The Role of MicroRNAs in Patients with Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a serious neurodegenerative disease that affects motor neurons and leads to death within 2 to 3 years after the first symptoms manifest. MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression in fundamental cellular processes and, post-transcriptionally, the translation levels of target mRNA transcripts. We searched PubMed for studies that examined miRNAs in ALS patients and attempted to group the results in order to find the strongest miRNA candidate for servings as an ALS biomarker. The studies on humans so far have been diverse, yielding considerably heterogeneous results, as they were performed on a wide variety of tissues and subjects. Among the miRNAs that were found consistently deregulated are miR-206, miR-133, miR-149, and miR-338-3p. Additively, the deregulation of some specific miRNAs seems to compose a miRNA expression profile that is specific for ALS. More research is required in order for the scientific community to reach a consensus.

Keywords
Amyotrophic lateral sclerosis · MicroRNA · Epigenetics · Biomarker · Neurodegeneration

Introduction

Amyotrophic lateral sclerosis (ALS) is considered to be the third most common neurodegenerative disease (Hardiman et al. 2017; Kruger et al. 2011). It is also the most frequent adult-onset motor neuron disease (Dardiotis et al. 2013a). Within 2 to 3 years after the first symptoms, respiratory failure leads to death of ALS patients, thus revealing the disease’s severity (Hardiman et al. 2017). The etiology of ALS still largely remains unknown (Dardiotis et al. 2018b). However, cumulative evidence suggests that genetic, epigenetic, and environmental factors may predispose to ALS (Dardiotis et al. 2018b; Mitropoulos et al. 2017; Nicolas et al. 2018).

Ten percent of all ALS cases are classified as familial, while sporadic forms account for 90% of all ALS cases (Nicolas et al. 2018). A few variants have been shown to modify the risk of ALS and neurodegenerative disorders through candidate gene association studies (CGASs) and genome-wide association studies (GWASs) (Evangelou et al. 2010; Mitropoulos et al. 2017; Nicolas et al. 2018).

Additionally, a few exogenous and environmental factors such as environmental toxicants, smoking, antioxidants, infections, body mass index, and traumatic brain injury among others...
MiRNAs are small non-coding RNA molecules, approximately 18–25 nucleotides long, that regulate gene expression in fundamental cellular processes and, post-transcriptionally, the translation levels of target mRNA transcripts (Abe and Bonini 2013; Campbell and Booth 2015; Cloutier et al. 2015; Goodall et al. 2013; Quinlan et al. 2017; Roshan et al. 2009). MiRNA transcribing genes are either localized in introns of protein-coding or non-coding genes or even in exons, overlapping with coding regions and consequently transcribed by the host promoter, or in intergenic regions with their own promoter (Goodall et al. 2013; Quinlan et al. 2017). In humans, the majority is transcribed independently and putative promoters have also been identified (Goodall et al. 2013). Numerous miRNAs are also temporally and spatially regulated with transcription factors or epigenetic mechanisms such as DNA methylation (Goodall et al. 2013).

In the canonical pathway, the primary transcript (pre-miRNA), a hundred-nucleotide-long product of RNA polymerase II, is processed by the nuclear microprocessor complex of the highly conserved RNase III Drosha and the DGCR8 (DiGeorge critical region 8)/Pasha protein in order to create the precursor miRNA (pre-miRNA), which leaves the nucleus and enters the cytoplasm (Allis and Jenuwein 2016; Goodall et al. 2013; Macciotta et al. 2013; Molasy et al. 2017; Quinlan et al. 2017; Roshan et al. 2009). Once in the cytoplasm, the pre-miRNA is further sliced by another RNase III protein, Dicer, to form a miRNA duplex (Abe and Bonini 2013; Goodall et al. 2013; Macciotta et al. 2013; Quinlan et al. 2017; Roshan et al. 2009). One of the strands is then chosen to “mature,” that is to bind, with Argonaute proteins (Ago), and the other is commonly degraded (Abe 2013, Goodall 2015). The “mature” strand binds with Ago-2, or Ago-1, and other proteins to form the miRNA-induced silencing complex (miRISC) (Abe and Bonini 2013; Goodall et al. 2013; Macciotta et al. 2013; Molasy et al. 2017; Quinlan et al. 2017; Shah et al. 2017), where the miRNA precisely indicates the target (Molasy et al. 2017; Quinlan et al. 2017). Apart from this canonical pathway, non-canonical pathways have also been described, bypassing the Drosha/Dicer process (Goodall et al. 2013; Quinlan et al. 2017).

More than half of protein-coding genes are thought to be regulated by miRNAs (Cloutier et al. 2015). The regulation occurs via the binding of the miRNA with corresponding regions of protein-coding mRNAs, interfering with the translation (Cloutier et al. 2015; Roshan et al. 2009). The downregulation can be mediated by an interference in the translation initiation or the elongation or by the recruiting of deadenylating enzymes (Roshan et al. 2009). They can even also cause target degradation (Roshan et al. 2009). The miRNAs recognize their targets mainly through complementarity in their “seed sequence,” which matches the mRNA (Abe and Bonini 2013; Goodall et al. 2013), located at nucleotides 2–8 of the miRNA 5’ end, with almost perfect complementation to the 3’UTR sequence (Abe and Bonini 2013; Macciotta et al. 2013; Molasy et al. 2017; Rinchetti et al. 2018). In the case of a perfect miRNA-mRNA match, degradation usually occurs; partial matches cause translation suppression (Goodall et al. 2013).

miRNAs also seem to play an important role in early development and cell differentiation (Campbell and Booth 2015; Roshan et al. 2009). A single miRNA can target multiple transcripts, and a single transcript can be targeted by a group of miRNAs; additionally, many miRNAs present tissue-specific expression, such as in neuron cells (Roshan et al. 2009). In neurons in particular, the mature miRNA is formed in specific intracellular compartments. Pre-miRNAs can be transferred through neuronal dendrites to dendritic spines and be processed by Dicer when increased neuronal activity is present (Quinlan et al. 2017). Finally, when glutamate or GABA receptors are activated, the calcium influx activates calpain, which in turn activates the Dicer enzyme (Quinlan et al. 2017).

The aim of the present review is to discuss the current knowledge, by focusing on miRNAs that have been linked to ALS development in humans and their possible application as biomarkers.

**Methods to Study Identification and Selection**

We searched PubMed for peer-reviewed articles, published in English until July 2018, concerning human studies on ALS and miRNAs. The articles were assessed for their relevance via their title and further assessed via their abstracts. Our search included “Amyotrophic Lateral Sclerosis” and “MicroRNAs,” as MeSH terms. Last literature search was performed on July 13, 2018. The reference lists of the selected articles were also examined in order to pinpoint relevant...
articles not appearing in our initial search. Published studies between 2010 and 2017 were included. Baseline characteristics from the included studies are summarized in Table 1.

Results and Discussion

Acknowledging the need of an accurate and efficient biomarker for ALS, we deemed it of greater importance to focus our review on studies performed on human subjects, following years of research on animal models. The available studies revealed a tremendous variety of deregulated miRNAs, as the studies were heavily differentiated as well: different tissues under examination, diverse number of patients, various stages of disease, and various study designs. We attempted to group these findings and will, firstly, individually present the data on common miRNAs inherent to multiple studies, in search of those that might serve as the optimal biomarkers of the disease.

The overall quantity of miRNAs has not been the main subject of interest for most studies. Some claim that it is increased (Pegoraro et al. 2017) or increased in later stages of disease (De Felice et al. 2014) and others, examining a wide variety of miRNAs in various tissues, report that the majority of them appear to be downregulated (Benigni et al. 2016; Campos-Melo et al. 2013; Emde et al. 2015; Wakabayashi et al. 2014). In general, the main question is whether specific miRNA molecules can help diagnose ALS and differentiate it from other pathologies, especially neurodegenerative ones (Theuns et al. 2014; Wang et al. 2015).

The miRNA that turned up the most in our research was miR-206. It is a skeletal muscle-specific miRNA and is widely expressed in new muscle fibers (de Andrade et al. 2016; De Felice et al. 2012; Pegoraro et al. 2017) since it regulates myogenesis (Goljanek-Whysall et al. 2012; Toivonen et al. 2014). It has also been shown to promote the formation of new neuromuscular junctions following nerve injury and therefore the reinnervation of denervated fibers (de Andrade et al. 2016; Tasca et al. 2016). In contrast to other miRNAs, miR-206 was consistently found to be upregulated in the studies we examined. Specifically, it was found to be overexpressed in muscle fibers of ALS patients (de Andrade et al. 2016; Pegoraro et al. 2017), their serum (de Andrade et al. 2016; Tasca et al. 2016; Toivonen et al. 2014; Waller et al. 2017), and in the muscles of rats that had been injected with cerebrospinal fluid (CSF) of ALS patients (Sumitha et al. 2014).

miR-206, due to its connection with muscle, seems to reflect the ongoing changes of the tissue during the course of the disease. Pegoraro et al. (2017) attribute the high levels of miR-206 they found, to the muscle remodeling process (atrophy in some fibers, compensating hypertrophy in others, reinnervation), in which this miRNA is naturally implicated (Pegoraro et al. 2017). De Andrade et al. (2016) showed that the levels of this miRNA did not progressively increase during follow-up of the patients examined and support the notion that its expression levels increase early in the disease course and then start to fall, thus mirroring the loss of muscle (de Andrade et al. 2016). Finally, Tasca et al. (2016) found higher levels of miR-206 in spinal ALS than in bulbar and as their muscle photometric analyses showed, bulbar cases presented higher atrophy rates (Tasca et al. 2016). This reinforces the theory that this miRNA might also have a protective role in ALS; in mice where it was deactivated, the disease manifested earlier (Brown 2009) and its levels were higher in long-term ALS survivors (Bruneteau et al. 2013).

Sumitha et al. (2014) injected CSF of ALS patients in murine muscles and found an upregulation of miR-206 in the gastrocnemius (hind muscles), but not in the biceps brachii (fore muscles) (Sumitha et al. 2014). Though both are considered fast twitch muscles, they present different metabolic needs and cell architecture, in animal models (Capitanio et al. 2012), and the researchers suggest that this finding is in accordance with the “hind limb onset” pattern of ALS, where hind limb muscles present atrophy sooner than fore limb ones (Sumitha et al. 2014).

Taking all of the above into consideration, miR-206 appears to be a promising biomarker for ALS, as several studies have also so far declared (de Andrade et al. 2016; Tasca et al. 2016; Toivonen et al. 2014; Waller et al. 2017). It can be assessed in easily attainable samples, such as serum, and it seems to correlate, to some degree, to disease characteristics: muscle atrophy and early stage. Therefore, further research on miR-206 is strongly recommended, particularly in large population cohorts, to better understand its utility to serve as a surrogate biomarker of ALS.

Alongside miR-206, some other miRNAs are also considered to be muscle specific, such as miR-133a/b, miR-1, and miR-27a, and are thought to be implicated in myogenesis, muscle growth, and heart function (McCarthy 2008; Toivonen et al. 2014; Vignier et al. 2013). MiR-133 was found to be higher in the serum of ALS patients that in controls (Tasca et al. 2016) and in muscle (Pegoraro et al. 2017), accompanied by miR-1 in one of the studies (Pegoraro et al. 2017). miR-133, like miR-206, was also reported more elevated in spinal than bulbar ALS (Tasca et al. 2016). miR-27a was found upregulated in CD14+ CD16− monocytes of ALS patients (Butovsky et al. 2012) and in muscle fibers of patients (Pegoraro et al. 2017), but downregulated in serum samples (Tasca et al. 2016). Pegoraro et al. (2017), as described in miR-206 as well, attribute the high levels of these miRNAs in the muscle fibers to the muscle remodeling process (Pegoraro et al. 2017), while Butovsky et al. (2012) claim that it is part of the “inflammatory signature” of patient monocytes, alongside miR-155, miR-146a, and miR-532-3p, while also not being expressed in healthy controls or MS subjects.
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<tr>
<td>Shioya et al. (2010)</td>
<td>Brain frontal cortex</td>
<td>6 ALS and 4 non-neurological controls</td>
<td>A trend for upregulation of miR-29a expression in ALS vs non-neurological controls ($p = 0.263$). No trend for the levels of miR-338-3p ($p = 0.956$).</td>
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<tr>
<td>Butovsky et al. (2012)</td>
<td>Blood</td>
<td>22 sALS, 3 fALS, 24 healthy controls, 8 RRMS</td>
<td>In CD14+/CD16+ blood monocytes (has-miR-340, has-miR-598, has-miR-15b, has-miR-29a, has-miR-24, has-miR-30b, has-miR-103, has-miR-26a, has-miR-21, has-miR-16, has-miR-142-5p, has-miR-223) were upregulated in ALS compared to HC, while (has-miR-7i, has-miR-23a, has-miR-494, has-miR-142-3p, has-miR-15a) were downregulated.</td>
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<td></td>
<td>CSF</td>
<td>10 sALS, 5 fALS, 10 volunteers without a history of neurologic disease or relatives with ALS, 10 PD</td>
<td>6 miRNAs (miR-27b, miR-150, miR-99b, miR-328, miR-146a and miR-532-3p) were significantly upregulated in sALS or fALS subjects. 3 miRNAs (miR-27b, miR-146a, and miR-532-3p) in common elevated in ALS CSF that were elevated both in monocytes and microglia from SOD1 mice and from human ALS subjects.</td>
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<tr>
<td>De Felice et al. (2012)</td>
<td>Leukocytes</td>
<td>8 sALS and 12 healthy controls (independent validation group of 14 sALS and 14 healthy controls)</td>
<td>8 miRNAs were significantly up- or downregulated in sALS compared to controls. More precisely, seven miRNA (hsa-miR-451, hsa-miR-1275, hsa-miR-328-5p, hsa-miR-638, hsa-miR-149, and hsa-miR-665) were downregulated and miR-338-3p was upregulated.</td>
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<td>Campos-Melo et al. (2013)</td>
<td>Spinal cord</td>
<td>5 sALS and 3 non-neurological controls</td>
<td>4 miRNAs (miR-558; miR-16-2*, miR-146a*; miR-508-5p) were expressed only in sALS. 7 miRNAs were expressed only in controls (miR-624; miR-520e, miR-524-5p, miR-548a-5p, miR-606, miR-612, miR-647). 245 miRNAs expressed in both sALS and controls, but only 6 (miR-373*, miR-551a; miR-506, miR-518a-5p, miR-518c*, and miR-890) of them were expressed in sALS at higher levels compared to controls. Only 10 miRNAs (form the 664 tested) were not expressed in sALS or control. The dysregulated miRNAs (miR-146a*, miR-524-5p and miR-582-3p) that were found in the sALS were capable of interacting with NFL mRNA 3′UTR.</td>
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<td>Zhang et al. (2013)</td>
<td>iPSC (induced pluripotent stem cell)-derived neurons</td>
<td>A 56-year-old male with the TARDBP M337V mutation, an FTD/ALS A90V TARDBP carrier, and a 65-year-old family member with no clinical symptoms of FTD, no TDP-43 or A90V mutations</td>
<td>The levels of miR-9 and its precursor pri-miR-9-2 were both reported lower in patient neurons, while remaining unaffected in controls, when the cells were exposed to staurosporine-induced oxidative stress. MiR-9-2 expression was also found lower pre- and post-stress in neurons with a different mutation, the TARDBP M337V.</td>
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<td>Koval et al. (2013)</td>
<td>Spinal cord</td>
<td>16 sALS or fALS and 12 non-ALS controls</td>
<td>Both in sALS and fALS patients, a significant increase of miR-155 was observed.</td>
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<td>Russell et al. (2013)</td>
<td>Muscle</td>
<td>14 sALS and 10 healthy controls</td>
<td>miR-23a, miR-29b, miR-206, miR-455, and miR-31 were significantly increased in ALS, when compared with the healthy controls.</td>
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<td>Freischmidt et al. (2013)</td>
<td>CSF and serum</td>
<td>22 sALS and 24 healthy controls</td>
<td>Expression levels of 5 (miR-132-5p, miR-132-3p, miR-143-3p, miR-143-5p, and miR-574-5p) TDP-43 binding microRNAs</td>
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<tr>
<td>Freischmidt et al. (2014)</td>
<td>Serum</td>
<td>Group 1: 9 fALS and 10 controls. Group 2: a validation cohort (13 fALS and 13 controls). Group 3: a second validation cohort (14 sALS and 14 controls). Group 4: 18 asymptomatic carriers of SOD1 mutations and 8 healthy controls</td>
<td>Overall, the downregulation of miRNAs seemed to be widely independent from the affected gene. Downregulation of 30 mature miRNAs was observed when fALS were compared to controls. 24 mature miRNAs were found to be significantly downregulated in presymptomatic ALS compared to controls. The downregulation of the four most statistical significant miRNAs in group 1 (miR-4745-5p, miR-1915-3p, miR-3665, miR-4530) was also observed in the group 2. In group 3, significant downregulation of three of the four miRNAs (miR-4745-5p, miR-3665, miR-4530) was also observed in sALS, while the miR-1915-3p showed a strong trend for association.</td>
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<td>Toivonen et al. (2014)</td>
<td>Serum</td>
<td>12 ALS and 12 healthy controls</td>
<td>4.3-fold upregulation of miR-206 ($p = 0.005$) and 2-fold upregulation of miR-106b ($p = 0.02$) in ALS vs controls serum samples. No significant changes in comparison between male ALS vs male controls (although miR-206 was close to significance. In the comparison between female ALS vs female controls, the significance was maintained.</td>
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<td>Wakabayashi et al. (2014)</td>
<td>Brain motor cortex</td>
<td>6 sALS and 4 neurologically normal controls and a validation cohort of 8 ALS and 8 normal controls</td>
<td>91 miRNAs were upregulated and 233 downregulated. miR-494 was the most highly differentially upregulated miRNA followed by miR-24-3p, miR-4299, and miR-1973. miR-4740-5p, miR-1290, miR-3619-3p, miR-1246, and miR-3180-3p were the top 5 downregulated miRNAs.</td>
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<td>De Felice et al. (2014)</td>
<td>Blood leukocytes (all participants), serum, and CSF (second group)and spinal cord (third group)</td>
<td>Group 1: 72 sALS and 62 unaffected healthy controls; group 2: 10 sALS and 10 unaffected healthy controls; group 3: 7 sALS and 3 unaffected healthy controls</td>
<td>Increased expression of the miR-338-3p in the blood leukocytes, serum, CSF, and spinal cord of ALS patients. There was signals that miR-338-3p is localized in the gray matter of spinal cord tissues from sALS autopsied patients.</td>
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<td>De Andrade et al. (2016)</td>
<td>Muscle and plasma</td>
<td>39 ALS and 39 healthy controls</td>
<td>11 miRNAs (hsa-miR-424, hsa-miR-503, hsa-miR-886-3p, hsa-miR-542-5p, hsa-miR-34a, hsa-miR-886-5p, hsa-miR-146b-5p, hsa-miR-504, hsa-miR-21, hsa-miR-214, and hsa-miR-183) differentially expressed in the muscle of ALS patients. hsa-miR424, hsa-miR-214, and has-miR-206 were validated by qPCR in muscle samples. Only miR-424 and miR-206 were overexpressed in their plasma. Higher baseline serum levels of both miR-424 and miR-206 predicted slower change in the MRC sum score.</td>
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<td>Ishtiaq et al. (2014)</td>
<td>Ventral lumbar spinal cord samples</td>
<td>3 sALS and 3 neurologically intact controls</td>
<td>The miR-b1336 and miR-b2403 were significantly downregulated in sALS and both stabilized NEFL miRNA.</td>
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<td>Sumitha et al. (2014)</td>
<td>CSF of ALS patients injected in murine muscles</td>
<td>5 ALS patients</td>
<td>Upregulation of miR-206 in the gastrocnemius (hind muscles), but not in the biceps brachii (fore muscles) of murines</td>
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<td>Bruneteau et al. (2013)</td>
<td>Muscle</td>
<td>11 ALS (rapidly progressive ALS n = 6, long-term ALS survivors n = 5)</td>
<td>MicroRNA-206 was upregulated in both patient groups, but did not correlate with disease progression or reinnervation.</td>
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<td>Takahashi et al. (2015)</td>
<td>Plasma</td>
<td>16 sALS and 10 healthy controls (independent validation group of 48 sALS and 47 healthy controls)</td>
<td>hsa-miR-4649-5p and hsa-miR-4299 were up- and downregulated, respectively, in plasma ALS compared to controls</td>
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<tr>
<td>Freischmidt et al. (2015)</td>
<td>Serum</td>
<td>18 sALS and 16 healthy controls</td>
<td>miR-1234-3p and miR-1825 were downregulated in sALS.</td>
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<td>Emdé et al. (2015)</td>
<td>Nervous tissue</td>
<td>8 sALS and 9 non-neurodegeneration controls/another cohort of 12 non-ALS and 12 sALS</td>
<td>Downregulation of miR-9 and miR-124 in patient tissue, relative to control tissue. The downregulation was also observed in two fALS SOD1 A4V mutation carriers. Additionally, No difference in expression for 27 long ncRNAs at nervous system in another cohort of 12 non-ALS and 12 sALS.</td>
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<td>Benigni et al. (2016)</td>
<td>CSF</td>
<td>24 ALS and 24 unaffected controls</td>
<td>miR-21-5p, miR-195-5p, let7a-5p, miR-148-3p, miR-15b-5p, let7b-5p, and let7f-5p were significantly downregulated, and the miRNA181a-5p was significantly upregulated in the CSF from ALS patients. No significant differences were observed between ALS patients with or without C9orf72 expansion. The highest diagnostic accuracy was revealed for the upregulated miR181a-5p and the downregulated miR21-5p and miR15b-5p.</td>
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<tr>
<td>Tasca et al. (2016)</td>
<td>Serum and plasma</td>
<td>14 sALS and 8 healthy controls</td>
<td>Increased expression of miR-206 and miR-133 and reduced expression of miR-27a in comparison: (a) ALS VS controls and (b) spinal-onset ALS VS onset bulbar ALS. Also, miRNA-146a and miRNA-149* were significantly reduced in ALS patients when compared to controls. Finally, miRNA-27a was significantly downregulated in all ALS patients when compared to controls.</td>
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<td>Chen et al. (2016)</td>
<td>Peripheral blood leukocytes</td>
<td>5 sALS and 5 healthy controls (independent validation group of 83 sALS and 61 healthy controls)</td>
<td>Four overexpressed (hsa-miR-34a, hsa-miR-100, hsa-miR-193b, hsa-miR-4485) and seven under-expressed (hsa-miR-3690, hsa-miR-124, hsa-miR-183, hsa-miR-3935, hsa-miR-451, hsa-miR-4538, and hsa-miR-4701) miRNAs were detected in sALS patients when compared to controls in the initial analysis. hsa-miR-183, hsa-miR-193b, hsa-miR-451, and hsa-miR-3935 under-expressed microRNAs, were confirmed in the analysis during the validation stage. This miRNA panel (hsa-miR-183, hsa-miR-193b, hsa-miR-451, and hsa-miR-3935) had a high diagnostic accuracy of sALS.</td>
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<td>Waller et al. (2017)</td>
<td>Serum</td>
<td>27 sALS and 25 healthy controls (validation group 23 sALS and 22 healthy controls)</td>
<td>miR-206, miR-143-3p, and miR-374b-5p are differentially expressed in the serum of sALS patients compared to control subjects. Moreover, miR-143-3p was significantly increased while miR-374b-5p was decreased in the serum of sALS patients over time identifying a continually altered miRNA profile associated with disease progression. Riluzole did no effect on the expression of the three previous referred miRNAs in the serum of sALS patients.</td>
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et al. (2016), however, found it to be significantly reduced in compared to controls; in CD14+ CD16 was also steadily reported to be overexpressed in ALS when (Butovsky et al. 2012), CSF (Butovsky et al. 2012), spinal cle fibers (de Andrade et al. 2016; Pegoraro et al. 2017). Tasca cords (Campos-Melo et al. 2013; Koval et al. 2013), and mus- thus prevents strong inflammation (Pegoraro et al. 2017). It downregulates the secretion of inflammatory cytokines and implicated in innate immunity; it candidate for a biomarker.

larger populations and better-standardized procedures (De

matter of the spinal cord tissues (De Felice et al. 2014). In fact, showed a localization of the miRNA in the dorsal horn of gray spinal cord of sALS patients, while in situ hybridization was also found to be elevated in the serum, the CSF and the was also found to be elevated, more than 2-fold, in all sporadic ALS (sALS) leukocyte samples (De Felice et al. 2012); a finding that was replicated by the group 2 yrs later (De Felice et al. 2014). In the same study, miR-338-3p was also found to be elevated in the serum, the CSF and the spinal cord of sALS patients, while in situ hybridization showed a localization of the miRNA in the dorsal horn of gray matter of the spinal cord tissues (De Felice et al. 2014). In fact, the difference between cases and controls was more than 2-fold for both serum and CSF (De Felice et al. 2014).

This data, consequently, suggests that miR-338-3p might be a suitable ALS biomarker (De Felice et al. 2012; Shioya et al. 2010). The fact that it can be assessed, like miR-206, from easily attainable tissues, facilitates future research in larger populations and better-standardized procedures (De Felice et al. 2012) while making it an even more attractive candidate for a biomarker.

miR-146a is an inflammatory miRNA (Pegoraro et al. 2017; Tasca et al. 2016), implicated in innate immunity; it downregulates the secretion of inflammatory cytokines and thus prevents strong inflammation (Pegoraro et al. 2017). It was also steadily reported to be overexpressed in ALS when compared to controls; in CD14+ CD16− monocytes (Butovsky et al. 2012), CSF (Butovsky et al. 2012), spinal cords (Campos-Melo et al. 2013; Koval et al. 2013), and muscle fibers (de Andrade et al. 2016; Pegoraro et al. 2017). Tasca et al. (2016), however, found it to be significantly reduced in the serum of ALS patients, both in bulbar and spinal types (Tasca et al. 2016). Campos-Melo et al. (2013) report that miR-146a, alongside miR-524-5p and miR-582-3p, which were found downregulated in their study, can interact with neurofilament (NFL) mRNA 3′UTR, in accordance with the lower mRNA levels in spinal neurons of sALS cases (Campos-Melo et al. 2013). It is also connected to NF-kB, which is associated with muscular atrophy and inflammatory cytokines, and researchers believe that the increase of inflammatory miRNAs, especially in cases with long duration of disease, is due to the activation of NF-kB, which regulates this miRNA (Pegoraro et al. 2017). miR-582-3p has also been shown to be associated with TGF-β (Butz et al. 2011) and could possibly promote cell death.

miR-149/149* is another inflammatory miRNA that was found to be consistently downregulated in serum (miR-149*) (Tasca et al. 2016), muscle fibers (miR-149*) (Pegoraro et al. 2017), leukocytes of sALS (alongside miR-451, miR-1275, miR-328-5p, miR-638, and miR-665) (De Felice et al. 2012) and in the serum of familial ALS cases (Freischmidt et al. 2014). Pegoraro et al. (2017) report that it was the only inflammatory miRNA found downregulated in their study (Pegoraro et al. 2017) and, regarding the familial cases, miR-149-3p seems to correlate to the clinical manifestation, as the downregulation was found stronger in symptomatic cases than in pre-manifestation pathogenic genomic variant carriers (Freischmidt et al. 2014).

Finally, miR-155 and miR-221 are also included in the inflammatory miRNAs (Pegoraro et al. 2017; Tasca et al. 2016). miR-155 regulates the innate and adaptive immune responses (Koval et al. 2013; O’Connell et al. 2010; Pegoraro et al. 2017; Rodriguez et al. 2007) and it was shown that an increase in miR-155 led to reduced TGF-β1 production (Butovsky et al. 2012). MiRNA-221 seems to participate in muscle differentiation and maturation (Cardinali et al. 2009) and inflammation processes (Pegoraro et al. 2017; Xia et al. 2016). Much like miR-146a, it might contribute to ALS development through its action on muscle growth/atrophy and inflammation, via NF-kB (Pegoraro et al. 2017).

The data concerning these two miRNAs seem to be less clear. More precisely, both of them were found significantly higher in ALS muscles than in controls (Pegoraro et al. 2017),

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<th>Tissue</th>
<th>Participants</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peogrado et al. (2017)</td>
<td>Muscle</td>
<td>13 sALS and healthy controls</td>
<td>Overexpression of all miRNAs in the following comparisons: (1) ALS vs controls; (2) male ALS vs females ALS; (3) ALS early onset vs ALS late onset; (4) ALS long disease duration vs ALS short duration.</td>
</tr>
</tbody>
</table>

ALS amyotrophic lateral sclerosis, sALS sporadic amyotrophic lateral sclerosis, fALS familial amyotrophic lateral sclerosis, CSF cerebrospinal fluid, PD Parkinson’s disease, RRMS relapsing-remitting multiple sclerosis, FTD frontotemporal dementia

(Butovsky et al. 2012). A closely related mature sequence, miR-27b, was also reported elevated in the CSF of ALS pa- tients in the same study (Butovsky et al. 2012). Combined, the findings provide preliminary evidence that ALS has a unique profile of miRNAs in its tissues, at least in regard to muscle, but further research is needed for a consensus to be reached.

miR-338-3p is another miRNA found consistently upregu- lated. It is said to suppress neuronal differentiation, by inhibiting the apoptosis-associated tyrosine kinase and the cytochrome oxidase complex IV (Aschrafi et al. 2008; Barik 2008). First Shioya et al. (2010) examined brain samples and identified its upregulation, although they could not validate the results by qRT-PCR (Shioya et al. 2010). Wakabayashi et al. (2014) also found it to be upregulated in brain biopsy samples (Wakabayashi et al. 2014). De Felice et al. (2012) reported that it was elevated, more than 2-fold, in all sporadic ALS (sALS) leukocyte samples (De Felice et al. 2012); a finding that was replicated by the group 2 yrs later (De Felice et al. 2014). In the same study, miR-338-3p was also found to be elevated in the serum, the CSF and the spinal cord of sALS patients, while in situ hybridization showed a localization of the miRNA in the dorsal horn of gray matter of the spinal cord tissues (De Felice et al. 2014). In fact, the difference between cases and controls was more than 2-fold for both serum and CSF (De Felice et al. 2014).
but no significant difference was ascertained vs. controls in serum (Tasca et al. 2016). miR-155 was further evaluated in several studies: higher in CD14+ CD16− monocytes (Butovsky et al. 2012) and spinal cords (Koval et al. 2013) of ALS cases. In spinal cords, it was significantly increased both in familial and sporadic cases of ALS (Koval et al. 2013). In serum samples, a significant difference was only found when spinal and bulbar ALS patients were examined separately; it was higher in bulbar patients than in spinal ones and lower in spinal cases when compared to controls (Tasca et al. 2016).

As noted by Butovsky et al. (2012), contemporary data consistently show that immune and inflammatory processes are involved in the motor neuron degeneration of ALS. In addition, patients’ monocytes present signs of a particular inflammatory phenotype (Butovsky et al. 2012). Although the data on inflammatory miRNAs do not seem to ascertain a definitive change, the majority of studies show significantly deregulated “inflammatory profile” for ALS patients.

Two miRNAs that target TGF-β1, miR-21 and miR-106b, were reported to be upregulated in CD14+ CD16− monocytes (Butovsky et al. 2012). miR-21 was also upregulated in ALS muscle samples (de Andrade et al. 2016), but not significantly in the serum, where miR-106b showed a 2-fold upregulation (Toivonen et al. 2014). miR-106b’s role in ALS has yet to be fully described, although it seems to partake in a plethora of other processes, such as neural stem cell proliferation and differentiation (Brett et al. 2011), and negative regulation of autophagy in cultured myoblasts (Wu et al. 2012). Toivonen et al. (2014) claimed that it might serve as an ALS biomarker alongside miR-206 (Toivonen et al. 2014). Benigni et al. (2016), on the other hand, examining CSF samples of ALS patients, found miR-21-5p to be downregulated, alongside miR-15b-5p and the upregulated miR-181a-5p (Benigni et al. 2016). Using these biomarkers, they calculated the diagnostic accuracy of the miR-181a-5p/miR-21-5p and miR-181a-5p/miR-15b-5p ratios; it detected ALS with 90% and 85% sensitivity and 87% and 91% specificity, respectively (Benigni et al. 2016). Therefore, these miRNAs may also prove useful biomarkers in ALS diagnosis.

The protein aggregates of TDP-43 are a pathological characteristic of ALS (Al-Chalabi et al. 2012; Arai et al. 2006); pathogenic genomic variants in the gene cause a type of familial ALS (Kabashi et al. 2008; Sreedharan et al. 2008) and the protein has been associated with miRNA processing (Gregory et al. 2004). TDP-43 binding miRNAs were examined by Freischmidt et al. (2013) and a disequilibrium between CSF and serum levels of sALS cases was reported; the majority of miRNAs presented an approximately 50-fold higher concentration in serum, while only three—miR-9-5p, miR-132-5p, and miR-558-3p—had higher concentrations, 2- to 3-fold, in the CSF (Freischmidt et al. 2013). Corroborating this finding, miR-558-3p was only found in sALS spinal cords in a different study and absent in controls (Campos-Melo et al. 2013). miR-143-3p and let7b were found decreased in the serum, alongside miR-132-3p, whereas miR-143-5p and miR-574-5p were significantly upregulated (Freischmidt et al. 2013). In the same study, a pathogenic genomic variant-dependent drop in miR-143-3p and miR-143-5p was found in lymphoblastoid cell lines of all ALS patients (Freischmidt et al. 2013). MiR-143-3p was found to be significantly increased in the serum of patients in a different study, alongside miR-206 and as opposed to the decreased miR-374b-5p (Waller et al. 2017). Waller et al. (2017) claimed, in fact, that the levels of these miRNAs correlated to the duration of the disease and miR-143-3p presented an increase in later-collected samples that only reached significance for lower limb-onset patients (Waller et al. 2017). As for let7b-5p, it was found to be significantly downregulated in the plasma of ALS patients, alongside miR-26b-5p, miR-4299, miR-4419a, miR-3187-5p, and miR-4496 (Takahashi et al. 2015), and in the CSF samples of cases (let7-a, let7b-5p, and let7f-5p) (Benigni et al. 2016). However, let-7b was found upregulated in CD14+ CD16− monocytes of ALS patients, contributing to the inflammatory profile inherent to these patients (Butovsky et al. 2012). Furthermore, as a miRNA, let-7f-5p is highly expressed in the brain, and the let7 family is assumed to partake in cell adhesion, muscle formation, and chemotaxis (Sokol et al. 2008; Thammaiah and Jayaram 2016). Takahashi et al. (2015), after reporting downregulation of this miRNA in spinal-onset patients, and a negative correlation between its levels the bulbar paralysis score, posited this miRNA is associated with the muscle mass of these patients (Takahashi et al. 2015).

Another TDP43-binding miRNA is miR-9. It is a brain-specific miRNA, whose sequence has been greatly preserved during evolution (Yuva-Aydemir et al. 2011). As previously mentioned, it was found 2–3 times higher in the CSF of ALS patients, than in the serum (Freischmidt et al. 2013). Zhang et al. (2013) studied iPSC (induced pluripotent stem cell)-derived neurons and compared those carrying the TARDBP p.A90V pathogenic genomic variant, from patients, and neurons from controls without it (Zhang et al. 2013). This pathogenic genomic variant is considered to confer susceptibility towards ALS (Chiang et al. 2012; Winton et al. 2008) and the researchers found a higher concentration of TDP-43 in the cytoplasm of patient neurons, although its total level was lower. The levels of miR-9 and its precursor pri-miR-9-2 were both reported lower in patient neurons, while remaining unaffected in controls, when the cells were exposed to staurosporine-induced oxidative stress (Zhang et al. 2013). Oxidative stress being one of the main risk factors for ALS (D’Amico et al. 2013; Turner et al. 2013). miR-9-2 expression was also found lower pre- and post-stress in neurons with a different pathogenic genomic variant, the TARDBP M337V (Zhang et al. 2013). Finally, miR-9 was reported decreased in...
The studies described to date are diverse, providing a plethora of data on the variety of miRNAs which might contribute to ALS etiology. We have, so far, covered the miRNAs that were found to be the most frequent and consistent, yet would next like to specifically address a few other miRNAs that might also be of potential use as biomarkers of disease.

De Andrade et al. (2016) have reported that of all the differentially expressed miRNAs in ALS muscle samples that could be validated, only miR-424, miR-206, and miR-214 were changed (with qPCR). miR-424 and miR-206 were found at higher levels in the plasma of patients as well, and the researchers claimed that miR-424 correlated inversely with disease progression and could also serve as a potential biomarker (de Andrade et al. 2016), although the same finding was not corroborated in other studies.

A large number of other miRNAs was also detected in the studies under discussion, but were not encountered in other studies. In spinal cord samples, miR-16-2, miR-508-5p, miR-373, miR-551a, miR-506, miR-518e, miR-890 (Campos-Melo et al. 2013), miR-b1123, miR-b2948, miR-b3263, miR-b5539, miR-b1217, and miR-b3998 (Ishiqia et al. 2014) were found upregulated in ALS, whereas miR-520e, miR-548-5p, miR-606-612, miR-647 (Campos-Melo et al. 2013), miR-b1336, miR-b2403, miR-b4652, and miR-b659 (Ishiqia 2014) were downregulated. Ishiqia et al. (2014) found that miR-b1336 and miR-b2403 stabilize NEFL mRNA, supporting the hypothesis that their down-regulation is involved in the instability of NEFL mRNA (Ishiqia et al. 2014), which is characteristic for spinal motor neurons in ALS (Kondo et al. 1986). In brain samples, Wakabayashi et al. (2014) reported an upregulation of miR-494, miR-425, and miR-1973 and a downregulation of miR-4740-5p, miR-3619-3p, miR-1246, miR-3180-3p, and miR-1290 (Wakabayashi et al. 2014). In the serum, Freischmidt et al. (2014) reported a down-regulation of miR-4745, miR-1915, miR-1825, miR-3613-3p, miR-3665, miR-3185, miR-4488, miR-3960, miR-4530, miR-1281, miR-4532, miR-4734, miR-477-5p, miR-4497, miR-3940-5p, miR-4466, miR-3196, miR-4270, miR-4507, miR-4505, miR-1469, miR-4741, miR-4787-5p, miR-371b-5p, miR-2861, miR-638, 149-3p, miR-4763-3p, and miR-4516 (Freischmidt et al. 2014). The same group of Freischmidt et al. (2015) reported a constant decrease of miR-1234-3p and miR-1825 in the serum of sporadic patients (Freischmidt et al. 2015).

Forms of familial ALS (fALS) stem from specific pathogenic genomic variants, such as those in the FUS/TLS gene (Kwiatkowski Jr. et al. 2009) and the TDP-43 (Kabashi et al. 2008; Sreedharan et al. 2008), whereas aggregations and/or cytoplasmic inclusions of TDP-43, SOD1, or FUS are characteristic of the vast majority of ALS cases (Freischmidt et al. 2013). Some of the studies included herein discussed the role of miRNAs in familial cases, as compared to sporadic ones. Butovsky et al. (2012) studied monocytes from four familial cases with SOD1 pathogenic genomic variants. Their miRNA profile was found similar to those of the SOD1 mice, which were the basis of the comparison for all ALS samples; the researchers believed there are common pathways disrupted in various forms of ALS (Butovsky et al. 2012). Emde et al. (2015) also found a similar downregulation of miRNAs in sALS and in familial SOD1 A4V pathogenic genomic variant carriers (Emde et al. 2015). Freischmidt et al. (2013), however, found a common link—the downregulation of miR-132-5p/3p and miR-574-5p/3p—between sALS, TARDBP, FUS, and C9ORF72, but not SOD1 pathogenic genomic variant-carrier patients (Freischmidt et al. 2013). The same group of
Freischmidt et al. (2014), reported a homogenous miRNA profile in fALS regardless of the mutated gene and found an overlap of 91.7% of the downregulated miRNAs between pre-manifest ALS pathogenic genomic variant carriers and fALS cases (Freischmidt et al. 2014). They also noticed a very early appearance of this miRNA deregulation, signifying that patients could be identified at a presymptomatic stage (Freischmidt et al. 2014).

Furthermore, another link between familial and sporadic ALS was detected by Koval et al. (2013); miR-155 was significantly increased in both groups (Koval et al. 2013). In general, though, as Freischmidt et al. (2015) reported, sALS presents a highly diverse miRNA profile, in contrast to fALS; 61% had miRNA “fingerprints” similar to fALS/pathogenic genomic variant carriers, while the rest were considerably different (Freischmidt et al. 2015). This finding supports the notion that genetic factors do in fact contribute, to a larger degree than previously thought, in sALS, and that a more complex molecular etiology is responsible for sALS, than genetic cases of the disease (Freischmidt et al. 2015).

Taken together, the discussion on miRNAs is at embryonic stages. The studies described herein have provided significant preliminary evidence on miRNAs’ use and importance as surrogate biomarkers of ALS, but several issues need to be addressed. The number of test subjects in the majority of the available studies has been relatively small; this comes as no surprise when one considers the small number of patients and the hardships of working on tissues, such as the spinal cord and brain. In those cases, the question of quality is also of concern; for instance, Wakabayashi et al. (2014) had several inclusion criteria concerning the preservation of their formalin-fixed paraffin-embedded specimens and came to several conclusions regarding the way preservation technique influences the RNA yield (Wakabayashi et al. 2014). On the other hand, miRNAs in body fluids have been shown to be quite stable and are also considered as biomarkers in several other diseases as well (Xu et al. 2012). Moreover, samples from easily attainable tissues, such as blood, could assist future research in larger populations and with better-standardized procedures (De Felice et al. 2012), making the study results more reliable.

Additional research on miRNAs as biomarkers is clearly timely, meritorious, and warranted. Results obtained to date are encouraging and merit future studies on the utility of miRNAs to serve as accurate and fast biomarkers for the diagnosis of ALS.

**Concluding Remarks**

ALS is a severe disease that rapidly leads patients to their deaths. Fast and accurate diagnosis at the disease’s earlier stages might allow a more timely intervention and prolong the patients’ survival. For that, a biomarker is needed. As far as miRNAs, several seem to be stronger candidates than others. MiR-206 has been the most frequently encountered one and it was found consistently upregulated in the studies, in the muscle and serum. Its role remains to be further elucidated, but several studies have corroborated its utility as a biomarker. The studies though, as a whole, have failed to reach a consensus; yet, there is one thing they seem to agree on, namely, that ALS patients present different and unique patterns of miRNA expression. More research on this topic is clearly warranted as to fully unveil the utility of various miRNAs to serve as accurate surrogate biomarkers of the disease. Studies with larger samples, from different populations, and with standardized and homogenous procedures are strongly recommended.

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