Published in final edited form as:

Neuromolecular Med. 2013 December ; 15(4): . doi:10.1007/s12017-013-8262-x.

Sumoylation of critical proteins in amyotrophic lateral sclerosis: emerging pathways of pathogenesis

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Abstract

Emerging lines of evidence suggest a relationship between amyotrophic lateral sclerosis (ALS) and protein sumoylation. Multiple studies have demonstrated that several of the proteins involved in the pathogenesis of ALS, including superoxide dismutase 1 (SOD1), fused in liposarcoma (FUS), and TAR DNA binding protein 43 (TDP43), are substrates for sumoylation. Additionally, recent studies in cellular and animal models of ALS revealed that sumoylation of these proteins impact their localization, longevity and how they functionally perform in disease, providing novel areas for mechanistic investigations and therapeutics. In this article, we summarize the current literature examining the impact of sumoylation of critical proteins involved in ALS and discuss the potential impact for the pathogenesis of the disease. In addition, we report and discuss the implications of new evidence demonstrating that sumoylation of a fragment derived from the proteolytic cleavage of the astroglial glutamate transporter, EAAT2, plays a direct role in downregulating the expression levels of full length EAAT2 by binding to a regulatory region of its promoter.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is an adult onset, ultimately fatal neurodegenerative disease, which primarily affects upper motor neurons in the motor cortex and lower motor neurons of the spinal cord and brain stem. The median age of diagnosis is 55 years and progression after diagnosis is swift, often culminating in death within 1–5 years (Haverkamp et al. 1995). The disorder has a focal onset followed by segmental spreading caused by the rapid degeneration of upper and lower motor neurons, resulting in muscle weakness and atrophy throughout the body and ultimately leading to death from respiratory failure (Haverkamp et al. 1995). There is no cure for ALS and the only FDA approved drug, Rilutek™ (Riluzole), only extends life by 3–6 months (Lacomblez et al. 1996) (Georgouloupoulou et al. 2013) (Miller et al. 2012).

One of the biggest challenges in the study of ALS pathogenesis is the complex nature of the disease. The etiology combines both environmental and genetic factors with 90–95% of cases having no known genetic cause or documented familial lineage (sporadic ALS or SALS). However, 5–10% of ALS cases are familial, and many have been linked to mutations in several genes (familial ALS or FALS) (Byrne et al. 2011). The first gene discovered carrying ALS-causative mutations was *superoxide dismutase 1 (SOD1)*. More

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Conflict of interest

The authors declare that they have no conflict of interest.

than 150 ALS-linked mutations have been found in *SOD1* over the course of 20 years, which are cumulatively responsible for 10–20% of FALS cases (Siddique and Deng 1996; Miller et al. 2012). The complexity of ALS is also seen in clinical manifestation. While ALS patients primarily suffer from progressive muscle wasting and weakness culminating in paralysis, some also develop dementia due to the clinical complication of frontotemporal lobar degeneration (FTLD). In 2006 and 2009 mutations in two RNA/DNA binding proteins, TAR DNA binding protein 43 (TDP43) and fused in sarcoma/translated in liposarcoma (FUS/TLS) were found to be ALS-causative (Neumann et al. 2006; Kwiatkowski et al. 2009). Mutations in the two genes have linked ALS and FTLD, as they cause a spectrum of disorders from pure ALS to pure FTLD with a large percentage of mixed cases (Geser et al. 2010). Indeed, overlap in clinical manifestations, neuroimaging and pathological findings suggest that ALS and FTLD are related diseases (Bigio et al. 2012; King et al. 2012; Lin and Dickson 2012; Whitwell et al. 2012). Furthermore, in some forms of familial ALS more recently linked to chromosome 9, affected family members develop ALS, FTLD or both (ALS/FTLD) with an autosomal dominant trait. In 2011, the genetic defect associated with chromosome 9 ALS/FTLD was identified as an aberrant number of expansions of a hexanucleotide sequence (GGGGCC) repeat in the non-coding region of the *c9orf72* gene. In addition to being involved in ~40% of familial cases, these intronic repeat expansions have been linked to ~10% of cases previously classified as sporadic (Majounie et al. 2012). Several other genes have been identified in which mutations can lead to non-traditional ALS or have been found in only a few families; including *VAPB* (Vesicle-associated membrane protein-associated protein B) (Nishimura et al. 2004), *ALS2* (alsin) (Yang et al. 2001), *VCP* (valosin-containing protein) (Johnson et al. 2010), *OPTN* (optineurin) (Maruyama et al. 2010), *UBQLN2* (ubiquilin 2) (Deng et al. 2011), *DAO* (D-amino acid oxidase) (Mitchell et al. 2010) and *SPG11* (Daoud et al. 2012). Most recently two additional genes, *hnRNPA2B1* and *hnRNPA1*, were linked to familial ALS (Kim et al. 2013).

Models of ALS and mechanisms of disease

The discovery of ALS-causative genetic mutations enabled the development of animal and cellular models to recapitulate, at least in part, the disease phenotypes, to study the disease mechanisms, and to develop potential therapeutics. The first mouse model of ALS, harboring the human *SOD1*-G93A mutation, was created in 1994 and found to recapitulate many aspects of the human disease (Gurney et al. 1994). Since then, other human *SOD1* mutations and some of the newly discovered gene mutations have been introduced into rodents and invertebrate models (Stieber et al. 2004; Watanabe et al. 2001; Popescu et al. 2013; Howland et al. 2002; Taes et al. 2013; Estes et al. 2013; Sasayama et al. 2012; Vaccaro et al. 2012; Swarup et al. 2012; McGoldrick et al. 2013). The introduction of induced pluripotent stem cell (iPSC) technology has permitted the generation of differentiated neural cells carrying disease-causative genetic mutation in the context of the genetic makeup of individual patients, allowing the creation of human cellular models (Han et al. 2011). The inclusion of iPS biology has also enabled the creation of sporadic neurodegeneration models, which had been not previously possible (Peitz et al. 2013).

Studies performed both in animal and cellular models have led to the conclusion that ALS is a multifactorial disease involving the dysfunction of many vital cellular processes and organelles. Excessive glutamate signaling leading to high intracellular Ca^{2+} can cause neuronal toxicity in a process known as excitotoxicity. Motor neurons in ALS are particularly vulnerable to calcium alterations, partly due to the overexpression of Ca^{2+} -permeable AMPA receptors (Kawahara et al. 2004; Yin et al. 2007), making them more susceptible to glutamate-induced excitotoxicity mediated by Ca^{2+} (Foran and Trotti 2009). Cellular systems, located at the interface between mitochondria and endoplasmic reticulum

that regulate intracellular Ca^{2+} levels are altered in ALS (Lautenschlager et al. 2013; Jaiswal et al. 2009), leading to elevated cytosolic Ca^{2+} . The anti-glutamatergic drug Riluzole can alleviate these excitotoxic changes by increasing glutamate uptake activity of astrocytic glutamate transporters, minimizing signaling at motor neurons and thus Ca^{2+} influx (Frizzo et al. 2004; Dall'igna et al. 2013; Cifra et al. 2011).

While it is known that motor neurons are the first and most affected cells of the CNS, recent work has indicated several other cell types are involved in ALS progression (Gentleman 2013; Evans et al. 2013). Decreases in expression and function of the astrocytic glutamate transporter, EAAT2, has been seen in animal models (Howland et al. 2002; Trotti et al. 1999; Warita et al. 2002), and evidence of protein decrease and decreased uptake has been seen in patients (Fizman et al. 2010; Rothstein et al. 1990; Bristol and Rothstein 1996). Synaptic glutamate mishandling eventually leads to an excitotoxic environment, which can be deleterious to the neighboring motor neurons.

Several lines of evidence have reported ALS primarily as a proteinopathy. Accumulation of ubiquitin positive aggregates in affected cells has been linked to dysfunction of the proteasomal degradation pathway (Takalo et al. 2013). SOD1 aggregates have been found in familial ALS patients with SOD1 mutations, in the SOD1-G93A mouse model, and in other models of ALS (Shaw et al. 2008; Wang et al. 2002a; Wang et al. 2002b). In addition, the ubiquitin E3 ligase, E6-AP, which binds WT and mutant SOD1 and whose overexpression leads to degradation of SOD1 protein and SOD1-G37R aggregates *in vitro*, was shown to be underexpressed in mutant SOD1-expressing cells and mice proceeding neurodegeneration (Mishra et al. 2013), further implicating a dysfunctional proteasomal system. TDP-43 and FUS/TLS have also been shown to form cytoplasmic inclusions in patients and models of ALS, suggesting proteinopathy in ALS not related to mutations in SOD1 (Arai et al. 2006; Rademakers et al. 2010). Similarly, ubiquitin positive aggregates of FUS/TLS (Suzuki et al. 2012; Fushimi et al. 2011), TDP43 (Nakamura et al. 2012), and SOD1 have been found in various models of ALS and in selected groups of patients (King et al. 2011).

The lines of evidence briefly summarized above all expose ALS as a complex, multifactorial disease, with many cellular abnormalities underlying motor neuron degeneration, and a multisystemic nature in which motor neuron death is the result of both intrinsic mechanisms and the contribution of different cell types including astrocytes and microglia.

Posttranslational modifications in ALS

The models based on genetic forms of ALS have been invaluable in elucidating disease mechanisms. Many of these models have shown an interaction between posttranslational modifications and proteins with disease-causative mutations or otherwise impacted by disease. Phosphorylation is one of the most common reversible posttranslational modifications and has far-reaching implications. Changes in the steady-state phosphorylation and glycosylation status of proteins have been seen in models of ALS (Hu et al. 2003). In the SOD1-G37R mouse model, for example, extensive hyperphosphorylation of neurofilaments has been reported (Farah et al. 2003), which in turn decreases O-glycosylation of these same neurofilaments in motor neurons of mice (Shan et al. 2012). The shift in the balance between phosphorylation and glycosylation of neurofilaments can contribute to slow axonal transport with strong implications for ALS pathophysiology (Williamson and Cleveland 1999).

Acetylation is another common posttranslational modification and promising work has been done with histone deacetylases (HDACs) and HDAC inhibitors in many neurodegenerative fields, including ALS. HDAC inhibitors have been tested with some success in preclinical trials for spinocerebellar ataxia type 3 (Yi et al. 2013), Parkinson's disease (Kidd and

Schneider 2011), ALS (Yoo and Ko 2011), and other neurodegenerative disorders (Ricobaraza et al. 2009; Chopra et al. 2012). In addition, mutant SOD1 increases tubulin acetylation through the downregulation of HDAC6, leading to increased SOD1 aggregation (Gal et al. 2013). Other posttranslational modifications of different proteins have been reported in ALS (Redler et al. 2011), including ubiquitinylation (Saxena et al. 2009) and nitrosylation (Schonhoff et al. 2006).

Sumoylation and ALS

Small ubiquitin-related modifier (SUMO), a ubiquitin-like protein and reversible post-translational protein modifier, can cause alterations in the localization, activity, and stability of modified proteins (Geiss-Friedlander and Melchior 2007). Sumoylation is a dynamic process involving the modification of lysine residues in often critical proteins. The rapidly reversible nature of sumoylation is vital for its role as a protein switch and for its ability to adjust to cellular changes (Schneider Aguirre and Karpen 2013). In the sumoylation process, an isopeptide bond is formed between the SUMO moiety and a lysine of the target protein in an ATP-dependent, multi-step process that resembles ubiquitylation (Tang et al. 2008). The heterodimeric activating protein E1 forms a thioester bond with the target protein, at which point the conjugating E2 (UBC9) enzyme binds. Often, a ligating E3 enzyme is required for ligation of SUMO (SUMO1-3) to the lysine of interest. In general, sumoylation occurs at a lysine residue within a consensus motif (Fuhs and Insel 2011; Arredondo et al. 2013), but exceptions have been found (Figueroa-Romero et al. 2009). Prior to sumoylation of the target protein, maturation of the SUMO moiety by SUMO-specific proteases with hydrolase activity, during which excess c-terminal amino acids are removed exposing the –GG active domain, is required (Xu and Au 2005). Several classes of desumoylating proteases (isopeptidase activity; SENPs, DeSI1-2, and USPL1) have been discovered and there is a great deal of specificity in removing the SUMO moiety from target proteins (Hickey et al. 2012). Most protein targets undergo rapid cycles of sumoylation and desumoylation. This often results in very low levels of the modified protein, making it difficult to detect. While sumoylation has not been extensively studied in ALS, the field is growing and links to disease are beginning to emerge. Reviewed below are several studies from the last few years describing the interaction of sumoylation and ALS-linked proteins and potential relations to disease.

Sumoylation and SOD1

SOD1 aggregates are a hallmark of mutant SOD1-linked ALS, found in patients and animal models of ALS (Shaw et al. 2008). The existence of hyper-oxidized SOD1 complex aggregates with toxic properties was also recently reported in sporadic ALS patient-derived cells, demonstrating a common SOD1-dependent toxicity in mutant SOD1-linked familial ALS and a subset of sporadic ALS (Guareschi et al. 2012). Fei et al. showed that SOD1 can be sumoylated by SUMO1 on lysine 9. Attachment of SUMO1 to SOD1 increases the propensity and stability of aggregation, regardless of whether or not the SOD1 is mutated. In addition, sumoylation of SOD1 increases the longevity of both wild type and mutated forms of SOD1, demonstrating that sumoylation has the potential to impact the severity of all forms of ALS (Fei et al. 2006).

Sumoylation and TDP43

Aggregated TDP-43 can also be seen in sporadic ALS and in mutant TDP43-linked ALS; hence, the aggregation of TDP-43 is an intriguing area of study for all ALS investigators. Cytoplasmic ubiquitin-positive TDP-43 aggregates have been seen in patients and models (Arai et al. 2006) and the mislocalization of TDP43 to the cytoplasm has been associated with motor neuron toxicity (Barmada et al. 2010). Recently, a spliced form of TDP-43 in

which most of the c-terminus is missing was found to be prone to aggregation. The aggregates formed by the spliced isoform are phosphorylated, ubiquitinated and sumoylated (SUMO2/3). These aggregates of TDP-43 are insoluble in buffer with a mild detergent and when cell lysates are treated with the desumoylation enzyme, SENP2, TDP-43 shows a decrease in the reactivity of SUMO2/3 in the insoluble fraction, indicating that a protein within the aggregate is directly sumoylated (Seyfried et al. 2010). The presence of SUMO2/3 in these aggregates could indicate that sumoylation contributes to the aggregation of TDP43, though it remains unclear if TDP-43 is itself sumoylated. While it is not known whether these aggregates are a toxic species, the high prevalence of TDP-43-positive aggregates makes it an area of strong interest.

FUS/TLS as an SUMO1 E3 ligase

FUS/TLS acts as an SUMO1 E3 ligase for the product of the tumor suppressor gene Ebp1, an ErbB3 binding protein. The subunit p43 of Ebp1 is sumoylated by FUS/TLS under normal conditions and levels of sumoylation are increased by DNA damage and other stress. There is also evidence that FUS/TLS is itself sumoylated (Oh et al. 2010), but how sumoylation affects FUS/TLS localization or function is not discussed. While it is not yet known if any of the ALS-causative mutations in FUS/TLS impact its ability to act as an E3 ligase, it is interesting to note that this possibility exists.

Sumoylation of the astroglial glutamate transporter EAAT2

The glutamate transporter EAAT2 is responsible for the clearance of the majority of synaptically released glutamate and expression levels of EAAT2 are decreased in the spinal cord of ALS patients (Bristol and Rothstein 1996). Decreases in EAAT2 expression and activity levels are also seen in the spinal cord of the SOD1-G93A mouse model of ALS during the symptomatic phase of the disease. This disease-driven decrease could presumably be responsible for a progressive impairment of the normal glutamatergic neurotransmission between upper and lower motor neurons, eventually contributing to the death of the spinal motor neurons by excitotoxicity (Howland et al. 2002). The involvement of EAAT2 in ALS, however, goes beyond the functional implication on glutamate homeostasis of the excitatory synapses of the spinal cord. A presumably non-apoptotic activation of caspase-3 occurring in astrocytes during disease in the SOD1-G93A mouse model of ALS leads to EAAT2 cleavage and the generation of a unique, proteolytic fragment composed of the cytosolic c-terminus domain of EAAT2, which was found to be sumoylated by SUMO1 (CTE-SUMO1; Figure 1) and to accumulate throughout disease progression in astrocyte nuclei, where it associates with promyelocytic leukemia (PML) nuclear bodies (Gibb et al. 2007). In confirmation of EAAT2 sumoylation, both SUMO1 and Ubc9, the E2-conjugating enzyme, were shown to selectively interact with the c-terminal domain of the transporter in a yeast two-hybrid screening assay. Moreover, ectopic expression of SUMO1 and Ubc9 was sufficient to drive sumoylation of EAAT2 and its c-terminus fragment (Gibb et al. 2007). Studies in the SOD1-G93A mouse showed that the number of astrocytes displaying nuclear accumulation of CTE-SUMO1 increases to up to ~20% of the GFAP⁺ cells of the ventral horn of the spinal cord at disease end-stage (Foran et al. 2011), suggesting that the accumulation of this sumoylated fragment was not a general response of astrocytes to disease progression but rather a phenomenon that was segregated to a subset of astrocytes, perhaps in response to a specific focal stimulus. Interestingly, *in vitro* experiments in a co-culture model system showed that astrocytes that display nuclear accumulation of CTE-SUMO1 can initiate a toxic response in motor neurons (Foran et al. 2011), suggesting that a similar non-cell autonomous mechanism of motor neuron toxicity plausibly could be occurring *in vivo* in ALS mouse models and patients. To explore the molecular pathway in astrocytes activated by CTE-SUMO1 that might be responsible for motor neuron impairment, genomic profiles of astrocytes transfected with either translationally fused

CTE-SUMO1 or unsumoylated CTE construct were analyzed. Changes in expression were found in several genes coding for growth factors, transcription regulators (including FUS/TLS and TARDBP/TDP43), and zinc ion binding proteins as well as genes involved in oxidative phosphorylation, mitochondrial function, cellular respiration, protein modification/ubiquitination, and protein processing, suggesting that the CTE-SUMO1 fragment could act as a generic dysregulator of the astrocyte physiology, which ultimately leads to death of motor neurons through a yet unidentified factor or combination of factors (Foran et al. 2011). Some of the discovered transcriptionally dysregulated proteins are currently being further investigated by the lab.

Transcriptional downregulation of the EAAT2 transporter by CTE-SUMO1 fragment

CTE-SUMO1 accumulation in PML nuclear bodies of astrocytes changes the transcription of numerous proteins. Most interestingly, we found that CTE-SUMO1 expression in spinal cord astrocytes caused a 4-fold decrease in EAAT2 protein levels (Figure 2A, B). No changes were found in the astrocytic glutamate transporter, EAAT1 (Figure 2A, B). Messenger RNA levels of EAAT2 were also downregulated, indicating that the negative regulatory impact of CTE-SUMO1 was at the level of the *EAAT2* gene transcription (Figure 2C). The regulatory effect of CTE-SUMO1 is extended to other glial transporters as well. The mRNA encoding the astrocytic glycine transporter, GlyT1 is upregulated (Figure 2C), while the mRNA encoding the glia GABA transporter, GAT4 is downregulated (not shown).

EAAT2 expression in astrocytes is regulated by the presence of motor neurons in culture. When astrocytes are cultured with neurons, kappa B-motif binding phosphoprotein (KBBP) binds the promoter of EAAT2 and increases EAAT2 transcription and translation (Yang et al. 2009). While expression of CTE-SUMO1 causes downregulation of EAAT2, it does not prevent the neuron-stimulated, KBBP-mediated transcriptional activation of EAAT2 (Figure 3) but rather acts independently. Addition of neurons to spinal cord astrocyte cultures increased EAAT2 mRNA expression in CTE-SUMO1 transduced astrocytes by 1.4-fold, similarly to what was seen in untreated or control transduced astrocytes (Figure 3). Chromatin immunoprecipitation (ChIP) analysis revealed an occupancy of CTE-SUMO1 on EAAT2 promoter (Figure 4 A,B). The ChIP assay was performed on chromatin fragments prepared from mouse spinal cords of non-transgenic, wild type SOD1 and SOD1-G93A mice at pre-symptomatic (50 days old) and symptomatic stage of disease (130 days old). EAAT2 promoter sites, shown as Probe A and Probe B (Figure 4A) were positive for binding tested with an anti-EAAT2 antibody directed toward its c-terminus CTE domain (Figure 1), indicating direct occupation of CTE-SUMO1 to these promoter sites. Interestingly, the binding had a tendency to increase as disease progressed to symptomatic stage. Of note, the binding of CTE-SUMO1 to the EAAT2 promoter did not prevent EAAT2 upregulation by neurons via KBBP. More studies are needed to unravel intimate mechanisms of this transcriptional regulation.

In summary, we have started to unravel additional mechanisms of dysregulation of the excitatory synapse homeostasis, mediated by a sumoylated fragment generated from the glia glutamate transporter, EAAT2, in ALS. Binding of CTE-SUMO1 to the EAAT2 promoter leads to the downregulation of EAAT2 mRNA and protein. Therefore, accumulation of CTE-SUMO1 in ALS could contribute to the disease pathogenesis by decreasing the transcription of EAAT2 gene, which ultimately leads to a decrease in EAAT2 protein levels and to glutamate-mediated excitotoxicity.

Sumoylation and other degenerative diseases of the motor neuron: spinal-bulbar muscular atrophy

Sumoylation has also been implicated in a disease with a very similar etiology to ALS, spinal-bulbar muscular atrophy (SBMA). SBMA is caused by the expansion of a CAG repeat in the n-terminal region of the androgen receptor. An X-linked adult onset degenerative disorder, SBMA causes the dysfunction of the spinobulbar motor neurons, leading to deficits in speech, chewing, swallowing and often atrophies of proximal limb muscles. It has been shown that the androgen receptor is sumoylated and that the sumoylation of the androgen receptor is increased in the presence of hormone (Kaikkonen et al. 2009). It is interesting that the sumoylation is hormone-dependent because it has been shown that expanded CAG-repeat dependent toxicity is also reliant on the presence of hormone (Chevalier-Larsen et al. 2004). However, the sumoylation of androgen receptor with the CAG expansion leads to the attenuation of protein aggregation (Mukherjee et al. 2009).

Similar to the study of sumoylation in ALS, the field of sumoylation effects in SBMA is in its infancy. That the primary protein is sumoylated under physiological conditions indicates a central role for the sumoylation, though the functional implications are not clear yet. However, unlike the sumoylation of TDP-43 and of SOD1, the sumoylation of the androgen receptor reduces the aggregation of the mutated protein. It may indicate that the prevention of sumoylation will not prove to be a panacea in all neurodegenerative disorders.

Conclusions

It is clear that alterations to posttranslational modifications in ALS are part of a growing field of research and, in particular, sumoylation has stood out as a convergent mechanism. The prevalence of sumoylation on disease causative proteins may prove to be a common target for familial and sporadic ALS. Increased protein longevity is central to proteinopathies like ALS, and longevity caused by sumoylation is troubling but may prove to be a pathway to therapeutics. While it is not known if the large compact aggregates are themselves toxic, the existence of long lasting proteins that harbor disease causative mutations is a significant area of concern.

The impact of sumoylation on protein localization has been well described. Several proteins that shuttle into the nucleus (Truong et al. 2012; Woeller et al. 2007) require SUMO attachment for proper function. In addition, sumoylation has been linked to localization and functional changes of ALS-linked proteins. The mislocalization of the DNA/RNA binding proteins FUS/TLS and TDP43 has been linked to disease and therefore could prove to be vital in disease pathology. In addition, the nuclear localization of the novel peptide CTE-SUMO1 has been directly linked to genetic alterations, which impact the fundamental biology of astrocytes. The study of sumoylation in ALS will continue to grow and will be an important aspect of future discoveries.

Acknowledgments

This work was supported by the National Institute of Health, grants RO1-NS44292 (to DT) and F31-NS073351 (to EF). The Weinberg Unit for ALS research is also supported by the Farber Family Foundation.

References

- Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun*. 2006; 351(3):602–611. [PubMed: 17084815]

- Arredondo C, Orellana M, Vecchiola A, Pereira LA, Galdames L, Andres ME. PIASgamma enhanced SUMO-2 modification of Nurr1 activation-function-1 domain limits Nurr1 transcriptional synergy. *PLoS One*. 2013; 8(1):e55035. [PubMed: 23358114]
- Barmada SJ, Skibinski G, Korb E, Rao EJ, Wu JY, Finkbeiner S. Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. *J Neurosci*. 2010; 30(2):639–649. [PubMed: 20071528]
- Bigio EH, Weintraub S, Rademakers R, Baker M, Ahmadian SS, Rademaker A, et al. Frontotemporal lobar degeneration with TDP-43 proteinopathy and chromosome 9p repeat expansion in C9ORF72: clinicopathologic correlation. *Neuropathology*. 2012
- Boston-Howes W, Gibb SL, Williams EO, Pasinelli P, Brown RH Jr, Trotti D. Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. *J Biol Chem*. 2006; 281(20):14076–14084. [PubMed: 16567804]
- Bristol LA, Rothstein JD. Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex. *Ann Neurol*. 1996; 39(5):676–679. [PubMed: 8619555]
- Byrne S, Walsh C, Lynch C, Bede P, Elamin M, Kenna K, et al. Rate of familial amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry*. 2011; 82(6):623–627. [PubMed: 21047878]
- Chevalier-Larsen ES, O'Brien CJ, Wang H, Jenkins SC, Holder L, Lieberman AP, et al. Castration restores function and neurofilament alterations of aged symptomatic males in a transgenic mouse model of spinal and bulbar muscular atrophy. *J Neurosci*. 2004; 24(20):4778–4786. [PubMed: 15152038]
- Chopra V, Quinti L, Kim J, Vollor L, Narayanan KL, Edgerly C, et al. The sirtuin 2 inhibitor AK-7 is neuroprotective in Huntington's disease mouse models. *Cell Rep*. 2012; 2(6):1492–1497. [PubMed: 23200855]
- Cifra A, Nani F, Nistri A. Riluzole is a potent drug to protect neonatal rat hypoglossal motoneurons in vitro from excitotoxicity due to glutamate uptake block. *Eur J Neurosci*. 2011; 33(5):899–913. [PubMed: 21324003]
- Dall'igna OP, Bobermin LD, Souza DO, Quincozes-Santos A. Riluzole increases glutamate uptake by cultured C6 astroglial cells. *Int J Dev Neurosci*. 2013; 31(7):482–486. [PubMed: 23777615]
- Daoud H, Zhou S, Noreau A, Sabbagh M, Belzil V, Dionne-Laporte A, et al. Exome sequencing reveals SPG11 mutations causing juvenile ALS. *Neurobiol Aging*. 2012; 33(4):839. e835–839. [PubMed: 22154821]
- Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature*. 2011; 477(7363):211–215. [PubMed: 21857683]
- Estes PS, Daniel SG, McCallum AP, Boehringer AV, Sukhina AS, Zwick RA, et al. Motor neurons and glia exhibit specific individualized responses to TDP-43 expression in a *Drosophila* model of amyotrophic lateral sclerosis. *Dis Model Mech*. 2013; 6(3):721–733. [PubMed: 23471911]
- Evans MC, Couch Y, Sibson N, Turner MR. Inflammation and neurovascular changes in amyotrophic lateral sclerosis. *Mol Cell Neurosci*. 2013; 53:34–41. [PubMed: 23110760]
- Farah CA, Nguyen MD, Julien JP, Leclerc N. Altered levels and distribution of microtubule-associated proteins before disease onset in a mouse model of amyotrophic lateral sclerosis. *J Neurochem*. 2003; 84(1):77–86. [PubMed: 12485403]
- Fei E, Jia N, Yan M, Ying Z, Sun Q, Wang H, et al. SUMO-1 modification increases human SOD1 stability and aggregation. *Biochem Biophys Res Commun*. 2006; 347(2):406–412. [PubMed: 16828461]
- Figuerola-Romero C, Iniguez-Lluhi JA, Stadler J, Chang CR, Arnoult D, Keller PJ, et al. SUMOylation of the mitochondrial fission protein Drp1 occurs at multiple nonconsensus sites within the B domain and is linked to its activity cycle. *FASEB J*. 2009; 23(11):3917–3927. [PubMed: 19638400]
- Fizman ML, Ricart KC, Latini A, Rodriguez G, Sica RE. In vitro neurotoxic properties and excitatory aminoacids concentration in the cerebrospinal fluid of amyotrophic lateral sclerosis patients. Relationship with the degree of certainty of disease diagnoses. *Acta Neurol Scand*. 2010; 121(2):120–126. [PubMed: 19804473]

- Foran E, Bogush A, Goffredo M, Roncaglia P, Gustincich S, Pasinelli P, et al. Motor neuron impairment mediated by a sumoylated fragment of the glial glutamate transporter EAAT2. *Glia*. 2011; 59(11):1719–1731. [PubMed: 21769946]
- Foran E, Trotti D. Glutamate transporters and the excitotoxic path to motor neuron degeneration in amyotrophic lateral sclerosis. *Antioxid Redox Signal*. 2009; 11(7):1587–1602. [PubMed: 19413484]
- Frizzo ME, Dall'Onder LP, Dalcin KB, Souza DO. Riluzole enhances glutamate uptake in rat astrocyte cultures. *Cell Mol Neurobiol*. 2004; 24(1):123–128. [PubMed: 15049516]
- Fuhs SR, Insel PA. Caveolin-3 undergoes SUMOylation by the SUMO E3 ligase PIASy: sumoylation affects G-protein-coupled receptor desensitization. *J Biol Chem*. 2011; 286(17):14830–14841. [PubMed: 21362625]
- Fushimi K, Long C, Jayaram N, Chen X, Li L, Wu JY. Expression of human FUS/TLS in yeast leads to protein aggregation and cytotoxicity, recapitulating key features of FUS proteinopathy. *Protein Cell*. 2011; 2(2):141–149. [PubMed: 21327870]
- Gal J, Chen J, Barnett KR, Yang L, Brumley E, Zhu H. HDAC6 regulates mutant SOD1 aggregation through two SMIR motifs and tubulin acetylation. *J Biol Chem*. 2013; 288(21):15035–15045. [PubMed: 23580651]
- Geiss-Friedlander R, Melchior F. Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol*. 2007; 8(12):947–956. [PubMed: 18000527]
- Gentleman SM. Review: microglia in protein aggregation disorders: friend or foe? *Neuropathol Appl Neurobiol*. 2013; 39(1):45–50. [PubMed: 23339288]
- Georgouloupoulou E, Fini N, Vinceti M, Monelli M, Vacondio P, Bianconi G, et al. The impact of clinical factors, riluzole and therapeutic interventions on ALS survival: A population based study in Modena, Italy. *Amyotroph Lateral Scler Frontotemporal Degener*. 2013; 14(5–6):338–345. [PubMed: 23373475]
- Geser F, Lee VM, Trojanowski JQ. Amyotrophic lateral sclerosis and frontotemporal lobar degeneration: a spectrum of TDP-43 proteinopathies. *Neuropathology*. 2010; 30(2):103–112. [PubMed: 20102519]
- Gibb SL, Boston-Howes W, Lavina ZS, Gustincich S, Brown RH Jr, Pasinelli P, et al. A caspase-3-cleaved fragment of the glial glutamate transporter EAAT2 is sumoylated and targeted to promyelocytic leukemia nuclear bodies in mutant SOD1-linked amyotrophic lateral sclerosis. *J Biol Chem*. 2007; 282(44):32480–32490. [PubMed: 17823119]
- Guareschi S, Cova E, Cereda C, Ceroni M, Donetti E, Bosco DA, et al. An over-oxidized form of superoxide dismutase found in sporadic amyotrophic lateral sclerosis with bulbar onset shares a toxic mechanism with mutant SOD1. *Proc Natl Acad Sci U S A*. 2012
- Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science*. 1994; 264(5166):1772–1775. [see comments] [published erratum appears in *Science* 1995 Jul 14;269(5221):149]. [PubMed: 8209258]
- Han SS, Williams LA, Eggan KC. Constructing and deconstructing stem cell models of neurological disease. *Neuron*. 2011; 70(4):626–644. [PubMed: 21609821]
- Haverkamp LJ, Appel V, Appel SH. Natural history of amyotrophic lateral sclerosis in a database population. Validation of a scoring system and a model for survival prediction. *Brain*. 1995; 118(Pt 3):707–719. [PubMed: 7600088]
- Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. *Nat Rev Mol Cell Biol*. 2012; 13(12):755–766. [PubMed: 23175280]
- Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, et al. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc Natl Acad Sci U S A*. 2002; 99(3):1604–1609. [PubMed: 11818550]
- Hu JH, Zhang H, Wagey R, Krieger C, Pelech SL. Protein kinase and protein phosphatase expression in amyotrophic lateral sclerosis spinal cord. *J Neurochem*. 2003; 85(2):432–442. [PubMed: 12675919]

- Jaiswal MK, Zech WD, Goos M, Leutbecher C, Ferri A, Zippelius A, et al. Impairment of mitochondrial calcium handling in a mtSOD1 cell culture model of motoneuron disease. *BMC Neurosci.* 2009; 10:64. [PubMed: 19545440]
- Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron.* 2010; 68(5):857–864. [PubMed: 21145000]
- Kaikkonen S, Jaaskelainen T, Karvonen U, Rytinki MM, Makkonen H, Gioeli D, et al. SUMO-specific protease 1 (SENP1) reverses the hormone-augmented SUMOylation of androgen receptor and modulates gene responses in prostate cancer cells. *Mol Endocrinol.* 2009; 23(3):292–307. [PubMed: 19116244]
- Kawahara Y, Ito K, Sun H, Aizawa H, Kanazawa I, Kwak S. Glutamate receptors: RNA editing and death of motor neurons. *Nature.* 2004; 427(6977):801. [PubMed: 14985749]
- Kidd SK, Schneider JS. Protective effects of valproic acid on the nigrostriatal dopamine system in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *Neuroscience.* 2011; 194:189–194. [PubMed: 21846494]
- Kim HJ, Kim NC, Wang YD, Scarborough EA, Moore J, Diaz Z, et al. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. *Nature.* 2013; 495(7442):467–473. [PubMed: 23455423]
- King A, Al-Sarraj S, Troakes C, Smith BN, Maekawa S, Iovino M, et al. Mixed tau, TDP-43 and p62 pathology in FTLD associated with a C9ORF72 repeat expansion and p.Ala239Thr MAPT (tau) variant. *Acta Neuropathol.* 2012
- King A, Maekawa S, Bodi I, Troakes C, Al-Sarraj S. Ubiquitinated, p62 immunopositive cerebellar cortical neuronal inclusions are evident across the spectrum of TDP-43 proteinopathies but are only rarely additionally immunopositive for phosphorylation-dependent TDP-43. *Neuropathology.* 2011; 31(3):239–249. [PubMed: 21118398]
- Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science.* 2009; 323(5918):1205–1208. [PubMed: 19251627]
- Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet.* 1996; 347(9013):1425–1431. [PubMed: 8676624]
- Lautenschlager J, Prell T, Ruhmer J, Weidemann L, Witte OW, Grosskreutz J. Overexpression of human mutated G93A SOD1 changes dynamics of the ER mitochondria calcium cycle specifically in mouse embryonic motor neurons. *Exp Neurol.* 2013; 247:91–100. [PubMed: 23578819]
- Lin W, Dickson DW. Ultrastructure of ubiquitin-positive, TDP-43-negative neuronal inclusions in cerebral cortex of C9ORF72-linked frontotemporal lobar degeneration/amyotrophic lateral sclerosis. *Neuropathology.* 2012; 32(6):679–681. [PubMed: 22394026]
- Majounie E, Traynor BJ, Chio A, Restagno G, Mandrioli J, Benatar M, et al. Mutational analysis of the VCP gene in Parkinson's disease. *Neurobiol Aging.* 2012; 33(1):209. e201–202. [PubMed: 21920633]
- Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, et al. Mutations of optineurin in amyotrophic lateral sclerosis. *Nature.* 2010; 465(7295):223–226. [PubMed: 20428114]
- McGoldrick P, Joyce PI, Fisher EM, Greensmith L. Rodent models of amyotrophic lateral sclerosis. *Biochim Biophys Acta.* 2013; 1832(9):1421–1436. [PubMed: 23524377]
- Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev.* 2012; 3 CD001447.
- Mishra A, Maheshwari M, Chhangani D, Fujimori-Tonou N, Endo F, Joshi AP, et al. E6-AP association promotes SOD1 aggregates degradation and suppresses toxicity. *Neurobiol Aging.* 2013; 34(4):1310, e1311–e1323. [PubMed: 23040663]
- Mitchell J, Paul P, Chen HJ, Morris A, Payling M, Falchi M, et al. Familial amyotrophic lateral sclerosis is associated with a mutation in D-amino acid oxidase. *Proc Natl Acad Sci U S A.* 2010; 107(16):7556–7561. [PubMed: 20368421]

- Mukherjee S, Thomas M, Dadgar N, Lieberman AP, Iniguez-Lluhi JA. Small ubiquitin-like modifier (SUMO) modification of the androgen receptor attenuates polyglutamine-mediated aggregation. *J Biol Chem*. 2009; 284(32):21296–21306. [PubMed: 19497852]
- Nakamura M, Kaneko S, Wate R, Asayama S, Nakamura Y, Fujita K, et al. Regionally different immunoreactivity for Smurf2 and pSmad2/3 in TDP-43-positive inclusions of amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol*. 2012
- Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis. *Science*. 2006; 314(5796):130–133. [PubMed: 17023659]
- Nishimura AL, Mitne-Neto M, Silva HC, Richieri-Costa A, Middleton S, Cascio D, et al. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet*. 2004; 75(5):822–831. [PubMed: 15372378]
- Oh SM, Liu Z, Okada M, Jang SW, Liu X, Chan CB, et al. Ebp1 sumoylation, regulated by TLS/FUS E3 ligase, is required for its anti-proliferative activity. *Oncogene*. 2010; 29(7):1017–1030. [PubMed: 19946338]
- Peitz M, Jungverdorben J, Brustle O. Disease-specific iPS cell models in neuroscience. *Curr Mol Med*. 2013; 13(5):832–841. [PubMed: 23642064]
- Popescu IR, Nicaise C, Liu S, Bisch G, Knippenberg S, Daubie V, et al. Neural progenitors derived from human induced pluripotent stem cells survive and differentiate upon transplantation into a rat model of amyotrophic lateral sclerosis. *Stem Cells Transl Med*. 2013; 2(3):167–174. [PubMed: 23413376]
- Rademakers R, Stewart H, DeJesus-Hernandez M, Krieger C, Graff-Radford N, Fabros M, et al. Fus gene mutations in familial and sporadic amyotrophic lateral sclerosis. *Muscle Nerve*. 2010; 42(2):170–176. [PubMed: 20544928]
- Redler RL, Wilcox KC, Proctor EA, Fee L, Caplow M, Dokholyan NV. Glutathionylation at Cys-111 induces dissociation of wild type and FALS mutant SOD1 dimers. *Biochemistry*. 2011; 50(32):7057–7066. [PubMed: 21739997]
- Ricobaraza A, Cuadrado-Tejedor M, Perez-Mediavilla A, Frechilla D, Del Rio J, Garcia-Osta A. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. *Neuropsychopharmacology*. 2009; 34(7):1721–1732. [PubMed: 19145227]
- Rothstein JD, Tsai G, Kuncel RW, Clawson L, Cornblath DR, Drachman DB, et al. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann Neurol*. 1990; 28(1):18–25. [PubMed: 2375630]
- Sasayama H, Shimamura M, Tokuda T, Azuma Y, Yoshida T, Mizuno T, et al. Knockdown of the Drosophila fused in sarcoma (FUS) homologue causes deficient locomotive behavior and shortening of motoneuron terminal branches. *PLoS One*. 2012; 7(6):e39483. [PubMed: 22724023]
- Saxena S, Cabuy E, Caroni P. A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. *Nat Neurosci*. 2009; 12(5):627–636. [PubMed: 19330001]
- Schneider Aguirre R, Karpen SJ. Inflammatory Mediators Increase SUMOylation of Retinoid X Receptor alpha in a c-Jun N-Terminal Kinase-Dependent Manner in Human Hepatocellular Carcinoma Cells. *Mol Pharmacol*. 2013; 84(2):218–226. [PubMed: 23690070]
- Schonhoff CM, Matsuoka M, Tummala H, Johnson MA, Estevez AG, Wu R, et al. S-nitrosothiol depletion in amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A*. 2006; 103(7):2404–2409. [PubMed: 16461917]
- Seyfried NT, Gozal YM, Dammer EB, Xia Q, Duong DM, Cheng D, et al. Multiplex SILAC analysis of a cellular TDP-43 proteinopathy model reveals protein inclusions associated with SUMOylation and diverse polyubiquitin chains. *Mol Cell Proteomics*. 2010; 9(4):705–718. [PubMed: 20047951]
- Shan X, Voadlo DJ, Krieger C. Reduced protein O-glycosylation in the nervous system of the mutant SOD1 transgenic mouse model of amyotrophic lateral sclerosis. *Neurosci Lett*. 2012; 516(2):296–301. [PubMed: 22521585]
- Shaw BF, Lelie HL, Durazo A, Nersissian AM, Xu G, Chan PK, et al. Detergent-insoluble aggregates associated with amyotrophic lateral sclerosis in transgenic mice contain primarily full-length, unmodified superoxide dismutase-1. *J Biol Chem*. 2008; 283(13):8340–8350. [PubMed: 18192269]

- Siddique T, Deng HX. Genetics of amyotrophic lateral sclerosis. *Human Molecular Genetics*. 1996; 5(Spec No):1465–1470. [PubMed: 8875253]
- Stieber A, Gonatas JO, Moore JS, Bantly A, Yim HS, Yim MB, et al. Disruption of the structure of the Golgi apparatus and the function of the secretory pathway by mutants G93A and G85R of Cu, Zn superoxide dismutase (SOD1) of familial amyotrophic lateral sclerosis. *J Neurol Sci*. 2004; 219(1–2):45–53. [PubMed: 15050437]
- Suzuki N, Kato S, Kato M, Warita H, Mizuno H, Kato M, et al. FUS/TLS-immunoreactive neuronal and glial cell inclusions increase with disease duration in familial amyotrophic lateral sclerosis with an R521C FUS/TLS mutation. *J Neuropathol Exp Neurol*. 2012; 71(9):779–788. [PubMed: 22878663]
- Swarup V, Audet JN, Phaneuf D, Kriz J, Julien JP. Abnormal regenerative responses and impaired axonal outgrowth after nerve crush in TDP-43 transgenic mouse models of amyotrophic lateral sclerosis. *J Neurosci*. 2012; 32(50):18186–18195. [PubMed: 23238732]
- Taes I, Timmers M, Hersmus N, Bento-Abreu A, Van Den Bosch L, Van Damme P, et al. Hdac6 deletion delays disease progression in the SOD1G93A mouse model of ALS. *Hum Mol Genet*. 2013; 22(9):1783–1790. [PubMed: 23364049]
- Takalo M, Salminen A, Soininen H, Hiltunen M, Haapasalo A. Protein aggregation and degradation mechanisms in neurodegenerative diseases. *Am J Neurodegener Dis*. 2013; 2(1):1–14. [PubMed: 23516262]
- Tang Z, Hecker CM, Scheschonka A, Betz H. Protein interactions in the sumoylation cascade: lessons from X-ray structures. *FEBS J*. 2008; 275(12):3003–3015. [PubMed: 18492068]
- Trotti D, Rolfs A, Danbolt NC, Brown RH Jr, Hediger MA. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat Neurosci*. 1999; 2(9):848. [PubMed: 10461226]
- Truong K, Lee TD, Li B, Chen Y. Sumoylation of SAE2 C terminus regulates SAE nuclear localization. *J Biol Chem*. 2012; 287(51):42611–42619. [PubMed: 23095757]
- Vaccaro A, Tauffenberger A, Aggad D, Rouleau G, Drapeau P, Parker JA. Mutant TDP-43 and FUS cause age-dependent paralysis and neurodegeneration in *C. elegans*. *PLoS One*. 2012; 7(2):e31321. [PubMed: 22363618]
- Wang J, Xu G, Borchelt DR. High molecular weight complexes of mutant superoxide dismutase 1: age-dependent and tissue-specific accumulation. *Neurobiol Dis*. 2002a; 9(2):139–148. [PubMed: 11895367]
- Wang J, Xu G, Gonzales V, Coonfield M, Fromholt D, Copeland NG, et al. Fibrillar inclusions and motor neuron degeneration in transgenic mice expressing superoxide dismutase 1 with a disrupted copper-binding site. *Neurobiol Dis*. 2002b; 10(2):128–138. [PubMed: 12127151]
- Warita H, Manabe Y, Murakami T, Shiote M, Shiro Y, Hayashi T, et al. Tardive decrease of astrocytic glutamate transporter protein in transgenic mice with ALS-linked mutant SOD1. *Neurol Res*. 2002; 24(6):577–581. [PubMed: 12238624]
- Watanabe M, Dykes-Hoberg M, Culotta VC, Price DL, Wong PC, Rothstein JD. Histological evidence of protein aggregation in mutant SOD1 transgenic mice and in amyotrophic lateral sclerosis neural tissues. *Neurobiol Dis*. 2001; 8(6):933–941. [PubMed: 11741389]
- Whitwell JL, Weigand SD, Boeve BF, Senjem ML, Gunter JL, DeJesus-Hernandez M, et al. Neuroimaging signatures of frontotemporal dementia genetics: C9ORF72, tau, progranulin and sporadics. *Brain*. 2012; 135(Pt 3):794–806. [PubMed: 22366795]
- Williamson TL, Cleveland DW. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat Neurosci*. 1999; 2(1):50–56. [PubMed: 10195180]
- Woeller CF, Anderson DD, Szebenyi DM, Stover PJ. Evidence for small ubiquitin-like modifier-dependent nuclear import of the thymidylate biosynthesis pathway. *J Biol Chem*. 2007; 282(24):17623–17631. [PubMed: 17446168]
- Xu Z, Au SW. Mapping residues of SUMO precursors essential in differential maturation by SUMO-specific protease, SENP1. *Biochem J*. 2005; 386(Pt 2):325–330. [PubMed: 15487983]

- Yang Y, Gozen O, Watkins A, Lorenzini I, Lepore A, Gao Y, et al. Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. *Neuron*. 2009; 61(6):880–894. [PubMed: 19323997]
- Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, Hirano M, et al. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat Genet*. 2001; 29(2):160–165. [PubMed: 11586297]
- Yi J, Zhang L, Tang B, Han W, Zhou Y, Chen Z, et al. Sodium valproate alleviates neurodegeneration in SCA3/MJD via suppressing apoptosis and rescuing the hypoacetylation levels of histone H3 and H4. *PLoS One*. 2013; 8(1):e54792. [PubMed: 23382971]
- Yin HZ, Tang DT, Weiss JH. Intrathecal infusion of a Ca(2+)-permeable AMPA channel blocker slows loss of both motor neurons and of the astrocyte glutamate transporter, GLT-1 in a mutant SOD1 rat model of ALS. *Exp Neurol*. 2007; 207(2):177–185. [PubMed: 17719032]
- Yoo YE, Ko CP. Treatment with trichostatin A initiated after disease onset delays disease progression and increases survival in a mouse model of amyotrophic lateral sclerosis. *Exp Neurol*. 2011; 231(1):147–159. [PubMed: 21712032]

Figure 1. Schematic representation of the glutamate transporter EAAT2 (a.k.a. Glt-1 in rodents) transmembrane topology

(A) Caspase-3 cleavage occurs at a conserved consensus site within the cytosolic c-terminus domain of EAAT2. (B) The site used by caspase-3 is unique within the EAAT2 sequence. Experiments with in vitro translated EAAT2 have shown that caspase-3 cleaves EAAT2 by generating only two fragments and that mutagenesis of the caspase-3 consensus site in the c-terminus abolishes this cleavage (Boston-Howes et al. 2006). The sumoylation consensus site is shown in the c-terminus domain of EAAT2 (CTE).

Figure 2. CTE-SUMO1 decreases mRNA and protein levels of the full-length transporter EAAT2

(A) Spinal cord astrocytes in culture were transduced with Adeno-Virus construct expressing CTE-SUMO1 fused protein (M.O.I. 3), as per our published protocol (Foran et al. 2011). AdV-empty vector and AdV-GFP (M.O.I. 3) treated astrocytes were used as controls. Homogenates were collected after 7 days of incubation post-treatment and western blot analysis was performed with an affinity-purified anti-EAAT2 polyclonal antibody recognizing an epitope in the c-terminus domain of EAAT2 downstream the caspase-3 cleavage consensus site (a.a. 518–536; anti-CTE domain), affinity purified anti-EAAT1 antibody (epitope a.a. 522–536; anti-EAAT1) and anti-GFAP antibodies. Intensity of the bands was quantified by densitometry using a Chemidoc system (Biorad). EAAT2 and EAAT1 signals were normalized against GFAP expression levels. The antibody anti-EAAT2 was produced by AffinityBioreagent (15 mg/ml) and used at 0.1. ug/ml. Anti-EAAT1 antibody was a generous gift from Dr. Danbolt (0.2 ug/ml, rabbit #8D0161). (B) Quantification of EAAT2 and EAAT1 changes as a result of CTE-SUMO1 transduction is

shown (** $P < 0.01$; Dunnett's multiple comparisons test). (C) Spinal cord astrocytes were transduced with AdV expressing CTE-SUMO1, GFP, or empty vector as control (MOI 1). Total RNA was collected after 7 days of incubation with virus and qPCR performed. First strand cDNA was synthesized by reverse transcription from 1 μ g of total RNA using High Capacity RNA-to-cDNA Master Mix (Applied Biosystems). The thermocycler parameters were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec, and 60 °C for 1 min. The results were analyzed by the comparative Ct method and normalized against GFAP expression levels (* $P < 0.05$; Dunnett's multiple comparisons test).

Figure 3. Astrocytic expression of CTE-SUMO1 does not block neuron-stimulated EAAT2 transcriptional activation

Astrocytes, grown in a 6 cm petri dish, were transduced with adenovirus expressing CTE-SUMO1, with AdV-empty for control (MOI=3) or left untreated. After 48 hours post-transduction, purified cortical neurons (8×10^4 cells) were added to the astrocytes and kept for the next 7 days. Total RNA was used for qPCR analysis. EAAT2 signal was normalized against GFAP mRNA levels (* $P < 0.05$; Dunnett's multiple comparisons test).

Figure 4. Occupancy of CTE-SUMO1 on EAAT2 promoter in spinal cord of SOD1-G93A mouse model of ALS increases in disease

(A) Schematic representation of a portion of the promoter region of the mouse EAAT2 gene is shown. Upstream Probe, Probe A, Probe B, and Downstream Probe indicate the locations of TaqMan probes used to amplify EAAT2 specific promoter sequences pulled down by chromatin immunoprecipitation (ChIP). (B) ChIP assay of EAAT2 promoter with antibody specific for EAAT2 c-terminus (CTE). For the preparation of chromatin samples, two spinal cords for each group were fixed in 1% formaldehyde solution for 10 min., and then a nuclear pellet was prepared following the protocol specified in the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific, Cat#78833). The pellet was resuspended in 700 l of shearing buffer and the chromatin fragments were generated according to a protocol specified in the ChIP-IT® Express Kit and Sonication Shearing Kit Manual (ActiveMotif, Cat#53008) by sonication. Sixty l of sheared chromatin was incubated with the anti-CTE antibody (epitope 518–536; 2 g in 200 l of ChIP reaction) and Protein G magnetic beads. Chromatin elution, cross-linking reversion and DNA isolation were done with reagents and according to protocol provided by ChIP-IT® Express Kit. The DNA that was bound to CTE was amplified by PCR with the 4 different TaqMan probes. Precipitated EAAT2 promoter segments were detected using quantitative real-time PCR and relative chromatin occupancy was calculated as fold increase over non-transgenic spinal cord ChIP. Control regions in the downstream and 1,280 bp upstream EAAT2 mRNA start transcription site (Upstream and Downstream probes) were also quantitated using real-time PCR in parallel as further demonstration of assay specificity. (* P<0.05; Dunnett's multiple comparisons test).