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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 lower 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

1. Introduction

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.

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, PFN1, 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 subunits (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 lncRNAs, 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 an 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- κ B, 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 understood. 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 mRNAs [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 **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 and co-authors, 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- κ B 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- β 1, **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 M337 V 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

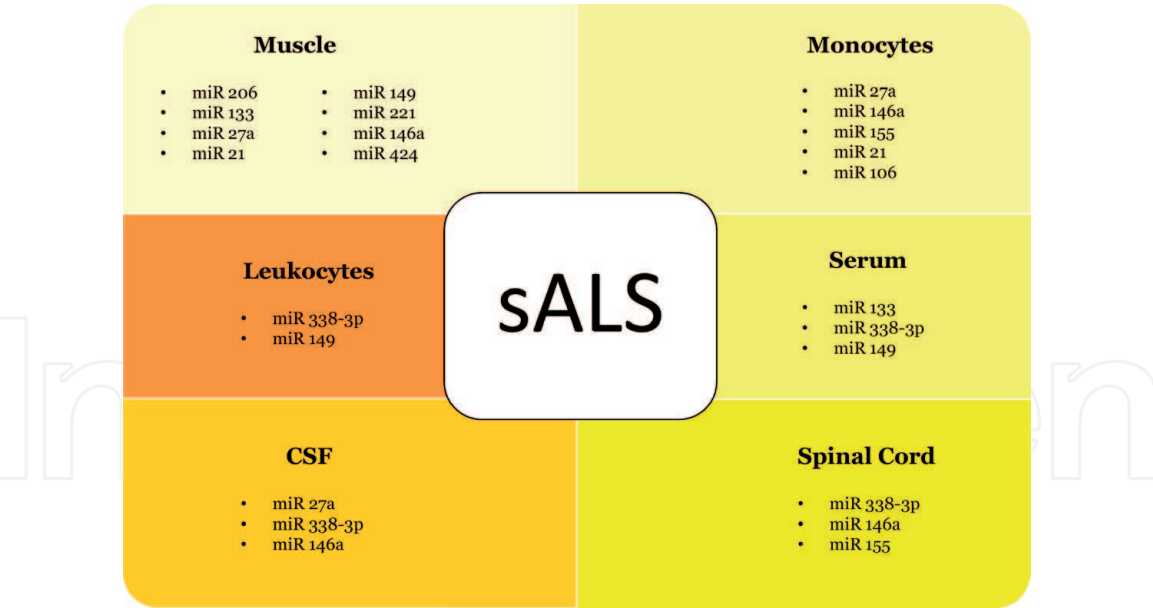


Figure 1.
Representation of the key miRNAs involved in amyotrophic lateral sclerosis sporadic patients (sALS).

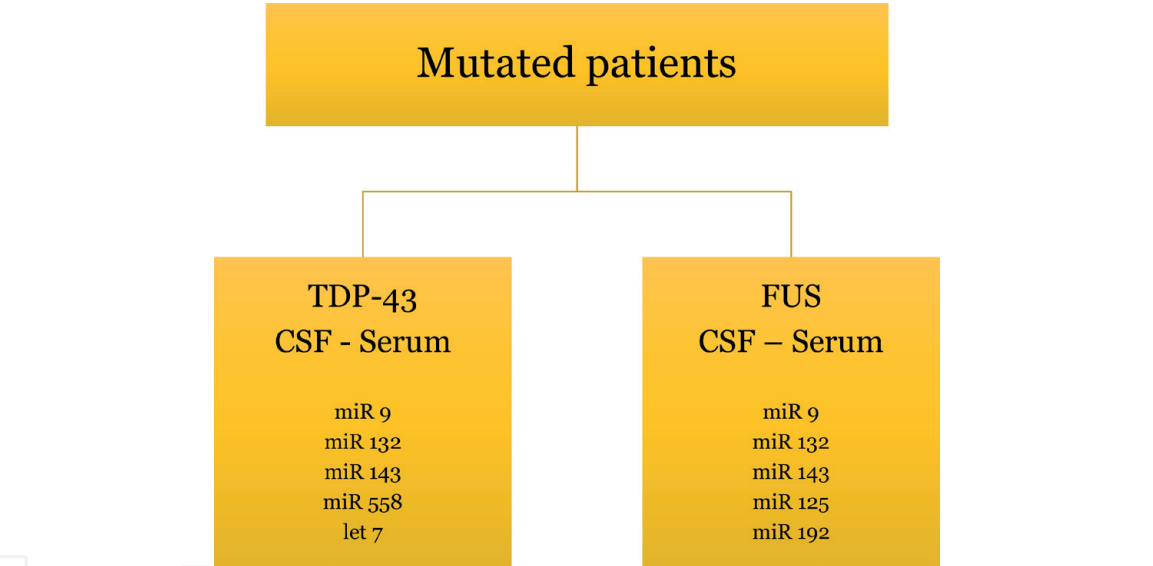


Figure 2.
Representation of the key miRNAs involved in amyotrophic lateral sclerosis mutated patients.

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

orientation to protein-coding genes or within intergenic regions. lncRNAs control the gene expression through different mechanisms, that is, epigenetic modulation 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, lncRNAs 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 pathogenesis [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 stimulates **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 paraspeckle 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 lncRNA. It is one of the first lncRNAs associated with human disease, and it is involved in alternative splicing, epigenetic modification of gene expression, synapse formation, and myogenesis. In NDs **MALAT1** is significantly increased in FTLD patients, where it recruits splicing factors to nuclear speckles and affects phosphorylation of SR proteins [13]. Some lncRNA transcripts have been associated to FUS, among these the **lncRNA CCND1** which binds to the FUS consensus sequence GGUG [73]. Data from Wang and colleagues suggested that FUS is a specific repressor of **CCND1**, which is downregulated in response to DNA damage signals. Until now the lncRNA **CCND1** has not been described in relation to ALS; but taken together, these observations point out that this lncRNA could be, at least partly, responsible in ALS and other neurodegenerative diseases.

Together with the lncRNA an increasing interest was addressed to the antisense (AS) noncoding transcripts. They are generated from the strand opposite the sense strand [74]. AS lncRNAs act by regulating chromatin, by controlling DNA methylation 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].

sALS	TARDBP mutation	FUS mutation
ATXN2-AS [76]	NEAT1 [22, 69–71]	lncCCND1 [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.

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

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.

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Abbreviations

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 containing 10
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
lncRNA	long noncoding RNA
lncRNAs	long noncoding RNAs
MALAT1	metastasis-associated lung adenocarcinoma transcript 1
MAP1B	microtubule-associated protein 1B
MAPT	microtubule-associated protein tau
MATR3	matrin 3

miRNAs	microRNAs
MN	motor neuron
ND	neurodegenerative disorder
NEAT1	nuclear-enriched autosomal transcript 1
NES	nuclear export signals
NF- κ B	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- β 1	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

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