Chapter

RNA Metabolism and Therapeutics in Amyotrophic Lateral Sclerosis

Orietta Pansarasa, Stella Gagliardi, Daisy Sproviero and Cristina Cereda

Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neuromuscular disorder characterized by the selective death of upper and lowers motor neurons in spinal cord, brain stem, and motor cortex, which leads to paralysis and death within 2–3 years of onset. Deeply sequencing technologies, to simultaneously analyze the transcriptional expression of thousands of genes, offered new possibilities to focus on ALS pathogenesis and, most notably, to find new potential targets for novel treatments. The present book chapter illustrates recent advances in transcriptomic studies in animal models and human samples and in new molecular targets related to ALS pathogenesis and disease progression. Additionally, new insights into the involvement of altered transcriptional profiles of noncoding RNAs (microRNA and lncRNA) and ALS-associated ribosomal binding proteins have been investigated, to understand the functional consequences of extensive RNA dysregulation in ALS. Attention has been also turned on how transcriptome alterations could highlight new molecular targets for drug development.

Keywords: ALS, RNA metabolism, transcriptomics, gene expression, noncoding RNA

Highlights

- Aberrant RNA metabolism is one of the major contributors to ALS pathogenesis.
- Understanding RNA-binding protein functions and identifying target RNA regulatory networks is crucial to deepen ALS knowledge and to develop new therapeutics.
- miRNAs are strongly linked to the development of ALS and are indicated as new potential biomarkers.
- lncRNAs have been recently indicated to play important roles in CNS in health and disease such as ALS.
- miRNA-based therapeutics as well as deregulated AS are considered important areas for therapeutic intervention.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder (ND) that affects the human motor system, that is, the lower and upper
motor neurons (MNs). Among the symptoms of ALS, there are progressive muscle weakness and paralysis, swallowing difficulties, and breathing impairment due to respiratory muscle weakness that finally causes death, within 2–5 years following clinical diagnosis [1]. Now, also extramotor systems are involved in ALS, thus providing new insight into the pathogenesis of the disease. So far, no effective therapy is available for ALS: Rilutek (riluzole) and Radicava (edaravone) are the only two drugs approved by the Food and Drug Administration for ALS treatment. Unfortunately their effect in slowing disease progression is very modest [2]. The majority of ALS cases, named as sporadic (sALS), has no a family history; a fraction of cases (about 5–10%) are considered familial (fALS) [3], because of mutations in genes involved in a wide range of cellular functions. 60–70% of fALS and 10% of sporadic ALS (sALS) cases can be ascribed to mutations in SOD1, TARDBP, FUS, VCP, C9ORF72, and OPTN [4]; further rare genetic variants have also been identified, MATR3, HNRNPA1, HNRNPA2/B1, EWSR1, TAF15, ANG, UBQLN2, VAPB, TBK1, SQSTM1, PFB1, TUBA4A, KIF5A, ANXA11, and CHCHD10 [5]. Although an in-depth understanding of the mechanisms underlying ALS has yet to be reached, a growing interest was addressed to the impairment of RNA metabolism as one of the major contributor to ALS pathogenesis. This concept is reinforced by the discovery of genetic mutations in FUS and in TARDBP genes coding for RNA binding proteins (RBPs), which play a multifaceted role in transcription and in maintaining RNA metabolism. Recent studies have reported that a substantial portion of the genome is actively transcribed as noncoding RNA molecules. These noncoding RNAs are fundamental key actors in the regulation of biological processes and function as a “fine switch” of gene expression. It is now recognized that dysregulations in the noncoding RNAs gene expression is a putative mechanism in several neurological disorders, including ALS. Moreover, noncoding RNAs are emerging as new potential biomarkers contributing to an early disease diagnosis and treatment follow-up. To date, miRNA have been one the main focus of most ALS studies. miRNAs are differentially expressed in several tissues (CSF, plasma and serum) in ALS patients compared to healthy controls.

In this chapter, we will focus on the involvement of altered transcriptional profiles of microRNAs (miRNAs) and long noncoding RNA (lncRNA) as well as on ALS-related RNA binding proteins. We also review biomarkers and potential therapeutic strategies based on the manipulation of noncoding RNAs.

2. Dysfunctions in RNA metabolism and RNA-binding protein

It is broadly recognized that an aberrant RNA metabolism may contribute to RNA toxicity, which is due to the accumulation of toxic RNAs and to the dysfunction of RBPs [6].

Messenger RNAs (mRNAs) are subjected to several processing steps including splicing, polyadenylation, editing, transport, translation, and turnover. All these processes are extremely dynamic and require the involvement of RBPs to coordinate both co- and posttranscriptional processing of transcripts. Understanding RBPs functions and identifying their target RNA regulatory networks are crucial to deepen the knowledge in NDs and to promptly develop new therapeutics.

Nussbacher and colleagues by a genome-wide approach, have shed a new light on how RBPs may affect the fate of their targets [7]. Considering the great impact of RBPs on the expression, splicing, and translation of multiple RNA targets, also little changes in their expression and/or activity have amplified effects. Moreover, an altered interaction between RBPs and their targets can induce serious pathological phenotypes, even if the exact mechanism is not clear. Briefly, we focus on RBPs,
TARDBP and FUS, and SOD1 and C9orf72 to highlight recent progresses on their involvement in RNA dysregulation.

**TDP-43** is a heterogeneous nuclear RBP of 414 amino acids that contains two RNA recognition motifs (RRM1-2), a glycine rich domain in the C-terminus and nuclear localization and export signals (NLS and NES) [8, 9]. TDP-43 is crucial in RNA processing, that is, RNA splicing, transcription, transport, stability, as well as miRNA production [10]. TDP-43 binds to more than 6000 RNA targets in the brain [11, 12]. TDP-43 binds to mRNA and regulates the expression of other proteins: FUS, Tau, ATXN2 CHMP2B, VAPB, and progranulin, all involved in ALS and in other NDs [12, 13]. Polymenidou and colleagues using an RNA-seq approach, demonstrated the involvement of TDP-43 in the regulation of the expression of 239 mRNAs, many of those encoding synaptic proteins including neurexin NRXN1-3, neuroligin NLGN1-2, Homer2, microtubule-associated protein 1B (MAP1B), GABA receptors subunits (GABRA2, GABRA3), AMPA receptor sub-units (GRIA3, GRIA4), syntaxin 1B, and calcium channels [11, 14–17]. Together these data suggest the involvement of TDP-43 in neuronal morphology, synaptic transmission, and neuronal plasticity likely through the regulation of RNA processing of synaptic genes [14]. TDP-43 is also a splicing regulator which decreases its own expression level by binding to the 3′-untranslated (UTR) region of its own pre-mRNA [18]. Moreover, its depletion or overexpression can influence the alternative splicing of specific targets genes, which are altered in ALS [11, 13, 19]. In 2012, Kawahara and Mieda-Sato also demonstrated the involvement of TDP-43 in miRNA biogenesis. TDP-43 helps the production of the precursor miRNAs (pre-miRNAs) through the interaction of the Drosha complex and the binding to the primary miRNAs (pri-miRNAs) [20]. An increased expression of miR-633 and a decreased expression of the let-7b miRNA have been observed when TDP-43 is downregulated [21]. Moreover, TDP-43 binds to IncRNAs, including the nuclear-enriched autosomal transcript 1 (NEAT1) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) [13]. Until now, the exact role of this interaction is unclear; however both NEAT1 and MALAT1 levels are enhanced in patients with frontotemporal lobar degeneration (FTLD) and ALS [12, 22]. Nishimoto and co-authors identified paraspeckles, that is, membraneless nuclear bodies, with high levels of NEAT1 and TDP-43 in MNs of patients in the early stage of the disease [22], thus interfering with TDP-43-mediated RNA processing and disrupting RNA homeostasis in ALS MNs.

**FUS** is an RBP of 526 amino acids mainly located in the nuclei. It is composed by a RNA recognition motif, a SYGQ (serine, tyrosine, glycine, and glutamine)-rich region, several RGG (arginine, glycine, and glycine)-repeat regions, a C2C2 zinc finger motif, and a nuclear localization signal (NLS) [23]. Similar to TDP-43, FUS has a key role in RNA processing. It is involved in transcriptional regulation, mRNA splicing, and miRNA production. FUS co-modulates certain transcription factors, including NF-kB, SPI1, and Runbox transcription factor (RUNX) [24, 25]. Genome-wide approaches have evidenced more than 5000 human RNA targets for FUS [26]. Considering that FUS is part of the hnRNP complex, it is crucial for the splicing mechanism [23], and it may affect the splicing mechanism of more than 900 mRNAs [26]. Among these, FUS may regulate the alternative splicing of genes related to cytoskeletal organization, axonal growth, and guidance such as the microtubule-associated protein tau (MAPT) [27], Netrin G1 (NTNG1) [28], neuronal cell adhesion molecule (NRCAM), and the actin-binding LIM (ABLIM1) [29]. Like TDP-43, FUS also binds to different mRNAs of ALS-related genes, VCP, VAPB, ubiquilin-2, and OPTN, thus modulating their expression [12, 26]. Furthermore, FUS is involved in the biogenesis of miRNA by recruiting Drosha to pri-miRNAs at their transcription sites and supports the biogenesis of a subset of...
miRNAs [30]. However, if FUS directly regulates the function of mature miRNAs remains to be understand. Finally, FUS is crucial for the regulation of NEAT 1 levels and paraspeckle formation. FUS nuclear deficiency, its loss of nuclear function, as well as its aggregation might cause sequestration of paraspeckle components into pathological inclusions. Thus, the interaction between FUS and NEAT 1 is involved in the development of neuronal dysfunction in ALS [31].

SOD1 is not an RBP; however, several authors demonstrated that mutant SOD1 has a role in RNA metabolism regulation [32, 33]. These authors reported that mutant SOD1 can bind mRNA species, that is, vascular endothelial growth factor (VEGF) and neurofilament light chain (NFL), and alter their expression, stabilization, and function [32, 33]. Mutant SOD1, by the direct bind to the 3’ UTR of VEGF mRNA, promotes the sequestration of other RBPs such as TIA-1-related protein (TIAR) and Hu antigen R (HuR) into insoluble aggregates. This, in turn, determines the impairment of HuR function and interferes with the HuR neuroprotective effect during stress responses [32]. Chen and colleagues further demonstrated that, through the modification of neurofilament (NF) stoichiometry, mutant SOD1 destabilizes NFL mRNA. Consequently, NFs aggregate in MNs and are considered a hallmark of ALS disease [33]. NFL mRNA stability could also be regulated by a common interaction between SOD1 and TDP-43 [34]. The exact mechanism is not completely understood; however, it is hypothesized that mutant SOD1 removes TDP-43 from the NFL mRNA, thus disturbing NFL mRNA metabolism and promoting the formation of aggregates.

In 2011, the large GGGGCC hexanucleotide repeat expansion of C9orf72 has been recognized as a new cause of ALS [35, 36], accounting for about 50% of fALS and 5–10% of sALS [37]. The C9orf72 repeat expansion is transcribed in both the sense and antisense directions and causes the accumulations of repeat containing RNA foci [38]. RNA foci formation allows the recruitment of RBPs and alter RNA metabolism [39]. Mori and co-authors observed that RNA foci can sequester the RBP hnRNP-A3 and can suppress its RNA processing function. Notably, RNA foci are also able to sequester nuclear proteins such as TDP-43 and FUS, thus affecting the expression of their RNA targets, mainly involved in RNA metabolism, stress response, and nuclear transport. Moreover, RNA-Seq data unveil new candidate genes, that is, genes involved in synaptic transmission, protein targeting, and cell–cell signaling; however future validation are required [40]. Moreover, poly-PR and poly-GR can alter the splicing patterns of specific RNAs. The poly-PR causes the exon skipping in RAN and PTX3 RNA [41]. Finally, C9orf72 repeats can interfere with transcription or splicing of C9orf72 transcripts and can disrupt the C9orf72 promoter activity [42, 43].

3. Dysfunctions in RNA metabolism and miRNA

miRNAs are short noncoding RNAs, approximately 18–25 nucleotides long, that play a key role in the regulation of gene expression in many fundamental cellular processes and, posttranscriptionally, at the translation levels of target mRNA transcripts [44, 45]. A high number of protein-coding genes have been demonstrated to be regulated by miRNA through base-pairing interactions within the UTR of the targeted miRNAs [46, 47]. Alongside their gene silencing functions, miRNAs can also induce upregulation of their targets [48]. An accurate regulatory pathway is fundamental to control and maintain the physiological processes of cells. However, when abnormalities occur, as in diseases, a complex dysregulation of the miRNA expression takes place. In this paragraph, we will focus on miRNAs which are linked to the development of ALS and miRNA with a potential role as biomarkers.

One of the most interesting miRNAs involved in ALS is \textit{miRNA206}. miRNA206 is skeletal muscle-specific, regulates myogenesis, and promotes the formation of
new neuromuscular junctions [49, 50]. Generally, the miRNA206 is overexpressed in muscle fibers and in serum of ALS patients [50, 51]. Pegoraro and co-authors associated the high levels of miRNA206 to the remodeling of the muscle, that is, atrophy, hypertrophy, and/or reinnervation of some fibers [51]. de Andrade et al. evidenced that miRNA206 increases early in the disease course and then decreases, thus suggesting its role during muscle loss [50]. miRNAs might also have a protective role in ALS; higher levels of miRNA206 were indeed observed in slow progressors, that is, in long-term ALS patients [52]. Thanks to the possibility to detect miRNA206 in accessible samples like serum and the correlation between miRNA206 levels and disease characteristics, miRNA206 could be indicated as a potential biomarker for ALS [53]. Other three miRNAs, miRNA133a/b, miRNA 1, and miRNA 27a, are indicated as muscle-specific. miRNA133 is higher in serum and muscle of ALS patients, and it is also higher in spinal ALS compared to bulbar ALS [51, 54]. An upregulation of miRNA27a was observed in CD14+ CD16− monocytes, in muscle fibers, and in CSF of ALS patients [51, 55], while a downregulation was reported in serum samples [54]. miRNA338-3p is another miRNA frequently upregulated. An increase of more than twofold was reported in leukocytes of sALS patients [56, 57]. Moreover, De Felice and co-authors showed an increase in miRNA338-3p in serum, CSF, and spinal cord of sALS patients. The evidence that it can be easily obtainable in body fluids suggested the possibility that miRNA338-3p might be a suitable biomarker for ALS. The inflammatory miRNA146a is overexpressed in CD14+ CD16− monocytes, CSF, spinal cord, and muscle fibers [55, 58]. miRNA146a can also interact with NFL mRNA 3′UTR, according to low mRNA levels observed in spinal neurons of sALS [58]. Tasca et al. on the other hand, identified a reduction of miRNA146a in serum of ALS patients, both bulbar and spinal [54]. Tasca et al. and Pegoraro et al. found a downregulation in serum, muscle fibers, and leukocytes of sALS of the inflammatory miRNA149/149*. Also miRNA221 seems to contribute to ALS development by acting on muscle growth and/ or atrophy and inflammation, through the NF-kB pathway [53, 54]. miRNA155 was evaluated in CD14+ CD16− monocytes [55] and spinal cords of ALS patients, and it increases both in fALS and sALS [59]. Two other miRNAs targeting TGF-β1, miRNA21, and miRNA106b were upregulated in CD14+ CD16− monocytes [55], and, at least for miRNA21, an upregulation was reported in muscle samples [50] in ALS patients even if its role in the pathology has yet to be fully explained. The same authors identified an inverse correlation between miRNA424 levels and disease progression, thus suggesting miRNA 424 as a potential biomarker [50] (Figure 1). The ALS genes, TDP-43 and FUS, play a role in miRNA biogenesis [60]. Mutations in TARDBP result in differential expression of miRNA9, miRNA132, miRNA143, miRNA558 [61], and let7 families [53], and differences between CSF and serum levels were observed. For instance, miRNA9, a brain-specific miRNA highly conserved during evolution is 2–3 times more elevated in CSF with respect to serum [62]. Differences are reported also for the presence of mutations. In induced pluripotent stem cell (iPSC)-derived neuron obtained from patients carrying the TARDBP p.A90V and the M337V mutation, miRNA9 and pri-miRNA9-2 levels were lower when compared to controls [61]). Likewise, miRNA9 also decreases in lumbar motor neurons of sALS and SOD1 A4V mutated patients [63]. Moreover, a correlation between these miRNAs and disease duration and site of onset was identified. Specifically, miRNA 143-3p levels increase in later-collected samples, and the increase becomes significant in lower limb-onset patients [53] (Figure 2).

Morlando and co-authors reported that, upon FUS depletion, the expression of miRNA9, miRNA132, miRNA143, miRNA125, and miRNA192 is altered [30]. The involvement of these miRNAs in motor neuron development and maintenance, axonal growth, and synaptic transmission accounts for their contribution to the ALS
pathological phenotype [64, 65]. In motor neurons progenitors derived from human ALS iPSCs, Rizzuti et al. observed that miRNA34a and miRNA504, involved in vesicle regulation and cell survival, were dysregulated [66]. Also miRNA1825 is downregulated in CNS of sALS and fALS patients, thus inducing depolymerization and degradation of tubulin alpha-4A (TUBA4A), which is encoded by the known ALS gene [67].

Taken together, these studies significantly contribute to evidence the importance of miRNAs, also as biomarkers for ALS. Despite these evidences, several issues need to be addressed mainly on the utility of miRNAs to serve as accurate and fast biomarkers for an early ALS diagnosis.

4. Dysfunctions in RNA metabolism and lncRNA

Long noncoding RNAs (lncRNAs) are transcripts, greater than 200 nucleotides in length, with no protein-coding potential which are found in sense or antisense
RNA Metabolism and Therapeutics in Amyotrophic Lateral Sclerosis
DOI: http://dx.doi.org/10.5772/intechopen.90704

orientation to protein-coding genes or within intergenic regions. IncRNAs control
the gene expression through different mechanisms, that is, epigenetic modula-
through chromatin remodeling, activation or repression of transcription,
posttranscriptional modifications of mRNA, and regulation of protein activity by
acting as scaffold to recruit RBPs and/or drive RBPs to DNA. Moreover, they can
compete for and disrupt protein-binding interactions or sponge miRNAs away from
their mRNA targets [68]. Recently, IncRNAs have been indicated to play important
roles in the CNS in health and disease such as ALS. Nishimoto and colleagues first
identify a relation between NEAT1 and ALS pathogeneses [22]. The NEAT1 gene
produces two transcripts, NEAT1_1 and NEAT1_2; NEAT1_2 expression is very
low in the adult nervous system, and it is the only one that forms paraspeckles [69].
Specifically, NEAT1 acts as a scaffold for paraspeckles thus enhancing their de novo
formation in spinal motor neurons in a cohort of sALS patients [22]. Paraspeckles
function through the retention of specific RNAs; the regulation of gene expression
by sequestration of transcription factors; and the modulation of miRNA biogenesis
and mitochondrial function [70]. Paraspeckles are enriched in pathological proteins
for ALS and are indicated as a hallmark of the disease [71]. Moreover, paraspeckle
proteins, including TDP-43 and FUS, are related to ALS and FTD. The increase in
paraspeckle formation in ALS could be triggered, at least in part, by the nuclear
depletion of TDP-43. TDP-43 binds NEAT1, and, in turn, its downregulation stimu-
lates NEAT1_2 accumulation and paraspeckle association in cultured cells [71].
Regarding FUS, in the spinal cord of FUS mutated ALS patients, Shelkovnikova and
co-authors reported the presence of pathological aggregation of NONO, a core para-
speckle protein [31]. This evidence allows to speculate that, considering that FUS
and NONO are both required to set up paraspeckles, the formation of paraspeckles
is disrupted in FUS mutated ALS patients. Also aberrant nuclear RNA foci formed
by the expanded C9ORF72 repeats sequester paraspeckle proteins including TDP-43
[72]. MALAT1 is abundantly expressed and evolutionarily conserved IncRNA. It
is one of the first IncRNAs associated with human disease, and it is involved in
alternative splicing, epigenetic modification of gene expression, synapse forma-
and myogenesis. In NDs MALAT1 is significantly increased in FTLD patients,
where it recruits splicing factors to nuclear speckles and affects phosphorylation of
SR proteins37 [13]. Some IncRNA transcripts have been associated to FUS, among
these the IncRNA CCND1 which binds to the FUS consensus sequence GGUG
[73]. Data form Wang and colleagues suggested that FUS is a specific repressor of
CCND1, which is downregulated in response to DNA damage signals. Until now the
IncRNA CCND1 has not been described in relation to ALS; but taken together, these
observations point out that this IncRNA could be, at least partly, responsible in ALS
and other neurodegenerative diseases.

Together with the IncRNA an increasing interest was addressed to the antisense
(AS) noncoding transcripts. They are generated from the strand opposite the sense
strand [74]. AS IncRNAs act by regulating chromatin, by controlling DNA methyla-
tion and/or histones modification, or by removing repressors. They promote sense
transcription by recruiting transcription factors, they also regulate the half-life
of their sense partners, and, in turn, they regulate gene expression [74]. About
70% of the human genome creates antisense transcripts with a great physiological
and pathological significance. Ataxin 2 (ATXN2) is a coding gene related to ALS
because of the association between the length of ATXN2 repeat expansion and the
disease risk of ALS [75]. In 2016, Li and co-authors described the ATXN2-AS [76].
ATXN2-AS with its CUG repeat expansion is neurotoxic and may contribute to ALS
pathogenesis. The CUG transcript toxicity is related to the structure formed by the
repeats; that is, the stems of hairpin structures act with sponge-like features,
sequester RBPs, and induce alterations of the RNA metabolism [77].
Thanks to the deep sequencing technologies which allow high-throughput massive RNA sequencing, a wide characterization of the transcriptome profile of cell populations and tissues is now available. Three different massive transcriptome profiles have been published in different tissues (spinal cord, monocytes, and peripheral blood mononuclear cells) of ALS patients, and matched controls reported a deregulation in expressed genes [78, 79] and in lncRNAs [80]. Differences in transcriptome profiles (coding and lncRNAs) were observed in PBMCs of unmutated sALS patients, SOD1, TARDBP, and FUS mutated ALS patients and healthy controls [80]. Specifically, the authors reported a remarkable AS deregulation of genes involved in the transcription regulation pathway such as ZEB1-AS and ZBTB11-AS in sALS patients. ZEB1 acts as a repressor or an activator of the transcription, that is, it may repress histone organization or activate chromatin regulators [81]. As regards ZBTB11-AS, it decreases in sALS patients compared to healthy controls. ZBTB11-AS is annotated as AS of Zinc finger and BTB domain-containing protein 11 (ZBTB11) gene, and it is reported to be a negative regulator of cell cycle; however its exact role has yet to be defined [82]. Moreover, Gagliardi and co-authors evidenced UBXN7-AS, ATG10-AS, and ADORA2A-AS in sALS patients, all related to NDs [83–85]. Specifically, the regulation of UBXN7, an ubiquitin protein bound by VCP a known ALS protein, through its AS controlled the ubiquitination in ALS disease. ATG10 is involved in the autophagy pathway, while ADORA2A is involved in Huntington’s disease and Parkinson’s disease in relation to defects in DNA methylation [84, 86] (Table 1).

5. Therapeutics

In the era of noncoding RNA, understanding the involvement of dysregulated miRNAs and of their targets in ALS disease is crucial to identify new pathways contributing to neurodegeneration that also offer novel opportunities for targeted intervention. miRNA-based therapeutics take advantages of two different approaches. The first involves the use of an anti-miRNA, that is, chemically modified antisense RNA, to decrease miRNA. Thus, miRNA duplex is not active and counteracts the negative regulatory effects of miRNA. This approach was first used to deliver the anti-miR-155 to the SOD1G93A transgenic mice via ventricular osmotic pumps; after this treatment the mortality was successfully delayed [59]. The second therapeutic approach using miRNA involves miRNA mimics, that is, small RNA molecules resembling miRNA precursors, that are reintroduced into cells exhibiting downregulation thus re-starting the key-related pathways [87]. Biomedical and nanoparticle engineering has begun to develop tools allowing

| sALS         | TARDBP mutation | FUS mutation |
|--------------|-----------------|--------------|
| ATXN2-AS [76]| NEAT1 [22, 69–71]| IncCCND1 [73]|
| ZEB1-AS [80] | MALAT1 [13]     |              |
| ZBTB11-AS [80]|              |              |
| UBXN7-AS [80] |              |              |
| ATG10-AS [80] |              |              |
| ADORA2A-AS [80]|              |              |

Table 1. List of lncRNAs related to ALS. In square bracket the relative references.
for this specific targeting. These second-generation miRNA-based therapeutics offer the potential for a greater delivery cargo to the tissue site while reducing RNA-mediated toxicity. Overall, the continued development of innovative RNA modifications and delivery items such as nanoparticles will aid in the development of future RNA-based therapeutics for a broader range of chronic disease.

Deregulated AS is considered an important area for therapeutic intervention. Particularly, gene therapy is an encouraging pharmacological approach for patients with diseases of genetic origins. This therapy is principally based on antisense oligonucleotides (ASOs), spliceosome-mediated RNA trans-splicing (SMaRT), or small interfering RNAs (siRNAs) [88]. ASOs, that is, synthetic single-stranded nucleic acids, bind the pre-mRNA intron/exon junctions and control the splicing through their action on enhancers or repressor sequences, thus determining the skipping of the exon or including alternatively spliced exons [89].

In ALS, one of the first ASO-based clinical trials was designed to silence SOD1. The intrathecal administration of this ASO pass with good results the phase I testing. Now a phase Ib/IIa trial is in process to assess safety, tolerability, and pharmacokinetics [90].

Among the ALS-related genes, C9orf72 is one of the best candidates for ASOs therapy. Early testing of ASO-based therapeutics for C9orf72 was performed on iPSC-derived neurons and fibroblasts [91]. Specifically, ASOs were designed to target the repeat expansion or within N-terminal regions of the mRNA transcript to destroy the transcript or to prevent the interaction between the repeat expansion and the RBPs, determining a decrease in RNA foci and dipeptide proteins and recovering the normal gene expression [91]. Other studies investigated the effects of ASO on the oligonucleotide backbone, sugar, and heterocycles to promote delivery, potency, and stability to target FUS. These studies evidenced that the affinities of nucleic acid binding domains depend on chemical changes and that the interaction between ASO and protein affects the localization of ASOs themselves [92]. These data strongly indicate that ASO-based therapy could be central in treating ALS-related genes, although there is great attention on the relation between the therapeutic outcomes and the stage of disease progression and on the time of intervention.

Also many novel lncRNAs have been discovered, and the potential to become therapeutic targets is gradually increasing. Considering that lncRNAs function as decoys, regulators of translation, and scaffolds directing chromatin-modifying enzymes to specific genomic loci, they are an attractive class of therapeutic targets. The relation between HOTAIR in breast cancer [93] and MALAT1 in metastatic lung cancer [94] is a remarkable example of this association. Therefore, there is enthusiasm about the possibility to develop therapeutic tools to modulate mis-regulated lncRNAs in diseases. Although lncRNAs represent appealing pharmacological and therapeutic targets, inhibiting lncRNAs in vivo remains a challenge. A possible approach could be the use of small molecules that disrupt the complex lncRNA-chromatin that alter the epigenetic state of the target cells. All these delivery efforts, along with further elucidation of lncRNA regulatory mechanisms, will ultimately lead to the development of effective therapeutic strategies that target lncRNAs in vivo.

6. Conclusion

The impairment in RNA regulation and processing is crucial in ALS pathogenesis. Defects at different steps of RNA processing alter both cellular function and survival; thus RNA metabolism can be an essential target for therapeutic intervention for ALS and for other NDs. The application of RNA-based therapies to modulate gene and protein expression is an interesting therapeutic strategy:
the preclinical application of RNA-based therapies targeting SOD1 and C9orf72 mutations are promising and pave the way to apply similar approaches for FUS and TDP-43 mutations. In conclusion, RNA-based therapies could be recommended for the future treatment of ALS.

**Funding**

Authors acknowledge the economic support of the Fondazione Regionale per la Ricerca Biomedica (FRRB): TRANS_ALS [2015-0023]; Finanziamento 5x1000 2016; Italian Ministry of Health GR-2016-02361552.

**Abbreviations**

| Abbreviation | Description                                      |
|--------------|--------------------------------------------------|
| ABLIM1       | actin-binding LIM                                |
| ADORA2A      | adenosine A2a receptor                           |
| ALS          | amyotrophic lateral sclerosis                    |
| ANG          | angiogenin                                       |
| ANXA11       | annexin A11                                      |
| AS           | antisense                                        |
| ASOs         | antisense oligonucleotides                       |
| ATG10        | autophagy related 10                             |
| ATXN2        | ataxin 2                                         |
| C9ORF72      | chromosome 9 open reading frame 72              |
| CCND1        | cyclin D1                                        |
| CHCHD10      | coiled-coil-helix-coiled-coil-helix domain       |
| CHMP2B       | charged multivesicular body protein 2B           |
| CNS          | central nervous system                           |
| CSF          | cerebrospinal fluid                              |
| EWSR1        | EWS RNA binding protein 1                        |
| fALS         | familial amyotrophic lateral sclerosis           |
| FTLD         | frontotemporal lobar degeneration                |
| FUS          | fused in sarcoma/translocated in liposarcoma     |
| GABRA2       | gamma-aminobutyric acid type A receptor alpha2 subunit |
| GABRA3       | gamma-aminobutyric acid type A receptor alpha3 subunit |
| GRIA3        | glutamate ionotropic receptor AMPA type subunit 3AMPA receptor subunits |
| GRIA4        | glutamate ionotropic receptor AMPA type subunit 4AMPA receptor subunits |
| HNRNPA1      | heterogeneous nuclear ribonucleoprotein A1       |
| HNRNPA2/B1   | heterogeneous nuclear ribonucleoprotein A2/B1    |
| Homer2       | homer scaffold protein 2                         |
| HOTAIR       | HOX transcript antisense RNA                     |
| HuR          | Hu antigen R                                    |
| iPSC         | induced pluripotent stem cell                    |
| KIF5A        | kinesin family member 5A                        |
| IncRNA       | long noncoding RNA                               |
| IncRNAs      | long noncoding RNAs                              |
| MALAT1       | metastasis-associated lung adenocarcinoma transcript 1 |
| MAP1B        | microtubule-associated protein 1B                |
| MAPT         | microtubule-associated protein tau               |
| MATR3        | matrin 3                                         |
| Acronym | Full Form |
|---------|-----------|
| miRNAs | microRNAs |
| MN | motor neuron |
| ND | neurodegenerative disorder |
| NEAT1 | nuclear-enriched autosomal transcript 1 |
| NES | nuclear export signals |
| NF-kB | nuclear factor kappa B subunit 1 |
| NFL | neurofilament light chain |
| NLGN1-2 | neuroligin |
| NLS | nuclear localization |
| NONO | non-POU domain-containing octamer-binding protein |
| NRCAM | neuronal cell adhesion molecule |
| NRXN1-3 | neurexin |
| NTNG1 | netrin G1 |
| OPTN | optineurin |
|PFN1 | profilin 1 |
| PTX3 | pentraxin 3 |
| RBP | RNA-binding proteins |
| RRM1-2 | RNA recognition motifs 1-2 |
| RUNX | Runbox transcription factor |
| sALS | sporadic amyotrophic lateral sclerosis |
| siRNAs | small interfering RNAs |
| SMaRT | spliceosome-mediated RNA trans-splicing |
| SOD1 | superoxide dismutase 1 |
| SPI1 | Spi-1 proto-oncogene |
| SQSTM1 | sequestosome 1 |
| SYGQ | N-terminal serine-tyrosine-glycine-glutamine |
| TAF15 | TATA-box binding protein-associated factor 15 |
| TARDBP | TAR DNA-binding protein |
| TBK1 | TANK-binding kinase 1 |
| TGF-β1 | transforming growth factor-beta |
| TIAR | TIA1 cytotoxic granule-associated RNA binding protein like 1 |
| TUBA4A | tubulin alpha 4a |
| UBQLN2 | ubiquilin 1 |
| UBXN7 | UBX domain protein 7 |
| UTR | 3’-untranslated |
| VAPB | vesicle-associated membrane protein-associated protein B/C |
| VCP | valosin-containing protein |
| VEGF | vascular endothelial growth factor |
| ZBTB11 | zinc finger and BTB domain-containing 11 |
| ZEB1 | zinc finger E-box-binding homeobox 1 |
Author details

Orietta Pansarasa*, Stella Gagliardi, Daisy Sproviero and Cristina Cereda
Genomic and Post-Genomic Center, IRCCS Mondino Foundation, Pavia, Italy

*Address all correspondence to: orietta.pansarasa@mondino.it

IntechOpen

© 2019 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. © BY
RNA Metabolism and Therapeutics in Amyotrophic Lateral Sclerosis
DOI: http://dx.doi.org/10.5772/intechopen.90704

References

[1] Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, et al. Amyotrophic lateral sclerosis. Lancet. 2011;377(9769):942-955. DOI: 10.1016/S0140-6736(10)6156-7

[2] Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. Nature Reviews. Disease Primers. 2017;3:17071. DOI: 10.1038/nrdp.2017.71

[3] Nguyen HP, Van Broeckhoven C, van der Zee J. ALS genes in the genomic era and their implications for FTD. Trends in Genetics. 2018;34(6):404-423. DOI: 10.1016/j.tig.2018.03.001

[4] Taylor JP, Brown RH Jr, Cleveland DW. Decoding ALS: From genes to mechanism. Nature. 2016;539(7628):197-206. DOI: 10.1038/nature20413

[5] Cook C, Petrucelli L. Genetic convergence brings clarity to the enigmatic red line in ALS. Neuron. 2019;101(6):1057-1069. DOI: 10.1016/j.neuron.2019.02.032

[6] Sicot G, Gomes-Pereira M. RNA toxicity in human disease and animal models: From the uncovering of a new mechanism to the development of promising therapies. Biochimica et Biophysica Acta. 2013;1832(9):1390-1409. DOI: 10.1016/j.bbadis.2013.03.002

[7] Nussbacher JK, Batra R, Lagier-Tourenne C, Yeo GW. RNA-binding proteins in neurodegeneration: Seq and you shall receive. Trends in Neurosciences. 2015;38(4):226-236. DOI: 10.1016/j.tins.2015.02.003

[8] Buratti E, Baralle FE. Characterization and functional implications of the RNA binding properties of nuclear factor TDP-43, a novel splicing regulator of CFTR exon 9. The Journal of Biological Chemistry. 2001;76:36337-36343. DOI: 10.1074/jbc.M104236200

[9] Winton MJ, Igaz LM, Wong MM, Kwong LK, Trojanowski JQ, Lee VM. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. The Journal of Biological Chemistry. 2008;283:13302-13309. DOI: 10.1074/jbc.M80342200

[10] Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Frontiers in Bioscience. 2008;13:867-878. DOI: 10.2741/2727

[11] Polymenidou M, Lagier-Tourenne C, Hutt KR, Huelga SC, Moran J, Liang TY, et al. Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. Nature Neuroscience. 2011;14:459-468. DOI: 10.1038/nn.2779

[12] Colombrita C, Onesto E, Megiorni F, Pizzuti A, Baralle FE, Buratti E, et al. TDP-43 and FUS RNA-binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells. The Journal of Biological Chemistry. 2012;287:15635-15647. DOI: 10.1074/jbc.M111.333450

[13] Tollervey JR, Curk T, Rogelj B, Briese M, Cereda M, Kayikci M, et al. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nature Neuroscience. 2011;14:452-458. DOI: 10.1038/nn.2778

[14] Sephton CF, Cenik C, Kucukural A, Dammer EB, Cenik B, Han Y, et al. Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. The Journal of Biological Chemistry. 2011;286:1204-1215. DOI: 10.1074/jbc.M110.190884

[15] Narayanan RK, Mangelsdorf M, Panwar A, Butler TJ, Noakes PG,
Wallace RH. Identification of RNA bound to the TDP-43 ribonucleoprotein complex in the adult mouse brain. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration. 2013;14:252-260. DOI: 10.3109/21678421.2012.734520

[16] Chang JC, Hazelett DJ, Stewart JA, Morton DB. Motor neuron expression of the voltage-gated calcium channel cacophony restores locomotion defects in a drosophila, TDP-43 loss of function model of ALS. Brain Research. 2014;1584:39-51. DOI: 10.1016/j.brainres.2013.11.019

[17] Coyne AN, Siddegowda BB, Estes PS, Johannesmeyer J, Kovalik T, Daniel SG, et al. Futsch/MAP1B mRNA is a translational target of TDP-43 and is neuroprotective in a drosophila model of amyotrophic lateral sclerosis. The Journal of Neuroscience. 2014;34:15962-15974. DOI: 10.1523/JNEUROSCI.2526-14.2014

[18] Ayala YM, De Conti L, Avendaño-Vázquez SE, Dhir A, Romano M, D’Ambrogiio A, et al. TDP-43 regulates its mRNA levels through a negative feedback loop. The EMBO Journal. 2011;30:277-288. DOI: 10.1038/emboj.2010.310

[19] Yang C, Wang H, Qiao T, Yang B, Aliaga L, Qiu L, et al. Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 2014;111:E1121-E1129. DOI: 10.1073/pnas.1322641111

[20] Kawahara Y, Mieda-Sato A. TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3347-3352. DOI: 10.1073/pnas.1112427109

[21] Buratti E, De Conti L, Stuani C, Romano M, Baralle M, Baralle F. Nuclear factor TDP-43 can affect selected microRNA levels. The FEBS Journal. 2010;277:2268-2281. DOI: 10.1111/j.1742-4658.2010.07643.x

[22] Nishimoto Y, Nakagawa S, Hirose T, Okano HJ, Takao M, Shibata S, et al. The long non-coding RNA nuclear-enriched abundant transcript 1_2 induces paraspeckle formation in the motor neuron during the early phase of amyotrophic lateral sclerosis. Molecular Brain. 2013;6:31. DOI: 10.1186/1756-6606-6-31

[23] Iko Y, Kodama TS, Kasai N, Oyama T, Morita EH, Muto T, et al. Domainarchitectureandcharacterization of an RNA-binding protein, TLS. The Journal of Biological Chemistry. 2004;279:44834-44840. DOI: 10.1074/jbc.M408552200

[24] Uranishi H, Tetsuka T, Yamashita M, Asamitsu K, Shimizu M, Itoh M, et al. Involvement of the pro-oncoprotein TLS (translocated in liposarcoma) in nuclear factor-k B p65-mediated transcription as a coactivator. The Journal of Biological Chemistry. 2001;276:13395-13401. DOI: 10.1074/jbc.M011176200

[25] Li X, Decker M, Westendorf JJ. TEThered to Runx: Novel binding partners for runx factors. Blood Cells, Molecules & Diseases. 2010;45:82-85. DOI: 10.1016/j.bcmd.2010.03.002

[26] Lagier-Tourenne C, Polymenidou M, Hutt KR, Vu AQ, Baughn M, Huelga SC, et al. Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nature Neuroscience. 2012;15:1488-1497. DOI: 10.1038/nn.3230

[27] Ishigaki S, Masuda A, Fujioka Y, Iguchi Y, Katsuno M, Shibata A, et al. Position-dependent FUS-RNA interactions regulate alternative splicing
events and transcriptions. Scientific Reports. 2012;2:529. DOI: 10.1038/srep00529

[28] Rogelj B, Easton LE, Bogu GK, Stanton LW, Rot G, Curk T, et al. Widespread binding of FUS along nascent RNA regulates alternative splicing in the brain. Scientific Reports. 2012;2:603. DOI: 10.1038/srep00603

[29] Nakaya T, Alexiou P, Maragkakis M, Chang A, Mourelatos Z. FUS regulates genes coding for RNA-binding proteins in neurons by binding to their highly conserved introns. RNA. 2013;19:498-509. DOI: 10.1261/rna.037804.112

[30] Morlando M, Dini Modigliani S, Torrelli G, Rosa A, Di Carlo V, Caffarelli E, et al. FUS stimulates microRNA biogenesis by facilitating co-transcriptional Drosha recruitment. The EMBO Journal. 2012;31:4502-4510. DOI: 10.1038/emboj.2012.319

[31] Shelkovnikova TA, Robinson HK, Troakes C, Ninkina N, Buchman VL. Compromised paraspeckle formation as a pathogenic factor in FUSopathies. Human Molecular Genetics. 2014;23(9):2298-2312. DOI: 10.1093/hmg/ddt622

[32] Lu L, Wang S, Zheng L, Li X, Suswam EA, Zhang X, et al. Amyotrophic lateral sclerosis-linked mutant SOD1 sequesters Hu antigen R (HuR) and TIA-1-related protein (TIAR): Implications for impaired posttranscriptional regulation of vascular endothelial growth factor. The Journal of Biological Chemistry. 2009;284:33989-33998. DOI: 10.1074/jbc.M109.067918

[33] Chen H, Qian K, Du Z, Cao J, Petersen A, Liu H, et al. Modeling ALS with iPSCs reveals that mutant SOD1 misregulates neurofilament balance in motor neurons. Cell Stem Cell. 2014;14:796-809. DOI: 10.1016/j.stem.2014.02.004

[34] Volkening K, Leystra-Lantz C, Yang W, Jaffee H, Strong MJ. Tar DNA binding protein of 43 kDa (TDP-43), 14-3-3 proteins and copper/zinc superoxide dismutase (SOD1) interact to modulate NFL mRNA stability. Implications for altered RNA processing in amyotrophic lateral sclerosis (ALS). Brain Research. 2009;1305:168-182. DOI: 10.1016/j.brainres.2009.09.105

[35] De Jesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011;72:245-256. DOI: 10.1016/j.neuron.2011.09.011

[36] Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011;72:257-268. DOI: 10.1016/j.neuron.2011.09.010

[37] Majounie E, Renton AE, Mok K, Dopper EG, Waite A, Rollinson S, et al. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: A cross-sectional study. Lancet Neurology. 2012;11:323-330. DOI: 10.1016/S1474-4422(12)70043-1

[38] Gendron TF, Bieniek KF, Zhang YJ, Jansen-West K, Ash PE, Caulfield T, et al. Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. Acta Neuropathologica. 2013;126:829-844. DOI: 10.1007/s00401-013-1192-8

[39] Mori K, Lammich S, Mackenzie IR, Forné I, Zilow S, Kretzschmar H, et al. hnRNP A3 binds to GGGGCC repeats
and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. Acta Neuropathologica. 2013a;125:413-423. DOI: 10.1007/s00401-013-1088-7

[40] Selvaraj BT, Livesey MR, Zhao C, Gregory JM, James OT, Cleary EM, et al. C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca2+-permeable AMPA receptor-mediated excitotoxicity. Nature Communications. 2018;9:347. DOI: 10.1038/s41467-017-02729-0

[41] Kwon I, Xiang S, Kato M, Wu L, Theodoropoulos P, Wang T, et al. Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. Science. 2014;345:1139-1145. DOI: 10.1126/science.1254917

[42] Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, et al. The C9orf72 GGGGCC repeat is translated into aggregating dipeptiderepeat proteins in FTLD/ALS. Science. 2013b;339:1335-1338. DOI: 10.1126/science.1232927

[43] Gijselinck I, Van Messevelde S, van der Zee J, Sieben A, Engelborghs S, De Bleecker J, et al. The C9orf72 GGGGCC repeat is translated into aggregating dipeptiderepeat proteins in FTLD/ALS. Science. 2013b;339:1335-1338. DOI: 10.1126/science.1232927

[44] Quinlan S, Kenny A, Medina M, Engel T, Jimenez-Mateos EM. MicroRNAs in neurodegenerative diseases. International Review of Cell and Molecular Biology. 2017;334:309-343. DOI: 10.1016/bs ircmb.2017.04.002

[45] Krokidis MG, Vlamos P. Transcriptomics in amyotrophic lateral sclerosis. Frontiers in Bioscience. 2018;10:103-121

[46] Roshan R, Ghosh T, Scaria V, Pillai B. MicroRNAs: Novel therapeutic targets in neurodegenerative diseases. Drug Discovery Today. 2009;14(23-24):1123-1129. DOI: 10.1016/j.drudis.2009.09.009

[47] Cloutier F, Marrero A, O’Connell C, Morin P Jr. MicroRNAs as potential circulating biomarkers for amyotrophic lateral sclerosis. Journal of Molecular Neuroscience. 2015;56(1):102-112. DOI: 10.1007/s12031-014-0471-8

[48] Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science. 2007;318:1931-1934. DOI: 10.1126/science.1149460

[49] Goljanek-Whysall K, Pais H, Rathjen T, Sweetman D, Dalmau T, Munsterberg A. Regulation of multiple target genes by miR-1 and miR-206 is pivotal for C2C12 myoblast differentiation. Journal of Cell Science. 2012;125:3590-3600. DOI: 10.1242/jcs.101758

[50] de Andrade HM, de Albuquerque M, Avansini SH, de S Rocha C, Dogini DB, Nucci A, et al. MicroRNAs-424 and 206 are potential prognostic markers in spinal onset amyotrophic lateral sclerosis. Journal of the Neurological Sciences. 2016;368:19-24. DOI: 10.1016/j.jns.2016.06.046

[51] Pegoraro V, Merico A, Angelini C. Micro-RNAs in ALS muscle: Differences in gender, age at onset and disease duration. Journal of the Neurological Sciences. 2017;380:58-63. DOI: 10.1016/j.jns.2017.07.008

[52] Bruneteau G, Simonet T, Bauché S, Mandjee N, Malfatti E, Girard E, et al. Muscle histone deacetylase 4 upregulation in amyotrophic lateral sclerosis: Potential role in reinnervation ability and disease progression. Brain. 2013;136(Pt 8):2359-2368. DOI: 10.1093/brain/awt164
[53] Waller R, Goodall EF, Milo M, Cooper-Knock J, Da Costa M, Hobson E, et al. Serum miRNAs miR-206, 143-3p and 374b-5p as potential biomarkers for amyotrophic lateral sclerosis (ALS). Neurobiology of Aging. 2017;55:123-131. DOI: 10.1016/j.neurobiolaging.2017.03.027

[54] Tasca E, Pegoraro V, Merico A, Angelini C. Circulating microRNAs as biomarkers of muscle differentiation and atrophy in ALS. Clinical Neuropathology. 2016;35:22-30. DOI: 10.5414/np300889

[55] Butovsky O, Siddiqui S, Gabriely G, Lanser AJ, Dake B, Murugaiyan G, et al. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. The Journal of Clinical Investigation. 2012;122(9):3063-3087. DOI: 10.1172/JCI62636

[56] De Felice B, Guida M, Guida M, Coppola C, De Mieri G, Cotrufo R. A miRNA signature in leukocytes from sporadic amyotrophic lateral sclerosis. Gene. 2012;508:35-40. DOI: 10.1016/j.gene.2012.07.058

[57] De Felice B, Annunziata A, Fiorentino G, Borra M, Biffali E, Coppola C, et al. miR-338-3p is over-expressed in blood, CFS, serum and spinal cord from sporadic amyotrophic lateral sclerosis patients. Neurogenetics. 2014;15(4):243-253. DOI: 10.1007/s10048-014-0420-2

[58] Campos-Melo D, Droppelmann CA, He Z, Volkening K, Strong MJ. Altered microRNA expression profile in amyotrophic lateral sclerosis: A role in the regulation of NFL mRNA levels. Molecular Brain. 2013;6:26. DOI: 10.1186/1756-6606-6-26

[59] Koval ED, Shaner C, Zhang P, du Maine X, Fischer K, Tay J, et al. Method for widespread microRNA-155 inhibition prolongs survival in ALS-model mice. Human Molecular Genetics. 2013;22(20):4127-4135. DOI: 10.1093/hmg/ddt261

[60] Da Cruz S, Cleveland DW. Understanding the role of TDP-43 and FUS/TLS in ALS and beyond. Current Opinion in Neurobiology. 2011;21:904-919. DOI: 10.1016/j.conb.2011.05.029

[61] Zhang Z, Almeida S, Lu Y, Nishimura AL, Peng L, Sun D, et al. Downregulation of microRNA-9 in iPSC-derived neurons of FTD/ALS patients with TDP-43 mutations. PLoS One. 2013;8(10):e76055. DOI: 10.1371/journal.pone.0076055

[62] Freischmidt A, Müller K, Ludolph AC, Weishaupt JH. Systemic dysregulation of TDP-43 binding microRNAs in amyotrophic lateral sclerosis. Acta Neuropathologica Communications. 2013;1:42. DOI: 10.1186/2051-5960-1-42

[63] Emde A, Eitan C, Liou LL, Libby RT, Rivkin N, Magen I, et al. Dysregulated miRNA biogenesis downstream of cellular stress and ALS-causing mutations: A new mechanism for ALS. The EMBO Journal. 2015;34(21):2633-2651. DOI: 10.15252/embj.201490493

[64] Luxenhofer G, Helmbrecht MS, Langhoff J, Giusti SA, Refojo D, Huber AB. MicroRNA-9 promotes the switch from early-born to late-born motor neuron populations by regulating Onecut transcription factor expression. Developmental Biology. 2014;386(2):358-370. DOI: 10.1016/j.ydbio.2013.12.023

[65] Edbauer D, Neilson JR, Foster KA, Wang CF, Seeburg DP, Batterton MN, et al. Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron. 2010;65(3):373-384. DOI: 10.1016/j.neuron.2010.01.005. Erratum in: Neuron. 2010;68(1):161
[66] Rizzuti M, Filosa G, Melzi V, Calandriello L, Dionisi L, Bollati V, et al. MicroRNA expression analysis identifies a subset of downregulated miRNAs in ALS motor neuron progenitors. Scientific Reports. 2018;8(1):10105. DOI: 10.1038/s41598-018-28366-1

[67] Helferich AM, Brockmann SJ, Reinders J, Deshpande D, Holzmann K, Brenner D, et al. Dysregulation of a novel miR-1825/TBCB/TUBA4A pathway in sporadic and familial ALS. Cellular and Molecular Life Sciences. 2018;75(23):4301-4319. DOI: 10.1007/s00018-018-2873-1

[68] Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell. 2009;136(4):629-641. DOI: 10.1016/j.cell.2009.02.006

[69] Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Molecular Cell. 2009;33:717-726. DOI: 10.1016/j.molcel.2009.01.026

[70] Hirose T, Virnicchi G, Tanigawa A, Naganuma T, Li R, Kimura H, et al. NEAT1 long noncoding RNA regulates transcription via protein sequestration within subnuclear bodies. Molecular Biology of the Cell. 2014;25(1):169-183. DOI: 10.1091/mbc.E13-09-0558

[71] An H, Williams NG, Shellkovnikova TA. NEAT1 and paraspeckles in neurodegenerative diseases: A missing lnc found? Non-coding RNA Research. 2018;3(4):243-252. DOI: 10.1016/j.jncrna.2018.11.003

[72] Česnik BA, Darovic S, Prpar Mihevc S, Štalekar M, Malnar M, Motaln H, et al. Nuclear RNA foci from C9ORF72 expansion mutation form paraspeckle-like bodies. Journal of Cell Science. 2019;132(5):jcs224303. DOI: 10.1242/jcs.224303

[73] Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, et al. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature. 2008;454(7200):126-130. DOI: 10.1038/nature06992

[74] Pelechano V, Steinmetz LM. Gene regulation by antisense transcription. Nature Reviews. Genetics. 2013;14(12):880-893. DOI: 10.1038/nrg3594

[75] Elden AC, Kim HJ, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature. 2010;466(7310):1069-1075. DOI: 10.1038/nature09320

[76] Li PP, Sun X, Xia G, Arbez N, Paul S, Zhu S, et al. ATXN2-AS, a gene antisense to ATXN2, is associated with spinocerebellar ataxia type 2 and amyotrophic lateral sclerosis. Annals of Neurology. 2016;80(4):600-615. DOI: 10.1002/ana.24761

[77] Nalavade R, Griesche N, Ryan DP, Hildebrand S, Krauss S. Mechanisms of RNA-induced toxicity in CAG repeat disorders. Cell Death & Disease. 2013;4:e752. DOI: 10.1038/cddis.2013.276

[78] D’Erchia AM, Gallo A, Manzari C, Raho S, Horner DS, Chiara M, et al. Massive transcriptome sequencing of human spinal cord tissues provides new insights into motor neuron degeneration in ALS. Scientific Reports. 2017;7(1):10046. DOI: 10.1038/s41598-017-10488-7

[79] Zhao W, Beers DR, Hooten KG, Sieglaff DH, Zhang A, Kalyana-Sundaram S, et al. Characterization of gene expression phenotype in
amotrophic lateral sclerosis monocytes. JAMA Neurology. 2017;74(6):677-685. DOI: 10.1001/jamaneurol.2017.0357

[80] Gagliardi S, Zucca S, Pandini C, Diamanti L, Bordoni M, Sproviero D, et al. Long non-coding and coding RNAs characterization in peripheral blood mononuclear cells and spinal cord from amyotrophic lateral sclerosis patients. Scientific Reports. 2018;8(1):2378. DOI: 10.1038/s41598-018-20679-5

[81] Li T, Xie J, Shen C, Cheng D, Shi Y, Wu Z, et al. Upregulation of long noncoding RNA ZEB1-AS1 promotes tumor metastasis and predicts poor prognosis in hepatocellular carcinoma. Oncogene. 2016;35(12):1575-1584. DOI: 10.1038/onc.2015.223

[82] Keightley MC, Carradice DP, Layton JE, Pase L, Bertrand JV, Wittig JG, et al. The Pu.1 target gene Zbtb11 regulates neutrophil development through its integrase-like HHCC zinc finger. Nature Communications. 2017;8:14911. DOI: 10.1038/ncomms14911

[83] Villar-Menéndez I, Porta S, Buira SP, Pereira-Veiga T, Díaz-Sánchez S, Albasanz JL, et al. Increased striatal adenosine A2A receptor levels is an early event in Parkinson's disease-related pathology and it is potentially regulated by miR-34b. Neurobiology of Disease. 2014;69:206-214. DOI: 10.1016/j.nbd.2014.05.030

[84] Lee JK, Shin JH, Lee JE, Choi EJ. Role of autophagy in the pathogenesis of amyotrophic lateral sclerosis. Biochimica et Biophysica Acta. 2015 Nov;1852(11):2517-2524. DOI: 10.1016/j.bbadis.2015.08.005

[85] Guo X, Qi X. VCP cooperates with UBXD1 to degrade mitochondrial outer membrane protein MCL1 in model of Huntington's disease. Biochimica et Biophysica Acta - Molecular Basis of Disease. 2017;1863(2):552-559. DOI: 10.1016/j.bbadis.2016.11.026

[86] Majidinia M, Mihanfar A, Rahbarghazi R, Nourazarian A, Bagca B, Avci ÇB. The roles of non-coding RNAs in Parkinson's disease. Molecular Biology Reports. 2016;43(11):1193-1204

[87] Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. Cancer Research. 2010;70(18):7027-7030. DOI: 10.1158/0008-5472.CAN-10-2010

[88] Arechavala-Gomeza V, Khoo B, Aartsma-Rus A. Splicing modulation therapy in the treatment of genetic diseases. The Application of Clinical Genetics. 2014;7:245-252. DOI: 10.2147/TACG.S71506

[89] Havens MA, Hastings ML. Splice-switching antisense oligonucleotides as therapeutic drugs. Nucleic Acids Research. 2016;44(14):6549-6563. DOI: 10.1093/nar/gkw533

[90] McCampbell A, Cole T, Wegener AJ, Tomassy GS, Setnicka A, Farley BJ, et al. Antisense oligonucleotides extend survival and reverse decrement in muscle response in ALS models. The Journal of Clinical Investigation. 2018;128(8):3558-3567. DOI: 10.1172/JCI99081

[91] Butti Z, Patten SA. RNA Dysregulation in amyotrophic lateral sclerosis. Frontiers in Genetics. 2019;9:712. DOI: 10.3389/fgene.2018.00712

[92] Bailey JK, Shen W, Liang XH, Crooke ST. Nucleic acid binding proteins affect the subcellular distribution of phosphorothioate antisense oligonucleotides. Nucleic Acids Research. 2017 Oct 13;45(18):10649-10671. DOI: 10.1093/nar/gkx709
[93] Han B, Peng X, Cheng D, Zhu Y, Du J, Li J, et al. Delphinidin suppresses breast carcinogenesis through the HOTAIR/microRNA-34a axis. Cancer Science. 2019;110(10):3089-3097. DOI: 10.1111/cas.14133

[94] Gomes CP, Nóbrega-Pereira S, Domingues-Silva B, Rebelo K, Alves-Vale C, Marinho SP, et al. An antisense transcript mediates MALAT1 response in human breast cancer. BMC Cancer. 2019;19(1):771. DOI: 10.1186/s12885-019-5962-0