MicroRNA Metabolism and Dysregulation in Amyotrophic Lateral Sclerosis

Paola Rinchetti[1] & Mafalda Rizzuti[1] & Irene Faravelli[1] & Stefania Corti[1]

Abstract MicroRNAs (miRNAs) are a subset of endogenous, small, non-coding RNA molecules involved in the post-transcriptional regulation of eukaryotic gene expression. Dysregulation in miRNA-related pathways in the central nervous system (CNS) is associated with severe neuronal injury and cell death, which can lead to the development of neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS). ALS is a fatal adult onset disease characterized by the selective loss of upper and lower motor neurons. While the pathogenesis of ALS is still largely unknown, familial ALS forms linked to TAR DNA-binding protein 43 (*TDP-43*) and fused in sarcoma (*FUS*) gene mutations, as well as sporadic forms, display changes in several steps of RNA metabolism, including miRNA processing. Here, we review the current knowledge about miRNA metabolism and biological functions and their crucial role in ALS pathogenesis with an in-depth analysis on different pathways. A more precise understanding of miRNA involvement in ALS could be useful not only to elucidate their role in the disease etiopathogenesis but also to investigate their potential as disease biomarkers and novel therapeutic targets.

Keywords Amyotrophiclateralsclerosis · ALS · microRNA · miRNA · Central nervous system · CNS

Paola Rinchetti and Mafalda Rizzuti are co-first authors.

✉ Stefania Corti
stefania.corti@unimi.it

[1] Dino Ferrari Centre, Neuroscience Section, Department of Pathophysiology and Transplantation (DEPT), Neurology Unit, IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Via Francesco Sforza 35, 20122 Milan, Italy

Introduction

Amyotrophic lateral sclerosis (ALS) represents one of the most common late-onset neurodegenerative disorders [1]. The neuropathological features are characterized by the progressive loss of somatic motor neurons in the spinal cord, which innervate all voluntary muscles in the body. This process clinically results in the progressive paralysis of the muscular functions. In addition, bulbar symptoms, such as dysphagia and dysarthria, related to the degeneration of lower brain stem motor neurons may arise during the disease course. Death usually occurs within a few years from onset due to respiratory failure [1, 2]. To date, the only approved compound for ALS treatment is riluzole that can only modestly increase survival by a few months [1].

ALS classified as sporadic (sALS) represents the majority of the diagnoses while familial ALS (fALS) accounts for only 10% of the cases [3, 4]. However, 10% of initially diagnosed sALS subjects display gene mutations [5]. The most common ALS-causative genes include chromosome 9 open reading frame 72 (*C9orf72*), Cu^{2+}/Zn^{2+} superoxide dismutase (*SOD1*), TAR DNA-binding protein 43 (*TARDBP*), and fused in sarcoma/translocated in liposarcoma (*FUS/TLS*) [4, 6, 7] (see Table 1 for the whole list). Interestingly, many ALS-linked genes, particularly *TARDBP* and *FUS*, are involved in RNA metabolism, including microRNA (miRNA) processing [44, 45].

MiRNAs are tissue-specific, small non-coding RNAs that are expressed in different viruses, animals, and plants [46–50]. They are widespread and highly conserved molecules representing approximately 1–2% of non-protein-coding genes [46, 47]. In particular, they are involved in the inhibition and degradation of messenger RNAs (mRNAs) thwarting their expression by pairing with them [46, 49]. Because of their involvement in the development, function, and survival of different types of mature neurons in organisms [51],

Table 1 Summary of the most common ALS causative genes

ALS-causative genes			References
Gene	Description	Functions/pathological mechanisms	
C9orf72	Chromosome 9 open reading frame 72	The repeat expansion (up to thousands of copies) of a non-coding hexanucleotide (GGGGCC) in the first intron of the gene has been associated with a decrease in the mRNA expression of C9orf72 transcripts. Repeat transcripts can induce the production of peptides that are prone to accumulation in specific foci, which can interfere with transcription and translation.	[8–11]
SOD1	Superoxide dismutase 1	The gene encodes for an antioxidant protein that produces hydrogen peroxide from superoxide radicals. Decreases in enzymatic dismutase activity have been linked to oxidative stress and excitotoxicity in motor neurons. Actually, such degeneration might be associated with mutant SOD1 aggregation and the resulting aberrant association with mitochondria. SOD1 promotes protein misfolding and aggregation processes.	[12–15]
TARDBP FUS	TAR DNA-binding protein FUS RNA-binding protein	TDP 43 and FUS are both members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family that is involved in multiple steps of RNA processing. They show notable structural and functional similarities, and the identification of TDP-43 as the main component of the ubiquitinated protein aggregates, as well as the discovery of mutations in the TARDBP gene, has supported the investigation of FUS through sequencing. TDP-43 and FUS mislocalizations have been observed in several disorders that lead to the development of specific proteinopathies.	[16–20]
HNRNPA1; HNRNPA2-B1	Heterogeneous nuclear ribonucleoprotein A1; A2/B1	RNA-binding proteins hnRNP A1 and hnRNP A2, as well as TDP 43, FUS, and SMN1 proteins, are recruited to stress granules under stress conditions. hnRNPA1 and hnRNPA2/B1 interact with TDP 43. Mutations in HNRNPA1 and HNRNPA2B1 have been associated with ALS etiology.	[21–23]
UBQLN2	Ubiquilin 2	Ubiquilin 2 plays a key role in the regulation of the ubiquitin–proteasome system and autophagy. Some UBQLN2 variants have been associated with ALS as well as ALS–FTD cases while histopathological analysis from ALS patients shows widespread ubiquilin 2-positive inclusions in affected neurons.	[23, 24]
MATR3	Matrin 3	MATR3 is an RNA/DNA-binding protein that interacts with TDP 43. Mutations in the MATR3 gene have been identified as a rare genetic cause of ALS confirming the role of RNA metabolism in the disease etiology.	[25, 26]
SETX	Senataxin	The encoded protein contains a DNA/RNA helicase domain, and it seems to be involved in nucleic acid processing. Mutations in SETX have been associated with juvenile-onset ALS. Phenotypes often overlap with ataxia and motor neuron disease.	[26, 27]
CHCHD10	Coiled-coil-helix–coiled-coil-helix domain containing 10	CHCHD10 is a coiled-coil helix–coiled-coil helix mitochondrial protein. It has been associated with neurological disorders and identified as a rare causative gene in FTD–ALS pathogenesis. Indeed, mitochondrial dysfunction plays a significant role in the evolution and progression of ALS disease.	[28, 29]
GRN	Granulin	Granulins are a group of peptides derived from a single precursor protein called progranulin. Mutations in GRN are associated with FTD with TDP 43 protein accumulation suggesting a link between GRN loss and TDP 43 pathology.	[30, 31]
ANG	Angiogenin	The ANG gene encodes for an angiogenic factor upregulated by hypoxia. The protein is involved in motor neuron development and maintenance. Mutations in the ANG gene seem to represent a risk factor for ALS occurrence.	[32, 33]
CHMP2B	Charged multivesicular body protein 2B	The protein is involved in autophagy and endolysosomal trafficking pathways. Pathogenic mutations in CHMP2B have been associated with FTD and ALS.	[34, 35]
PFN1	Profilin 1	Profilin 1 is an actin-binding protein involved in the regulation of actin polymerization. Mutations in PFN1 inhibit the axon outgrowth and alter stress granule dynamics contributing to ALS pathogenesis.	[36, 37]
VCP	Valosin-containing protein	VCP belongs to chaperone-like family proteins which are involved in different biological pathways including the ubiquitin–proteasome system. VCP mutations have been associated with FTD and ALS.	[38, 39]
TBK1	TANK-binding kinase 1	TBK1 gene has been identified as possibly linked to ALS and FTD. The protein interacts with proteins related to autophagy and innate immunity, such as p62 and OPTN.	[40, 41]
OPTN	Optineurin	OPTN is a ubiquitously expressed cytosolic protein involved in many cellular pathways and signaling. Genetic data show OPTN mutations are associated with ALS pathogenesis and neurodegenerative processes.	[42, 43]

Most common causative genes are here summarized including their potential role in ALS pathogenesis

miRNAs may play an important role in the etiology and progression of neurodegenerative disorders, such as ALS [52]. Indeed, miRNA dysfunction has been associated with a progressive loss of specific neuronal populations, such as motor neurons in ALS [53–55]. Therefore, neurodegenerative diseases can also be considered as RNA disorders in which the dysregulation of miRNAs is striking because of their ability to regulate different pathways associated with the onset and progression of disorders [56].

In the context of ALS, a global dysregulation of miRNAs has been described as a common feature underlying different forms of the disease [57]. It is also worthy to consider that the ability to detect changes in miRNA expression profiles could be a useful tool as a diagnostic biomarker to identify the onset and progression of the disease [2, 58]. Finally, the identification of misregulated miRNAs could potentially represent a tool for developing therapeutic approaches to treat ALS.

The Biology of MiRNAs

Classification of Non-coding RNAs

The entire human genome is extremely rich in non-coding RNAs (ncRNAs), which might represent a way for cells belonging to the same organ to develop specific identities and functions [59]. In the heterogeneous group of ncRNAs, different subsets of functional molecules should be recognized according to their lengths and functions, such as long non-coding RNAs (lncRNAs), which are typically greater than 200 nucleotides, and small RNAs [60].

Several functions have been identified for lncRNAs, such as targeting proteins associated with specific transcription patterns, interfering with translation and DNA methylation, altering the activity of protein-binding partners and chromatin, or acting as precursors for small RNAs [61]. Small RNAs are processed from longer precursors to carry out post-transcriptional gene silencing of target RNA transcripts. They can be clustered as heterochromatic small interfering RNAs [62], small temporal RNAs (stRNAs) [63], tiny non-coding RNAs [64, 65], and a group of very small RNAs that include short interfering RNAs (siRNAs) [66], PIWI-interacting RNAs (piRNAs) [67], and the well-known miRNAs [68]. MiRNAs were first described in 1993 [69] and are defined by their lengths, ranging from 20 to 30 nucleotides, and their interactions with Argonaute proteins (AGO and PIWI) [70].

MiRNA Biogenesis, Metabolism, and Biological Function

MiRNAs are short and evolutionarily conserved RNA sequences that are transcribed from specific genes or from the introns of protein-coding genes [71]. In humans, most of the canonical miRNAs are encoded by intronic regions. Often, the loci of different miRNAs belong to the same polycistronic transcription unit and are usually co-transcribed even if an additional single miRNA regulation can be performed post-transcriptionally [72]. Approximately 60% of all protein-coding genes seem to be regulated by miRNAs [73].

MiRNAs inhibit gene expression mainly through highly specific binding to complementary sequences in the three prime untranslated regions (3′-UTRs) of target mRNAs. The pairing with the target regions leads to downregulation of the corresponding mRNA through its destabilization or impedes processes at the protein level through translational inhibition [74]. While miRNA-binding sites are generally sited in the 3′-UTR domain of target mRNAs, the short nucleotide region located in the 5′ end of the miRNA called the ʙmiRNA seed^ (nucleotides 2–7) has been determined to be very important for defining the miRNA function and evolution and determining the target recognition [21]. Indeed, miRNAs that display identical sequences at nucleotides 2–8 are usually considered to belong to the same family, even if some miRNA molecules display a common origin but a different miRNA seed [75].

The complexity of gene expression regulation by miRNAs has been depicted in a lot of studies, which show that a single miRNA can target many different genes. It can also occur that a set of miRNAs cooperate in an additive or synergistic way in order to exert control over a single gene expression [76]. In particular, while some individual miRNAs may account for the expression of several tissue-specific genes [77, 78] the specific expression of a single target gene seems to be regulated by a network of interactive miRNA molecules [79, 80].

Each miRNA locus produces two mature molecules that arise from the 5′ strand or from the 3′ terminal. Nevertheless, one arm called ʙthe guide strand^ is the more biologically active and accounts for 96–99% of the total mature functional miRNA molecules [70]. The other strand, which is known as the ʙpassenger^ or miRNA*, is generally thought to be degraded during the biogenesis process. Actually, the passenger strand has also been identified as a potential biological regulator with the ability to modulate gene expression. In the context of different pathologies, miRNAs* were demonstrated to be able to actively target specific mRNAs and therefore do not behave as simple, passive bystanders [81–83].

The majority of miRNAs are transcribed by RNA polymerase II [71], whereas others are transcribed by RNA polymerase III [84]. RNA polymerase III can also transcribe viral miRNAs and some endogenous miRNA-like small RNAs derived from transfer RNAs (tRNAs) [85, 86].

In addition, the miRNA transcription process is regulated by different RNA polymerase II-associated transcription factors and is subjected to epigenetic control [71, 87–90].

The primary transcripts (pri-miRNAs, in which miRNA molecules are embedded) are then processed through different maturation steps. In the nucleus, the pri-miRNA is specifically recognized by the microprocessor enzymatic complex composed

of a double-stranded RNA-binding protein named DGCR8, which identifies the stem-loop structure, and the nuclear ribonuclease III Drosha, which processes the pri-miRNA to generate the 70-nucleotide-long precursor form (pre-miRNA)[91].

Additional sequence motifs that reside in the pri-miRNA structure seem to be involved in the maturation process in order to improve the efficiency of processing primary transcripts [92, 93]. Following the microprocessor processing, the resulting pre-miRNA is translocated to the cytoplasm by exportin-5 through the nuclear pore complex in a Ran GTP-dependent process [94]. The pre-miRNA is then released into the cytosol, where it is cleaved by another RNase III-type endonuclease termed Dicer to produce a mature 20-bp miRNA duplex intermediate [95]. In this processing step, the endoribonuclease Dicer is associated with the transactivation response RNA-binding protein (TRBP) and the protein activator of the interferon-induced protein kinase (PACT) in a proteic complex [96]. The small RNA duplex is then loaded onto an AGO protein to shape the RNA-induced silencing complex (RISC) [97]. Notably, among the four AGO protein families in humans, only AGO2 can process perfectly matched target mRNAs [74]. The functional core of the RISC complex consists of AGO2, which has endonuclease activity responsible for mRNA silencing, and of 182-kDa glycine–tryptophan proteins (GW182), which are essential for miRNA-mediated translational repression and transcript decay. Moreover, additional proteins, such as fragile X mental retardation 1 (FMRP), Mov10 RISC complex RNA helicase (MOV10), and Hu antigen R (HuR), join the RISC enzymatic complex, and the inclusion of the GW182 paralogue trinucleotide repeat-containing gene 6A protein (TNRC6) can trigger deadenylation, decapping, and decay of mRNAs [96, 98] (Fig. 1).

Thus, the RISC assembly initially involves the RNA duplex association with AGO proteins to generate the pre-RISC enzymatic complex. Subsequently, the removal of the passenger strand from the duplex determines the development of the mature RISC, which requires the contribution of only the guide strand [89]. Typically, the choice of the guide strand is established by the thermodynamic stability of the RNA duplex, even if the passenger strand displays a weaker silencing ability [99–101].

Finally, miRNA-loaded RISC guides the enzymatic complex toward the target mRNA based on the complementarity sequence for the 3′-UTR region. The miRNA-RISC examines the pool of cytoplasmic transcripts to find the potential complementary targets. The degree of miRNA target complement determines the fate of the target mRNA; a perfect match leads to transcript degradation through AGO2 enzymatic activity, whereas incomplete base-pairing triggers mRNA silencing by translational repression, mRNA degradation, or sequestration in cytoplasmic structures (P-bodies) [98, 102].

The expression of miRNAs is subjected to close regulation from their biogenesis to their decay. Actually, the stability of miRNAs seems to be associated with endogenous factors, such as specific exoribonuclease (XRN1, XRN2), and is affected by the binding to their target mRNAs. Environmental factors may show an influence on the stability of these small RNA sequences [96]. Moreover, miRNAs seem to have intrinsic elements capable of modulating their stability in cells[103].

Modifications in the RNA sequence or structure influence miRNA processing and turnover. The intrinsic regulation of miRNAs can be affected by different biological occurrences, such as the existence of single nucleotide polymorphisms (SNPs) in miRNA genes, which have been associated with miRNA biogenesis or altering the target specificity.

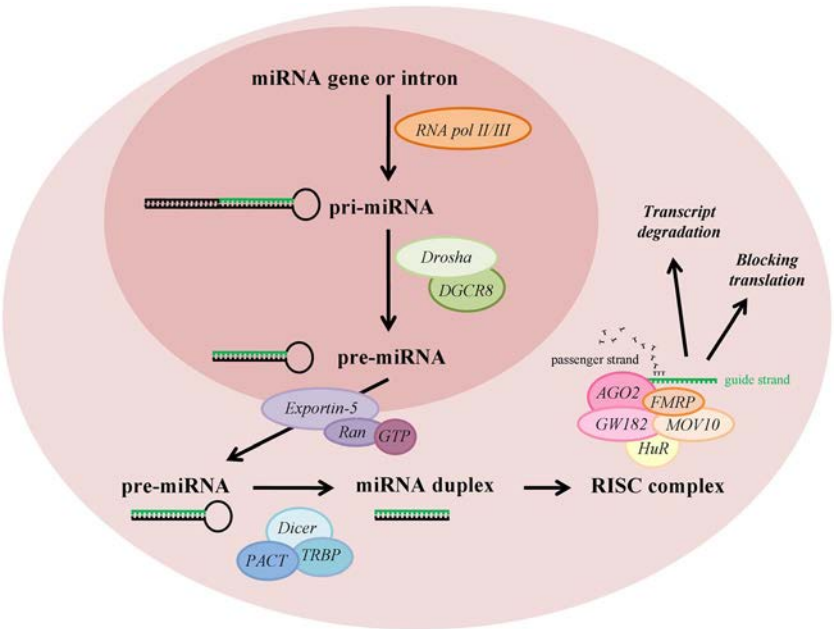

Fig. 1 MiRNA biogenesis. The biogenesis process of miRNAs starts in the nucleus with the formation of pri-miRNA. This pri-miRNA is processed by *Drosha* and transported in the cytoplasm by *Exportin-5*. In the cytoplasm, *Dicer* binds pre-miRNA, forming the miRNA duplex. At this point, the guide strand of the duplex is incorporated into the RISC complex, whereas the other strand is typically degraded

Furthermore, in addition to regulation through miRNA stability, as described above, other methods of modifying RNA molecules that affect biogenesis include miRNA tailing, RNA editing, and RNA methylation [70].

MiRNAs regulate different cellular processes including growth, differentiation, and signaling. They are involved in the control of gene expression as post-transcriptional regulators in animals, plants, and viruses [104, 105]. After matching with the mRNA target sequence, miRNAs prompt either the induction of mRNA decay or the inhibition of the translational process.

As regards mRNA decay mechanism, the miRNA–mRNA interaction causes target deadenylation; in eukaryotes, the removal of Poly-A tails starts with the Poly(A)-specific ribonuclease complex, PARN2–PARN3, and proceeds to the CAF1–CCR4–NOT complex. After deadenylation, the decapping is carried out by Decapping 1 (DCP1) and Decapping 2 (DCP2) enzyme, followed by 5′–3′ exonucleolytic digestion by the 5′–3′ exoribonuclease 1 (XRN1) [106–108].

As mentioned above, miRNAs are also able of inhibiting the translation of mRNA targets at different steps of the process [109], even if this process occurs in only a small percentage of cases (11–16%) [77]. The mRNA repression can be associated with the recruitment of competent ribosomes or the promotion of the ribosomal drop-off during the elongation step. Finally, the eukaryotic translation initiation factor 4F (eIF4F) cap recognition can be inhibited [77, 110–112].

To summarize, miRNA generation encompasses complex biological mechanisms, strictly regulated through different steps. MiRNA role is crucial in determining cell homeostasis and biological fate.

The Role of MiRNAs in ALS Pathogenesis

The importance of miRNAs in ALS was unraveled for the first time by the observation of differential miRNA profiles in ALS patients compared to healthy controls (Fig. 2). MiRNAs are highly stable in serum and other bodily fluids, but readily subjected to decay in the postmortem brain; thus, the feasibility of directly analyzing CNS tissues is limited. However, analyses on biological samples, including blood and cerebrospinal fluid (CSF), showed a different expression of miRNAs between healthy controls and ALS patients' samples, indicating that these small RNAs could be involved in the pathogenesis of ALS [58, 113–115]. Several miRNAs associated with nervous system maintenance and cell death pathways were deregulated on human samples isolated from the spinal cord of ALS patients [116].

Overall, a global reduction of miRNA levels could be observed in both familial and sporadic ALS in comparison with healthy controls and other neurodegenerative patients [57, 116, 117]. Characterizing miRNA biogenesis and investigating the potential mechanisms underlying miRNA

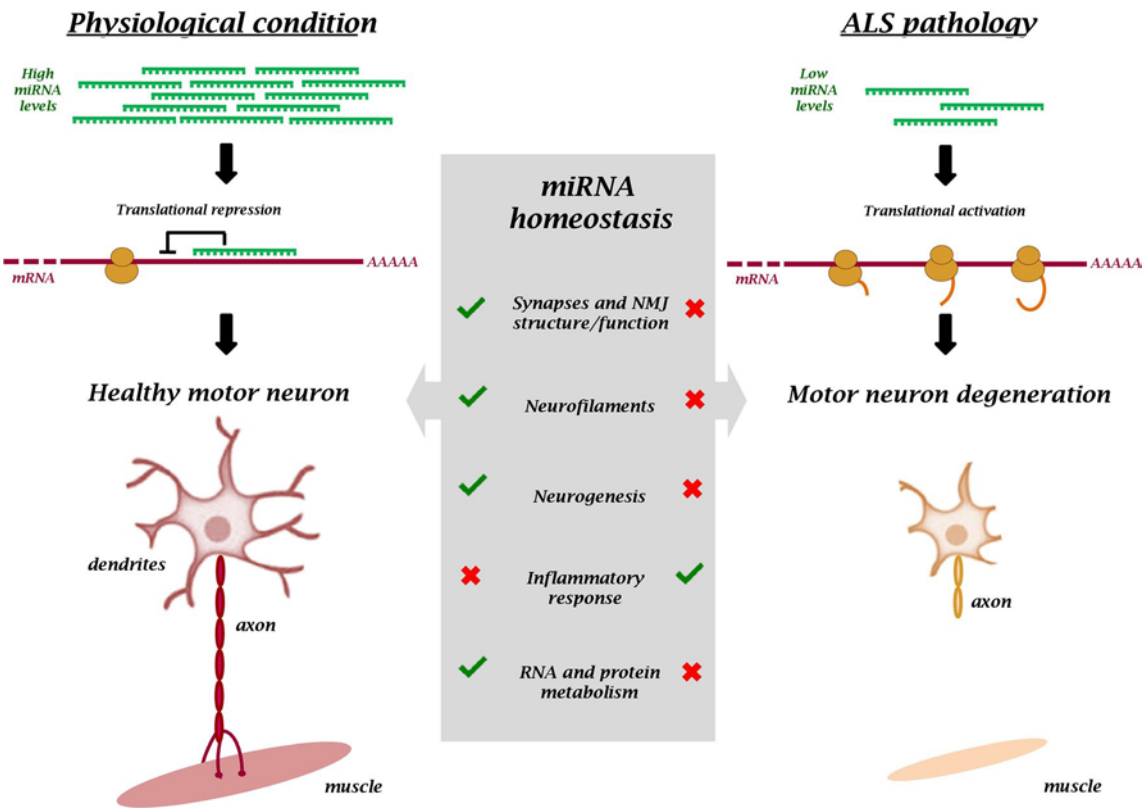

Fig. 2 Role of miRNAs in motor neuron physiology and degeneration. The principal functions exerted by miRNA in motor neuron homeostasis (on the *left*) and pathology (on the *right*) are here represented

dysregulation could offer a promising tool both in understanding their involvement in ALS pathogenesis and in developing future therapeutic approaches.

We will further describe different roles played by miRNAs in ALS pathogenesis in the next few paragraphs, which have been subdivided accordingly (for a detailed overview, refer to Table 2).

Table 2 Summary of miRNAs involved in ALS and their role in the disease progression

	MiRNAs	Up/downregulated in ALS	Involvement in ALS pathogenesis	Reference
Synapses and NMJ	miR-206	Upregulated	miRNA expresses specifically in skeletal muscle. Deficiency in ALS model causes acceleration of disease progression	[118]
			Regulates the expression of HDAC4, which is involved in neuromuscular gene expression	[119]
			Increased expression after denervation near synaptic sites	[120]
	miR-23		Overexpression causes the reduction of PGC1α	[121]
	miR-31		Induces cell proliferation	[122]
	miR-29b		Increases in skeletal muscle in ALS patients	[121]
	miR-455			
	miR-338-3p		Detected in cerebrospinal fluid and in the spinal cord gray matter of sALS patients; involved in excitotoxicity	[114]
	miR-451	Downregulated	Detected in cerebrospinal fluid and in the spinal cord gray matter of sALS patients;	
	miR-1275			
	miR-328			
	miR-638			
	miR-149			
	miR-665			
	miR-583			
	miR-218		Expressed only in motor neurons and involved in their differentiation	[123] [124]
	miR-124a	Upregulated	Low level in spinal cord of SOD1 mice; involved in GLUT expression	[125]
Neurofilaments	miR-146a	Upregulated	Involved in the regulation of NFL mRNA expression in ALS	[116]
	miR-524-5p	Downregulated		
	miR-582-3p			
	miR-b1336		Low expression causes destabilization of neurofilament mRNAs at the neuromuscular junction level	[126]
	miR-b2403			
	miR-1		Involved in myelination process in the spinal cord of the ALS animal model	[127]
	miR-330			
	miR-29			
	miR-133			
	miR-9			
Neurogenesis	miR-9	Downregulated	Both in vitro and in vivo is involved in NSC proliferation, distribution, and differentiation	[128] [58] [129] [130]
	miR-124a	Upregulated		
	miR-19a	Up/downregulated[a]	Involved in the cell cycling	[129]
	miR-19b			
	miR-29a	Upregulated	Involved in ER stress	[131, 132]
	miR-29b	Downregulated	Dysregulation of NAV3 (regulator of axon guidance)	[133]
	miR-125	Up/downregulated[a]	Involved in astrocyte and oligodendrocyte regulation, neuronal morphogenesis, and synaptic plasticity	[134]
	miR-134			
	miR-219			
Neuroinflammation	miR-155	Upregulated	Involved in the control of innate immuno system; treating SOD1 mutant mice with anti-miR-155 reduces mortality	[135] [113]
	let-7		The biogenesis of this miRNA is regulated by TDP-43	[117]
	miR-146a		Regulator of Ly6Chi monocyte	[113]
	miR-223		Increased in Ly6Chi cells in the spleen of SOD1 mice	
	miR-27a			
	miR-142-5p			[117]
	miR-365		Negatively regulates interleukin-6 (IL-6) increasing the expression of TNFα	[136]
	miR125b		Negatively regulates STAT3 increasing the expression of TNFα	
	miR-24		Regulator of T-cells in vitro	[135]
	miR-148b-5p	Downregulated	Involved in regulation of genes associated with neurodegeneration on ALS	[117]
	miR-577			
	miR133b			
	miR-140-3p			

[a] This miRNA is observed as being upregulated or downregulated, depending on the different areas of the brain that are analyzed

MiRNAs, Cytoplasmatic Inclusions, and Stress Granules

In ALS and frontotemporal dementia (FTD) disorders, ubiquitin-positive inclusions in neurons and glia typically restrain the DNA-binding proteins TDP-43 or FUS [16]. In most fALS cases where *TARDBP* is not mutated, TDP-43 aggregates may be detected, whereas FUS inclusions are less common [16, 17, 137].

Under pathological conditions, such as cellular stress, mutant TDP-43 and FUS can interact with different proteins associated with RNA metabolism, leading to the development of protein aggregates and the formation of stress granules (SGs). It has been suggested that SGs could be precursor structures of the pathological protein inclusions observed in neurodegenerative disorders [138, 139]. Notably, SG assembly starts with the phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α), the modulation of which is associated with neurotoxicity in ALS animal models [140, 141]. The SGs recruit many RBPs that are prone to aggregation such as TDP-43 and FUS, which are involved in RNA metabolism [18, 142]. TDP-43 promotes the process of interacting with the nuclear Drosha and the cytoplasmic Dicer complexes [44], and FUS enhances miRNA production through Drosha [143], thus providing functional links among the disease, dysregulated miRNA biogenesis, and SG-related RBPs. TDP-43 plays a key role also in the post-transcriptional maturation of a subset of miRNA molecules, both in the nucleus and in the cytoplasm. Consequently, mislocalization of the TDP-43 protein in cytoplasmic aggregates seems to be associated with reduction in Drosha and Dicer processing of TDP-43-regulated miRNAs [44].

The observed impairment in miRNA biogenesis has been related to the stress response induced by mutations in ALS-related genes, such as TDP-43, FUS, and SOD1. Overall, these findings suggest a potential link between defective miRNA biogenesis and ALS due to impaired Dicer processing. Therefore, the latter may be a promising target for the development of therapeutic approaches for a wide range of disorders resulting from dysregulated miRNA expression [144].

MiRNAs and Neuromuscular Junctions

A group of miRNAs, usually referred as myomiRs, is expressed mostly in the muscular tissue [145] and includes miR-1, miR-133, miR-206, miR-208 a/b, miR-486, and miR-499 [146]. Although these miRNAs are expressed both in cardiac and skeletal muscle (except for miR-208, which is expressed only in cardiac muscle), miR-206 is expressed especially in skeletal muscles, and in physiological conditions, it is involved in the maintenance of neuromuscular synapses and regeneration of neuromuscular junctions after injury, and it regulates myoblast differentiation [118, 147]. miR-206 importance during myogenesis is supported by a study by Grifone and colleagues, who demonstrated that skeletal muscle-specific Dicer-1 knockout mice have a significant reduction in muscle mass due to hypoplasia [148]. miR-206 seems to negatively control the expression of histone deacetylase 4 (HDAC4), which is involved in the control of neuromuscular gene expression [149, 150]. In particular, miR-206 is not involved in the pathogenesis, but it plays a crucial role in the organism's ability to restore normal NMJ formation after injury [120].

miR-23 acts as a negative regulator of the peroxisome proliferator-activated receptor-gamma coactivator alpha (PGC-1α) signaling [121]. It is already known that skeletal muscle mitochondrial dysfunction may be implicated in the severity and progression of ALS and since PGC-1α is involved in mitochondrial biogenesis and function, the inhibition of this miRNA could be used to develop a therapeutic strategy to rescue PGC-1α activity in ALS subjects [121].

MiRNAs and Neuroinflammation

In ALS pathology, neuroinflammation and the immune system play an important role in the disease progression through microglial activation, dysregulation of immune-related genes, and recruitment of monocytes to affected tissues.

Interestingly, miR-155 seems to promote tissue inflammation by enhancing the generation of Th17 cells and recruiting macrophages as a part of the immune response. In addition, miR-155 is also implicated in the increase of proinflammatory cytokine secretion by binding to suppressor of cytokine signaling 1 (SOCS1) mRNAs [151–153]. Koval and collaborators showed that the level of miR-155 in both ALS human and mouse CSF is increased twofold and fivefold, respectively. Moreover, the anti-miR-155 was able to promote a significant extension in survival time of affected animals [135]. Several dysregulated miRNAs, such as let-7, miR-148b-5p, miR-577, miR-133b, and miR-140-3p, seem to be involved in the regulation of genes implicated in inflammatory pathways in the ALS context.

Another group thoroughly investigated the role of miR-125b in the modulation of NF-kb signaling in microglia [136, 154]. In a first study, the authors evaluated the miRNA expression profile of SOD1G93A mouse microglia after inflammatory activation. They identified that both miR-365 and miR-125b seem to be involved in the proinflammatory signal. In microglia, miR-365 and miR-125b negatively regulate interleukin-6 (IL-6) and signal transducer and activator of transcription 3 (STAT3), respectively. Downregulation of IL-6 and STAT3 pathways causes activation of proinflammatory signals through an increase in tumor necrosis factor-alpha (TNFα) expression [136]. In a more recent study, the same group investigated the molecular role of miR-125b in the neuroinflammatory pathway, directly relating miR-125b to

NF-kb signaling. The action exerted by this miRNA prolongs the activation of NF-kb in microglia with a toxic effect on surrounding motor neurons. These results highlight the fundamental role played by miRNA in the complex interplay between microglia and motor neurons, which appears as a strong contributor to motor neuron degeneration in ALS and other neurological disorders [154].

MiRNAs and Endoplasmic Reticulum Stress

As mentioned above, among the different mechanisms underlying ALS pathogenesis, defects in protein folding or degradation of proteins leads to increase and accumulation of aggregated or misfolded proteins in the endoplasmic reticulum (ER) lumen, resulting in a change in ER homeostasis called ʙER stress,^ which culminates with apoptosis [155].

Nolan and colleagues analyzed the dysregulation of the miRNA pathway after the induction of ER stress. They identified both in vitro and in vivo an increase in miR-29a [131, 132, 155]. Interestingly, an increase in miR-29a expression could be observed in the lumbar spinal cords of ALS mice at post-natal day 70 compared to controls [131]. Moreover, the increase in miR-29 led to a decrease in induced myeloid leukemia cell differentiation protein (Mcl-1) [131] involved in the apoptosis pathway [156].

In a more recent study, they demonstrated that ER stress-induced transcription factor activating transcription factor-4 (ATF4) enhanced the expression of miR-29a increasing through this mechanism the sensitivity of motor neurons to ER stress-induced apoptosis [132].

In conclusion, the authors hypothesized that during the progression of ALS, motor neurons undergo ER stress conditions leading to apoptosis [131].

MiRNAs as Disease Biomarkers and Novel Therapeutic Targets

So far, specific disease biological markers of ALS or effective therapies have not been identified. The diagnosis and follow-up still relies upon clinical criteria, and, despite the intense efforts, there are still no established biomarkers clinically applicable [157]. In particular, the research of valuable ALS biomarkers has been the focus of several studies aiming to direct the therapeutic research and instruct the clinical trial enrollment (for a detailed review on the more recent studied biomarkers in the ALS field, refer to [157]).

Recent evidence from several findings suggests that ALS patients show a dysregulation of gene expression profiles including miRNAs [2, 58, 98, 158–162].

Interestingly, the different miRNA expression patterns observed in ALS subjects could represent a disease signature and thus be useful both for improving the diagnosis of the disease by using them as potential biomarkers and for the development of new miRNA-based therapeutics. MiRNAs are expressed in a tissue-specific manner, and they can be released as circulating molecules in several bodily fluids, which suggests that there are differences between the profiles of affected subjects and healthy controls. Furthermore, they seem to be stable in body fluids, such as CSF, blood, and urine, because of their incorporation in exosomes, protein complexes similar to Argonaute proteins and lipoproteins, which confer resistance to RNase in the circulating environment [163–165]. These differences make them appealing as potential ALS peripheral biomarkers [166].

MiRNAs could be also used as therapeutic molecules; a tested approach to reduce the upregulated miRNAs includes the use of antagomirs and locked nucleic acids (LNAs). These molecules have the same conformation of RNA, and they are characterized by high stability and great affinity for the RNA targets. Therefore, they are able to prevent the binding of the miRNA to its target and at the same time they can reduce miRNA levels [167].

In contrast, a complementary approach aims to increase the expression of downregulated miRNAs through replacement with miRNA mimics. This type of miRNA has the same sequence of the dysregulated miRNA, and its mRNA target is the same as the endogenously depleted miRNA. Thus, this methodology is set up on the hypothesis that decreasing the target protein levels could be useful for the development of a protective therapeutic strategy [168]. Unfortunately, miRNA mimics have a limited half-life; thus, a repetitive administration would be necessary to maintain constant effects [169]. To overcome this problem, a viral vector could be used with the major challenge of delivering the selected miRNAs to the proper cells and crossing the blood–brain barrier (BBB). The discovery of adeno-associated virus AAV9 ability of crossing the BBB after systemic administration opened new expectations for the development of gene therapy approaches for neurological disorders [170]. Interestingly, recent studies investigated the therapeutic potential of developing AAV-mediated RNAi gene therapy for ALS [171].

Stoica et al. evaluated the therapeutic efficacy of delivering an AAV9 construct encoding an artificial microRNA against the human SOD1 (amiR^{SOD1}) in an ALS mouse model. They performed bilateral intracerebro ventricular (ICV) injections in ALS SOD1^{G93A} pups at postnatal day 1 (P1) observing a 50% increase in survival and a satisfactory preservation of the motor functions. ICV administration efficiently ensured gene delivery to both cortical and spinal cord motor neurons. Overall, AAV9 treatment was able to delay but not to prevent ALS progression, maybe due to the residual level of mutant hSOD1 expression [172]. Borel et al. tested the therapeutic efficacy of an artificial miRNA specific to SOD1 systemically delivered using the serotype rh.10 (rAAVrh10–miR–SOD1) in early symptomatic adult mice. Treated animals (P56–68)

showed an increased lifespan by 21%, preserving muscle strength and both motor and respiratory functions [173]. However, AAV9 and AAVrh10 target mostly glial than motor neurons when delivered in adult mice, so current AAV vectors do not seem to be efficient enough to cross the BBB and to transduce the sufficient amount of motor neurons in order to achieve good results in terms of therapeutic efficacy. Despite this, promising results were obtained in lower motor neurons by Borel et al. in non-human primates delivering the same rAAVrh10–miR–SOD1 vector [173]. The potential use of artificial miRNAs to induce RNAi against hSOD1 employing specific AAV serotypes [174] is now being extended to other ALS-associated genes such as C9ORF72 hexanucleotide expansion [175].

Thus, even if more studies need to be performed, the results suggested that miRNA silencing/upregulation could be used to develop new potential therapeutic strat- egies for ALS.

Conclusions and Future Perspectives

In the broad scenario of neurodegenerative disorders, ALS remains one of the most dramatically untreatable conditions. Because no effective treatment is available, investigating and defining previously unrecognized molecular mechanisms underlying the disease, such as miRNAs, could lead to the establishment of new potentially therapeutic targets. The central role of miRNAs as key regulators of several important biological pathways supports their involvement in the insurgence and progression of neurodegenerative disorders. Many research studies are currently ongoing that highlight the dysregulation of miRNA expression in ALS models and patients. Besides, broad dysregulation due to perturbation of proteins critical in miRNA biogenesis, defect in single specific miRNAs that govern pathways, and genes critical for motor neuronal function can play an essential pathogenetic role in ALS.

Alterations in this pattern of expression could represent a potential diagnostic biomarker capable of supplying important information about the onset or the progression of the disease. Furthermore, new therapeutic strategies could be developed to restore the physiological levels of miRNA expression. In particular, miRNA-based therapeutic treatment could be developed to overexpress miRNAs that are downregulated and vice versa.

Despite the fact that a large group of miRNAs have already been described in the literature as dysregulated in ALS, several miRNAs need yet to be explored in such a role, as reviewed in this study. Thus, this area of research requires further investigation toward a clinically meaningful application.

Acknowledgements The authors wish to thank the Associazione Centro Dino Ferrari for their support. The financial support of research grant to S.C. is gratefully acknowledged: AriSLA smallRNALS grant.

References

1. Bucchia M, Ramirez A, Parente V, Simone C, Nizzardo M, Magri F, Dametti S, Corti S (2015) Therapeutic development in amyotrophic lateral sclerosis. Clin Ther. doi:10.1016/j.clinthera.2014.12.020
2. Cloutier F, Marrero A, O'Connell C, Morin PJ (2014) MicroRNAs as potential circulating biomarkers for amyotrophic lateral sclerosis. Journal of molecular neuroscience : MN. doi:10.1007/s12031-014-0471-8
3. Ajroud-Driss S, Siddique T (2015) Sporadic and hereditary amyotrophic lateral sclerosis (ALS). Biochim Biophys Acta 1852(4): 679–684. doi:10.1016/j.bbadis.2014.08.010
4. Rizzo F, Riboldi G, Salani S, Nizzardo M, Simone C, Corti S, Hedlund E (2014) Cellular therapy to target neuroinflammation in amyotrophic lateral sclerosis. Cellular and molecular life sciences : CMLS 71(6):999–1015. doi:10.1007/s00018-013-1480-4
5. Chio A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, White LA (2013) Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 41(2):118–130. doi:10.1159/000351153
6. Andersen PM, Al-Chalabi A (2011) Clinical genetics of amyotrophic lateral sclerosis: what do we really know? Nat Rev Neurol 7(11):603–615. doi:10.1038/nrneurol.2011.150
7. Bigio EH, Weintraub S, Rademakers R, Baker M, Ahmadian SS, Rademaker A, Weitner BB, Mao Q et al (2013) Frontotemporal lobar degeneration with TDP-43 proteinopathy and chromosome 9p repeat expansion in C9ORF72: clinicopathologic correlation. Neuropathology : official journal of the Japanese Society of Neuropathology 33(2):122–133. doi:10.1111/j.1440-1789.2012.01332.x
8. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H et al (2011) Ahexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72:257–268
9. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA et al (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72:245–256
10. Ratti A, Corrado L, Castellotti B, Del Bo R, Fogh I, Cereda C, Tiloca C, D'Ascenzo C et al (2012) C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect. Neurobiology 33(2528):e2527–e2514
11. Ash PE, Bieniek KF, Gendron TF, Caulfield T, Lin WL, Dejesus-Hernandez M, van Blitterswijk MM, Jansen-West K et al (2013) Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron 77:639–646
12. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW (2006) Onset and progression in inherited ALS determined by motor neurons and microglia. Science 312:1389–1392
13. Okado-Matsumoto A, Fridovich I (2002) Amyotrophic lateral sclerosis: a proposed mechanism. Proceedings of the National Academy of Sciences of the United States of America 99:9010–9014

14. Liu J, Lillo C, Jonsson PA, VandeVelde C, Ward CM, Miller TM, Subramaniam JR, Rothstein JD et al (2004) Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. Neuron 43:5–17
15. Polymenidou M, Lagier-Tourenne C, Hutt KR, Bennett CF, Cleveland DW, Yeo GW (2012) Misregulated RNA processing in amyotrophic lateral sclerosis. Brain Res 1462:3–15
16. Ling SC, Polymenidou M, Cleveland DW (2013) Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. Neuron 79(3):416–438. doi:10.1016/j.neuron.2013.07.033
17. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T et al (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314(5796):130–133. doi:10.1126/science.1134108
18. Lagier-Tourenne C, Cleveland DW (2010) Neurodegeneration: an expansion in ALS genetics. Nature 466(7310):1052–1053. doi:10.1038/4661052a
19. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J et al (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323:1205–1208
20. Bentmann E, Neumann M, Tahirovic S, Rodde R, Dormann D, Haass C (2012) Requirements for stress granule recruitment of fused in sarcoma (FUS) and TAR DNA-binding protein of 43 kDa (TDP-43). J Biol Chem 287:23079–23094
21. Kim YK, Wee G, Park J, Kim J, Baek D, Kim JS, Kim VN (2013) TALEN-based knockout library for human microRNAs. Nat Struct Mol Biol 20(12):1458–1464. doi:10.1038/nsmb.2701
22. Honda, H., Hamasaki, H., Wakamiya, T., Koyama, S., Suzuki, S.O., Fujii, N., Iwaki, T., 2015. Loss of hnRNPA1 in ALS spinal cord motor neurons with TDP-43-positive inclusions
23. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F et al (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477:211–215
24. Zhang KY, Yang S, Warraich ST, Blair IP (2014) Ubiquilin 2: a component of the ubiquitin-proteasome system with an emerging role in neurodegeneration. Int J Biochem Cell Biol 50:123–126
25. Johnson JO, Pioro EP, Boehringer A, Chia R, Feit H, Renton AE, Pliner HA, Abramzon Y et al (2014) Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. Nat Neurosci 17:664–666
26. Leblond CS, Gan-Or Z, Spiegelman D, Laurent SB, Szuto A, Hodgkinson A, Dionne-Laporte A, Provencher P et al (2016) Replication study of MATR3 in familial and sporadic amyotrophic lateral sclerosis. Neurobiol Aging 37(209):e217–e221
27. Hirano M, Quinzii CM, Mitsumoto H, Hays AP, Roberts JK, Richard P, Rowland LP (2011) Senataxin mutations and amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases 12:223–227
28. Bannwarth S, Ait-El-Mkadem S, Chaussenot A, Genin EC, Lacas-Gervais S, Fragaki K, Berg-Alonso L, Kageyama Y et al (2014) A mitochondrial origin for frontotemporal dementia and amyotrophic lateral sclerosis through CHCHD10 involvement. Brain : a journal of neurology 137:2329–2345
29. Ronchi D, Riboldi G, Del Bo R, Ticozzi N, Scarlato M, Galimberti D, Corti S, Silani V et al (2015) CHCHD10 mutations in Italian patients with sporadic amyotrophic lateral sclerosis. Brain : a journal of neurology 138:e372
30. Zhang YJ, Xu YF, Dickey CA, Buratti E, Baralle F, Bailey R, Pickering-Brown S et al (2007) Progranulin mediates caspase-dependent cleavage of TAR DNA binding protein-43. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:10530–10534
31. Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, Lee SK, Shankar P et al (2007) Transvascular delivery of small interfering RNA to the central nervous system. Nature 448:39–43
32. Greenway MJ, Andersen PM, Russ C, Ennis S, Cashman S, Donaghy C, Patterson V, Swingler R et al (2006) ANG mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. Nat Genet 38:411–413
33. Gellera C, Colombrita C, Ticozzi N, Castellotti B, Bragato C, Ratti A, Taroni F, Silani V (2008) Identification of new ANG gene mutations in a large cohort of Italian patients with amyotrophic lateral sclerosis. Neurogenetics 9:33–40
34. Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS et al, Study M.R.C.P.i.A., Consortium, F.R (2006) ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). Neurology 67:1074–1077
35. Cox LE, Ferraiuolo L, Goodall EF, Heath PR, Higginbottom A, Mortiboys H, Hollinger HC, Hartley JA et al (2010) Mutations in CHMP2B in lower motor neuron predominant amyotrophic lateral sclerosis (ALS). PLoS One 5:e9872
36. Wu CH, Fallini C, Ticozzi N, Keagle PJ, Sapp PC, Piotrowska K, Lowe P, Koppers M et al (2012) Mutations in the profilin1 gene cause familial amyotrophic lateral sclerosis. Nature 488:499–503
37. Figley MD, Bieri G, Kolaitis RM, Taylor JP, Gitler AD (2014) Profilin 1 associates with stress granules and ALS-linked mutations alter stress granule dynamics. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:8083–8097
38. Bersano A, Del Bo R, Lamperti C, Ghezzi S, Fagiolari G, Fortunato F, Ballabio E, Moggio M et al (2009) Inclusion body myopathy and frontotemporal dementia caused by a novel VCP mutation. Neurobiol Aging 30:752–758
39. Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, Gibbs JR, Brunetti M (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron 68:857–864
40. Cirulli ET, Lasseigne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, Couthouis J, Lu YF et al (2015) Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science 347:1436–1441
41. Freischmidt A, Wieland T, Richter B, Ruf W, Schaeffer V, Muller K, Marroquin N, Nordin F et al (2015) Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Nat Neurosci 18:631–636
42. Bury JJ, Highley JR, Cooper-Knock J, Goodall EF, Higginbottom A, McDermott CJ, Ince PG, Shaw PJ et al (2016) Oligogenic inheritance of optineurin (OPTN) and C9ORF72 mutations in ALS highlights localisation of OPTN in the TDP-43-negative inclusions of C9ORF72-ALS. Neuropathology : official journal of the Japanese Society of Neuropathology 36:125–134
43. Goldstein O, Nayshool O, Nefussy B, Traynor BJ, Renton AE, Gana-Weisz M, Drory VE, Orr-Urtreger A (2016) OPTN 691_692insAG is a founder mutation causing recessive ALS and increased risk in heterozygotes. Neurology 86:446–453
44. Kawahara Y, Mieda-Sato A (2012) TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. Proc Natl Acad Sci U S A 109(9):3347–3352. doi:10.1073/pnas.1112427109
45. Lagier-Tourenne C, Polymenidou M, Cleveland DW (2010) TDP-43 and FUS/TLS: emerging roles in RNA processing and neurodegeneration. Hum Mol Genet 19(R1):R46–R64. doi:10.1093/hmg/ddq137
46. Ason B, Darnell DK, Wittbrodt B, Berezikov E, Kloosterman WP, Wittbrodt J, Antin PB, Plasterk RH (2006) Differences in vertebrate microRNA expression. Proc Natl Acad Sci U S A 103(39):14385–14389. doi:10.1073/pnas.0603529103

47. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297
48. Cullen BR (2006) Viruses and microRNAs. Nat Genet 38(Suppl): S25–S30. doi:10.1038/ng1793
49. Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A et al (2007) A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129(7): 1401–1414. doi:10.1016/j.cell.2007.04.040
50. Mallory AC, Vaucheret H (2006) Functions of microRNAs and related small RNAs in plants. Nat Genet 38(Suppl):S31–S36. doi: 10.1038/ng1791
51. Sun K, Lai EC (2013) Adult-specific functions of animal microRNAs. Nat Rev Genet 14(8):535–548. doi:10.1038/nrg3471
52. Tan JY, Marques AC (2014) The miRNA-mediated cross-talk between transcripts provides a novel layer of posttranscriptional regulation. Adv Genet 85:149–199. doi:10.1016/B978-0-12-800271-1.00003-2
53. Al-Chalabi A, Hardiman O (2013) The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 9(11): 617–628. doi:10.1038/nrneurol.2013.203
54. Hardiman O, van den Berg LH, Kiernan MC (2011) Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat Rev Neurol 7(11):639–649. doi:10.1038/nrneurol.2011.153
55. Robberecht W, Philips T (2013) The changing scene of amyotrophic lateral sclerosis. Nat Rev Neurosci 14(4):248–264. doi:10.1038/nrn3430
56. Johnson R, Noble W, Tartaglia GG, Buckley NJ (2012) Neurodegeneration as an RNA disorder. Prog Neurobiol 99(3): 293–315. doi:10.1016/j.pneurobio.2012.09.006
57. Emde A, Eitan C, Liou LL, Libby RT, Rivkin N, Magen I, Reichenstein I, Oppenheim H et al (2015) Dysregulated miRNA biogenesis downstream of cellular stress and ALS-causing mutations: a new mechanism for ALS. EMBO J 34(21):2633–2651. doi:10.15252/embj.201490493
58. Freischmidt A, Muller K, Zondler L, Weydt P, Volk AE, Bozic AL, Walter M, Bonin M et al (2014) Serum microRNAs in patients with genetic amyotrophic lateral sclerosis and pre-manifest mutation carriers. Brain : a journal of neurology 137(Pt 11):2938–2950. doi:10.1093/brain/awu249
59. Lipovich L, Johnson R, Lin CY (2010) MacroRNA underdogs in a microRNA world: evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA. Biochim Biophys Acta 1799(9):597–615.doi:10.1016/j.bbagrm.2010.10.001
60. Costa FF (2007) Non-coding RNAs: lost in translation? Gene 386(1–2):1–10. doi:10.1016/j.gene.2006.09.028
61. Ponting CP, Oliver PL, Reik W (2009) Evolution and functions of long noncoding RNAs. Cell 136(4):629–641. doi:10.1016/j.cell.2009.02.006
62. Reinhart BJ, Bartel DP (2002) Small RNAs correspond to centromere heterochromatic repeats. Science 297(5588):1831. doi:10.1126/science.1077183
63. Pasquinelli AE, Reinhart BJ, Slack F, Martindale MQ, Kuroda MI, Maller B, Hayward DC, Ball EE et al (2000) Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. Nature 408(6808):86–89. doi:10.1038/35040556
64. Ambros V, Lee RC, Lavanway A, Williams PT, Jewell D (2003) MicroRNAs and other tiny endogenous RNAs in *C. elegans*. Current biology : CB 13(10):807–818
65. Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science 294(5543):858–862. doi:10.1126/science.1065062
66. Elbashir SM, Lendeckel W, Tuschl T (2001) RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev 15(2):188–200
67. Ghildiyal M, Zamore PD (2009) Small silencing RNAs: an expanding universe. Nat Rev Genet 10(2):94–108. doi:10.1038/nrg2504
68. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. Science 294(5543):853–858. doi:10.1126/science.1064921
69. Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5):843–854
70. Ha M, Kim VN (2014) Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15(8):509–524. doi:10.1038/nrm3838
71. Krol J, Loedige I, Filipowicz W (2010) The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11(9):597–610. doi:10.1038/nrg2843
72. Lee Y, Jeon K, Lee JT, Kim S, Kim VN (2002) MicroRNA maturation: stepwise processing and subcellular localization. EMBO J 21(17):4663–4670
73. Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19(1):92–105. doi:10.1101/gr.082701.108
74. Huntzinger E, Izaurralde E (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 12(2):99–110. doi:10.1038/nrg2936
75. Bartel DP (2009) MicroRNAs: target recognition and regulatory functions. Cell 136(2):215–233. doi:10.1016/j.cell.2009.01.002
76. Thomson DW, Bracken CP, Goodall GJ (2011) Experimental strategies for microRNA target identification. Nucleic Acids Res 39(16):6845–6853. doi:10.1093/nar/gkr330
77. Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466(7308):835–840. doi:10.1038/nature09267
78. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS et al (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433(7027):769–773.doi:10.1038/nature03315
79. Peter ME (2010) Targeting of mRNAs by multiple miRNAs: the next step. Oncogene 29(15):2161–2164. doi:10.1038/onc.2010.59
80. Wu S, Huang S, Ding J, Zhao Y, Liang L, Liu T, Zhan R, He X (2010) Multiple microRNAs modulate p21Cip1/Waf1 expression by directly targeting its 3′ untranslated region. Oncogene 29(15): 2302–2308. doi:10.1038/onc.2010.34
81. Shan SW, Fang L, Shatseva T, Rutnam ZJ, Yang X, Du W, Lu WY, Xuan JW et al (2013) Mature miR-17-5p and passenger miR-17-3p induce hepatocellular carcinoma by targeting PTEN, GalNT7 and vimentin in different signal pathways. J Cell Sci 126(Pt 6): 1517–1530. doi:10.1242/jcs.122895
82. Wu C, Arora P (2014) MicroRNA passenger strand: orchestral symphony of paracrine signaling. Circ Cardiovasc Genet 7(4): 567–568. doi:10.1161/CIRCGENETICS.114.000805
83. Yang X, Du WW, Li H, Liu F, Khorshidi A, Rutnam ZJ, Yang BB (2013) Both mature miR-17-5p and passenger strand miR-17-3p target TIMP3 and induce prostate tumor growth and invasion. Nucleic Acids Res 41(21):9688–9704. doi:10.1093/nar/gkt680
84. Borchert GM, Lanier W, Davidson BL (2006) RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 13(12): 1097–1101. doi:10.1038/nsmb1167
85. Babiarz JE, Blelloch R (2008) Small RNAs - their biogenesis, regulation and function in embryonic stem cells. In: StemBook Cambridge (MA). doi:10.3824/stembook.1.47.1
86. Pfeffer S, Lagos-Quintana M, Tuschl T (2005) Cloning of small RNA molecules. Current protocols in molecular biology / edited by Frederick M Ausubel [et al] Chapter 26:Unit 26 24. doi:10.1002/0471142727.mb2604s72
87. Cai X, Hagedorn CH, Cullen BR (2004) Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 10(12):1957–1966. doi:10.1261/rna.7135204

88. Davis-Dusenbery BN, Hata A (2010) Mechanisms of control of microRNA biogenesis. J Biochem 148(4):381–392. doi:10.1093/jb/mvq096
89. Kim W, Benhamed M, Servet C, Latrasse D, Zhang W, Delarue M, Zhou DX (2009) Histone acetyltransferase GCN5 interferes with the miRNA pathway in Arabidopsis. Cell Res 19(7):899–909. doi:10.1038/cr.2009.59
90. Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN (2004) MicroRNA genes are transcribed by RNA polymerase II. EMBO J 23(20):4051–4060. doi:10.1038/sj.emboj.7600385
91. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R (2004) The microprocessor complex mediates the genesis of microRNAs. Nature 432(7014):235–240. doi:10.1038/nature03120
92. Aghamaleky Sarvestany A, Hunter G, Tavendale A, Lamont DJ, Llavero Hurtado M, Graham LC, Wishart TM, Gillingwater TH (2014) Label-free quantitative proteomic profiling identifies disruption of ubiquitin homeostasis as a key driver of schwann cell defects in spinal muscular atrophy. J Proteome Res 13(11):4546–4557. doi:10.1021/pr500492j
93. Auyeung VC, Ulitsky I, McGeary SE, Bartel DP (2013) Beyond secondary structure: primary-sequence determinants license pri-miRNA hairpins for processing. Cell 152(4):844–858. doi:10.1016/j.cell.2013.01.031
94. Lund E, Guttinger S, Calado A, Dahlberg JE, Kutay U (2004) Nuclear export of microRNA precursors. Science 303(5654):95–98. doi:10.1126/science.1090599
95. Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293(5531):834–838. doi:10.1126/science.1062961
96. Kye MJ, Goncalves Ido C (2014) The role of miRNA in motor neuron disease. Front Cell Neurosci 8:15. doi:10.3389/fncel.2014.00015
97. Kawamata T, Tomari Y (2010) Making RISC. Trends Biochem Sci 35(7):368–376. doi:10.1016/j.tibs.2010.03.009
98. Paez-Colasante X, Figueroa-Romero C, Sakowski SA, Goutman SA, Feldman EL (2015) Amyotrophic lateral sclerosis: mechanisms and therapeutics in the epigenomic era. Nat Rev Neurol 11(5):266–279. doi:10.1038/nrneurol.2015.57
99. Chiang HR, Schoenfeld LW, Ruby JG, Auyeung VC, Spies N, Baek D, Johnston WK, Russ C et al (2010) Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. Genes Dev 24(10):992–1009. doi:10.1101/gad.1884710
100. Hu HY, Yan Z, Xu Y, Hu H, Menzel C, Zhou YH, Chen W, Khaitovich P (2009) Sequence features associated with microRNA strand selection in humans and flies. BMC Genomics 10:413.doi:10.1186/1471-2164-10-413
101. Khvorova A, Reynolds A, Jayasena SD (2003) Functional siRNAs and miRNAs exhibit strand bias. Cell 115(2):209–216
102. Roberts TC, Blomberg KE, Smith CI, El Andaloussi S, Wood MJ (2016) mRNA and microRNA transcriptomics analyses in a murine model of dystrophin loss and therapeutic restoration. Genomics data 7:88–89. doi:10.1016/j.gdata.2015.11.025
103. Bail S, Swerdel M, Liu H, Jiao X, Goff LA, Hart RP, Kiledjian M (2010) Differential regulation of microRNA stability. RNA 16(5):1032–1039. doi:10.1261/rna.1851510
104. Dong H, Xu L, Wu L, Wang X, Duan W, Li H, Li C (2014) Curcumin abolishes mutant TDP-43 induced excitability in a motoneuron-like cellular model of ALS. Neuroscience 272:141–153. doi:10.1016/j.neuroscience.2014.04.032
105. Nielsen AF, Leuschner PJ, Martinez J (2007) Not miR-ly a splicing factor: hnRNP A1 succumbs to microRNA temptation. Nat Struct Mol Biol 14(7):572–573. doi:10.1038/nsmb0707-572
106. Lykke-Andersen J (2002) Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay. Mol Cell Biol 22(23):8114–8121
107. Parker R, Song H (2004) The enzymes and control of eukaryotic mRNA turnover. Nat Struct Mol Biol 11(2):121–127. doi:10.1038/nsmb724
108. van Dijk E, Cougot N, Meyer S, Babajko S, Wahle E, Seraphin B (2002) Human Dcp2: a catalytically active mRNA decapping enzyme located in specific cytoplasmic structures. EMBO J 21(24):6915–6924
109. Petersen CP, Bordeleau ME, Pelletier J, Sharp PA (2006) Short RNAs repress translation after initiation in mammalian cells. Mol Cell 21(4):533–542. doi:10.1016/j.molcel.2006.01.031
110. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. Cell 122(4):553–563. doi:10.1016/j.cell.2005.07.031
111. Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. Dev Biol 216(2):671–680. doi:10.1006/dbio.1999.9523
112. Pillai RS, Bhattacharyya SN, Artus CG, Zoller T, Cougot N, Basyuk E, Bertrand E, Filipowicz W (2005) Inhibition of translational initiation by Let-7 microRNA in human cells. Science 309(5740):1573–1576. doi:10.1126/science.1115079
113. Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, Doykan CE, Wu PM et al (2012) Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J Clin Invest 122(9):3063–3087. doi:10.1172/JCI62636
114. De Felice B, Guida M, Guida M, Coppola C, De Mieri G, Cotrufo R (2012) A miRNA signature in leukocytes from sporadic amyotrophic lateral sclerosis. Gene 508(1):35–40. doi:10.1016/j.gene.2012.07.058
115. Wakabayashi K, Mori F, Kakita A, Takahashi H, Utsumi J, Sasaki H (2014) Analysis of microRNA from archived formalin-fixed paraffin-embedded specimens of amyotrophic lateral sclerosis. Acta neuropathologica communications 2:173. doi:10.1186/s40478-014-0173-z
116. Campos-Melo D, Droppelmann CA, He Z, Volkening K, Strong MJ (2013) Altered microRNA expression profile in amyotrophic lateral sclerosis: a role in the regulation of NFL mRNA levels. Molecular brain 6:26. doi:10.1186/1756-6606-6-26
117. Figueroa-Romero C, Hur J, Lunn JS, Paez-Colasante X, Bender DE, Yung R, Sakowski SA, Feldman EL (2015) Expression of microRNAs in human post-mortem amyotrophic lateral sclerosis spinal cords provides insight into disease mechanisms. Mol Cell Neurosci 71:34–45. doi:10.1016/j.mcn.2015.12.008
118. Toivonen JM, Manzano R, Olivan S, Zaragoza P, Garcia-Redondo A, Osta R (2014) MicroRNA-206: a potential circulating biomarker candidate for amyotrophic lateral sclerosis. PLoS One 9(2):e89065. doi:10.1371/journal.pone.0089065
119. Williams AH, Valdez G, Moresi V, Qi X, McAnally J, Elliott JL, Bassel-Duby R, Sanes JR et al (2009) MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326(5959):1549–1554. doi:10.1126/science.1181046
120. Valdez G, Heyer MP, Feng G, Sanes JR (2014) The role of muscle microRNAs in repairing the neuromuscular junction. PLoS One 9(3):e93140. doi:10.1371/journal.pone.0093140
121. Russell AP, Wada S, Vergani L, Hock MB, Lamon S, Leger B, Ushida T, Cartoni R et al (2013) Disruption of skeletal muscle mitochondrial network genes and miRNAs in amyotrophic lateral sclerosis. Neurobiol Dis 49:107–117. doi:10.1016/j.nbd.2012.08.015

122. Liu X, Cheng Y, Chen X, Yang J, Xu L, Zhang C (2011) MicroRNA-31 regulated by the extracellular regulated kinase is involved in vascular smooth muscle cell growth via large tumor suppressor homolog 2. J Biol Chem 286(49):42371–42380. doi:10.1074/jbc.M111.261065
123. Amin ND, Bai G, Klug JR, Bonanomi D, Pankratz MT, Gifford WD, Hinckley CA, Sternfeld MJ et al (2015) Loss of motoneuron-specific microRNA-218 causes systemic neuromuscular failure. Science 350(6267):1525–1529. doi:10.1126/science.aad2509
124. Thiebes KP, Nam H, Cambronne XA, Shen R, Glasgow SM, Cho HH, Kwon JS, Goodman RH et al (2015) miR-218 is essential to establish motor neuron fate as a downstream effector of Isl1-Lhx3. Nat Commun 6:7718. doi:10.1038/ncomms8718
125. Morel L, Regan M, Higashimori H, Ng SK, Esau C, Vidensky S, Rothstein J, Yang Y (2013) Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter GLT1. J Biol Chem 288(10):7105–7116. doi:10.1074/jbc.M112.410944
126. Ishtiaq M, Campos-Melo D, Volkening K, Strong MJ (2014) Analysis of novel NEFL mRNA targeting microRNAs in amyotrophic lateral sclerosis. PLoS One 9(1):e85653. doi:10.1371/journal.pone.0085653
127. Dobrowolny G, Bernardini C, Martini M, Baranzini M, Barba M, Musaro A (2015) Muscle expression of SOD1(G93A) modulates microRNA and mRNA transcription pattern associated with the myelination process in the spinal cord of transgenic mice. Front Cell Neurosci 9:463. doi:10.3389/fncel.2015.00463
128. Zhang Z, Pinto AM, Wan L, Wang W, Berg MG, Oliva I, Singh LN, Dengler C et al (2013) Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. Proc Natl Acad Sci U S A 110(48):19348–19353. doi:10.1073/pnas.1319280110
129. Marcuzzo S, Kapetis D, Mantegazza R, Baggi F, Bonanno S, Barzago C, Cavalcante P, Kerlero de Rosbo N et al (2014) Altered miRNA expression is associated with neuronal fate in G93A-SOD1 ependymal stem progenitor cells. Exp Neurol 253:91–101. doi:10.1016/j.expneurol.2013.12.007
130. Zhou F, Guan Y, Chen Y, Zhang C, Yu L, Gao H, Du H, Liu B et al (2013) miRNA-9 expression is upregulated in the spinal cord of G93A-SOD1 transgenic mice. Int J Clin Exp Pathol 6(9):1826–1838
131. Nolan K, Mitchem MR, Jimenez-Mateos EM, Henshall DC, Concannon CG, Prehn JH (2014) Increased expression of microRNA-29a in ALS mice: functional analysis of its inhibition. Journal of molecular neuroscience : MN 53(2):231–241. doi:10.1007/s12031-014-0290-y
132. Nolan K, Walter F, Tuffy LP, Poeschel S, Gallagher R, Haunsberger S, Bray I, Stallings RL et al (2016) Endoplasmic reticulum stress-mediated upregulation of miR-29a enhances sensitivity to neuronal apoptosis. Eur J Neurosci 43(5):640–652. doi:10.1111/ejn.13160
133. Shioya M, Obayashi S, Tabunoki H, Arima K, Saito Y, Ishida T, Satoh J (2010) Aberrant microRNA expression in the brains of neurodegenerative diseases: miR-29a decreased in Alzheimer disease brains targets neurone navigator 3. Neuropathol Appl Neurobiol 36(4):320–330. doi:10.1111/j.1365-2990.2010.01076.x
134. Marcuzzo S, Bonanno S, Kapetis D, Barzago C, Cavalcante P, D'Alessandro S, Mantegazza R, Bernasconi P (2015) Up-regulation of neural and cell cycle-related microRNAs in brain of amyotrophic lateral sclerosis mice at late disease stage. Molecular brain 8:5. doi:10.1186/s13041-015-0095-0
135. Koval ED, Shaner C, Zhang P, du Maine X, Fischer K, Tay J, Chau BN, Wu GF et al (2013) Method for widespread microRNA-155 inhibition prolongs survival in ALS-model mice. Hum Mol Genet 22(20):4127–4135. doi:10.1093/hmg/ddt261
136. Parisi C, Arisi I, D'Ambrosi N, Storti AE, Brandi R, D'Onofrio M, Volonte C (2013) Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. Cell Death Dis 4:e959. doi:10.1038/cddis.2013.491
137. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K et al (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 351(3):602–611. doi:10.1016/j.bbrc.2006.10.093
138. Bentmann E, Haass C, Dormann D (2013) Stress granules in neurodegeneration—lessons learnt from TAR DNA binding protein of 43 kDa and fused in sarcoma. FEBS J 280(18):4348–4370. doi:10.1111/febs.12287
139. Wolozin B (2012) Regulated protein aggregation: stress granules and neurodegeneration. Mol Neurodegener 7:56. doi:10.1186/1750-1326-7-56
140. Kim HJ, Raphael AR, LaDow ES, McGurk L, Weber RA, Trojanowski JQ, Lee VM, Finkbeiner S et al (2014) Therapeutic modulation of eIF2alpha phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. Nat Genet 46(2):152–160. doi:10.1038/ng.2853
141. Saxena S, Cabuy E, Caroni P (2009) A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. Nat Neurosci 12(5):627–636. doi:10.1038/nn.2297
142. Aulas A, Vande Velde C (2015) Alterations in stress granule dynamics driven by TDP-43 and FUS: a link to pathological inclusions in ALS? Front Cell Neurosci 9:423. doi:10.3389/fncel.2015.00423
143. Morlando M, Dini Modigliani S, Torrelli G, Rosa A, Di Carlo V, Caffarelli E, Bozzoni I (2012) FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. EMBO J 31(24):4502–4510. doi:10.1038/emboj.2012.319
144. Bicker S, Schratt G (2015) MicroRNAs in ALS: small pieces to the puzzle. EMBO J 34(21):2601–2603. doi:10.15252/embj.201592805
145. Jeng SF, Rau CS, Liliang PC, Wu CJ, Lu TH, Chen YC, Lin CJ, Hsieh CH (2009) Profiling muscle-specific microRNA expression after peripheral denervation and reinnervation in a rat model. J Neurotrauma 26(12):2345–2353. doi:10.1089/neu.2009.0960
146. Ma G, Wang Y, Li Y, Cui L, Zhao Y, Zhao B, Li K (2015) MiR-206, a key modulator of skeletal muscle development and disease. Int J Biol Sci 11(3):345–352. doi:10.7150/ijbs.10921
147. Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V (2004) Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biol 5(3):R13. doi:10.1186/gb-2004-5-3-r13
148. Grifone R, Demignon J, Houbron C, Souil E, Niro C, Seller MJ, Hamard G, Maire P (2005) Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. Development 132(9):2235–2249. doi:10.1242/dev.01773
149. Cohen TJ, Waddell DS, Barrientos T, Lu Z, Feng G, Cox GA, Bodine SC, Yao TP (2007) The histone deacetylase HDAC4 connects neural activity to muscle transcriptional reprogramming. J Biol Chem 282(46):33752–33759. doi:10.1074/jbc.M706268200
150. Tang H, Macpherson P, Marvin M, Meadows E, Klein WH, Yang XJ, Goldman D (2009) A histone deacetylase 4/myogenin positive feedback loop coordinates denervation-dependent gene induction and suppression. Mol Biol Cell 20(4):1120–1131. doi:10.1091/mbc.E08-07-0759
151. Ceppi M, Pereira PM, Dunand-Sauthier I, Barras E, Reith W, Santos MA, Pierre P (2009) MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells. Proc Natl Acad Sci U S A 106(8):2735–2740. doi:10.1073/pnas.0811073106

152. O'Connell RM, Kahn D, Gibson WS, Round JL, Scholz RL, Chaudhuri AA, Kahn ME, Rao DS et al (2010) MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity 33(4):607–619. doi:10.1016/j.immuni.2010.09.009
153. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D (2007) MicroRNA-155 is induced during the macrophage inflammatory response. Proc Natl Acad Sci U S A 104(5):1604–1609. doi:10.1073/pnas.0610731104
154. Parisi C, Napoli G, Amadio S, Spalloni A, Apolloni S, Longone P, Volonte C (2016) MicroRNA-125b regulates microglia activation and motor neuron death in ALS. Cell Death Differ 23(3):531–541. doi:10.1038/cdd.2015.153
155. Kiskinis E, Sandoe J, Williams LA, Boulting GL, Moccia R, Wainger BJ, Han S, Peng T et al (2014) Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1. Cell Stem Cell 14(6):781–795. doi:10.1016/j.stem.2014.03.004
156. Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G et al (1993) bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74(4):597–608
157. Benatar M, Boylan K, Jeromin A, Rutkove SB, Berry J, Atassi N, Bruijn L (2016) ALS biomarkers for therapy development: state of the field and future directions. Muscle Nerve 53(2):169–182. doi:10.1002/mus.24979
158. Blasco H, Corcia P, Moreau C, Veau S, Fournier C, Vourc'h P, Emond P, Gordon P et al (2010) 1H-NMR-based metabolomic profiling of CSF in early amyotrophic lateral sclerosis. PLoS One 5(10):e13223. doi:10.1371/journal.pone.0013223
159. Pradat PF, Dib M (2009) Biomarkers in amyotrophic lateral sclerosis: facts and future horizons. Molecular diagnosis & therapy 13(2):115–125. doi:10.2165/01250444-200913020-00005
160. Rothstein JD, Kuncl R, Chaudhry V, Clawson L, Cornblath DR, Coyle JT, Drachman DB (1991) Excitatory amino acids in amyotrophic lateral sclerosis: an update. Ann Neurol 30(2):224–225. doi:10.1002/ana.410300223
161. Shaw PJ, Forrest V, Ince PG, Richardson JP, Wastell HJ (1995) CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. Neurodegeneration : a journal for neurodegenerative disorders, neuroprotection, and neuroregeneration 4(2):209–216
162. Spreux-Varoquaux O, Bensimon G, Lacomblez L, Salachas F, Pradat PF, Le Forestier N, Marouan A, Dib M et al (2002) Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. J Neurol Sci 193(2):73–78
163. Arroyo JD, Chevillet JR, Kroh EM, Ruf IK, Pritchard CC, Gibson DF, Mitchell PS, Bennett CF et al (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc Natl Acad Sci U S A 108(12):5003–5008. doi:10.1073/pnas.1019055108
164. Gallo A, Tandon M, Alevizos I, Illei GG (2012) The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. PLoS One 7(3):e30679. doi:10.1371/journal.pone.0030679
165. Wagner J, Riwanto M, Besler C, Knau A, Fichtlscherer S, Roxe T, Zeiher AM, Landmesser U et al (2013) Characterization of levels and cellular transfer of circulating lipoprotein-bound microRNAs. Arterioscler Thromb Vasc Biol 33(6):1392–1400. doi:10.1161/ATVBAHA.112.300741
166. Jin H, Li C, Ge H, Jiang Y, Li Y (2013) Circulating microRNA: a novel potential biomarker for early diagnosis of intracranial aneurysm rupture a case control study. J Transl Med 11:296. doi:10.1186/1479-5876-11-296
167. Jadhav VM, Scaria V, Maiti S (2009) Antagomirzymes: oligonucleotide enzymes that specifically silence microRNA function. Angew Chem 48(14):2557–2560. doi:10.1002/anie.200805521
168. Bader AG, Brown D, Winkler M (2010) The promise of microRNA replacement therapy. Cancer Res 70(18):7027–7030. doi:10.1158/0008-5472.CAN-10-2010
169. Junn E, Mouradian MM (2012) MicroRNAs in neurodegenerative diseases and their therapeutic potential. Pharmacol Ther 133(2):142–150. doi:10.1016/j.pharmthera.2011.10.002
170. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK (2009) Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol 27(1):59–65. doi:10.1038/nbt.1515
171. Stoica L, Sena-Esteves M (2016) Adeno associated viral vector delivered RNAi for gene therapy of SOD1 amyotrophic lateral sclerosis. Front Mol Neurosci 9:56. doi:10.3389/fnmol.2016.00056
172. Stoica L, Todeasa SH, Cabrera GT, Salameh JS, ElMallah MK, Mueller C, Brown RH Jr, Sena-Esteves M (2016) Adeno-associated virus-delivered artificial microRNA extends survival and delays paralysis in an amyotrophic lateral sclerosis mouse model. Ann Neurol 79(4):687–700. doi:10.1002/ana.24618
173. Borel F, Gernoux G, Cardozo B, Metterville JP, Toro Cabreja GC, Song L, Su Q, Gao GP et al (2016) Therapeutic rAAVrh10 mediated SOD1 silencing in adult SOD1(G93A) mice and nonhuman primates. Hum Gene Ther 27(1):19–31. doi:10.1089/hum.2015.122
174. van Zundert B, Brown RH Jr (2017) Silencing strategies for therapy of SOD1-mediated ALS. Neurosci Lett 636:32–39. doi:10.1016/j.neulet.2016.07.059
175. Peters OM, Cabrera GT, Tran H, Gendron TF, McKeon JE, Metterville J, Weiss A, Wightman N et al (2015) Human C9ORF72 hexanucleotide expansion reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice. Neuron 88(5):902–909. doi:10.1016/j.neuron.2015.11.018