Heterogeneity in motoneuron disease

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Recently, mutations in several genes have been identified as primary causes for the degeneration of motoneurons and their axons. Strikingly, mutations in the same genes were associated with clinically different motoneuron syndromes. The identity of these genes also shed light on the mechanisms of motoneuron degeneration and revealed that overlapping motoneuron phenotypes might be caused by heterogeneous molecular mechanisms. Overall, these findings have challenged the diagnostic classification system set by clinical judgement and triggered the notion of heterogeneity in motoneuron disease. It will now be especially relevant to identify the mechanisms and principles that motoneuron diseases have in common, as this will allow us to identify the most relevant therapeutic targets. On the other hand, heterogeneity in motoneuron disease also implies that finding a monotherapy cure for motoneuron disease will be challenging and that pre-clinical testing of therapeutic targets should not be limited to a single animal model.

Introduction

The selective death of motoneurons is the pathological hallmark of motoneuron disorders (MNDs). The best characterized of these is amyotrophic lateral sclerosis (ALS). Despite intensive efforts, ALS is still incurable, depriving afflicted individuals of movement, breath and, eventually, life. Only two percent of ALS cases are attributable to mutations in Cu-Zn superoxide dismutase (SOD1), the etiology of most other cases remains largely enigmatic. Most previous reviews on ALS have focused on SOD1 as the prototype disease-causing gene. However, an increasing number of motoneuron genes is being discovered and, as a result, novel molecular pathways of motoneuron vulnerability are emerging. Here, we provide a general overview on these genes and their pathways, while focusing on the common principles with other MNDs.

Heterogeneous phenotypes, common genotypes?

Motoneuron disorders have traditionally been categorized according to the type of motoneuron (upper or lower) affected, the involvement of additional types of neurons (sensory or cortical neurons), and the site of the neuron (cell body or axon) primarily affected. Using this neuropathological pattern of vulnerability as a guideline, the French neurologist Charcot described in 1869 ALS as a disease caused by death of upper and lower motoneuron cell bodies; the classification of other common MNDs is shown in the Glossary.

Over the past five years, numerous MND genes have been discovered. Initially, mutations in a gene were each associated with a single clinical syndrome, but over time, it became evident that MNDs, previously classified as distinct clinical entities, are in fact caused by mutations in the same gene and thus likely belong to the same molecular disease class. Phenotypic differences have even been observed in patients with SOD1 mutations, in which motor and sensory neurons, spino-cerebellar and corticospinal tracts can be variably affected. Examples of genes causing MND according to this concept of ‘divergent clinical spectrum–convergent molecular etiology’ are listed in Box 1.

There is also considerable heterogeneity in the molecular pathways that trigger specific motoneuron phenotypes, as well as in the susceptibility of motoneurons to degeneration. For instance, histopathological studies can distinguish sporadic ALS from ALS caused by SOD1 mutations, thereby suggesting that patients with SOD1 mutations might not represent the full spectrum of sporadic ALS [1]. In addition, different types of motoraxons in SOD1 mice also exhibit a distinct vulnerability:

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**Glossary: different types of motoneuron syndromes**

**Amyotrophic lateral sclerosis (ALS):** A syndrome of upper and lower motoneuron dysfunction, without involvement of any other part of the nervous system. Typical ALS patients develop an adult-onset disease rapidly progressing to death within three to five years after onset. ALS has a lifetime risk of one in two thousand, but owing to the aggressive disease course, the prevalence of ALS, estimated to be six to eight per 100 000 people, is relatively low compared to the other less progressive MNDs. In 10% of patients ALS is a familial disease, in all other cases the etiology is sporadic. In up to 15% of patients, ALS can occur in association with frontotemporal dementia (FTD). Several other variants of ALS exist: primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA), respectively pure upper and pure lower MND.

**Charcot-Marie-tooth’s disease (CMT):** A heterogeneous group of peripheral neuropathies involving both motor- and sensory axons, leading to muscular weakness and atrophy in the lower extremities and impaired sensation. Based on motor nerve conduction velocities, CMT can be divided into a demyelinating type and an axonal type, but intermediate forms exist as well. CMT has a prevalence of 30–40 per 100 000 inhabitants.

**Hereditary spastic paraplegia (HSP):** An MND characterized by spasticity in the lower extremities and caused by axonal degeneration of the corticospinal tracts. Ascending sensory (dorsal column fibers) and spino-cerebellar axons are affected to a variable but limited degree. Sometimes ataxia is a prominent feature of HSP and thus, the term spastic ataxia is used. HSPs have a prevalence of around 1.5–9.6 per 100 000 people.

**Spinal muscular atrophies (SMA):** A heterogeneous group of MNDs caused by degeneration of lower motoneurons in the spinal cord. They are usually subdivided in proximal SMA (i.e. classical spinal muscular atrophy or SMA), which is characterized by proximal muscle weakness, and distal SMA, also called distal hereditary motor neuropathy (dHMN) or spinal forms of Charcot-Marie-Tooth disease. The estimated prevalence of proximal and distal SMA are ten and four per 100 000 inhabitants, respectively.

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motoneurons innervating fast-fatigable muscle fibers responsible for brief bursts of forceful muscle contractions are affected long before disease symptoms appear [2], and much earlier than motoneurons innervating slow-twitch muscle fibers [2,3].

Thus, clinically different MNDs might share common molecular grounds, whereas vice versa, clinically similar MNDs might be caused by diverting molecular mechanisms. We will therefore now discuss these MNDs according to their common molecular mechanisms (Figure 1), rather than their clinical phenotype.

Mechanisms of motoneuron degeneration
Motoneurons are polarized cells, consisting of a soma with short dendrites and a single axon that might extend over one meter in length. The ability of a motoneuron to maintain this specialized morphology depends on the organization of its cytoskeleton, i.e. complex networks of microtubules, actin filaments and neurofilaments. Most of the proteins in the synaptic terminals are synthesized in the soma and transported along the axon by specialized organelles. We will now discuss how disturbances in these axonal ‘highways’ cause motoneuron death.

Defects in the neuronal cytoskeleton
Several lines of evidence illustrate the importance of a functional cytoskeleton in preserving motoneuron function. Neurofilaments (NFs) are the most abundant structural proteins in neurons and exist as light (NFL), medium (NFM) and heavy (NFH) subunits, which assemble into a filamentous structure, that determines axon calibar and cell integrity. Because NF subunits are obligate heteropolymers, it is intriguing that mutations in the various subunits cause distinct MNDs. Indeed, heterozygous mutations in NFL cause early-onset Charcot-Marie Tooth (CMT), whereas heterozygous deletions or insertions in NFH can cause ALS [4]. Aggregation of NFs in cell bodies and proximal axons of motoneurons is also a prominent pathological feature of ALS, whereas mouse models overexpressing NFH, wild-type or mutant NFL accumulate NFs in neuronal cell bodies and develop atrophy of motor-axons [4]. Changing the NF stoichiometry thus leads to misassembly, aggregation and accumulation of NFs, thereby triggering neuronal death. Counterintuitively, accumulation of NFs in the soma enhanced the survival of SOD1 mice [4]. Two models have been proposed to explain these unexpected observations. The first model proposes that accumulation of NFs in the soma relieves the crippled axonal transport machinery in SOD1 mice from an overload of cargo [5]. In the second model, misassembled NFs in the soma function as sinks, buffering the toxic effects of mutant SOD1 [6].

Peripherin (PRPH), an intermediate filament related to NF, has also been associated with motoneuron death, as heterozygous frameshift mutations and homozygous loss-of-function mutations occur in ALS patients [7]. Overexpression of PRPH caused late-onset motoneuron death in mice, the onset of which was accelerated in the absence of NFL. Thus, correct PRPH subunit stoichiometry is crucial for proper neurofilament assembly, and a deviation of this equilibrium predisposes to motoneuron death. Nonetheless, the overall importance of PRPH dysfunction in the pathogenesis of ALS still remains puzzling, as neither its loss nor overexpression affects SOD1 mice [8].

Figure 1. Areas of motoneuron vulnerability. With the increasing number of genes known to cause motoneuron disease, the areas of motoneuron vulnerability are being revealed. Critical processes that are disrupted include those involved in the intracellular trafficking processes from motoneurons (i.e. endosomal trafficking and axonal transport by molecular motors), the correct assembly of the cytoskeleton, mitochondrial functioning as well as RNA processing. Recent data also provided evidence that the ‘environment’ of motoneurons, which consists of the neighboring ( moto)neurons, glial cells and muscle cells, critically influences the severity of the motoneurodegenerative phenotype.
Motoneuron death is also associated with other genes involved in assembling the neuronal cytoskeleton. Missense mutations in vesicle-trafficking protein-B (VAPB) cause a dominant atypical form of ALS, accompanied by unusual tremor. DVAP33A, the fruitfly homologue of VAPB, is required for the organization of the synaptic microtubule cytoskeleton: overexpression of DVAP33A increases, whereas its loss decreases the number of neuromuscular junctions [9]. Another cytoskeleton gene implicated in MND is spastin (SPG4). Loss-of-function mutations in spastin are a frequent cause of hereditary spastic paraplegia (HSP). In motor cell lines, spastin is required for the assembly of the synaptic microtubule cytoskeleton, resulting in defective synaptic growth, impaired neurotransmission and severe movement defects [11]. Abnormalities in synaptic microtubule network formation might thus contribute to spastin- and VAPB-associated MND.

As a member of the rho family of GTPases, RAC1 (ras-related C3 butulinum substrate 1) regulates actin dynamics within growth cones. Loss-of-function mutations in ALSIN, a guanine-nucleotide exchange factor that activates RAC1 activity [12], occur in several MNDs (Box 1), thereby suggesting that ALSIN mutations contribute to motoneuron demise by disrupting RAC1-regulated actin dynamics (Box 2).

All these studies highlight the sensitivity of motoneurons to misassembly of their cytoskeleton. Building and maintaining the axonal highway infrastructure is thus obviously of crucial importance.

Molecular motors of axonal transport

By moving along microtubular tracks, the molecular motors of the kinesin and dynein superfamilies execute axonal transport. Defects in axonal transport contribute to motoneuron degeneration. For instance, KIF1B, which is a member of the kinesin superfamily (KIF), transports synaptic vesicle precursors anterogradely along microtubules. A loss-of-function mutation in the KIF1B gene has been identified in one family with CMT (but not in another family linked to the same genetic locus [13]), whereas mice lacking a KIF1B allele develop a neuropa thy-like phenotype [14]. Dominant-negative mutations in another kinesin, KIF5A, occur in individuals with HSP [15], thereby underscoring that kinesins cause MND. Retrograde transport is also critical for neuronal subsistence. In humans, MND is linked to dynactin (DCTN1) mutations that impair the activity of dynein [16], the major motor complex for retrograde axonal transport. In mice, disruption of dynein also results in motoneuron loss [17]. It is well established that neurons depend for their survival on neurotrophins, which are released by the target organs that are innervated by these neurons. According to the ‘signaling endosome model’, the target-derived neurotrophin binds its receptor, the receptor-ligand complex is internalized through clathrin-mediated endocytosis and then retrogradely translocated in a dynein-dependent fashion to the soma, where it induces survival. A shortage of neurotrophic support might thus explain why mutations that impair dynein transport cause late-onset MND.

Box 1. Mutations in the same gene are associated with different MNDs, thereby crossing the borders set by clinical judgement (the possibility that these differences were due to different diagnostic criteria in clinical centers or countries was excluded)

- Two percent of ALS patients have SOD1 mutations and suffer from upper and lower motoneuron degeneration. However, in patients with an A4V or G93C mutation, upper motoneurons are only negligibly affected [48,49]. In other cases, not only motoneurons, but also sensory neurons and spino cerebellar tracts degenerate [50]; for instance, patients with a G93C mutation exhibit severe spino cerebellar tract abnormalities [49]. By contrast, prominent corticospinal tract degeneration is also present in patients with L3BV or homozygous D90A mutations [51];
- Identical missense mutations in the VAPB gene have been identified in families, in which disease ranged from typical ALS to mild late-onset SMA [52];
- Homozygous mutations in ALSIN have been found in a family with juvenile-onset ALS, but also with juvenile-onset PLS or infantile-onset HSP [53];
- Dominant mutations in dynactin were first identified in a family with a lower MND starting with vocal cord paralysis, but subsequent studies also revealed mutations in ALS patients and a family with ALS and FTD [54,16];
- Missense mutations in senataxin have been identified in patients with HMN displaying some upper motoneuron involvement (and therefore classified as ALS by others [29]), whereas other mutations give rise to a recessive form of ataxia with oculomotor apraxia type-2 (AOA2), which is characterized by cerebellar atrophy, peripheral neuropathy and oculomotor apraxia;
- Mutations in GARS and HSP22 give rise to hHN in some individuals, and CMT in others [55];
- spastin mutations account for 40% of HSP, but can also result in PLS [56];
- ALS phenotypes exhibit considerable overlap with ubiquitine-positive FTLD: mutations in CHMP2B, dynactin and progranulin (PGRN) have been found in ALS and FTLD patients [57,58].

Box 2. Mice lacking ALSIN display subtle motor deficits and neuropathological abnormalities, but fail to develop motoneuron degeneration as seen in humans [4].

- The pathogenic mechanism of ALSIN-deficiency for MNDs still remains unclear. So far, ALSIN has been implicated in regulating actin dynamics, as well as in regulating trafficking of receptor complexes.
- ALSIN is a guanine-nucleotide exchange factor that activates RAC1; RAC1 is a regulator of actin dynamics in the axon growth cone [59].
- Knockdown of ALSIN and expression of dominant-negative RAC1 reduce axon outgrowth of motoneurons, whereas increased expression of ALSIN or expression of constitutively-active RAC1 increase the outgrowth [59].
- ALSIN also activates RAB5; RAB5 is an orchestrator of endosome dynamics [12].
- Loss of ALSIN impairs the uptake of activated receptor complexes of insulin-like growth factor-1 (IGF1) [60].
- ALSIN interacts with the glutamate receptor interacting protein-1 (GRIP1), which regulates transport and anchorage of the glutamate receptor subunit-2 (GLUR2) in the postsynaptic membrane of dendrites [61]. GLUR2 is important for motoneurons because its deficiency or overexpression aggravates or attenuates SOD1-mediated ALS, respectively [62]. In motoneurons lacking ALSIN, the subcellular distribution of GRIP1 is altered resulting in reduced GLUR2 expression in synapses.
Surprisingly, however, disruption of dynein attenuates motoneuron loss in SOD1 mice [18]. As for NFs, this paradox might be explained by the fact that a reduction in retrograde axonal transport reduces transport overload in mutant SOD1 motoneurons.

Although several questions are still outstanding (Box 3), these studies implicate various MND genes in regulating axonal transport.

**Disruption of endosomal trafficking**

During endocytosis, internalized receptors and transmembrane proteins are delivered to endosomes, from where they are targeted to other cellular components for the propagation of downstream signaling or to lysosomes for degradation. Opposite outcomes can thus result from trafficking through the endocytic pathway: signal propagation and attenuation. Members of the RAB (Ras-related protein or RAB GTPase family) GTPase superfamily are essential for endocytosis. RAB5, for instance, orchestrates the initial steps of early endosome formation. Interestingly, ALSIN activates RAB5, thereby stimulates the initial steps of early endosome formation. Interest-

gingly, ALSIN activates RAB5, thereby stimulates endosome formation and regulates uptake, transport and anchorage of activated receptor complexes (Box 2). Intriguingly, heterozygous missense mutations in RAB7, essential for biogenesis of lysosomes, cause CMT [19]. Both impaired signal propagation and attenuation due to disruptions in endosomal trafficking thus induce MND.

Other genes involved in endosomal trafficking are also important for motoneuron homeostasis. Recessive mutations in *vacuolar protein sorting 54 homolog (VPS54)*, required for retrograde trafficking of cargoes from endosomes to the Golgi network, cause motoneuron death in the wobbler mouse [20]. Retrograde axonal transport is also impaired in these mice, which suggests that disruptions in retrograde endosomal trafficking cause MND. Furthermore, mutations in *chromatin-modifying protein 2B (CHMP2B)*, a human ortholog of yeast VPS2, occur in patients with frontotemporal dementia (FTD) and ALS [21]. Transfection of plasmids that encode these mutations caused dysfunction of endosomal sorting complexes by impairing the internalization of membrane-bound cargoes in neurons [21].

Although several questions remain outstanding (Box 3), these mutations underline the emerging recognition that defects in membrane trafficking represent a significant cause of MND.

**Mitochondrial toxicity**

By generating ATP and regulating cytoplasmic calcium, mitochondria are obviously of vital importance for neurons. Mitochondria abundantly accumulate in synaptic terminals, which is thought to reflect the higher energy and calcium buffering needs of these tip structures. Because mitochondria have a half-life of one month in the brain, an intense axonal transport secures the supply of nuclear-encoded proteins to the synapse for their assembly, whereas retrograde transport of mitochondria might serve to replenish some of these building blocks. It is thus not surprising, that mitochondrial damage or disruption of their transport have been implicated in a wide variety of neurological diseases.

Mutations in genes encoded by the mitochondrial genome result in major neurological syndromes, but rarely in MNDs. By contrast, mutations in nuclear-encoded mitochondrial genes cause MNDs. Mice lacking *paraplegin* (*SPG7*), a gene mutated in HSP, develop a distal axonopathy characterized by accumulation of mitochondria in axon tips [22]. Paraplegin proteolytically processes the mitochondrial ribosomal protein L32 (MRPL32), a subunit of mitochondrial ribosomes implicated in ribosome assembly [23]. Dominant-negative mutations in another nucleus-encoded mitochondrial gene, *mitofusin-2 (MFN2)*, cause various forms of CMT [13]. MFN2, which is located in the outer mitochondrial membrane, regulates fusion-and-fission events that allow mitochondria to change their size and morphology according to the physiological state of the cell [13]. In neurons expressing disease-mutated forms of MFN2, the transport of mitochondria in axons was impaired. This defect was not attributable to diminished ATP levels or oxidative respiration, thereby suggesting that abnormal mitochondrial trafficking itself caused axon degeneration [24].

Damage to mitochondria also triggers motoneuron death in SOD1 mice. Indeed, mitochondria become vacuolated early in the disease course [25]. Mutant SOD1 has also been found in the mitochondrial intermembrane space and matrix, where it can interfere with proper mitochondrial functioning by: affecting the translocation machinery; generating toxic free radicals; forming aggregates, which promote outer membrane vacuolization; and decreasing respiratory activity [25]. Also, mutant SOD1 can aggregate with the anti-apoptotic mitochondrial protein, B-cell lymphoma protein-2 (BCL-2) [26]. Together, all these effects might result in abnormal mitochondrial energy metabolism and the release of apoptotic factors. Intriguingly, both in rodent models and patient samples, mutant SOD1 is present in fractions enriched for mitochondria from
affected, but not from unaffected tissues. Moreover, wild-type SOD1 appears to be largely excluded from these mitochondrial preparations. Where exactly this dual selectivity comes from, remains however an unresolved key question.

Driving the RNA machinery
Motoneurons also display a unique sensitivity towards defects in the RNA machinery. For instance, loss of the survival motoneuron (SMN) gene, involved in RNA splicing, gives rise to spinal muscular atrophy (SMA), whereas

Figure 2. The motoneuron environment determines motoneuron degeneration. (a) The healthy motoneuron environment consists of healthy motoneurons and supporting astrocytes and microglia. Supportive astrocytes prevent the accumulation of neuotoxic glutamate through their glutamate transporter excitatory amino acid transporter-2 (EAAT2) (shown by the red arrow pointing through the red channels). Evidence that microglia positively contribute to the motoneuron environment comes from SOD1 mice, in which wild-type bone-marrow derived microglial cells significantly delayed disease onset and prolonged disease duration compared with mice receiving mutant SOD1 transplants [65]. Bone marrow (BM)-derived stem cells implanted intraperitoneally into SOD1 mice also significantly increased lifespan [66]. Tracing studies in the spinal cord of these mice revealed that most BM-derived cells had differentiated into macrophages and microglia, suggesting that these cell types were responsible for the observed therapeutic effects. Moreover, microglia and astrocytes can also release neurotrophic molecules, which in combination with other molecules might be responsible for the protective effect of microglia. (b) A detrimental motoneuron environment in which molecular signals exchanged between mutant SOD1 motoneurons and glial cells trigger the activation of a vicious cycle. Recently, an initial stimulus for microglial activation in SOD1 mutant mice was identified: chromogranins, which are components of neurosecretory vesicles, bind mutant but not wild-type SOD1, and act as chaperone-like molecules to promote secretion of toxic SOD1 protein from motoneurons, thereby initiating inflammatory responses in microglia [67]. Once activated, microglia start to produce deleterious pro-inflammatory mediators such as TNF-α and interleukins (e.g. IL-1β and IL-6) at the expense of neurotrophic factors. In response to additional stimuli, possibly derived from degenerating motoneurons, microglia are further activated to become amoeboid cells with a fully phagocytic potential. Under these conditions, they produce massive amounts of pro-inflammatory cytokines and pro-apoptotic activators, and contribute to oxidative stress damage by releasing nitric oxide and peroxynitrite. An important regulator of oxidative stress during inflammation is NADPH oxidase. In SOD1 mice, the expression of the NADPH oxidase subunit gp91phox co-localized with signs of oxidative damage in microglia [68]. The detrimental role of neuro-inflammation in ALS is further evidenced by the observation that several molecules known to temper the neuroinflammatory responses delay disease onset and slow disease progression in ALS mouse models [25]. Astrocytes also enhance excitotoxic damage by decreasing EAAT2 transporter expression, which is responsible for the clearance of excess glutamate, and thereby aggravate motoneuron death. In addition, astrocytes expressing mutant SOD1 adversely affect the survival of primary motoneurons, but not that of other neuronal cell types, thereby suggesting that astrocytes release soluble factors, which are toxic for motoneurons [69,70]. This effect was specific for motoneurons, as microglia, fibroblasts, cortical neurons and myocytes did not elicit such neurotoxicity.

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abnormal SMN copy numbers resulting in lower SMN levels are also a susceptibility factor for ALS [27]. Recent studies demonstrate that SMN promotes axon outgrowth by interacting and translocating β-actin mRNA to the growth cones of motoneurons [28]. Several other genes involved in RNA processes also cause MND. SMA with respiratory distress type-1 results from recessive mutations in the immunoglobulin μ-binding protein (IGHMBP2) gene, whereas dominant mutations in setaxin (SETX) occur in some atypical ALS families [29,30]. Both genes contain a DNA-binding domain and co-localize with the RNA machinery (Box 3). Heterozygous missense mutations in glycyl and tyrosyl tRNA synthase (GARS and YARS), which enable tRNAs to associate with their cognate amino acids, cause various types of SMA and CMT [31]. A mutation in the isoleucine-tRNA synthetase has also been discovered in a patient with atypical ALS [32]. Because lysi-l-tRNA synthetase interacts with mutant but not wild-type SOD1, defective RNA translation might contribute to ALS. As disease-associated GARS mutations suggest a loss-of-function effect [33], tRNA charging deficits thus seem to be involved in the pathogenesis of MNDs.

The motoneuron environment

Non-motoneuron cell types have also been involved in motoneuron degeneration. This has been best documented for SOD1 mice, which we will therefore discuss as an example. Chimeric mice, containing normal and mutant SOD1-expressing motoneurons, develop a less aggressive form of paralysis when mutant motoneurons are surrounded by wild-type neurons [25]. Expression of mutant SOD1 by various neuron-specific promoters was insufficient to provoke motoneuron death, whereas selective removal of mutant SOD1 from motoneurons, delayed, but did not prevent paralysis [34].

If not only the motoneuron itself is important, which other cell types then contribute to disease? An early hallmark of ALS is activation of astrocytes and microglia. These cells might exert both beneficial and toxic effects on motoneurons (Figure 2). Skeletal muscles also provide a source of signals that influence axonal growth and maintenance of synaptic connections. Indeed, muscle-restricted overexpression of insulin-like growth factor-1 (IGF1) in SOD1 mice maintained muscle integrity and regeneration, stabilized NMJs and delayed motoneuron death [35]. A similar effect was reported for glial cell line-derived neurotrophic factor (GDNF) as its overexpression in muscle, but not in astrocytes, delayed disease onset [36].

Overall, ALS is best considered a disease, in which alterations in different cell types might act synergistically to exacerbate or delay the disease. It remains, however, outstanding whether these cell types play a similar role in other MNDs (Box 3).

On track for better therapies in ALS

Despite intensive research, MNDs are still incurable. In contrast to intense therapeutic efforts devoted towards prolonging survival of SOD1 mice, there have been relatively few attempts in other motoneurodegenerative models. We will now discuss three therapeutic strategies that have dominated efforts in SOD1 mice.

First, many researchers have focused on Food and Drug Administration (FDA)-approved, orally available molecules capable of prolonging survival of SOD1 mice; some of these compounds successfully delayed disease by several weeks (Table 1). Nonetheless, caution is warranted, as illustrated by the recent disappointing trial with celecoxib (Pfizer, www.celbrex.com). Second, numerous neurotrophic factors have been tested in SOD1 mice and humans, mostly by systemic injection, but with little success. At least part of the reason for these failures is attributable to inappropriate delivery as systemically-delivered proteins are immunogenic, rapidly cleared, and poorly cross the blood–brain barrier [37]. As a means of ensuring adequate delivery into the central nervous system, several other approaches have been explored recently, including direct delivery into cerebrospinal fluids, delivery using encapsulated xenogeneic cells and gene transfer using various viral vectors and delivery methods. Third, with the identity of many MND genes being discovered, strategies to knockdown or restore expression of disease genes, in case of a toxic gain-of-function or loss-of-function effect, respectively, offer promising opportunities. The therapeutic efficacies of the latter two strategies are closely linked to the efficiency of the delivery methods, as discussed below.

Gene therapy for MND

To reach motoneurons, viral vectors have been developed that infect nerve terminals at the NMJ and ‘hijack’ the retrograde axonal transport system to transduce the motoneuron soma. Two such types of vectors have been optimized: lentiviral vectors pseudotyped with rabies-G envelopes (EIAV) and adeno-associated adenviral vectors (AAV). We will here restrict our discussion to a brief description of the most robust effects obtained in MND animal models.

In SOD1 mice, the most robust therapeutic effects were obtained with EIAV vectors expressing an interfering RNA inhibiting human SOD1 (EIAV-hSODi). Intramuscular delivery of EIAV-hSODi increased the lifespan of SOD1

Table 1. Promising therapeutic approaches in SOD1 mice using orally available molecules

<table>
<thead>
<tr>
<th>Compound</th>
<th>Delay in survival time</th>
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<tbody>
<tr>
<td>Cyclooxygenase-2 (COX2) inhibitors</td>
<td>26 days</td>
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<tr>
<td>Celecoxib</td>
<td>26 days</td>
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<tr>
<td>Rofecoxib</td>
<td>24 days</td>
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<td>Inhibitors of tumor necrosis factor-α (TNF-α) and other inflammatory cytokines</td>
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<tr>
<td>Thalidomide</td>
<td>21 days</td>
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<tr>
<td>Lenalidomide</td>
<td>24 days</td>
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<tr>
<td>Minocycline</td>
<td>11–21 days</td>
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<tr>
<td>Minocycline-riluzole-nimodipine</td>
<td>42 days</td>
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<tr>
<td>Compounds targeting excitotoxicity</td>
<td></td>
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<tr>
<td>Ceftriaxone</td>
<td>10 days</td>
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<tr>
<td>AMPA receptor antagonists</td>
<td>±20 days</td>
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<tr>
<td>Riluzole</td>
<td>10–15 days</td>
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<tr>
<td>Anti-oxidants</td>
<td>20 days</td>
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<td>AEOL 10150</td>
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*Reviewed in [4,64].
mice by as much as 80% [38]. Silencing of the endogeneous SOD1 would not be desirable, as mice lacking SOD1 develop various defects, including age-associated loss of neuromuscular synapses [39]. In humans, individual point mutations in the SOD1 protein will thus need to be targeted, which will be challenging given the numerous different mutations identified. In a model of SMA, intramuscular delivery of EIAV-SMN2 increased survival by five days [40]. Although this might seem a modest effect, it is relevant considering that the time window to obtain full expression of the transgene was relatively short (EIAV-SMN2 was administered after birth, whereas mice already died at day 13).

AAV vectors are also effective in delivering transgenes to motoneurons after intramuscular injection. Injection of AAV2-IGF1 vectors increased the lifespan of SOD1 mice by 30% [41]. In the same study, delivery of AAV2-GDNF had only modest effects, indicating that not only the efficiency of delivery, but also the activity of IGF1 itself was responsible for the observed effects. In the brain, IGF1 affects various cell types, including motoneurons. IGF1 also enhances axon outgrowth and improves neuromuscular function and muscle strength [42]. All these pleiotrophic effects together might have contributed to its therapeutic success. The potential of AAV-mediated gene therapy was confirmed recently in another MND model: intramuscular administration of AAV-SPG7 to SPG7-deficient mice retrogradely transduced motoneurons, thereby halting the progression of neuropathological changes and improving motor performance [43].

A VEGF story

Another molecule, which was particularly effective in SOD1 models, is the vascular endothelial growth factor (VEGF), widely recognized as a key regulator of angiogenesis. Recent findings revealed, however, another previously unrecognized role of VEGF as a neurotrophic factor: transgenic mice with a targeted deletion in the VEGF promoter resulting in reduced VEGF expression, developed adult-onset, progressive motoneurodegeneration [44]. Lowering VEGF levels in SOD1 mice also aggravated the disease [44], whereas neuronal overexpression of VEGF or its fetal-liver kinase-1 (Flk1) receptor prolonged survival [45]. When EIAV-VEGF vectors were intramuscularly injected into SOD1 mice, lifespan was lengthened by 38 days [46], thereby resembling the effect of AAV2-IGF1 as the most effective therapy in the field. Delivery of recombinant proteins is clinically attractive, as it offers a flexible control over dose and duration of the administered factor. Intracerebroventricular (ICV) delivery of VEGF protein to SOD1 rats extended survival significantly and changed disease subtype from a severe form, in which rats suffered from forelimb paralysis, to a milder form [45]. The more pronounced effect on forelimb muscles was likely attributable to the higher concentration of VEGF closer to the infusion site.

The discovery of the link between VEGF and ALS has also stimulated interest in evaluating the role of other angiogenic factors in ALS. Recently, missense mutations were identified in angiogenin (ANG) [47]. Although the precise biological properties of ANG remain to be defined, ANG might act downstream of VEGF, as documented in endothelial cells (Box 3).

Concluding remarks

Recently, the discovery of novel MND genes provided insights into the pathogenesis of motoneuron vulnerability. But what else did we learn from their identity and the phenotypes they cause? First of all, clinical entities previously classified as distinct, are caused by mutations in the same gene, thereby illustrating that MND is phenotypically heterogeneous. Second, mutations in different genes can give rise to an identical phenotype, indicating that there is substantial genetic heterogeneity in clinical entities of MND. Third, mutations in different MND genes result in disregulation of multiple but distinct cellular processes, suggesting that overlapping phenotypes are caused by heterogeneous molecular mechanisms. Importantly, heterogeneity in MND has huge therapeutic implications as well. Indeed, if MND is such a heterogeneous disease, finding a monotherapy cure will be challenging, or perhaps even impossible. Also, heterogeneity implies that pre-clinical testing of therapeutic targets should not be limited to a single MND model. In addition, other available pre-clinical models might be used [4]. Pre-clinical models that target the MND genes that have been most recently identified also promise to be of great therapeutic value.

The challenge will be to further define the heterogeneous molecular basis of MND and to study the therapeutic potential of novel candidates in a more integrated manner. This will involve studies in which therapeutic candidates are delivered alone or in combination with other molecules, to SOD1 and other MND models.

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References

2 Pun, S. et al. (2006) Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. Nat. Neurosci. 9, 408–419