
Motoneuron Disease

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Abstract

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) represent the two major forms of motoneuron disease. In both forms of disease, spinal and bulbar motoneurons become dysfunctional and degenerate. In ALS, cortical motoneurons are also affected, which contributes to the clinical phenotype. The gene defects for most familial forms of ALS and SMA have been discovered and they point to a broad spectrum of disease mechanisms, including defects in RNA processing, pathological protein aggregation, altered apoptotic signaling, and disturbed energy metabolism. Despite the fact that lack of neurotrophic factors or their corresponding receptors are not found as genetic cause of motoneuron disease, signaling pathways initiated by neurotrophic factors for motoneuron survival, axon growth, presynaptic development, and synaptic function are disturbed in ALS and SMA. Better understanding of how neurotrophic factors and downstream signaling pathways interfere with these disease mechanisms could help to develop new therapies for motoneuron disease and other neurodegenerative disorders.

Keywords

Amyotrophic lateral sclerosis • Superoxide dismutase • Mitochondria • Endoplasmic reticulum stress • RNA metabolism • Survival motoneuron protein • Frontotemporal lobar degeneration • TDP-43 gene • Ciliary neurotrophic factor • Cardiotropin-1 • Leukemia-inhibitory factor • Neurotrophins • BDNF • Glial-derived neurotrophic factor • Neurodegeneration

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1 Introduction

During development of higher vertebrates, many types of neurons are generated in excess, and about half of the newly generated neurons undergo cell death. Spinal and bulbar motoneurons have been a central focus of research to understand the underlying mechanisms. These neurons become postmitotic at early stages of development; they grow out axons and make functional contacts with skeletal muscle, before this phase of physiological cell death occurs. Pioneering work by Viktor Hamburger and Rita Levi-Montalcini has shown that limiting amounts of survival factors from target tissue play a central regulatory role in this context, and this observation has been the basis for the discovery of neurotrophic factors in the twentieth century. It has long been suspected that dysregulation of neurotrophic signaling could also underlie the degeneration of motoneurons in amyotrophic lateral sclerosis and spinal muscular atrophy, the two major forms of human motoneuron disease. During the last three decades, gene defects underlying monogenic forms of these disorders have been identified, and none of these gene defects point to a lack of neurotrophic factors or defective receptors as cause of motoneuron disease. Motoneurons that are isolated from mouse embryos and cultured *in vitro* also depend on neurotrophic factors for their survival. These cultures are a useful tool for studying signaling pathways for motoneuron survival, but also signaling for axon growth, presynaptic differentiation, dendrite growth and stabilization of neurites, and synaptic contacts. These parameters represent targets of motoneuron disease processes, and recent research has indicated that neurotrophic factor signaling also interferes with these mechanisms. The analysis of disease processes and mechanisms how neurotrophic factors interfere could help to develop new therapeutic strategies for amyotrophic laterals sclerosis and spinal muscular atrophy.

2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder causing dysfunction and death of lower motoneurons in the spinal cord and brain stem and of upper motoneurons in the motor cortex (Kiernan et al. 2011). This results in progressive dysfunction of neuromuscular innervation that normally causes death due to respiratory failure. The incidence of ALS is approximately 2 per 100,000 individuals worldwide, the mean age of onset is 55–60 years, and the disease more commonly affects men than women. Average survival from symptom onset is approximately 3 years, although some forms of the disease also have a much slower disease course, allowing patients to survive for several decades (Wood-Allum and Shaw 2010). Traditionally, ALS has been considered as a pure motor disorder. However, it has become increasingly evident that also other types of neurons are affected and that some forms of ALS are coupled with prefrontal dementia or with degeneration of dopaminergic neurons. Even in patients in which dysfunction of the motor systems predominates the clinical phenotype, histopathological alterations

are also found in many types of neurons, including hippocampus and basal ganglia (Al-Sarraj et al. 2011). Therefore, ALS is now regarded as a more general neurodegenerative disorder in which the motor phenotype predominates the clinical picture.

In more than 90 % of all cases, ALS appears sporadic. Only 5–10 % of all cases are familial. In these cases, an autosomal-dominant inheritance is predominant (Andersen and Al-Chalabi 2011). Even this subset of familial ALS is highly heterogeneous on a genetic basis, and the so far identified genetic defects underlying familial forms of ALS point to multifactorial pathogenic processes (Table 1).

3 fALS with Mutations in the SOD-I Gene

The first identified gene defect which accounts for about 10–20 % of familial ALS were point mutations in the gene for Cu²⁺/Zn²⁺-dependent superoxide dismutase (SOD-I) (NM 000454) (Rosen et al. 1993). So far, more than 50 different mutations in this gene have been identified. Clinically, there seems to be no clear correlation between disease onset or severity with specific mutation in the SOD-I gene (Andersen and Al-Chalabi 2011), and the clinical appearance of ALS does not differ from the majority of sporadic cases in this disease. Some types of mutations in the SOD-I gene are prone to cause a severe and rapid course of disease, in particular the A4V mutation; other forms, i.e., E21G, G37R, D40A, G93C, I104F, L144S, and I151C, are associated with survival times that can exceed 10 years (reviewed in Ferraiuolo et al. 2011). Not all of these mutations are associated with loss of enzymatic function. In particular the G37A mutation which has been intensively studied in transgenic mouse models, but also the A90V or D91A mutations, does not primarily affect enzyme activity (reviewed in Al-Chalabi et al. 2012).

The SOD-I protein plays a central role in detoxifying superoxide radicals from the cell and preventing the generation of hydroxyl radicals that react with a great variety of molecules, in particular polyunsaturated fatty acids in cell membranes, but also proteins and nucleic acids. Cerebrospinal fluid (CSF), but also serum and urine, show elevated markers of free radical damage in patients with ALS (Smith et al. 1998; Simpson et al. 2004), and this does not only apply to patients and mouse models of fALS with mutations in the SOD-I gene. Interestingly, knockout of the SOD-I gene in mice does not result in motoneuron disease (Reaume et al. 1996), whereas transgenic overexpression of mutant SOD-I in general causes rapid and severe forms of the disease (Gurney et al. 1994). Mouse models overexpressing A4V or G93A mutant SOD-I molecules have most commonly been used in studies on the pathophysiological consequences of these mutations. The observation that SOD-I gene knockout does not lead to motoneuron disease in mice and that many of the mutations identified in patients with fALS do not show reduced enzyme activity points to pathogenic mechanisms other than loss of enzyme activity. These include actions of the mutant protein in cell types that appear not primarily affected such as microglia and astrocytes. Mutant SOD-I in microglia increases NADPH

Table 1 Established ALS associated genes

Genetic subtype	Gene	Chromosomal locus	Mouse	Onset/inheritance	Phenotype	References
ALS1	<i>SOD1</i>	21q22.1	Chr 16	Adult/AD	Cognitive impairment (rare)	Rosen et al. (1993)
ALS2	<i>ALS2</i> <i>Alsin</i>	2q33.2	Chr 1	Juvenile/AR		Hadano et al. (2001)
ALS3	<i>Unknown</i>	18q21		Adult/AD		Hand et al. (2002)
ALS4	<i>SETX</i> <i>Senataxin</i>	9q34	Chr 2	Juvenile/AD	Cerebellar ataxia, motor neuropathy	Chen et al. (2004)
ALS5	<i>SPG11</i> <i>Spatacsin, Spastic paraplegia 11</i>	15q21.1	Chr 2	Juvenile/AR		Orlacchio et al. (2010)
ALS6	<i>FUS</i> <i>Fused in sarcoma</i>	16p11.2	Chr 7	Adult/AD	Frontotemporal dementia (FTD), Parkinsonism	Vance et al. (2009), Kwiatkowski et al. (2009)
ALS7	<i>Unknown</i>	20p13		Adult/AD		Sapp et al. (2003)
ALS8	<i>VAPB</i> (vesicle associated membrane protein) associated <i>protein and C</i>	20q13.3	Chr 2	Adult/AD		Nishimura et al. (2004)
ALS9	<i>ANG</i> <i>Angiogenin ribonuclease, RNaseA family 5</i>	14q11.2	Chr 14	Adult/AD	Parkinsonism	Greenway et al. (2006) Rayaprolu et al. (2012)
ALS10	<i>TARDBP</i> <i>TAR DNA-binding protein</i>	1p36.2	Chr 4	Adult/AD	FTD, Parkinsonism	Sreedharan et al. (2008), Kabashi et al. (2008)
ALS11	<i>FIG4</i> <i>SAC domain containing lipid phosphatase</i>	6q21	Chr 10	Adult/AD	Charcot-Marie-Tooth disease 4J	Chow et al. (2009)
ALS12	<i>OPTN</i> <i>Optineurin</i>	10p13	Chr 2	Adult/AD and AR		Maryama et al. (2010)
ALS13	<i>ATXN2</i> <i>Ataxin 2</i>	12q24	Chr 5	Adult/AD		Elden et al. (2010)

Associated or putatively associated with ALS

	<i>C9orf72</i> <i>Chromosome 9 open reading frame 72</i>	9p21.2	Chr 4	Adult/AD, sporadic	Also in FTD, common	Renton et al. (2011), DeJesus-Hernandez et al. (2011)
ALS14	<i>VCP</i> <i>Valosin containing protein</i>	9p13.3	Chr 4	Adult/AD	Rare, described in one family, FTD, Paget disease, inclusion body myopathy	Johnson et al. (2010)
ALS15	<i>UBQLN2</i> <i>Ubiquitin 2</i>	Xp11.23-p11.1	Chr X	Juvenile and Adult/x linked	Rare, described in one family	Deng et al. (2011)
ALS16	<i>SIGMAR1</i> <i>Non opioid intracellular receptor 1</i>	8p13.3	Chr 4	Juvenile/AR	Rare, described in few families	Luty et al. (2010)
ALS17	<i>CHMP2B</i> <i>Charged multivesicular body protein 2B</i>	3p11	Chr 16	Adult/AD sporadic	Also in FTD, rare	Parkinson et al. (2006)
ALS18	<i>PFN1</i> <i>Profilin 1</i>	17p13.3	Chr 11	Adult/AD sporadic	Rare	Wu et al. (2012)

oxidase-mediated superoxide production (Harraz et al. 2008), resulting in prolonged ROS production. Mutant SOD-I protein has been found to interact with chromogranins (Urushitani et al. 2006) and by this way appears to be released from astrocytes and interneurons. Thus, extracellular mutant SOD-I can activate microglia and possibly also promote direct toxic effects on motoneurons. In chimeric mice expressing mutant SOD-I in astrocytes, motoneurons degenerate and show ALS pathology (Clement et al. 2003). Furthermore, mice in which the mutant allele encoding SOD-I G37R is deleted from motoneurons using Cre-lox technology (Boillee et al. 2006) show delayed disease onset, but no alteration in the disease course once first symptoms have become apparent. Also in cell culture, toxic effects of astrocytes expressing mutant SOD-I have been demonstrated when these cells are cocultured with primary motoneurons from embryonic mouse (Nagai et al. 2007) or human stem cell-derived motoneurons (Di Giorgio et al. 2008). This toxic effect of mutant astrocytes has been shown to involve the deregulation of glutamate receptor 2 (GluR2) in motoneurons, as a consequence of mutant SOD-I expression in astrocytes (Van Damme et al. 2007). Taken together, these findings indicate that non-neuronal cells expressing mutant forms of SOD-I exert toxic effects on motoneurons and contribute to disease.

Both in patients and mouse models with mutations in the SOD-I gene, protein inclusions are found in motoneurons and other types of neurons, but these inclusions differ from inclusions found in the vast majority of sporadic ALS patients and other forms of familial ALS because they do not include the TDP43 protein (Maekawa et al. 2009). Some of the protein aggregates that include the mutant SOD-I protein are associated with mitochondria and thus could contribute to mitochondrial dysfunction. The mutated SOD-I protein seems to aggregate in vacuoles in the mitochondrial intermembrane space (Wong et al. 1995), and this finding together with other reports showing interaction of mutant SOD-I protein with bcl-2 (Pasinelli et al. 2004) gives further support to the idea that the mutant SOD-I protein causes mitochondrial dysfunction and defective respiratory chain activity. These findings also correlate with observations that the calcium buffering capacity is impaired in mitochondria isolated from neural tissues of SOD-I mutant mice (Damiano et al. 2006; Grosskreutz et al. 2010). The altered calcium homeostasis caused by this defect might also correlate with susceptibility for glutamate-mediated excitotoxicity and ER stress, which is also observed in motoneurons from SOD-I mutant mouse models.

Mitochondrial dysfunction and morphological alterations such as vacuolation occur early during presymptomatic disease stages in mouse models, and they are thought to contribute to defective axonal transport of mitochondria (De Vos et al. 2007). It is thought that a reduction in the mitochondrial content in axon terminals could be a major mechanism for dying-back axonopathy, which is generally observed in ALS.

Because of the multitude of pathological mechanisms that apparently contribute to motoneuron disease in SOD-I mutant mouse and cell culture models, no clear conclusions can be drawn on which signaling pathways downstream of neurotrophic factor receptors are most important to interfere with pathomechanisms

in this form of familial ALS. Overexpression of mutant SOD-I in motoneurons causes cytoplasmic aggregation of the enzyme, and neurons with such aggregates subsequently undergo apoptotic cell death (Durham et al. 1997). When SOD-I G93A mice are crossed with *bcl-2*-overexpressing mice, onset of disease is delayed (Kostic et al. 1997), suggesting that inhibition of classical pathways for apoptotic cell death interferes with the disease. However, *bcl-2* overexpression cannot prevent disease. Similar observations were made when SOD-I G93A mutated mice were crossed with mice overexpressing a dominant-negative ICE isoform, which prevents caspases from activation of cell death pathways (Friedlander et al. 1997). Thus, interference with classical apoptotic signaling pathways apparently has some impact, but the effects are not sufficient to prevent disease in this mouse model of familial motoneuron disease. This indicates that interference with motoneuron cell death programs is not sufficient for therapy and that additional pathomechanisms, ranging from dysfunction of neuromuscular transmission to destabilization of axonal processes and depletion of dendritic synaptic inputs, also need to be targeted, at least in this form of motoneuron disease.

4 Inclusions and Altered RNA Metabolism in ALS: TDP-43, FUS, C9orf72

Alterations in RNA metabolism have been suspected to contribute to ALS pathophysiology for a long time. Since the discovery that fragile X syndrome is caused by altered expression of FMR-1, a member of a large family of RNP proteins that are involved in RNA binding and transport (Ashley et al. 1993), the potential impact of defective RNA processing to neurodegeneration has increasingly become a focus of interest. However, the mechanisms how altered RNA metabolism could contribute to the pathomechanisms of ALS have only become more concrete in the last few years. An important finding in this context was the identification of the TAR DNA-binding protein-43 (TDP-43) protein as a major component of ubiquitin-positive cellular inclusions (Neumann et al. 2006). These inclusions have the appearance of threads, skeins, or compact bodies and are located in nuclei and soma of neurons, including proximal dendrites and axons. TDP-43-positive inclusions have also been found in other neurodegenerative disorders such as FTLD (Buratti and Baralle 2008). They have also been described in *postmortem* brain of patients with Huntington's disease (Schwab et al. 2008), Alzheimer's disease, and dementia with Lewy body inclusions (Higashi et al. 2007).

The cellular function of TDP-43 is not fully understood. TDP-43 is a 414-amino-acid protein of the hnRNP family (Krecic and Swanson 1999), with two RNA recognition motifs (RRM1 and RRM2) and a C-terminal glycine-rich domain, and thus resembles many other RNA-binding proteins such as fused in sarcoma (FUS) and hnRNP-R, the latter having been previously identified to interact with the survival motoneuron (*Smn*) protein (Rossoll et al. 2002), the central protein of the *Smn* complex that is deficient in spinal muscular atrophy.

After the identification of TDP-43 protein as the major component of proteinaceous inclusions in sporadic ALS and other neurodegenerative disorders, mutations in the gene encoding for TDP-43 were found in some ALS patients (Rutherford et al. 2008; Mackenzie et al. 2010), and also in patients with frontotemporal lobar degeneration (FTLD, new nomenclature FTLD-TDP). About 4 % of patients with familial ALS and 1.5 % of patients with sporadic ALS have mutations in the TDP-43 gene. All of the so far identified mutations in familial ALS are autosomal dominant, and most of them encode for a missense mutation within the C-terminal domain which encodes the glycine-rich domain (Pesiridis et al. 2009), a part of the protein that is important for interaction with other proteins and molecules, but it does not directly interact with RNA. Based on these data, it has been suggested that the mutations in the C-terminus are sufficient to induce neurodegeneration.

The TDP-43-positive inclusions are strongly ubiquitinated and phosphorylated. It is not the full-length TDP-43 which is found in inclusions, but a truncated 20–25 kDa C-terminal fragment (Pesiridis et al. 2011). So far it is still unclear whether the associated loss of TDP-43 function, due to the cleavage of the N-terminus, which contains the RRM1 domain, is causative for neurodegeneration or a loss of function due to depletion of TDP-43 from the nucleus and other cytoplasmic regions where TDP-43 functions are necessary for neuronal maintenance or gain of function by the cleaved fragments.

TDP-43 interactions with RNA have been studied in detail, and these studies have revealed functions of TDP-43 in several aspects of RNA metabolism. The RRM1 domain of this protein binds to single-stranded RNA (Buratti and Baralle 2001), in particular to regions containing UG repeats. These UG regions are contained in many RNAs, and this fits with the observation that several thousand different RNA species can interact with the protein (Tollervey et al. 2011; Polymenidou and Lagier-Tourenne 2011), in particular intronic regions, but also 3'untranslated regions (UTRs), and also noncoding RNAs. The association of TDP-43 with intronic sequences and its predominant nuclear localization implicates TDP-43 in early steps of pre-mRNA processing in the nucleus. These functions could include transcriptional regulation, alternative splicing (Buratti et al. 2001), and in particular micro-RNA (miRNA) processing. Defects in miRNA malfunction have been shown to result in motoneuron disease (Haramati et al. 2010). Thus, TDP-43 (Buratti and Baralle 2010a) and other members of the hnRNP protein family (Pascale and Govoni 2012) like FUS (Morlando et al. 2012) could also contribute to motoneuron maintenance by regulating miRNA function.

Whether motoneuron injury is caused by loss of such nuclear function of TDP-43 is still not fully understood. Among the mRNAs that interact with TDP-43 are those encoding for FUS, VCP (Sephton et al. 2011), and TDP-43 mRNA itself (Buratti and Baralle 2010b). TDP-43 regulates processing of its own transcript by interaction of the protein with the 3'UTR of TDP-43 mRNA, leading to alternative splicing of the 3'UTR. As a consequence, high levels of TDP-43 cause reduced translation of TDP-43 mRNA. These functions involve interaction of TDP-43 with other proteins that bind to the C-terminus, in particular other members of the hnRNP family. These include hnRNP-A2/B1 (Buratti et al. 2005), hnRNP-

A1, hnRNP-A3, and hnRNP-C1-C2 (Ling et al. 2010), but possibly also other members of this large family (Freibaum et al. 2010).

Blocking TDP-43 expression with antisense oligonucleotides in adult mouse brain alters the expression levels of more than 600 mRNA transcripts and changes splicing of more than 900 transcripts (Polymenidou and Lagier-Tourenne 2011), including such transcripts which are also relevant for motoneuron function, such as choline acetyl transferase, and transcripts for other RNA-binding proteins for which mutations lead to degeneration, such as FUS and progranulin.

Several animal models have been developed to study TDP-43 dysfunction, including mouse models in which the gene is knocked out or overexpressed in mutant form, but also *Drosophila*, zebrafish, and *C. elegans* models (reviewed in Wegorzewska and Baloh 2011). Many models available so far cannot give final hints about the pathomechanistic contributions of mutant TDP-43 to motoneuron disease, because those overexpressing TDP-43 might also lead to dysregulation of RNAs simply as a consequence of the TDP-43 overexpression itself, and a good example for this problem is the processing of the TDP-43 mRNA by TDP-43. Another potential problem is the interaction of the TDP-43 with intronic sequences, which are highly different between species, and interaction of TDP-43 with intronic sequences in human genes might not be found in mouse, fish, and *C. elegans* models, because the intronic sequences differ more than coding sequences between these species. Moreover, by overexpression of mutant TDP-43, the RRM1 domains are mostly preserved, allowing functions in pre-mRNA processing that either resemble the physiological function or alter these functions in a dominant-negative manner, for example, when the C-terminal mutations lead to altered distribution of the protein. Thus, the normal function of TDP-43 in regulating expression of mRNA levels of cyclin-dependent kinase 6 (Ayala et al. 2008), histone deacetylase 6 (Fiesel et al. 2010, 2011), low molecular weight neurofilament (Strong et al. 2007), or other transcripts with essential functions in motoneurons could contribute to the pathophysiology and generate additional pathological features in these mouse models that do not necessarily exist in humans with mutant TDP-43.

So far, the mechanisms that lead to altered subcellular distribution of TDP-43 and translocation between the nucleus and the cytoplasm are not fully understood. Different types of cell stress lead to TDP-43 redistribution from the nucleus to the cytoplasm (Moisse et al. 2009a, b), and the protein is then found within stress granules (Freibaum et al. 2010; Dewey et al. 2011; Kiebler and Bassell 2006), which are thought to stabilize mRNAs and prevent translation under these specific cellular conditions (Kiebler and Bassell 2006). This function seems to be central for understanding the role of TDP-43 in motoneuron disease. After axotomy, TDP-43 translocates to cytosolic compartments, and this translocation seems to be functionally connected with caspase-3 activation (Moisse et al. 2009a). Also after oxidative insult, TDP-43 is recruited to stress granules (Colombrita et al. 2009). There are also reports indicating that TDP-43 could interact with SOD-1 and 14-3-3 proteins in the cytosol and thus modulate the stability of mRNAs such as the neurofilament-L chain mRNA (Volkening et al. 2009). Moreover, stress granule dynamics seems to be influenced by TDP-43 (Dewey et al. 2011; McDonald et al. 2011) under

conditions such as oxidative stress or sorbitol-induced osmotic stress. It is still unclear at which stage of disease such stress granules occur and whether TDP-43 inclusion in stress granules is a consequence of other pathophysiological mechanisms, whether such stress granules are fully reversible, or whether such stress granules can give rise to insoluble proteinaceous aggregates. As a common observation made under different types of cell stress, TDP-43 seems to be redistributed from the nucleus to the cytosol. Sporadic ALS patients with slow progression of the disease have been reported to exhibit a relatively low number of TDP-43 inclusions (Nishihira et al. 2009), and this points to a correlation between the number of these aggregates in motoneurons and severity of disease. Thus, TDP-43 aggregates apparently do not protect neurons from degeneration. It remains to be shown whether these TDP-43 aggregates are toxic and contribute to the neurodegenerative process.

The TDP-43 protein also interacts with another RNA-binding protein named fused in sarcoma (FUS) (Zinszner et al. 1994) or translocated in liposarcoma (TLS) (Freibaum et al. 2010). TDP-43 and FUS are related (Drepper et al. 2011) and both are members of the hnRNP protein family with two RRM (RRM1 and RRM2) motifs. This protein is also involved in transcriptional regulation and mRNA processing. Mutations in the FUS genes are found in 4 % of fALS cases (Kwiatkowski et al. 2009) and only rarely (probably less than 1 %) in sporadic ALS cases (Ferraiuolo et al. 2011; Mackenzie et al. 2010). Similar to TDP-43, most mutations associated with ALS are found in the C-terminal regions containing the glycine-rich domain. Some of these mutations seem to disrupt a nuclear translocation signaling, thus leading to cytoplasmic accumulation of the FUS protein within cytoplasmic granules (Ito et al. 2011; Dormann et al. 2010). Alternatively, these mutations in the C-terminus could also disturb protein interaction in particular with other members of the hnRNP family, so that the altered subcellular distribution of the FUS protein in these ALS patients could also be caused by such defects. Similar to TDP-43, it is still not resolved whether loss of a physiological function of FUS due to instability of the protein, cellular misdistribution, or decreased stability causes motoneuron degeneration or a toxic gain of function.

Gene knockout mice have been generated that lack FUS gene function (Kuroda et al. 2000). These mice show male sterility and increased sensitivity to ionizing radiation, but no phenotype that could help to understand the role of this protein in neurons and in neurodegeneration.

The last major gap in the identification of gene defects responsible for familial ALS was closed by two independent groups identifying a hexanucleotide (GGGGCC) repeat expansion in the first intron of the C9ORF72 gene on human chromosome 5 as a frequent genetic cause of ALS (Renton et al. 2011; DeJesus-Hernandez et al. 2011). These mutations, which are associated with both frontotemporal dementia and ALS, cause disease with high penetrance with autosomal-dominant inheritance. There are first indications that this pathophysiology also influences RNA metabolism and that the expanded pre-mRNA also binds to members of the hnRNP family, in particular hnRNP-A3 (Mori et al. 2013a). The repeat domain forms a G-quadruplex structure in the corresponding mRNA (Fratta

et al. 2012), exactly the same as those found in specific mRNAs that are highly sorted in neurons such as the mRNA for PSD95 and CaMKIIa (Subramanian et al. 2011; Drepper and Sendtner 2011). It is possibly that this structure encoded by mutant C9orf72 transcripts disturbs transport and sorting of mRNAs from the nucleus to the cytoplasm and subsequently into axons and dendrites. As an alternative disease mechanism, the formation of proteinaceous aggregates has been suggested. The (GGGGCC) repeat expansion seems to be translated, and the corresponding protein products are found as poly-(Gly-Ala) or poly-(Gly-Pro) or poly-(Gly-Arg) dipeptide repeat proteins in nuclei and the cytoplasm of neuronal cells (Ash et al. 2013; Mori et al. 2013b). These are presumably generated by novel translation initiation sites allowing the expanded GGGGCC repeat to be translated into proteins. Whether altered RNA metabolism by interaction of the corresponding mRNAs with hnRNP proteins or the formation of a quadruplex structure is the primary pathomechanism, or the formation of protein aggregates, and how these mechanisms relate to TDP-43 and FUS pathomechanisms is currently unknown.

Besides TDP-43, FUS, and C9orf72, two other proteins play a role in neurodegenerative disorders like FTLN and in ALS: progranulin and sortilin: Reduced progranulin levels and activity are thought to be of broad relevance for these diseases (Hu et al. 2010). Recently, sortilin was identified as a key progranulin-binding partner on the surface of cortical neurons. In the stressed nervous system, progranulin is not expressed in neurons, but in nearby glial cells. Sortilin rapidly internalizes progranulin to lysosomes. Mice that do not express Sortilin exhibit high levels of extracellular progranulin. Importantly, mice with a progranulin deficiency similar to that seen in FTLN were fully normalized with regard to progranulin levels when sortilin was deleted. These findings implicate sortilin-mediated progranulin endocytosis in FTLN and ALS pathophysiology and identify sortilin binding as a potential therapeutic site to alter progranulin pathology. Sortilin is also a co-receptor for the p75 neurotrophin receptor (P75NTR) and modifies a broad spectrum of actions through this receptor. In addition, sortilin is also involved in subcellular transport of complexes including BACE and other membrane proteins relevant for APP processing. Therefore, the interaction of p75NTR with sortilin could be a modifier for progranulin actions and thus modify disease mechanisms in those forms of motoneuron disease that suffer from altered progranulin metabolism.

5 Spinal Muscular Atrophy

Spinal muscular atrophy is the most common form of motoneuron disease in children and young adults (Hausmanowa-Petrusewicz 1978; Crawford and Pardo 1996). In contrast to amyotrophic lateral sclerosis, more than 90 % of all cases of this disease are caused by homozygous deletion or in rare cases mutation of the *SMN1* gene on human chromosome 5. In contrast to most forms of familial ALS, this form of motoneuron disease is autosomal recessive and represents one of the rare cases where loss of function of a specific gene and the corresponding protein is responsible for the disease. The Smn protein is a central component of a complex

that is necessary for assembly of spliceosomal snRNP particles (Pellizzoni 2007) and the regeneration of such particles in the nucleus. This so-called Snn complex has been characterized in much detail, the interaction partners of Snn called gemins have been identified, and the structural basis of the interaction and of the function of these components in the assembly of snRNP particles investigated. However, the Snn protein is also localized in axons (Rossoll et al. 2002) and axon terminals of motoneurons that are very distant from the cell body in which the assembly of spliceosomal snRNP particles normally occurs. This has led to the conclusions that, in addition to its role in the assembly of snRNP particles, the Snn protein could serve an additional function in RNA metabolism in axons and axon terminals (Sendtner 2001; Burghes and Beattie 2009).

In contrast to the human genome which contains two copies of *SMN* called *SMN1* and *SMN2*, both of which are expressed, the mouse genome only contains one copy of the *Snn* gene. Conventional gene knockout of *Snn* in the mouse is embryonic lethal at early developmental stages (Schrank et al. 1997), before blastocysts form. This is consistent with an essential role of the Snn protein in the assembly of spliceosomes: Abolishing pre-mRNA splicing and nuclear processing is considered not to be compatible with life. Interestingly, when the *SMN2* gene, which is still present in patients with this disease, is overexpressed on a *Snn* knockout background in mice (Monani et al. 2000), these mice develop to term and then show typical signs of the disease. The *SMN1* and *SMN2* genes differ only by five nucleotide exchanges (Wirth 2000), two of them within exons. A translationally silent cytosine to thymidine exchange at position 6 of exon 7 is responsible for the skipping of exon 7 in the majority of transcripts from the *SMN2* gene. It has been shown that this mutation abolishes an exonic splice enhancer site (Cartegni and Krainer 2002) and generates a new exonic splicing silencer domain (Kashima and Manley 2003) for the last coding exon of the *SMN2* gene. Therefore, at least 80 % of the resulting SMN protein from *SMN2* transcripts lack the C-terminal 16 amino acids which are replaced by four amino acids encoded by exon 8 sequences. As a consequence, the corresponding protein is unstable (Cho and Dreyfuss 2010), the truncated SMN protein with altered C-terminus cannot self-associate, and thus it is less active in forming SMN complexes and probably also less active in a putative axonal function.

A large variety of animal models has been generated in which the consequences of Snn deficiency have been investigated (reviewed in Burghes and Beattie 2009). In all of these organisms, complete loss of Snn is lethal, and the time point of lethality depends on the levels of maternal Snn protein. For example, this explains why death in Snn-deficient *Drosophila melanogaster* (Chan et al. 2003) occurs later during development than in early mouse embryos. Interestingly, expression of a high number of *SMN2* gene copies in *Snn*^{-/-} mice completely reverses the phenotype (Monani et al. 2000). Such mice appear healthy, indicating that high expression of SMN2 can fully restore function.

Overexpression of Snn via the prion promoter only in the nervous system in mice with low Snn expression in non-neuronal tissues also has a major effect on survival of these animals (Gavrilina et al. 2008). Thus, low levels of functional Snn

proteins produced from at least two copies of *SMN2* gene are sufficient for normal function in most organs and cell types. Apparently, motoneurons need more SMN protein than other types of neurons and non-neuronal cell types, and this could explain why the disease expresses itself as a relatively pure motoneuron disease. Interestingly, efforts to restore *Smn* expression in muscle in mouse models with reduced *Smn* expression had much less effect than restoring *Smn* in neurons (Gavrillina et al. 2008). This correlates with observations that isolated motoneurons from *Smn*^{-/-}*SMN2tg* mice already show a clear dysmorphic phenotype in cell culture (Rossoll et al. 2003). Survival of *Smn*-deficient motoneurons in cell culture is normal: No difference in neuronal numbers is observed in the presence or absence of neurotrophic factors when *Smn*^{-/-}*SMN2tg* and control motoneurons are compared in culture. However, axon growth is altered (Rossoll et al. 2003). Within the first 3 days in culture, these motoneurons show normal axon growth, but further extension of the axons between day 3 and 7 is severely disturbed (Jablonka et al. 2007). Axonal growth cones are smaller, and a specific lack of actin protein is observed in axon terminals. This correlates with the finding that actin mRNA levels are highly reduced in axons of *Smn*-deficient motoneurons (Rossoll et al. 2003).

The *Smn* protein itself does not interact with specific mRNAs such as the β -actin mRNA. However, the *Smn* protein does not only bind to components of the classical *Smn* complex (Gubitza et al. 2004), but also with other proteins of the hnRNP family, in particular hnRNP-R (Rossoll et al. 2002; Mourelatos et al. 2001). There are also reports that the *Smn* protein interacts with TDP-43 (Wang et al. 2002) and also with the FUS protein (Yamazaki et al. 2012). However, in the case of TDP-43 and FUS, it is still not fully resolved whether these interactions occur directly in postnatal motoneurons, or whether the *Smn* interacts with hnRNP complexes containing TDP-43, FUS, and other members of the hnRNP family. Studies aimed at identifying TDP-43-binding partners in nuclear and cytosolic extracts point to the fact that the TDP-43 and FUS proteins are normally present in large protein complexes involving several members of the hnRNP family (Ling et al. 2010; Freibaum et al. 2010). Therefore, it is possible that *Smn* does not directly interact with each of these proteins, but with different preference to individual members of the hnRNP family. This needs further experimental analyses.

Smn and hnRNP-R proteins are co-localized in axons of motoneurons (Rossoll et al. 2002). The hnRNP-R protein is capable of directly interacting with the β -actin mRNA, and this interaction is reduced when the *Smn*-binding domain of hnRNP-R is deleted (Rossoll et al. 2003). The consequences of reduced interaction of *Smn* with hnRNP-R are not known. However, the observation that *Smn*-deficient motoneurons show reduced β -actin translocation into axons indicates that the *Smn* protein could play a role for axonal translocation of this and probably also other mRNAs into axons. Knockdown of hnRNP-R in isolated motoneurons or in zebrafish embryos (Glinka et al. 2010) leads to a similar phenotype as *Smn* deficiency. This points to an involvement of *Smn* in the formation and function of hnRNP complexes for axonal translocation of specific mRNAs. Whether *Smn* plays an essential role in the assembly of hnRNP complexes in the nucleus, in

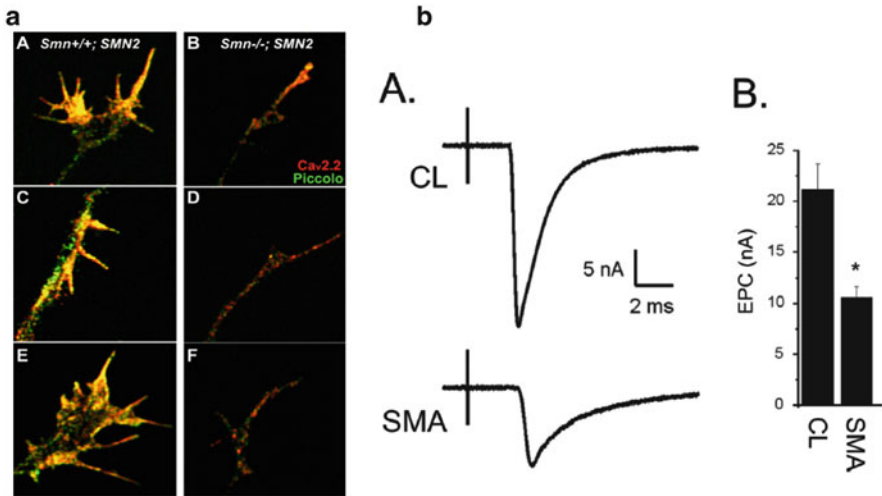


Fig. 1 Axonal defects in *Smn*-deficient motoneurons. (a) *Snn*^{-/-}–*SMN2*tg motoneurons show defects in formation of presynaptic structures. They lack the accumulation of voltage-gated calcium channels (*Cav2.2*) in the tip of axonal growth cones where active zones form. This is also reflected by reduced colocalization with other proteins of the active zone, such as piccolo (green). Reproduced from Jablonka et al. (2007) (b) Diminished neuromuscular endplate currents (EPC) in tibialis anterior muscle of postnatal *Smn*-deficient (SMA) mice. The deficit in neurotransmission is caused by a deficit in release of synaptic vesicles. CL control; **P* < 0.01. Reproduced from Kong et al. (2009)

nuclear exports of such mRNA transport complexes, and in the translocation of these protein/mRNA complexes to axons, and whether it also has a role in regulating the translation of these mRNAs in the axon terminals, remains to be determined.

Reduced axon growth has also been observed in zebrafish embryos in which *Smn* has been knocked down by Morpholino technologies (McWhorter et al. 2003). Axons are shorter and many of them are truncated or branched, so that they do not reach their physiological target muscles. There is no evidence that axon growth is reduced in *Smn*-deficient mouse models *in vivo* (McGovern et al. 2008). However, motor axons grow out very early during embryonic development, and reduced speed of axon elongation could be compensated *in vivo*, so that even motoneurons with reduced axon growth rates in cell culture reach their target and make synaptic connections. *Smn*-deficient motoneurons in cell culture show altered growth behavior on laminin- β 2/merosin (Jablonka et al. 2007). Wild-type motoneurons normally exhibit reduced axon growth on the synapse-specific form of laminin, but *Smn*-deficient motoneurons do not. This is due to altered distribution of $Ca_v2.2$ voltage-gated Ca^{2+} channels in axon terminals of *Smn*-deficient motoneurons (Fig. 1). The altered distribution of these voltage-gated Ca^{2+} channels correlates with altered excitability and altered Ca^{2+} influx after the initiation of action potentials in the cell body of *Smn*-deficient motoneurons (Jablonka et al. 2007).

Only a small proportion of action potential-like depolarizations in isolated Smn-deficient motoneurons leads to Ca^{2+} transients in axon terminals of motoneurons from the SMA mouse model. These alterations predict defects in presynaptic function and neurotransmission at the neuromuscular endplates. Indeed, such defects are also observed in mouse models. Reduced folds at neuromuscular junctions have been observed in Smn-deficient mouse models during postnatal development, and neurotransmission at the synapses is also altered (Kong et al. 2009; Torres-Benito et al. 2012; Ruiz et al. 2010) (Fig. 1).

Additional defects affecting excitability and neurotransmission have been discovered in Smn-deficient mouse and *Drosophila* models. In Smn-deficient mice, synaptic input to spinal motoneurons is reduced (Mentis et al. 2011), and the majority of the proprioceptive sensory afferents that normally make direct synaptic contact with spinal motoneurons are defective. It is still not clear whether this sensory defect is a consequence of altered excitability of motoneurons or reflects a primary defect in sensory neurons (Gogliotti et al. 2012). Such alterations in sensory afferents have also been observed in fly models. Smn deficiency in *Drosophila melanogaster* leads to aberrant splicing of stasimon in cholinergic sensory neurons and interneurons (Imlach et al. 2012; Lotti et al. 2012), due to severely impaired U12 splicing in Smn-deficient neurons, including neural cell types other than motoneurons. This leads to decreased excitation of motoneurons and thus possibly to malfunction and degeneration.

Taken together, the cellular basis of spinal muscular atrophy is complex. Smn deficiency on the one side could lead to altered splicing of gene products that are important for the function of neurons that project to motoneurons and are necessary for giving them excitatory signals. Furthermore, Smn deficiency in motoneurons could impair axon growth and presynaptic differentiation, resulting in impaired neurotransmission at neuromuscular endplates. In any case, therapy has to focus on restoration of Smn function, and this could be through increasing full-length Smn expression in neurons, i.e., through strategies that improve the inclusion of exon 7-encoded domains from the SMN2 gene, or in increasing promoter activity for the SMN2 gene with the aim to increase overall transcript levels of the Smn mRNA (Sendtner 2010). In addition, strategies to restore physiological innervation of motoneurons, motoneuron excitability, and neurotransmission at neuromuscular endplates appear essential. Such strategies could go beyond increasing the levels of functional Smn protein in motoneurons. Evidence for this has been given by depleting PTEN in Smn-deficient motoneurons in cell culture and in vivo in mouse models. PTEN depletion leads to a normalization of axon elongation, increases axonal growth cone size, and restores excitability of Smn-deficient motoneurons (Ning et al. 2010). These changes are associated with increased pAKT and p70S6 levels in Smn-deficient motor axons. This treatment also restores actin protein levels in axonal growth cones of Smn-deficient motoneurons. In vivo, the injection of siPTEN constructs in limb muscles of Smn-deficient motoneurons increases motoneuron survival (Ning et al. 2010). The hypothesis that defective actin cytoskeleton in axon terminals contributes to disease is also supported by genetic evidence in humans. Plastin-3, a protein that stabilizes filamentous actin, has

been shown as modifier of SMA in patients (Oprea et al. 2008). This genetic observation in patients has recently been confirmed by a corresponding mouse model (Ackermann et al. 2013), and this opens perspectives for therapeutic strategies that stabilize the actin cytoskeleton in presynaptic compartments of neuromuscular endplates as another target for therapy development.

6 Developmental Motoneuron Cell Death

Neuronal cell death is often considered as a pathological feature, disregarding that many neurons undergo cell death during normal development. Although some observations on this phenomenon go back to the early twentieth century, it was the work of Viktor Hamburger (1934, 1975) and other pioneer researchers who discovered the principles and physiological meaning of this phenomenon. Spinal motoneurons played a central role in this discovery process. Viktor Hamburger and his colleagues showed that developmental motoneuron cell death is guided by influences provided from target tissue. Removal of limb buds in developing chick embryos enhances massively developmental motoneuron cell death and transplantation of an additional limb reduces the number of dying motoneurons. This kind of plasticity does not only allow the individual organism to react to deviations from genetically determined developmental programs, it also allows plasticity to generate an individual architecture of the nervous system in response to environmental cues, and thus might have contributed during evolution to the generation of a highly complex nervous system in higher vertebrates. On the other hand, the complex nature of such regulatory mechanisms also implies vulnerability and any disturbance of the regulatory processes theoretically could lead to pathological losses of neurons and neuronal function. Since the cloning of BDNF (Leibrock et al. 1989) and CNTF (Stockli et al. 1989) in 1989, a broad variety of neurotrophic factors were identified that can support motoneuron survival. At least three neurotrophins, brain derived neurotrophic factor, neurotrophin-4, and neurotrophin-3, but not NGF, support motoneuron survival. The CNTF/LIF family, which mediates pro-survival actions through a cytokine receptor involving LIFR- β and gp130, also includes several members, besides CNTF leukemia-inhibitory factor (LIF), cardiotrophin-1 (CT-1) (Pennica et al. 1996), and cardiotrophin-1-like cytokine (CLC) (Elson et al. 2000).

Survival of cultured motoneurons is also supported by members of the glial-derived neurotrophic factor (GDNF) gene families. Factors supporting motoneuron survival include GDNF (Henderson et al. 1994), neurturin (Klein et al. 1997), persephin (Milbrandt et al. 1998), and artemin (Baloh et al. 1998), and these molecules mediate their survival effects through C-Ret-tyrosine kinase and specific α -receptors. Motoneurons are also supported by insulin-like growth factor 1 and 2 (Arakawa et al. 1990). In cultures of isolated embryonic chick spinal motoneurons, the survival-promoting effect of IGF is relatively low. However, when IGFs are combined with other neurotrophic factors such as CNTF, this leads to supra-additive survival effects, indicating that neurotrophic factors

potentate each other. The relatively low survival effect of IGF-1 on chick embryonic motoneurons could be due to cell culture conditions that include serum in culture medium with inhibitory insulin-like growth factor-binding proteins. Not very much is known on how insulin-like growth factor-binding proteins modulate the action of IGFs on motoneuron survival during development and in the adult.

Also other types of pluripotent growth factors support motoneuron survival such as members of the FGF family (Arakawa et al. 1990; Hughes et al. 1993a), members of the vascular endothelial growth factor (VEGF) family (Poesen et al. 2008; Azzouz et al. 2004; Carmeliet and Storkebaum 2002), or hepatocyte growth factor family (Yamamoto et al. 1997). HGF is a heterodimer protein with similarities to plasminogen. However, it lacks the enzymatic activity of plasminogen (Weidner et al. 1991). Interestingly, only lumbar motoneurons from 5-day-old chick embryos survive with HGF, but not motoneurons from thoracic or cervical spinal cord (Novak et al. 2000). In developing chick embryos, the c-met tyrosine kinase is expressed in lumbar but not in thoracic motoneurons between embryonic day 5 and 10 during the period of physiological motoneuron cell death. Additional experiments have shown that the expression of c-met in lumbar motoneurons seems to be regulated by target tissue-derived factors other than HGF. This was concluded from experiments showing that the massive cell death of motoneurons in the lumbar spinal cord after limb removal cannot be rescued by HGF treatment because the receptor was downregulated by target deprivation. HGF thus represents another neurotrophic factor that influences survival of only specific subpopulations of motoneurons and needs cooperation with other signals in order to exert a survival-promoting effect. Together with the observation that IGF acts in a supra-additive way with other factors on cultured motoneurons, this supports the conclusion that motoneuron survival during development is regulated by a complex orchestra of many factors that play together in supporting survival, presynaptic differentiation, and maturation of neuromuscular endplates, regulating preservation and stabilization of axons and by this way also the long-term functionality of these cells in the nervous system.

Such interactions are also observed experimentally after lesion in peripheral nerves. For example, when the facial nerve is transected in newborn rats, individual application of CNTF (Sendtner et al. 1990) or BDNF (Sendtner et al. 1992a) supports survival, but does not prevent atrophy of motoneuron cell bodies. Atrophy is significantly reduced when both factors are applied (Gravel et al. 1997). Not all of these factors that support survival of isolated embryonic motoneurons are also expressed in developing skeletal muscle. For example, CNTF is not expressed in muscle. The high expression found in adult mice is confined to myelinating Schwann cells, and expression of this factor only starts in the postnatal period in rodents when the period of physiological cell death is over. Similarly, only low quantities of BDNF are found in skeletal muscle (Hughes et al. 1993b). Levels of BDNF expression are much higher in Schwann cells after nerve lesion (Meyer et al. 1992). Gene knockout experiments have been performed and it has been shown that depletion of BDNF and/or NT-4 does not increase developmental cell death of motoneurons (Liu et al. 1995). The same is true in animal models lacking

CNTF and/or LIF (Holtmann et al. 2005). Only in mouse models in which GDNF is depleted (Oppenheim et al. 2000) or cardiotrophin-1 (Oppenheim et al. 2001), subsets of motoneurons are lost during this physiological cell death period. Also these genetic data point to a collaboration of several neurotrophic factors in developmental maintenance and regulation of survival during the period of physiological cell death.

These data also show that Schwann cells play a role in motoneuron maintenance. Mice in which Schwann cell-derived CNTF and LIF are eliminated show progressive loss of motoneurons and of motoneuron functions, which correlates with loss of muscle strength in adult mice (Holtmann et al. 2005). Similarly, mice deficient for erb-B3, the receptor for glial growth factor (GGF), which exhibit severely disturbed development of Schwann cells, show as a consequence significant reduction (79 %) in motoneurons (Riethmacher et al. 1997). Thus, Schwann cells apparently do not only play a role as source of survival and maintenance factor in the adult peripheral nervous system, but apparently also during development. Developing Schwann cells either play a role in helping motoneurons to contact skeletal muscle and to become functionally active, which then leads to upregulation of neurotrophic support from skeletal muscle, or alternatively, they could provide trophic support in addition to that of motoneurons, and only those motoneurons that receive sufficient signals from developing Schwann cells and muscles are supported, and those that do not receive the support are eliminated. When limb buds are completely removed from chick embryos, motoneuron survival is severely impaired (Oppenheim 1985). Similar observation is made when only skeletal muscle is destroyed (Grieshammer et al. 1998), indicating that the remaining Schwann cells are not sufficient to support survival, and therefore, additional support from muscle is necessary.

7 Interactions of Neurotrophic Signaling with Pathomechanisms of Motoneuron Disease

The identification of underlying gene defects for most of the familial forms of spinal muscular atrophy and amyotrophic lateral sclerosis has pointed to a large variety of disease mechanisms. There are two major groups of pathomechanisms that have emerged: On the one side, dysfunctional RNA processing in spinal muscular atrophy and familial forms with mutations in TDP-43, FUS, and abnormal protein aggregates and dysfunctional signaling pathways for mitochondrial metabolisms due to mutations in the SOD-I genes and potentially also the c9Orf72 gene. These two groups of pathomechanisms do not exclude each other: TDP-43 C-terminal fragments are a major component of inclusions in most cases of ALS, including the majority of sporadic ALS. Axonal swellings containing protein aggregates and dysmorphic mitochondria are commonly found in motoneurons and also in mouse models of motoneuron disease such as pmn mutant mice (Bommel et al. 2002; Selvaraj et al. 2012) (Fig. 2). None of the gene products named above are directly connected to neurotrophic factors or their receptors. Therefore,

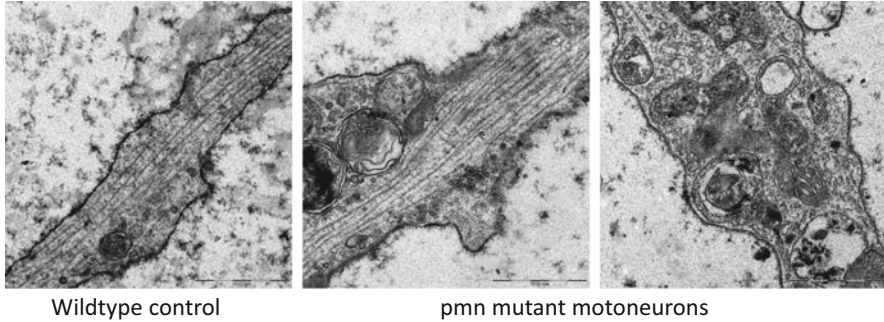


Fig. 2 Axonal swellings in isolated motoneurons from progressive motor neuropathy (pmn) mutant mice. Based on a mutation in the *TBCE* gene (Bommel et al. 2002) motoneurons develop axonal swellings containing protein aggregates and dysmorphic mitochondria. Scale bar: 1,000 nm

deficiency of individual neurotrophic factors that lead to enhanced developmental cell death, i.e., in the case of CT-1, GDNF, or HGF, or progressive postnatal motoneuron loss after depletion of CNTF, LIF, or IGF-1, apparently does not seem to be a primary cause of motoneuron disease, at least on a genetic level. Nevertheless, the signaling pathways exerted downstream from receptor for neurotrophic factors for motoneuron survival, for axon maintenance and regeneration, and for presynaptic function and stabilization of neuromuscular endplates apparently seem to be disturbed are not fully functional in motoneuron disease, and several possibilities exist that need to be considered.

First, neurotrophic factors could play a role in compensating for neurodegeneration of spinal motoneurons by promoting sprouting. The central role of neurotrophic factors in axonal and terminal sprouting has been known for a long time (Caroni 1997). Indeed, in a mouse model of a mild form of spinal muscular atrophy, *Smn*^{+/-} mice that exhibit a reduction of *Smn* protein by only 50 % and thus resemble mild forms of spinal muscular atrophy in humans do not show an overt phenotype (Simon et al. 2010). Nevertheless, there is progressive loss of motoneurons that reaches more than 50 % at an age of 1 year in this mouse model. For comparison, *Smn*^{-/-}*SMN2*^{tg} mice, the mouse model for the severe form of SMA type I only exhibits a loss of about 20 % above control (Monani et al. 2000) when mice are terminally sick and completely paralyzed early after birth. This indicates that loss of motor function does not necessarily correlate with the loss of motoneurons, in particular not in cases with slowly progressive forms of motoneuron disease that allow remaining motoneurons to sprout and compensate for lost neurons by reinnervating denervated skeletal muscle fibers. Indeed, massive sprouting occurs in *Smn*^{+/-} mice and this explains the lack of any loss of muscle strength in these mice. Electrophysiological analysis shows an increase of motor units by a factor of at least 2, and morphological analysis provides evidence for massive sprouting, including terminal sprouting and axonal sprouting to reinnervate neuromuscular endplates in different muscle groups. This type of sprouting depends

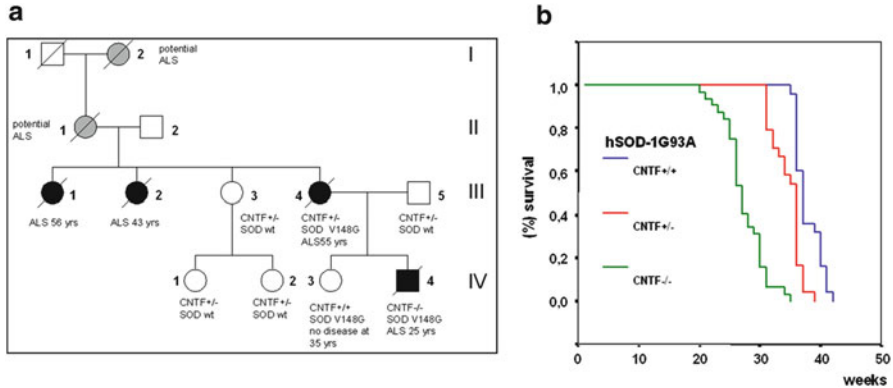


Fig. 3 Modifier effect of *CNTF* in a family with fALS patients (SOD V148G) and in SOD G93A tg mice: (a) A family with autosomal-dominant ALS with SOD-I V148G mutation showed highly variable disease onset, ranging from 25 to 56 years. Search for candidate modifier gene defects revealed a homozygous *CNTF* null mutation in the patient with early onset at 25 years. (b) Depletion of *CNTF* from SOD G93A mice confirms that *CNTF* deficiency leads to earlier disease onset in mice. Reproduced from (Giess et al. 2002)

on neurotrophic factors provided from myelinating Schwann cells, in particular *CNTF*. When these *Smn*^{+/-} mice are crossbred with *CNTF*-deficient mice, sprouting does not occur, and the compensatory increase in motor unit size detected by electromyographical analysis is also not found. Thus, neurotrophic signaling could help to compensate for loss of motoneuron function over prolonged periods, and it is possible that this contributes to the observation that most forms of ALS only become apparent at higher age.

This is also suggested by experiments when SOD-1 G93A mice are crossbred with *CNTF*-deficient mice (Giess et al. 2002) (Fig. 3). When *CNTF* is lacking, disease onset occurs earlier, providing evidence that this and probably also other factors contribute to plasticity that helps animal models or individuals with SOD-I gene defects to maintain motor function before disease finally becomes apparent. Also in patients with ALS, the presence or absence of *CNTF* seems to play a role. Due to an abundant polymorphism in the splice acceptor site of exon 2 of the *CNTF* gene, about 2 % of the population worldwide is homozygous *CNTF* deficient and express only a truncated *CNTF* protein without function. Average disease onset in such patients is at least 10 years earlier, and in one family with an SOD-I mutation, the additional homozygous deletion of *CNTF* leads to very early disease onset, whereas other members of the same family with the same SOD-I mutation develop the disease only 20 years later (Giess et al. 2002).

The question is open as to which parameters determine the time point when compensation is lost and disease becomes apparent. In SOD-I mutant mice, depletion of synaptic vesicles in presynaptic motor terminals at neuromuscular junctions precedes the loss of presynaptic branches (Pun et al. 2006) and the progressive degeneration of the motoneurons. Interestingly, when *CNTF* was injected, the

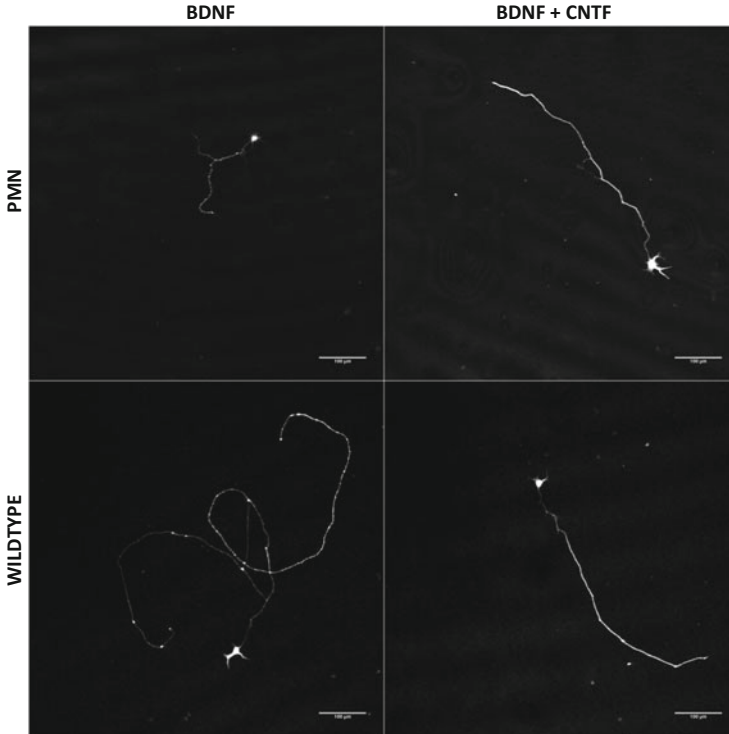


Fig. 4 CNTF rescues defective axon elongation in pmn mutant motoneurons (Bommel et al. 2002; Selvaraj et al. 2012; Sendtner et al. 1992b): Wild-type and pmn mutant motoneurons were cultured for 5 days in the presence of BDNF or BDNF+CNTF and stained with alpha-tubulin. Pmn mutant motoneurons cultured with BDNF have shorter axons when compared to wild-type controls. CNTF application restores axon elongation in pmn mutant motoneurons. *Scale bar:* 100 μ m

depletion of synaptic vesicles and pruning of nerve terminals are delayed. Even more interestingly, the injection of the neurotrophic factor GDNF was without effect in this context. This is interesting insofar as both CNTF and GDNF are potent survival factors for developing motoneurons, but apparently, they seem to differ with respect to their function in maintaining nerve terminals.

Such differences between different groups of neurotrophic factors have also been observed in other mouse models of motoneuron disease. For example, in pmn mutant mice, which develop a motoneuron disease on the basis of a gene defect in tubulin-specific chaperone-E gene (Bommel et al. 2002), CNTF can delay disease onset and prolong survival (Sendtner et al. 1992b), whereas treatment with GDNF (Sagot et al. 1996a) or BDNF cannot. The mutation in the TBCE gene leads to instability of microtubules that correspond to defective axon growth in isolated pmn mutant motoneurons in cell culture (Fig. 4).

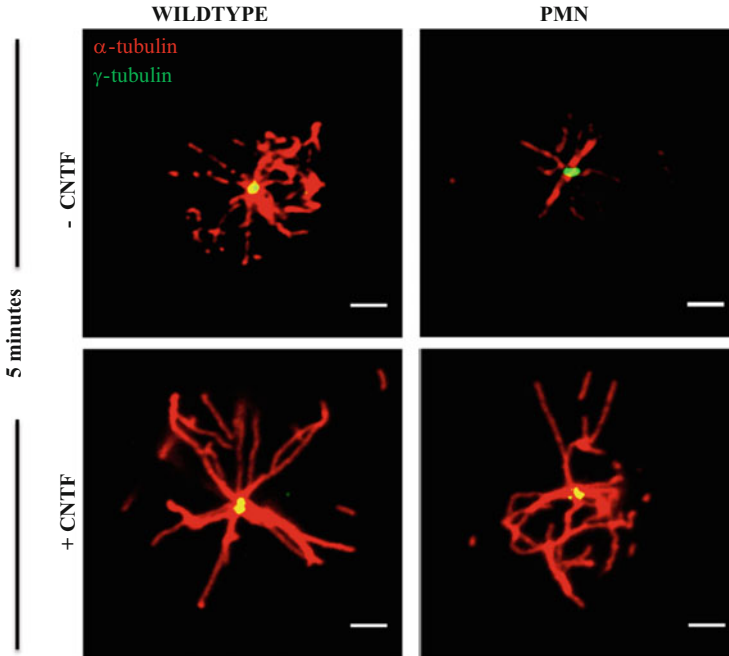


Fig. 5 CNTF promotes microtubule polymerization: Primary motoneurons isolated from wild-type and *pmn* mutant embryos were treated with nocodazole for 6 h to depolymerize the microtubule network. Nocodazole was washed out and microtubule regrowth was analyzed at 5 min after CNTF application. Polymerized microtubules were labeled with antibodies against α -tubulin (red) and microtubule organization center was labeled with antibodies against γ -tubulin (green). Number of microtubules and length of microtubules formed in *pmn* mutant motoneurons were significantly less when compared to wild-type motoneurons. Application of CNTF increased the number of microtubules and length of microtubules formed in *pmn* mutant motoneurons (Selvaraj et al. 2012). Scale bar: 2 μ m

Survival of *pmn* mutant motoneurons is primarily not affected, but axons are shorter and exhibit swellings that contain dysmorphic filaments and accumulations of mitochondria (Fig. 2). Microtubule stability is altered in *pmn* mutant motoneurons (Selvaraj et al. 2012): There is an increase of tyrosinated highly dynamic microtubules, and this correlates with reduced axonal transport of mitochondria. Interestingly, CNTF, but not GDNF or BDNF, can rescue this axonal phenotype. The CNTF effect is mediated by the activation of STAT-3, which exerts a local, non-transcriptional function in the axon via interaction with Stathmin, a microtubule-destabilizing protein. Destabilization of microtubules in cultured motoneurons shows that the capacity to regrow microtubules is highly reduced in isolated motoneurons from this mouse model of motoneuron disease and that CNTF treatment can help *pmn* mutant motoneurons to regrow stable microtubules (Fig. 5). Similarly, Stathmin knockdown also rescues the phenotype. Stabilization of microtubules with Taxol has a similar effect. Treatment of *pmn* mutant mice with CNTF delays disease onset (Sendtner et al. 1992b), but transgenic overexpression of *bcl-2* (Sagot et al. 1996b) or

treatment with GDNF only rescues cell bodies without any effect on axons, and the consequence is that onset and course of the disease are not altered by *bcl-2* overexpression or GDNF treatment. Thus, the local effect of neurotrophic signaling on axon stability, in the case of CNTF, via STAT-3 and Stathmin seems to be more important for modulating disease than the classical neurotrophic signaling pathways for neuronal survival, and it is possible that similar effects are also contributing to the modulatory effect of CNTF in ALS with mutations in the SOD-1 gene.

Some neurotrophic factors, in particular members of the neurotrophin family and their receptors, could also mediate additional effects via the p75 neurotrophin receptor (p75^{NTR}). This transmembrane protein shares structural and functional similarities with other transmembrane molecules of the FAS/APO-1 CD95 and TNF-receptor-1 family (Chao 2003). In a variety of cellular contexts *in vitro* and *in vivo*, p75^{NTR} mediates cell death after binding of neurotrophins and in particular pro-neurotrophins, in particular when Trk receptors are not expressed, and binding of pro-neurotrophins to p75^{NTR} has been shown in a variety of physiological contexts to destabilize neurites and cause neuronal cell death. Injection of neutralizing antibodies against p75^{NTR} into the eye of early chick embryos has shown that early developmental cell death of retinal ganglion cells can be mediated through this receptor (Frade et al. 1996). Some mediators of p75^{NTR} signaling also specifically destabilize axons (Plachta et al. 2007). Activation of p75^{NTR} upregulates expression of the sugar-binding protein galectin-1. Increased amounts of galectin-1 destroy neuronal processes, both in cell culture and *in vivo*. The p75^{NTR} receptor is highly upregulated in degenerating motoneurons in ALS (Kerckhoff et al. 1991; Seeburger et al. 1993), and it is likely that activation of this receptor contributes to the degenerative effects, in particular pruning of neuromuscular synapses and degeneration of neural processes (Singh et al. 2008).

In summary, neurotrophic factors modulate motoneuron disease on several levels. On the one side, they play a role in compensating the loss of motoneurons at early stages by sprouting, by stabilizing neuromuscular synapses, by stabilizing axons, and also by acting on motoneuron survival. On the other side, p75^{NTR} signaling could contribute to degenerative mechanisms responsible for denervation of neuromuscular endplates and axon destruction, possibly even motoneuron cell death at later stages of disease. This offers many options how neurotrophic factors and their signaling pathways could be used as targets for therapy. So far, clinical trials with CNTF, IGF-1, and BDNF have not been successful in motoneuron disease (Thoenen and Sendtner 2002), but this is mainly due to side effects in the case of CNTF and adverse pharmacokinetic properties in the case of BDNF and possibly also IGF-1. Future developments to overcome these problems could help to develop new therapies for motoneuron disease. Similarly, approaches that inhibit potential destructive signaling through p75^{NTR} could also be of benefit for treatment of motoneuron disease. These strategies could be even more efficient when combined with therapies aiming at counteracting the consequences of primary causes of motoneuron disease, such as *Smn* deficiency in spinal muscular atrophy, or of altered TDP-43, Fus, or C9orf72 processing in familial or sporadic forms of motoneuron disease.

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