enzyme called Cre recombinase. Breeding mice carrying the modified lox–gene–lox with mice expressing the Cre recombinase under a tissue-specific transcriptional promoter generates the gene disruption only in the tissues that express the Cre recombinase. (b) Gene insertion: ‘transgenic’ mouse. An exogenous gene is inserted randomly into the mouse genome after direct injection of the gene into the one cell stage fertilized egg. After implantation into a foster mother, the oocyte matures into mice whose cells all carry the transgene.
Spinal muscular atrophy  After cystic fibrosis, spinal muscular atrophy (SMA) is the most common autosomal recessive disorder in humans and represents the most common genetic cause of infant mortality. In this disorder, specific neuronal loss of lower motor neurons in the spinal cord results in atrophy of proximal muscles of the trunk and the limbs. SMA cases are classified into three groups: Type I SMA is the most severe phenotype, with an age of onset before 6 months and death occurring before 2 years of age; type II SMA is intermediate, with an age of onset between 6 and 18 months; and type III SMA, which initiates after 18 months, is the mildest form, slowly progressing with a normal life span.

Identifying the genetic cause of SMA was challenging because of the complex and unstable nature of the region of human chromosome 5 where the defective gene resides. For all disease types, symptoms are caused by inactivating mutations in the SMN1 (survival of motor neuron 1) gene, but the variability in the disease severity depends on the number of copies (ranging from one to five) of an almost identical copy gene, the SMN2, located just next to SMN1. Each copy of the SMN2 gene produces only a small amount of functional product so that only a single copy leads to fatal, severe type I disease, and five copies lead to the more benign type III disease. Although the role of SMN protein in lower motor neuron survival is not fully understood, it has been implicated in processing within the nucleus of initial RNA copies of each expressed gene into their mature forms capable of translation into the corresponding proteins. A second likely function is as an RNA-binding protein for RNA transport into axons.

Because the mouse genome contains only the SMN1 gene and no SMN2 gene, mouse models for SMA have been attempted by deleting the SMN1 locus. These animals are not viable, demonstrating an essential role for SMN1 in cell survival. Selective deletion of the SMN1 gene solely from neurons (Figure 2, box 1) produced mice with motor abnormalities and skeletal muscle denervation secondary to motor axon loss, culminating in death at a mean age of 25 days. This is not a very satisfying model, but it does demonstrate an essential role for SMN1 in neurons. SMN1 expression has also been modified only in muscle cells. This has revealed that SMN expression in muscle prevents the SMA phenotype and increases the life span of the animals. This supports targeting muscle as a therapeutic strategy in SMA. Because in human the severity of disease is modulated by the SMN2 gene, the SMN2 gene has been introduced (by a transgene) to the mice with the 'neuronal' deletion of SMN1. This showed that SMN2 gene number ameliorates SMN1 absence, closely mimicking what occurs in human disease.

Motor and Sensory Disease

Charcot–Marie–Tooth  Charcot–Marie–Tooth (CMT) diseases refer to a heterogeneous class of neuropathies that affect not only motor but also sensory nerves in the peripheral nervous system. In addition to the weakness and atrophy of the distal limb muscles, patients experience impaired sensation and absence of deep tendon reflexes. Representing the most common inherited disorder of the peripheral nervous system, this group of diseases, which are sometimes transmitted through dominant, recessive, or X-linked modes of inheritance, has been classified into two subgroups. The demyelinating form (also called CMT1) results in an impairment of the myelin sheath produced by Schwann cells. CMT2 forms are characterized by degeneration of the axon. The genetic heterogeneity of the disease has led to the identification of at least 17 genes involved in both demyelinating and axonal forms of CMT. The CMT1-causing genes (PMP22, MPZ, LITAF/SIMPLE, and EGR2) have revealed an essential role of myelin compaction, the regulation of myelin protein degradation, and the transcriptional control of myelination-specific genes in demyelination.

Axonal survival has been shown to depend on many different pathways. Among these, mutation in the gene encoding the mitochondrial GTPase mitofusin 2 as a cause of CMT has proven an essential role of mitochondrial fusion/transport in axon survival. Maintenance of axonal architecture and transport (for motor neuron survival) is particularly important for sustaining neuron function. Forms of CMT2 are also caused by mutation in the neurofilament subunit NF-L or the motor kinesin KIF1bβ. Impaired intracellular trafficking is also a common theme in axonal degeneration, as revealed by mutation in the regulator of vesicle trafficking Rab7 and the phosphatase MTMR2, which modulate membrane trafficking.

CMT1A is the most frequent form of CMT. Instead of a typical disease-causing mutation that alters or eliminates the encoded protein, CMT1A disease is caused by a duplication of the genomic region surrounding an otherwise normal PMP22 gene so that affected individuals have an extra copy of the normal gene. Transgenic rat and mouse models containing multiple copies of the PMP22 gene have proven that overexpression of only this gene is sufficient to cause peripheral demyelination and the symptoms associated with the human disease. This also demonstrated an increased disease severity with increased expression of PMP22. These animal models have been shown to be very useful to test the first rational experimental therapies of CMT1A. Indeed, a synthetic antagonist of the nuclear progesterone receptor was shown to reduce PMP22 expression and to
Ameliorate the clinical severity in those animals. Moreover, administration of ascorbic acid, an essential factor of *in vitro* myelination (already approved by the US Food and Drug Administration for other clinical indications), has prolonged survival and restoration of myelination in those models, suggesting its use in human clinical trials for CMT1A.

**Giant axonal neuropathy** Factors that establish the cytoarchitecture of the axon and its associated axonal transport components are critical for neuronal survival. Disorganized cytoskeletal intermediate filaments constitute the hallmark of another neurodegenerative disease termed giant axonal neuropathy (GAN). This is a rare, recessively inherited condition characterized by a structural deficit in both the central nervous system and the peripheral motor and sensory axons. With disease onset in infancy and marked by gait instability and frequent falls, patients develop diminution of deep tendon reflexes, muscle atrophy, and muscle weakness that progressively evolves to sensory loss and loss of ambulation. Other symptoms, including ataxia, language deficits, and mental retardation, reveal a later impairment of the central nervous system.

GAN is a progressive, fatal disease, with life expectancy of less than 30 years. Identification of the GAN gene led to recognition that its encoded protein, gigaxonin, plays a critical role in the ubiquitination machinery whose function, among others, is to tag proteins for degradation. Other errors in other components of this degradation apparatus are implicated in several neurodegenerative disorders, the most prominent of which is Parkinson’s disease. Gigaxonin seems to link degradation and impairment of the axonal cytoskeleton. Loss of gigaxonin prevents normal degradation of proteins involved in the assembly and dynamics of microtubules, the protein polymers that serve as the tracks for transport of components up and down the axon. Whether altered microtubules properties are the disease-causing damage and, if so, how this provokes the specific aggregation of intermediate filaments seen in patients is not known.

Developing animal models for rare disorders such as GAN is particularly crucial since insights from direct inspection of patient materials are very limited. The study of the mechanisms underlying neurodegeneration through construction and analysis of genetic models mimicking the loss of gigaxonin is at its earliest stages. Mice deleted of the GAN gene develop a progressive deterioration of motor function and ataxia, a sign of central nervous system impairment. As in human disease, the absence of gigaxonin generates axonal loss and neurofilament aggregation. Alteration of cytoskeleton architecture has been further confirmed by the decreased density of axonal microtubules and the increased abundance of proteins that affect microtubule assembly.

**Conclusion**

Since essentially all forms of motor or sensory neuron degeneration are incurable, mouse and rat models represent an indispensable resource to address the origin of neuronal dysfunction, the mechanisms of degeneration, and the determinants of the selectivity of death of subpopulations of neurons. In turn, these key issues will power the design of strategies to ameliorate these life-long, frequently fatal conditions. Thus, for example, for ALS, this fundamental research has led to the development of many experimental therapies in these animals, including anti-glutamate drugs, neurotrophic growth factor delivery, SOD1 silencing, and stem cell therapy, which will be tested in human clinical trials. Identifying the pathways of neuron survival will help in understanding the basis of motor/sensory neuron diseases in humans for which the majority of genetic causes remain unknown.

*See also:* Amyotrophic Lateral Sclerosis (ALS): Disease Mechanisms; Amyotrophic Lateral Sclerosis (ALS): Axonal Transport and ALS; Neurofilaments: Organization and Function in Neurons.

**Further Reading**


