

Mitochondrial dysfunction as a cause of ALS

A. PIZZUTI, S. PETRUCCI

Università di Roma "Sapienza", Dipartimento di Medicina Sperimentale,
Istituto CSS-Mendel, Rome, Italy

ABSTRACT

Recent studies on patients with sporadic ALS and on in vitro and in vivo models of mendelian diseases have been addressed toward the unravelling of the mitochondrial behaviour in ALS, whether as a primarily pathogenic factor, or as a fundamental contributor to the cell death. Morphological evidence suggests mitochondria pathology in ALS and many physiological mechanisms involving these organelles appear deranged in ALS, such as energy production, apoptotic triggering, calcium homeostasis and axonal transport of mitochondria. The article briefly addresses recent advances on this field.

Key words

Mitochondrion • SOD1 • ALS

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive disease with motor neuron death as a main pathological feature. Ten percent of all the ALS cases are familial, and in mendelian cases copper-zinc superoxide dismutase type 1 (*SOD1*) gene (Rosen et al., 1993) mutations have been demonstrated to be the most prevalent. Mutations of other genes have also been found in other families with motor neuron diseases of the ALS type such as a potential G-protein exchange factor ALS2 (Yang et al., 2001), vesicle associated membrane protein B (VAMPB) (Nishimura et al., 2004), senataxin (Chen et al., 2004), the p150 subunit of dynactin (DCTN1) (Puls et al., 2004), angiogenin (Greenway et al., 2006), and the recently discovered RNA processing proteins TDP-43 (Sreedharan et al., 2008) and FUS/TLS (Kwiatkowski et al., 2009).

ALS and mitochondria

Many different theories have been put forward to explain the pathogenesis of ALS (Rothstein, 2009), by the derangements of several different neuronal or glial sub-cellular components. One of the most invoked mechanism is the triggering of the apoptotic machinery, where the cell kills itself using the same pathways seen in the embryo-fetal programmed cell death (Okouchi et al., 2007). However, oxidative and glutamate receptor mediated toxicity (Shi et al., 2010), energy production and energy failure, and axonal transport derangement are variously advocated as a pathogenic mechanisms in ALS. Mitochondrial damage may be regarded as a unique ending point in motor neuro-degeneration, common to all these possible mechanisms (Barber and Shaw, 2010). As we will try to show, mitochondrial damage is well demonstrated in both human ALS and animal models. Mitochondrial damage may be the very link between all the above mentioned cell

stress phenomena and the irreversible motor neuron derangement and death.

Energy metabolism

The mitochondrion is needed for many cell functions. Mitochondria are the primary site of ATP production, maintain calcium homeostasis, participate in calcium signalling, and regulate intrinsic apoptosis. Therefore, mitochondrial function derangement may act through many basic cellular mechanisms able to cause damage and death, in particular to very energy requiring cells as motoneurons are.

Morphological evidences of mitochondrial damage have been for long time demonstrated in ALS, as subsarcolemmal aggregates in motor plaques and enlarged motor-neuron mitochondria (Atsumi, 1981; Sasaki and Iwata, 1996; Siklos et al., 1996). Similar electron microscopy features recur in mice ALS models carrying mutations in the *SOD1* gene. Here we can find the same dilated mitochondria with disruption of the crista system (Wong, 1995), internal clumps of SOD1 in mitochondria and abnormal links between mitochondria themselves and peroxisomes. In SOD1 mutant mice biochemical evidences of mitochondrial dysfunction have also been demonstrated, such as complex I and IV respiratory chain deficiency, which get worse after a long follow up (Wiedemann et al., 1998; Vielhaber et al., 2000). Another very interesting feature in SOD1 mutated mice is the decreased amount of calcium ion concentration within mitochondria compared to the cytoplasm (Kruman et al., 1999). In the end, mitochondrial pathology may cause energy production deficiency, calcium homeostasis alterations and motor neuron death. Interestingly, a patient with both motor neuron disease and cytochrome c oxidase subunit I gene microdeletion has been described (Comi et al., 1998). Another patient with motor neuron disease had a mutation in a mitochondrial tRNA gene (Borthwick et al., 2006).

SOD1 gene mutations are the most prevalent cause of mendelian ALS, segregating as a dominant trait in most individuals and as a recessive trait in a few of them. In the mice models of SOD1 ALS, SOD1 protein, which is mostly a cytosolic component (there is another strictly mitochondrial SOD) can be also found in other sub-cellular compartments such

as the endoplasmic reticulum and even mitochondria (Higgins et al., 2002). In mitochondria SOD1 and its mate protein copper chaperone for SOD1 (CCS) (Kawamata and Manfredi, 2010) are localized in the inter-membrane space (Okado-Matsumoto and Fridovich, 2001). Actually, a small amount of SOD1 is normally found in mitochondria. Unconjugated SOD1 (without Cu and Zn) freely enters mitochondria and during protein maturation (S-S mediated dimerization and ion charging) it associates to CCS, which maintains SOD1 in the inter-membrane space, until the proteins separate and SOD1 can go back to the cytosol (Field et al., 2003). As above mentioned, in SOD1 mice, the protein seems unable to be released, and aggregates in the mitochondria. These mitochondria show permeability transition, swelling and damage (Xu et al., 2004). Mitochondria swelling primarily affects inter-membrane compartments where SOD1 accumulates, as also shown in ALS patients. Mitochondria are very important in calcium ion homeostasis, checking any rapid increment in ion calcium concentration within the cell (Kawamata and Manfredi, 2010) and buffering intracellular surges of Ca^{2+} in excitable cells. Mitochondria can pump Ca^{2+} from the cytosol to the mitochondrial matrix by the Ca^{2+} uniporter and eject Ca^{2+} from the matrix by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (Cai and Lytton, 2004) and the mitochondrial permeability transition pore (Peng and Jou, 2010). When cytoplasmic Ca^{2+} is elevated, (free Ca^{2+} concentration above $0.5 \mu\text{M}$) mitochondria accumulate Ca^{2+} . Ca^{2+} filled mitochondria undergo permeability transition and swelling and rupture of the outer membrane. Mitochondria within synapses are more susceptible than non-synaptic mitochondria to Ca^{2+} overload. Ca^{2+} is also an ubiquitous second messenger and participates in many signalling pathways that are crucial for cell survival.

Calcium homeostasis

These are the main reasons why mitochondria accumulate in regions with abundant calcium flux, such as the intercellular junctions. SOD1 mutated mice actually show increased intra-neuronal calcium ion concentration and decreased homeostatic ability of mitochondria (Kawamata and Manfredi, 2010). Increased Ca^{2+} concentration and mitochondrial damage were found also in ALS patients.

As we know, calcium stimulates enzymes which can generate reactive oxygen species (ROS) and in turn can damage membrane permeability and again determine progressive calcium leakage (Adam-Vizi and Starkov, 2010). Increased cellular calcium also amplifies the glutamate mediated toxicity, which also causes calcium influx in the cell (Grosskreutz et al., 2010). ALS patients have reduced levels of synaptosomal high-affinity glutamate uptake and astroglial glutamate transporter excitatory amino acid transporter 2 (EAAT2 or GLT1) in motor cortex and spinal cord increasing the extracellular concentrations of glutamate at synapse (Yang, 2010). Motor neurons might be particularly sensitive to glutamate excitotoxicity because they have a low proportion of GluR2-edited or under-edited AMPA subtype glutamate receptor, risking excess Ca^{2+} , mitochondrial ROS production, and mitochondrial trafficking abnormalities. Markers of oxidative stress and ROS damage are elevated in human ALS, such as protein carbonyls and tyrosine nitration.

Mitochondrial permeability transition brings to cytochrome c extrusion into the cytoplasm, a *primum movens* of the apoptotic cascade, as well as increased cytoplasmic pro-apoptotic molecules Bad and Bax, and decreased anti-apoptotic Bcl-2 family proteins. Moreover, mutated SOD1 is able to lock Bcl-2 activity, activate permeability transition and accelerate cytochrome c leakage from mitochondria, resulting in apoptotic cell death (Okouchi et al., 2007). Mutant SOD1 can sequester anti-apoptotic protein Bcl-2, reduce mitochondrial membrane potential, and cause cytochrome c leakage. It follows activation of caspases. In fact intraventricular administration of minocycline, which inhibits cytochrome c release from mitochondria, delays disease onset and lengthen the survival time in ALS models as well as overexpression of anti-apoptotic protein Bcl-2 and intraventricular administration of the caspase inhibitor zVAD-fmk (Siciliano et al., 2010). Cross mate SOD1 transgenic mice and Bax knockout mice show denervation and mitochondrial vacuolization but no apoptotic death. Presynaptic terminals accumulate mutant SOD1 before the axons detach from the neuromuscular junction and show mitochondrial abnormalities at the neuromuscular junction (Gould et al., 2006). Transgenic mice experiments point to the “dying back” hypothesis, the motor neuron death resulting from damages to the distal motor axon

rather than the activation of the cell death pathway in cell bodies.

Axonal transport derangement

Mitochondrial dysfunction is also linked to the axonal transport system failure, another supposed ALS pathogenic mechanism. Mitochondria are very abundant in nerve cell axonal terminals where they provide energy by ATP and calcium flux regulation. Synaptic activity primes mitochondrion movement, distribution, ATP synthesis and dendritogenesis (Cai and Lytton, 2009). Transgenic mice with muscular overexpression of uncoupling protein 1 (UCP1), a potent mitochondrial uncoupler, display age-dependent deterioration of the neuro-muscular junction, terminal axonal degeneration and a mild late-onset motor neuron pathology (Dupuis et al., 2009). The effect is probably due to defects in the axonal transport systems. Anterograde axonal transport is mediated by kinesin proteins, while retrograde transport uses the dynein-dynactin system. By hydrolyzing ATP, kinesin 1 moves cargo toward the plus end of microtubules (anterograde transport). To date, more than 45 kinesin have been identified in humans. Most of them transport in the anterograde direction, a few function in the retrograde direction, whereas others play a role in regulating microtubule dynamics (Hirokawa et al., 2009).

Dynein is a large complex (approximately two million daltons) and consists of two dynein heavy chains (DHC), two dynein intermediate chains (DIC), four dynein light intermediate chains (DLIC), and various dynein light chains (DLC). DHC dimers are the core of the dynein complex. DHC form a globular head as the motor domain and a moving stalk involved in dimerization of the two heavy chains and in the interaction with other dynein subunits. Dynein requires dynactin which increases the motor efficiency of dynein and cross-links dynein to many transported substrates (McGrath, 2005). Dynactin is made of multiple subunits that form a filament base and a projecting sidearm linked to the base by a shoulder domain (Morfini et al., 2009). Microtubule associated transport systems can move mitochondria to distant sites in the neuron axonal tree. It is well known that mutations in genes involved in axon transport systems may cause neurodegeneration.

For instance, mutation in kinesin 5A causes hereditary spastic paraparesis type 10 (Blair et al., 2006). Mutations in dynein-dynactin retrograde system protein genes has been demonstrated to be linked to neuronal degenerations in both humans and animal models (Teuling et al., 2008). SOD1 mutated mice show a precocious impairment of retrograde transport system. Mutated SOD1 could impair retrograde transport in several ways: by physical blockade of dynein by mutant SOD1 aggregates (mutated SOD1 interacts with dynein more stably than WT SOD1), disruption of microtubule formation or stability, disturbance of dynein motor activity, disruption of the dynein-dynactin complex integrity, disruption of dynein-dynactin microtubule interaction, or masking of cargo-binding sites. One hypothesis states that the dynein mutations alter intracellular transport and thereby change the subcellular localization of SOD1 or the interaction of SOD1 with other proteins or organelles. Mutated SOD1 in mice models is able to link kinesin-2 through KAP3 protein (kinesin associated protein 3) as well as the retrograde system dynein-dynactin proteins (Tateno et al., 2009). A genomewide SNP analysis in sporadic ALS revealed that a variant of the KAP3 gene is associated with decreased KAP3 expression and increased survival in sporadic ALS.

G93A transgenic mice show inhibition of retrograde survival signaling (P-Trk and p-Erk1/2) and increase of stress signalling (P-JNK, caspase-8 and p75^{NTR}). Therefore suppression of dynein-mediated retrograde transport can produce damage to motor neurons (Perlson et al., 2009). Moreover p38 stress-activated kinase was activated and phosphorylated neurofilaments (NFM and NFH) in G93A transgenic mice. p38 has been shown to be involved in regulating fast axonal transport including transport of mitochondria. In SOD1 mutated mice, axonal transport is clearly defective and this dysfunction is a very early event. Mitochondria are transported by specific kinesines, together with other proteins belonging to a multi-meric complex, among them the miro and the milton proteins (Wang and Schwarz, 2009). Milton is a molecular adapter for the microtubular transport of mitochondria. It links directly to the kinesin heavy chain and through the miro protein, to the mitochondrion. Miro mutants in *Drosophila* show mitochondria clumping in the neuronal perikarion, less mitochondria in the neuropilus, and abnormal anterograde trans-

port of mitochondria, reminiscent of SOD1 mutated mice. Miro possesses a couple of calcium binding sites. Calcium saturated miro directly links kinesines (without milton crosslink) and it determines kinesine detachment from the microtubule. A microtubule transported mitochondrion can be therefore stopped at high calcium concentration sites such as the neuromuscular junction. High levels of intracellular calcium as observe in ALS models neurons may interfere with mitochondria movement toward the extremities of long axons causing cell retrograde degeneration.

Mitochondrial biogenesis

As above mentioned, mitochondria reproduce themselves through fusion, fission, and clearance by autophagy. In order to fuse they are transported on microtubules. Genes involved include Mfn1, Mfn2, and OPA1 GTPases all targeted to mitochondria. Mutation in Mfn2 cause Charcot-Marie-Tooth neuropathy type 2A (Zuchner et al., 2004) and mutations in OPA1 cause hereditary optic atrophy (Alexander et al., 2000). Fission genes on the other hand include Drp1 and Fis1. Overexpression of Fis1 leads to mitochondrial fragmentation, release of cytochrome c, and apoptosis. Mitochondrial clustering and fragmentation have been reported in SOD1 mice. Moreover, during apoptosis, mitochondrial fusion and fission balance is altered, with fragmentation of the mitochondria. Abnormally fragmented mitochondria do not work very well in Ca²⁺ buffering. Thus, defects in mitochondrial fission and fusion can result in the malfunction of mitochondria. As we have seen in this brief review many mechanisms can implicate mitochondria in ALS motor neuron degeneration, as a common last node in the degenerative process. Future progress in research will tell us how these evidences will be fruitful for a complete understanding of motor neuron diseases and their treatment.

Summary

Recent evidences from animal models of mendelian ALS shed light on the likely involvement of mitochondrial function failure as a central issue in ALS pathogenesis. Morphological hints of mitochondrial

pathology in patient muscle have been described time ago, and the same alteration have been reported in SOD1 mutate mice, such as swelling of sub-sarcolemmal aggregates of mitochondria in motor plaques, suggesting energy failure. Actually, respiratory complexes derangement are evident in such mouse models, and in at least one instance of human ALS, mitochondrial genome mutations have been demonstrated. In SOD1 mice, mutated SOD1 seems to trigger mitochondrial permeability transition, a critic event in apoptotic death of motor-neurons. Most theories accounting for the mitochondrial mechanism of motor-neuron degeneration involve calcium ion leakage. Increased calcium ion concentration in the cell stimulates enzymes which can generate toxic reactive oxygen species, and amplifies the glutamate mediated toxicity. Finally, mitochondrial dysfunction has been demonstrated to be able to determine and be determined by both anterograde and retrograde axonal flux derangement. Whether mitochondrial failure is the primer or just adds up to the ALS pathogenesis, the identification of its contribution may be fundamental in the search for new therapeutic strategies.

References

- Adam-Vizi V. and Starkov A.A. Calcium and mitochondrial reactive oxygen species generation: how to read the facts. *J. Alzheimers Dis.*, **20**: S413-S426, 2010.
- Alexander C., Votruba M., Pesch U.E.A., Thiselton D.L., Mayer S., Moore A., Rodriguez M., Kellner U., Leo-Kottler B., Auburger G., Bhattacharya S.S., Wissinger B. OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nature Genet.*, **26**: 211-215, 2000.
- Atsumi T. The ultrastructure of intramuscular nerves in amyotrophic lateral sclerosis. *Acta Neuropathol. (Berl.)*, **55**: 193-198, 1981.
- Barber S.C. and Shaw P.J. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. *Free Radic. Biol. Med.*, **48**: 629-641, 2010.
- Blair M.A., Ma S., Hedera P. Mutation in KIF5A can also cause adult-onset hereditary spastic paraplegia. *Neurogenetics*, **7**: 47-50, 2006.
- Borthwick G.M., Taylor R.W., Walls T.J., Tonska K., Taylor G.A., Shaw P.J., Ince P.G., Turnbull D.M. Motor neuron disease in a patient with a mitochondrial tRNA^{Ile} mutation. *Ann. Neurol.*, **59**: 570-574, 2006.
- Cai X. and Lytton J. Molecular cloning of a sixth member of the K(+)-independent Na⁺/Ca²⁺-exchanger gene family, NCKX6. *J. Biol. Chem.*, **279**: 5867-5876, 2004.
- Cai Q. and Sheng Z.H. Mitochondrial transport and docking in axons. *Exp. Neurol.*, **218**: 257-267, 2009.
- Chen Y.-Z., Bennett C.L., Huynh H.M., Blair I.P., Puls I., Irobi J., Dierick I., Abel A., Kennerson M.L., Rabin B.A., Nicholson G.A., Auer-Grumbach M., Wagner K., De Jonghe P., Griffin J.W., Fischbeck K.H., Timmerman V., Cornblath D.R., Chance P.F. DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.*, **74**: 1128-1135, 2004.
- Comi G.P., Bordoni A., Salani S., Franceschina L., Sciacco M., PELLE A., Fortunato F., Zeviani M., Napoli L., Bresolin N., Moggio M., Ausenda C.D., Taanman J.W., Scarlato G. Cytochrome c oxidase subunit I microdeletion in a patient with motor neuron disease. *Ann. Neurol.*, **43**: 110-116, 1998.
- Dupuis L., Gonzalez de Aguilar J.L., Echaniz-Laguna A., Eschbach J., Rene F., Oudart H., Halter B., Huze C., Schaeffer L., Bouillaud F., Loeffler J.P. Muscle mitochondrial uncoupling dismantles neuromuscular junction and triggers distal degeneration of motor neurons. *PLoS One*, **4**: e5390, 2009.
- Field L.S., Furukawa Y., O'Halloran T.V., Culotta V.C. Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria. *J. Biol. Chem.*, **278**: 28052-28059, 2003.
- Gould T.W., Buss R.R., Vinsant S., Prevette D., Sun W., Knudson C.M., Milligan C.E., Oppenheim R.W. Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J. Neurosci.*, **26**: 8774-8786, 2006.
- Greenway M.J., Andersen P.M., Russ C., Ennis S., Cashman S., Donaghy C., Patterson V., Swingler R., Kieran D., Prehn J., Morrison K.E., Green A., Acharya K.R., Brown R.H. Jr., Hardiman O. ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. *Nature Genet.*, **38**: 411-413, 2006.
- Grosskreutz J., Van Den Bosch L., Keller B.U. Calcium dysregulation in amyotrophic lateral sclerosis. *Cell Calcium*, **47**: 165-174, 2010.
- Higgins C.M., Jung C., Ding H., Xu Z. Mutant Cu,Zn superoxide dismutase that causes motoneu-

- ron degeneration is present in mitochondria in the CNS. *J. Neurosci.*, **22**: RC215, 2002.
- Hirokawa N., Noda Y., Tanaka Y., Niwa S. Kinesin superfamily motor proteins and intracellular transport. *Nat. Rev. Mol. Cell Biol.*, **10**: 682-696, 2009.
- Kawamata H. and Manfredi G. Import, Maturation, and Function of SOD1 and Its Copper Chaperone CCS in the Mitochondrial Intermembrane Space. *Antioxid. Redox Signal*, Epub ahead of print, 2010.
- Kawamata H. and Manfredi G. Mitochondrial dysfunction and intracellular calcium dysregulation in ALS. *Mech Ageing Dev.*, Epub ahead of print, 2010.
- Kwiatkowski T.J. Jr., Bosco D.A., LeClerc A.L., Tamrazian E., Vanderburg C.R., Russ C., Davis A., Gilchrist J., Kasarskis E.J., Munsat T., Valdmanis P., Rouleau G.A., et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science*, **323**: 1205-1208, 2009.
- Kruman W.A., Pedersen J.E., Springer E., Mattson M.P. ALS-linked Cu/Zn-SOD mutation increases vulnerability of motor neurons to excitotoxicity by a mechanism involving increased oxidative stress and perturbed calcium homeostasis. *Exp. Neurol.*, **160**: 28-39, 1999.
- McGrath J.L. Dynein motility: four heads are better than two. *Curr. Biol.*, **15**: R970-972, 2005.
- Morfini G.A., Burns M., Binder L.I., Kanaan N.M., LaPointe N., Bosco D.A., Brown R.H. Jr., Brown H., Tiwari A., Hayward L., Edgar J., Nave K.A., Garber J., Atagi Y., Song Y., Pigino G., Brady S.T. Axonal transport defects in neurodegenerative diseases. *J. Neurosci.*, **29**: 12776-12786, 2009.
- Nishimura A.L., Mitne-Neto H., Silva H.C.A., Richieri-Costa A., Middleton S., Cascio D., Kok F., Oliveira J.R.M., Gillingwater T., Webb J., Skehel P., Zatz M. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.*, **75**: 822-831, 2004.
- Okado-Matsumoto A. and Fridovich I. Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu,Zn-SOD in mitochondria. *J. Biol. Chem.*, **276**: 38388-38393, 2001.
- Okouchi M., Ekshyyan O., Maracine M., Aw T.Y. Neuronal apoptosis in neurodegeneration. *Antioxid. Redox Signal*, **9**: 1059-1096, 2007.
- Peng T.I. and Jou M.J. Oxidative stress caused by mitochondrial calcium overload. *Ann. N.Y. Acad. Sci.*, **1201**: 183-188, 2010.
- Perlson E., Jeong G.B., Ross J.L., Dixit R., Wallace K.E., Kalb R.G., Holzbaur E.L. A switch in retrograde signaling from survival to stress in rapid-onset neurodegeneration. *J. Neurosci.*, **29**: 9903-9917, 2009.
- Puls I., Jonnakuty C., LaMonte B.H., Holzbaur E.L.F., Tokito M., Mann E., Floeter M.K., Bidus K., Drayna D., Oh S.J., Brown R.H. Jr., Ludlow C.L., Fischbeck K.H. Mutant dynactin in motor neuron disease. *Nature Genet.*, **33**: 455-456, 2003.
- Rosen D.R., Siddique T., Patterson D., Figlewicz D.A., Sapp P., Hentati A., Donaldson D., Goto, J., O'Regan J.P., Deng H-X., Rahmani Z., Krizus A., et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature*, **362**: 59-62, 1993.
- Rothstein J.D. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann. Neurol.*, **65** Suppl 1: S3-S9, 2009.
- Sasaki S. and Iwata M. Impairment of fast axonal transport in the proximal axons of anterior horn neurons in amyotrophic lateral sclerosis. *Neurology*, **47**: 535-540, 1996.
- Shi P., Gal J., Kwinter D.M., Liu X., Zhu H. Mitochondrial dysfunction in amyotrophic lateral sclerosis. *Biochim. Biophys. Acta*, **1802**: 45-51, 2010.
- Siciliano G., Carlesi C., Pasquali L., Piazza S., Pietracupa S., Fornai F., Ruggieri S., Murri L. Clinical trials for neuroprotection in ALS. *CNS Neurol. Disord. Drug Targets*, **9**: 305-313, 2010.
- Siklos L., Engelhardt J., Harati Y., Smith R.G., Joo F., Appel S.H. Ultrastructural evidence for altered calcium in motor nerve terminals in amyotrophic lateral sclerosis. *Ann. Neurol.*, **39**: 203-216, 1996.
- Sreedharan J., Blair I.P., Tripathi V.B., Hu X., Vance C., Rogelj B., Ackerley S., Durnall J.C., Williams K.L., Buratti E., Baralle F., de Belleruche J., Mitchell J.D., Leigh P.N., Al-Chalabi A., Miller C.C., Nicholson G., Shaw C.E. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science*, **319**: 1668-1672, 2008.
- Tateno M., Kato S., Sakurai T., Nukina N., Takahashi R., Araki T. Mutant SOD1 impairs axonal transport of choline acetyltransferase and acetylcholine release by sequestering KAP3. *Hum. Mol. Genet.*, **18**: 942-955, 2009.
- Teuling E., van Dis V., Wulf P.S., Haasdijk E.D., Akhmanova A., Hoogenraad C.C., Jaarsma D. A novel mouse model with impaired dynein/dynactin function develops amyotrophic lateral sclerosis (ALS)-like features in motor neurons and

- improves lifespan in SOD1-ALS mice. *Hum. Mol. Genet.*, **17**: 2849-2862, 2008.
- Vielhaber S., Kunz D., Winkler K., Wiedemann F.R., Kirches E., Feistner H. Mitochondrial DNA abnormalities in skeletal muscle of patients with sporadic amyotrophic lateral sclerosis. *Brain*, **123**: 1339-1348, 2000.
- Wang X. and Schwarz T.L. The mechanism of Ca²⁺-dependent regulation of kinesin-mediated mitochondrial motility. *Cell*, **136**: 163-174, 2009.
- Wiedemann F.R., Winkler K., Kuznetsov A.V., Bartels C., Vielhaber S., Feistner H., Kunz H. Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. *J. Neurol. Sci.*, **156**: 65-72, 1998.
- Wong P.C., Pardo C.A., Borchelt D.R., Lee M.K., Copeland N.G., Jenkins N.A., Sisodia S.S., Cleveland D.W., Price D.L. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron*, **14**: 1105-1116, 1995.
- Xu Z., Jung C., Higgins C., Levine J., Kong J. Mitochondrial degeneration in amyotrophic lateral sclerosis. *J. Bioenerg. Biomembr.*, **36**: 395-399, 2004.
- Yang Y., Hentati A., Deng H.-X., Dabbagh O., Sasaki T., Hirano M., Hung W.-Y., Ouahchi K., Yan J., Azim A.C., Cole N., Gascon G., Yagmour A., Ben-Hamida M., Pericak-Vance M., Hentati F., Siddique T. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nature Genet.*, **29**: 160-165, 2001.
- Yang Y., Gozen O., Vidensky S. Robinson M.B., Rothstein J.D. Epigenetic regulation of neuron-dependent induction of astroglial synaptic protein GLT1. *Glia*, **58**: 277-286, 2010.
- Züchner S., Mersiyanova I.V., Muglia M., Bissar-Tadmouri N., Rochelle J., Dadali E.L., Zappia M., Nelis E., Patitucci A., Senderek J., Parman Y., Evgrafov O., Jonghe P.D., Takahashi Y., Tsuji S., Pericak-Vance M.A., Quattrone A., Battaloglu E., Polyakov A.V., Timmerman V., Schröder J.M., Vance J.M. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nature Genet.*, **36**: 449-451, 2004.