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MicroRNAs as Potential Circulating Biomarkers for Amyotrophic Lateral Sclerosis

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Abstract Amyotrophic lateral sclerosis (ALS) is a condition primarily characterized by the selective loss of upper and lower motor neurons. Motor neuron loss gives rise to muscle tissue malfunctions, including weakness, spasticity, atrophy, and ultimately paralysis, with death typically due to respiratory failure within 2 to 5 years of symptoms' onset. The mean delay in time from presentation to diagnosis remains at over 1 year. Biomarkers are urgently needed to facilitate ALS diagnosis and prognosis as well as to act as indicators of therapeutic response in clinical trials. MicroRNAs (miRNAs) are small molecules that can influence posttranscriptional gene expression of a variety of transcript targets. Interestingly, miRNAs can be released into the circulation by pathologically affected tissues. This review presents therapeutic and diagnostic challenges associated with ALS, highlights the potential role of miRNAs in ALS, and discusses the diagnostic potential of these molecules in identifying ALS-specific miRNAs or in distinguishing between the various genotypic and phenotypic forms of ALS.

Keywords Amyotrophic lateral sclerosis · Biomarker · MicroRNA

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A Brief Overview of ALS

Although juvenile forms exist, amyotrophic lateral sclerosis (ALS) is primarily an adult-onset neurodegenerative disorder characterized by the progressive death of motor neurons in the cortex, brain stem, and spinal cord. Consequently, an irreversible downhill deterioration of muscle functions manifested by skeletal muscle weakness and wasting, dysphagia, dysarthria, and respiratory impairment occurs. It is the third most common neurodegenerative conditions of "middle age", affecting individuals in the 40–60 years old age group (after dementia and Parkinson's disease). In addition, a significant proportion of cases presents with cognitive involvement, including executive function impairment or frontotemporal dementia (FTD) (Goldstein and Abrahams 2013). Death typically occurs within 2–5 years after onset, usually as a result of respiratory failure. Riluzole, the only FDA-approved compound to treat ALS, only slows disease progression and extends survival for 2 to 3 months. ALS has a low incidence of 1–2 cases per 100,000 per year, but the cumulative lifetime risk has been shown to be as high as 1 in 338 (Johnston et al. 2006). The incidence rate increases with age, with a peak incidence rate observed in the 55–75 years old age group that can reach 13/100,000 (Chio et al. 2013). With the aging of the global population, a likely increase in diagnosed cases of ALS is foreseen.

Approximately 5–10 % of ALS cases are the familial form with a Mendelian pattern of inheritance. To date, 13 genes and loci of major effects have been identified (reviewed in (Leblond et al. 2014)). The most commonly mutated loci in adult-onset ALS are: SOD1, FUS (Kwiatkowski et al. 2009; Vance et al. 2009), TAR DNA-binding protein 43 (TDP-43) (Kabashi et al. 2008; Sreedharan et al. 2008), and C9ORF72 (Hosler et al. 2000; Morita et al. 2006; Vance et al. 2006; Valdmanis et al. 2007; DeJesus-Hernandez et al. 2011; Renton et al. 2011; Gijselinck et al. 2012). Mutations in C9ORF72

account for about 40 % of familial cases and 5–7 % of sporadic cases. Mutations in SOD1 account for about 20 % of familial cases and 2–7 % of sporadic cases. FUS mutations account for approximately 5 % of familial cases and less than 1 % of sporadic cases, while TARDBP mutations account for approximately 3 % of familial cases and 1.5 % of sporadic cases. The majority of ALS cases are sporadic (sALS) with no clear genetic linkage; the etiology of which remains unknown. A growing list of potential environmental risk factors for ALS has been proposed, including exposure to cyanobacteria, heavy metals, pesticides, intense physical activity, head injury, cigarette smoking, electromagnetic fields, and electrical shocks (Gawel et al. 1983; Deapen and Henderson 1986; Johansen 2000; Hakansson et al. 2003; Morahan and Pamphlett 2006; Qureshi et al. 2006; Steenland et al. 2006; Johnson and Atchison 2009; Sutedja et al. 2009; Weisskopf et al. 2009; Vanacore et al. 2010; Callaghan et al. 2011; Bradley et al. 2013); although at present, there is no ascertained causal link between environmental toxicants and ALS pathogenesis. Some of these risk factors for ALS deserve more attention. For instance, while initial reports revealed that Gulf War veterans may be at increased risk for ALS (Haley 2003; Horner et al. 2003; Weisskopf et al. 2005), recent studies with longer follow-up and thorough analysis called for additional studies that can address the limitations of the first studies such as lack of clinical data and low statistical power (Barth et al. 2009; Beard and Kamel 2014). Another interesting observation is the high prevalence for ALS in Guam (Kurland and Mulder 1954; Mulder and Kurland 1987) where a particular case of ALS-like conditions (ALS/Parkinsonism dementia complex) appear to be linked to the neurotoxic nonprotein amino acid, beta-N-methylamino-L-alanine (BMAA) (Spencer et al. 1987; Bradley and Mash 2009). BMAA is consumed by Chamorros through multiple dietary sources including cycad flour, flying foxes (a type of fruit bat), and other animals that feed on cycad seeds (Cox and Sacks 2002; Banack and Cox 2003; Murch et al. 2004). Finally, a recent epidemiological study revealed that more cases of ALS were associated with prior diagnosis of autoimmune disease raising the possibility of shared genetic or environmental risk factors (Turner et al. 2013).

The specific mechanisms underlying the selective degeneration of motor neurons also remain elusive. Nonetheless, the general consensus within the field is an agreement that the cause of ALS is multifactorial, and a number of possible pathological mechanisms have been put forward. Excitotoxicity, oxidative stress, aberrant protein aggregation, defective axonal transport, mitochondrial dysfunction, and altered RNA metabolism have notably been implicated in one way or another in the molecular and/or cellular pathways leading to ALS (Barber and Shaw 2010; Bogaert et al. 2010; Cozzolino and Carri 2012; Blokhuis et al. 2013; Fischer-Hayes et al. 2013). The number of proposed contributing factors reinforces the fact that ALS is a complex disorder wherein multiple pathways converge to give rise to the selective death of motor neurons.

It seems that the anatomical origin of the first dying motor neurons can originate either from the frontal cortex, the brain stem, or multiple regions within the spinal cord. This translates into a number of clinical or phenotypic presentations of ALS: Limb-onset ALS (symptoms first presenting in an arm/or leg) is the most common presentation accounting for 70 % of the incidence. Bulbar-onset ALS (first presentation involving speech, swallowing functions, and/or pseudobulbar features) is the second most common presentation at 25 %. Both limb- and bulbar-onset patterns include features of upper and lower motor neuron involvement (see Table 1) and require the presence of both in more than two regions of the body to make at least a probable diagnosis (Brooks et al. 2000). ALS with cognitive impairment (ALSci) or frontotemporal demential (ALS-FTD) represent phenotypes with multisystem involvement. Less common variants include primary lateral sclerosis with primarily upper motor neuron (UMN) involvement, progressive muscular atrophy, with primarily lower motor neuron (LMN) involvement (Gordon et al. 2006). In all cases, symptoms progress to include more extensive involvement of all body regions, with death resulting from respiratory compromise in the vast majority. While the clinical picture of ALS can be identified in its more advanced stages, a significant challenge remains the early and efficient diagnosis of these various forms supporting the need to identify clinically relevant biomarkers.

Diagnostic Challenges Associated with ALS

Early diagnosis and management in specialized ALS clinics providing multidisciplinary patient care has been shown to positively impact quality of life and prolong survival of ALS patients (Andersen et al. 2012). Unfortunately, several inherent challenges associated with early diagnosis of ALS exist. In a recent paper, more than half of the ALS patients received an alternative diagnosis, and each patient saw an average of three different physicians before ALS diagnosis was confirmed (Paganoni et al. 2014). The diagnosis and subsequent monitoring of ALS is based on clinical assessment that follows the EI Escorial criteria (Brooks et al. 2000). This involves the use of a combination of UMN and LMN signs to establish levels of diagnostic certainty. Although the disease is easily recognized in its full-blown presentation, retrospective reviews have highlighted a delay from symptom onset to diagnosis that has remained unchanged for more than a decade and that ranges from 8.0 to 15.6 months (Cellura et al. 2012). The delay can be longer (2.5 years) for limb-onset patients, for slow progression disease (up to 45 months), or for people in rural areas where there are fewer neurologists (Williams et al.

Table 1 Lower motor neuron and upper motor neuron signs in four CNS regions

	Brain stem	Cervical	Thoracic	Lumbosacral
Lower motor neuron signs				
Weakness, atrophy, fasciculations, hyporeflexia	Jaw, face tongue, palate, larynx	Neck, arm, hand, diaphragm	Back, abdomen	Back, abdomen, leg, foot
Upper motor neuron signs				
Pathologic spread of reflexes, clonus, weakness, emotional lability, loss dexterity	Clonic jaw jerk, gag reflex, exaggerated snout reflex, (pseudobulbar features), forced yawning, spastic tone, pathologic DTRs	Pathologic DTRs, spastic tone, clonic DTRs, Hoffman reflex, preserved reflex in weak and wasted limb	Pathologic DTRs, spastic tone, loss of superficial abdominal reflexes	Pathologic DTRs, spastic tone, clonic DTRs, preserved reflex in weak and wasted limb, extensor plantar responses

DTRs deep tendon reflexes

2013; Nzwalo et al. 2014; Sato et al. 2014). False diagnosis occurs as well and can range from 8 to 44 % (Belsh and Schiffman 1996; Davenport et al. 1996; Traynor et al. 2000). These challenges can direct patients towards the wrong treatments or even lead to unnecessary surgery. Indeed, there are reports highlighting how frequent ALS patients can undergo surgeries that were not required (Srinivasan et al. 2006; Kraemer et al. 2010). It is also important to point out that the delay observed for definitive diagnosis of ALS can push the patient beyond the window of therapeutic opportunity. Any new drug therapy for ALS would probably have its major impact in the early phase of the disease further reinforcing the need for early diagnosis. Overall, clear advantages are associated with an earlier diagnosis of ALS and identifying biomarkers to reach this goal is of great interest. The only treatment available, riluzole, an inhibitor of glutamate release, is a disease-modifying (neuroprotective) therapy for patients with ALS. In four large randomized controlled trials, riluzole extended survival or prolonged time to ventilation need of patients by an average of 2–3 months (Bensimon et al. 1994; Lacomblez et al. 1996; Miller et al. 2012) over the duration of the trial. Earlier diagnoses with robust biomarkers could benefit this therapeutic approach.

The Chase for ALS Biomarkers

Over the last two decades, tremendous efforts have been made worldwide to find reliable biomarkers for ALS. Interesting correlations have been observed and a plethora of candidates has been proposed as potential biomarkers. Reviewed by Robelin et al. (Robelin and Gonzalez De Aguilar 2014), biomarkers related to excitotoxicity, oxidative stress, inflammation, neurodegeneration, and others have been investigated. Unfortunately, none of these biomarkers has yet to translate into a clinically relevant tool. Several reasons can explain, albeit in part, these observations including contradictory results or weak statistical power in selected studies. Examples of selected biomarkers are reviewed here. In relation to excitotoxicity-associated biomarkers, while several molecules have been shown to be cytotoxic, the glutamate-induced excitotoxicity hypothesis has been well characterized as underlying the cascade of events that leads to motor neuron death. Glutamate release and reuptake imbalance can lead to disproportionate glutamate-induced calcium influx which subsequently triggers a cascade leading to neurotoxicity and death (Bogaert et al. 2010). Elevated glutamate concentration in the cerebrospinal fluid (CSF) could thus represent an interesting biomarker. Although elevated glutamate concentrations have been reported in CSF of ALS patients (Rothstein et al. 1991), other studies have showed elevated CSF glutamate concentrations only in a subset of them (Shaw et al. 1995; Spreux-Varoquaux et al. 2002), whereas other studies have shown that glutamate levels remained unchanged (Perry et al. 1990; Camu et al. 1993). Using a more sensitive and specific method, Fiszman et al. (Fiszman et al. 2010) revealed elevated glutamate levels in 28 out of 29 patients with definite, probable, or possible ALS. The same authors found no difference in glutamate concentrations when the three clinical forms of the disease were compared and concluded that glutamate levels may not influence the degree of diagnosis certainty or lesion extension. Besides glutamate, other metabolites present in the CSF have diagnostic potential. Using high-throughput techniques, including metabolomics of the CSF, as well as leveraging the 309 identified metabolites by the Human Metabolome Project (www.hmdb.ca), some groups revealed promising combinations of metabolites as diagnostic markers for ALS (Pradat and Dib 2009; Wuolikainen et al. 2009; Blasco et al. 2010; Wuolikainen et al. 2011; Wuolikainen et al. 2012).

Regarding inflammatory factors, while ALS is not primarily perceived as an inflammatory or immune-mediated

disease, immune mechanisms appear to play a role in this pathogenesis. In both ALS patients and animal models, inflammatory responses are observed (for reviews (McGeer and McGeer 2002; Bowerman et al. 2013)). A plethora of factors linked to inflammation can be followed in the periphery as potential biomarkers (reviewed in (Robelin and Gonzalez De Aguilar 2014)). Unfortunately, there is still inconsistency across laboratories, and published studies have included only a limited number of patients. An additional issue revolves around the lack of specificity for a given biomarker to discriminate between ALS and other types of neurodegenerative diseases (Bowser et al. 2011; Kiernan et al. 2011; Robelin and Gonzalez De Aguilar 2014). Immune cells partake in the inflammatory process and may represent the future for biomarkers since they can present unique molecular signatures for specific diseases. Particularly, the group of Weiner (Butovsky et al. 2012) demonstrated that ALS patients have analogous monocytes ($CD14^{+}CD16^{-}$) which exhibited an ALS-specific microRNA inflammatory signature similar to the one observed in the ALS mouse model, linking the animal model to the human disease. In parallel, the group of De Felice revealed a unique microRNA signature in leukocytes from ALS patients (De Felice et al. 2012; De Felice et al. 2014). According to these studies, the underlying role of microRNAs in ALS does warrant a closer investigation.

MicroRNAs Underlying Neurodegenerative Diseases

MicroRNAs (miRNAs) are short evolutionarily conserved non-coding RNA molecules involved in post-transcriptional regulation of gene expression. This regulation is achieved via pairing of miRNAs with complementary sequences located on targeted mRNAs (Bartel 2009). MiRNA/mRNA binding leads to downregulation of the corresponding mRNA and/or protein levels due to mRNA destabilization or translational inhibition. Recent evidence gained through simultaneous mRNA and proteomic/ribosomal profiling suggests that the former is dominant in mammalian cells (Baek et al. 2008; Selbach et al. 2008; Guo et al. 2010). Approximately 60 % of all protein-coding genes are thought to be regulated by miRNAs (Friedman et al. 2009) and such vast regulation allows miRNAs to be involved in a variety of cellular and pathophysiological processes (Bartel 2009). Accordingly, multiple miRNAs have been reported as deregulated in neurodegenerative diseases. MiR-34a and members of the miR-20a family were shown to regulate the expression of tau and amyloid precursor protein, respectively, two key factors linked to Alzheimer's disease (AD) pathogenesis (Hebert and De Strooper 2009; Dickson et al. 2013). Additional members of the miR-34 family of miRNAs, mir-34b and miR-34c, were deregulated in early stages of brain samples collected from patients diagnosed with Parkinson's disease (PD) (Minones-Moyano et al. 2011). Selected miRNAs, such as miR-9, have been reported as deregulated in different neurodegenerative diseases including AD and Huntington's disease (HD) (Cogswell et al. 2008; Packer et al. 2008), while miR-29a was differentially expressed in PD and HD (Johnson et al. 2008; Margis et al. 2011). Interestingly, the underlying roles of key proteins involved in miRNA biogenesis have also been explored in selected CNS diseases. A transgenic mice model for Dicer, a key enzyme involved in miRNA synthesis, displayed a neurodegenerative phenotype as well as tau hyperphosphorylation when Dicer expression was specifically ablated from the forebrain (Hebert et al. 2010). Another study demonstrated altered miRNA biogenesis, notably through altered Dicer expression, in two models of HD transgenic mice (Lee et al. 2011). MiRNAs are thus involved in several CNS diseases, and it is not surprising to find them regulating expression of key processes in ALS.

Deciphering the Roles of miRNAs in ALS

Several research groups acted as pioneers in the early characterization of miRNA involvement in ALS. Confronted with challenges that included sufficient patient enrollment for the various subforms of ALS, research performed in recent years has nevertheless yielded a clearer picture of the likely involvement of miRNAs in ALS as well as their potential usefulness as biomarkers for this condition. Several studies have notably leveraged the SOD1-G93A mouse model for ALS (Gurney et al. 1994). A study performed in this model of familial ALS and subsequently validated in human ALS spinal cord tissues notably demonstrated strong expression of miR-155 (Koval et al. 2013). Furthermore, downregulation of miR-155 in ALS mice using oligonucleotide-based miRNA inhibitors or anti-miRs significantly prolonged survival. Profiling primary microglia cell cultures purified from the model also revealed a plethora of differentially expressed miRNAs including miR-22, miR-155, miR-125b, and miR-146b (Parisi et al. 2013). The group notably highlighted the miR-125b-based modulation of TNFα in ALS. A recent study using the SOD1-G93A model reported strong expression of miR-29 in ALS brain and spinal cord even though its knockdown did not lead to significant improvements in ALS-associated clinical endpoints (Nolan et al. 2014).

Studies on human samples have also been conducted and have revealed the potential importance of miRNAs in ALS. First, a postmortem analysis of tissues isolated from the spinal cord at the lumbar level by the group of Michael Strong (Campos-Melo et al. 2013) revealed that the expression of numerous miRNAs was altered in ALS patients. Pathway analysis showed that these miRNAs were implicated in nervous system functions and cell death. The use of two prediction algorithms revealed three miRNAs (miR-146a*, miR-

524-5p, and miR-582-3p) capable of interacting with the 3′ UTR of the human low molecular weight neurofilament (NEFL) mRNA. A subsequent study from the same group revealed two additional miRNAs, miR-b1336 and miR-b2403, capable of stabilizing NEFL transcripts in ventral lumbar spinal cord samples obtained from ALS patients (Ishtiaq et al. 2014). The presence of intraneuronal neurofilamentous aggregates is a neuropathological hallmark of ALS, and reduced NEFL mRNA levels have been observed in degenerating spinal motor neurons (Bergeron et al. 1994; Wong et al. 2000; Menzies et al. 2002). The discovery of this new set of NEFL-associated miRNAs has put the light on an additional layer of NEFL expression regulation in spinal motor neurons in ALS.

Motor neurons from the frontal cortex are also affected in ALS, and miRNA-related research to understand the role of these molecules in these cells is slowly emerging. Samples isolated form postmortem frontal cortex tissues of three ALS patients notably revealed an upregulation of miR-29a, miR-29b, and miR-338-3p (Shioya et al. 2010). However, due to a significant inter-individual variation, results were not subsequently validated by quantitative RT-PCR. Nevertheless, miR-338-3p upregulation in ALS patients has been also observed in blood leukocytes, CSF, serum, and spinal cord (De Felice et al. 2014). It is important to point out that skeletal muscle tissue represents another interesting source of potential biomarkers. Skeletal muscle mitochondrial dysfunction is believed to play a role in the progression and severity of ALS, and Russell et al. (Russell et al. 2013) showed that miR-23a, miR-29b, miR-206, and miR-455 expressions were increased in skeletal muscle of ALS patients. Histone deacetylase 4 (HDAC4) is an important mediator neural activity action on muscle gene expression, and HDAC4 expression is dramatically induced in this tissue in response to denervation in ALS mice (Cohen et al. 2007). MiR-206 has been proposed as a potential regulator of HDAC4. Bruneteau et al. (Bruneteau et al. 2013) investigated the role of the miRNA-206-HDAC4 axis and showed that miR-206 was upregulated in ALS long-term survivors but it did not correlate with disease progression or reinnervation.

Clearly, several miRNAs seem to underlie the pathogenesis associated with ALS. It is only logical to wonder if any of those could potentially be leveraged as non-invasive circulating biomarkers to diagnose ALS and its various subsets.

MicroRNAs as Appealing Biomarkers for ALS

Interestingly, living neurons and other CNS cells secrete miRNAs and other small non-coding RNAs into the extracellular space packaged in exosomes, microvesicles, or lipoprotein complexes. In addition, several studies have successfully isolated and quantified miRNAs from a variety of human body fluids including plasma or serum, urine, and saliva (Mitchell et al. 2008; Park et al. 2009; Hanke et al. 2010). Other factors positioning miRNAs as appealing biomarkers notably include their significant stability in body fluids as well as the relative ease of their detection given their well-conserved sequences (Chen et al. 2008; Jin et al. 2013). These characteristics, coupled with the rapidly evolving improvements in technologies that allow for detection of RNA species from small amounts of biological material, have contributed to the strong interest dedicated towards the study of extracellular RNAs as potential biomarkers for CNS disorders including multiple sclerosis, AD, PD, and ALS (Vella et al. 2008; Galimberti et al. 2014; Honardoost et al. 2014). In a recent study, using the ALS mouse model SOD1-G93A, miR-206, involved in the maintenance of neuromuscular connectivity in ALS, was flagged as a potential circulating biomarker candidate as it exhibited strong upregulation in the serum of mice and ALS patients (Williams et al. 2009; Toivonen et al. 2014). MiR-206 upregulation was almost statistically significant in the presymptomatic stages of SOD1-G93A mice making it an interesting biomarker candidate for early diagnosis of ALS. A drawback associated with miR-206 as an ALS biomarker is the fact that similar increases have been observed in a wide range of conditions and pathologies including the Duchenne Muscular Dystrophy (Roberts et al. 2013), AD, cerebral ischemia (Jeyaseelan et al. 2008; Shioya et al. 2010), schizophrenia (Hansen et al. 2007), and in cytotoxic insult from exposure to environmental toxins (Zhang and Pan 2009). This further reinforces the importance and challenges of identifying ALS-specific circulating biomarkers to properly discriminate ALS from other CNS conditions. Recent work demonstrated that let-7 and miR-92 could notably differentiate ALS patients from patients diagnosed with relapsing-remitting multiple sclerosis, but not secondary progressive multiple sclerosis suggesting the latter possesses features present in other neurodegenerative diseases (Gandhi et al. 2013).

TDP-43 aggregates are observed in most ALS cases (Neumann et al. 2006; Ince et al. 2011; Al-Chalabi et al. 2012), and identifying a biomarker associated with this target has been explored by several research teams. Freischmidt et al. (Freischmidt et al. 2013) reported altered expression levels of five out of nine TDP-43-binding miRNAs in CSF and serum samples of sALS cases including miR-143-5p/3p. However, these authors found a poor correlation between CSF and serum levels of these miRNAs suggesting an independent regulation of TDP-43-binding microRNAs in the serum and CSF. Nonetheless, as proposed by these authors, these findings might be relevant for an easily accessible biological assessment of TDP-43 levels as well as of miRNAs regulating its expression.

With the aim to find blood miRNAs specific to ALS and that correlates between CSF and serum, the group of De Felice

(De Felice et al. 2012) first investigated the changes in miRNA expression profiles in leukocytes from ALS patients using a microarray strategy. Several miRNAs were differentially expressed including miR-149, miR-328, miR-338-3p, miR-451, miR-583, miR-638, miR-665, and miR-1275. As mentioned previously, miR-338-3p overexpression was reported in frontal cortex tissues collected from three ALS patients (Shioya et al. 2010). Subsequent work was undertaken in a large cohort and showed an overexpression of miR-338-3p in blood leukocytes, CSF, serum, and spinal cord obtained from sALS patients (De Felice et al. 2014). MiR-308-3p expression was higher in ALS patients compared to healthy patients as well as to patients suffering from other neurodegenerative disorders like PD, AD, and HD. Interestingly, miR-338-3p might relate with the higher glutamate levels observed in CSF of ALS patients described above. Indeed, one putative targets of deregulated miR-338p is the membrane-bound protein SLC1A2 which is the principal transporter that clears the excitatory neurotransmitter glutamate from the extracellular space at synapses in the CNS. Mutations and decreased expression of this protein are associated with certain forms of ALS (Rothstein et al. 1995).

Around the same time, the group of Weiner (Butovsky et al. 2012) demonstrated that recruitment of inflammatory monocytes into the CNS played an important role in ALS progression. A thorough characterization of monocyte population was undertaken, and a unique miRNA signature within $CD14^{+}CD16^{-}$ monocytes isolated from ALS patients with the SOD1 familial form was identified. This signature was similar to the one found in Ly6Chi monocytes from the mouse SOD1 model (Butovsky et al. 2012). MiRNAs such as miR-27a, miR-155, miR-142-5p, miR-223, and miR-532-3p were highly expressed in ALS patients compared to healthy controls or patients diagnosed with multiple sclerosis. MiR-27a could differentiate multiple sclerosis from ALS patients even though this miRNA is also known to be modulated in carcinogenesis and other pathological processes. (Gottardo et al. 2007; Mertens-Talcott et al. 2007; Wang et al. 2008; Guttilla and White 2009; Liu et al. 2009). Nonetheless, the same group showed that three miRNAs: miR-27b, miR-146a, and

Table 2 Differentially expressed miRNAs in ALS patients

miRNAs	Sample	Patients	Site of disease onset	Method	References
miR-27a↑, miR-55↑, miR-142-5p↑, miR-223↑, and miR-532-3p↑ miR-27b↑, miR-146a↑, miR-532-3p↑	Blood/monocytes CSF	18 sALS (%) 4 fALS (%)	B (18), C (55), L (27) L (100)	qRT-PCR	(Butovsky et al. 2012)
		10 sALS (%) 5 fALS (%)	B (10), C (30), L (50), U (10) L (60), U (40)	TaqMan real-time PCR	
miR-149↓, miR-328↓, miR-338-3p↑, miR-451↓, miR-638↓, miR-665↓, miR-1275↓	Blood/leukocytes	8 sALS 14 sALS	No mention	Microarray, qRT-PCR TacMan	(De Felice et al. 2012)
miR-29a↑, miR-29b↑, miR-338-3p↑	Frontal cortex	3 6	No mention	Microarray RT-PCR	(Shioya et al. 2010)
miR-146a*↑, miR-524-5p↓, miR-582-3p↓	Spinal cord	5 sALS	2 B, 1 S, 1 UL, and 1 U	TaqMan miRNA microarray, qRT-PCR	(Campos-Melo et al. 2013)
miR-132-3p↓, miR-132-5p↓, miR-143-3p↓, miR-143-5p↓↑, let-7b↓-	Serum, CSF	22 sALS	No mention	qRT-PCR	(Freischmidt et al. 2013)
miR132-5p/3p↓, miR-574-5p/3p↓	LCLs	3–8 fALS	No mention	qRT-PCR	(Freischmidt et al. 2013)
miR-24-2*↑, miR-142-3p↑, miR-142-5p↑, miR-146b↑, miR-155↑, miR-1461↑	Spinal cord	16	No mention	TaqMan miRNA assay	(Koval et al. 2013)
miR-338-3p↑	Blood/leukocytes, CSF, serum, spinal cord	72 sALS (%) 10 sALS (%) 7 sALS (%)	B (33), L (67) B (20), L (80) B (30), L (70)	qRT-PCR	(De Felice et al. 2014)
miR-sb659*↓, miRb1123↑, miR-sb1217*↑, miR-b1336↓, miR-b2403↓, miR-b2948↑, miR-b3265↑, miR-sb3998↑, miR-b4652↓, miR-b5539↑	Spinal cord	3 sALS 5 sALS	2 B and 1 UL 2 B, 1 L, and 2 U	Small RNA library qRT-PCR	(Ishtiaq et al. 2014)
miR-106b↑, miR-206↑	Serum	12	No mention	qRT-PCR	(Toivonen et al. 2014)

Studies highlighting deregulated miRNAs in primary human ALS samples collected from different sources. The diagnostic relevance of these modulated miRNAs is discussed in the text

B bulbar, *C* cervical, *f* familial, *L* limb, *LCLs* immortalized lymphoblast cell lines, *s* sporadic, *U* unknown, *UL* upper limb

miR532-3p that were commonly elevated in CSF samples of ALS patients, in monocytes and microglia from SOD1 mice and from human ALS patients with the sporadic and familial forms (Butovsky et al. 2012). This finding highlights that a combination of miRNAs could represent a more plausible signature instead of only one miRNA.

Need for Biomarkers that can Differentiate the Subforms of ALS and ALS-like Forms

Differentially expressed miRNAs in ALS patients are summarized in Table 2. It is important to mention that several studies did not specify the subforms of ALS associated with the patient population investigated as well as it did not attempt to establish any correlations between altered miRNAs and subforms of ALS. Only the studies of Freischmidt (Freischmidt et al. 2013) and Butovsky (Butovsky et al. 2012) highlighted differences between genotypic forms of familial ALS. It is probably too early as the potential of miRNAs as biomarker has just been undertaken, but the identification of miRNA-specific footprints for each ALS subform will be beneficial to direct the patients toward the appropriate therapeutic regimen.

Although ALS presents as a motor disorder, it is now well recognized as a multisystem disease with neuropsychological impairments in an estimated 20–50 % of patients. Furthermore, there are ALS-like forms including Kennedy's disease or primary lateral sclerosis. Despite advances in histopathology techniques, neurophysiology and neuroimaging diagnosis is made clinically "at the bedside". The variability in clinical findings early in the course of ALS and the lack of biological diagnostic marker make absolute diagnosis difficult and compromise the certainty of diagnosis in clinical practice, therapeutic trials, and other research purposes. Furthermore, genotypic and phenotypic variability support the concept of ALS as a spectrum of disease, and it is realistic to expect that previous trials, not differentiated between the various types of ALS, resulted in negative outcomes. In fact, there remains only one treatment, to date, approved for treatment of ALS (riluzole), which did show trend to be somewhat more effective in those with bulbar-onset pattern. Targeting treatment in research and clinical trials to specific subforms of ALS, particularly early in disease course, is a promising strategy to discovery of effective treatments.

Outlook

ALS is a neurodegenerative disorder for which therapeutic and diagnostic options remain limited. While early diagnosis of ALS is crucial to best manage this condition, biomarkers are lacking to allow rapid identification or distinguish between its various subforms. It is not surprising that miRNAs, with their capabilities of silencing a broad array of transcripts and their appearances in various body fluids, have garnered greater interest from the ALS community for their potential roles and clinical usefulness. Looking ahead, a detailed assessment of circulating miRNAs associated with the different forms of ALS as well as a careful monitoring of modulated miRNAs over time in ALS patients will provide crucial insights on the diagnostic relevance of these molecules in ALS. It is expected that the identification of ALS-associated miRNA signatures, whether for early diagnosis of patients or to assess therapeutic response, will be of significant help to better manage patients diagnosed with ALS.

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