Free-Circulating miRNAs as Biomarkers in Amyotrophic Lateral Sclerosis and Hereditary Spastic Paraplegias

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ABSTRACT

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The development of biomarker research is essential to enhance the diagnostic workup for neurodegenerative disorders and to facilitate the monitoring of effective disease-modifying therapies. While DNA testing remains a predominant biomarker for identifying mutations in familial forms of neurodegenerative diseases, various types of RNA have more recently been linked to both familial and sporadic forms of these diseases. miRNAs, a subset of endogenous non-coding RNA, bind to partially complementary sequences in mRNAs and inhibits mRNA translation by either blocking translational machinery or degrading mRNAs. They are involved in a variety of cellular processes, including cell cycle, development, metabolism, and synaptic plasticity. Dysregulation of miRNA expression and function has been reported in various neurological disorders (NDs) of the central nervous system, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and hereditary spastic paraplegias (HSP). If a specific profile of dysregulated miRNAs is identified in patients suffering from NDs, this could serve as a biomarker for earlier diagnosis and monitoring of the disease progression. Identifying the role of miRNAs in normal cellular processes and understanding how miRNA expression is linked to their neurological effects are also critical in developing new therapeutic strategies for NDs. In this review, we will focus on free-circulating miRNA and their association between ALS and HSP, as both diseases share the same neurodegeneration process of the upper and lower motor neurons. This suggests that they may also share the same miRNA pattern, which might be considered a shared biomarker.

Key words: *miRNA, biomarkers, motor neuron disorders, neurodegeneration, rare disease*

STRESZCZENIE

Wolne krążące miRNA jako biomarkery w stwardnieniu bocznym zanikowym i dziedzicznej spastycznej paraplegii

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Rozwój badań w dziedzinie badań biomarkerów jest niezbędny do usprawnienia diagnostyki chorób neurodegeneracyjnych i skutecznego monitorowania terapii modyfikujących przebieg choroby. Chociaż badania DNA pozostają dominującym biomarkerem pozwalającym na identyfikację mutacji w chorobach neurodegeneracyjnych, ostatnio powiązano różne typy RNA zarówno z rodzinnymi, jak i sporadycznymi postaciami tych chorób. miRNA, endogenne niekodujące cząsteczki RNA, wiąże się z częściowo komplementarnymi sekwencjami w mRNA i hamuje translację mRNA albo poprzez blokowanie mechanizmu translacji, albo przez degradację mRNA. miRNA, bierze udział w różnych procesach komórkowych, w tym w cyklu komórkowym, rozwoju, metabolizmie i plastyczności synaptycznej. Potwierdzono ich udział w zaburzeniach ekspresji i funkcji miRNA w różnych schorzeniach neurologicznych (ND) ośrodkowego układu nerwowego, takich jak m.in. choroba Alzheimera (AD), choroba Parkinsona (PD), choroba Huntingtona (HD), stwardnienie boczne zanikowe (ALS) i dziedziczne spastyczne paraplegie (HSP). Jeśli u pacjentów cierpiących na choroby neurodegeneracyjne zostanie zidentyfikowany specyficzny profil ekspresji miRNA, może on posłużyć jako biomarker do wcześniejszej diagnozy i monitorowania postępu choroby. Identyfikacja roli miRNA w prawidłowych procesach komórkowych i zrozumienie, w jaki sposób ekspresja miRNA jest powiązana z ich skutkami, ma również kluczowe znaczenie w opracowywaniu nowych strategii terapeutycznych dla ND. W tym artykule skupiamy się na wolno krążących cząsteczkach miRNA i ich związku pomiędzy ALS i HSP, ponieważ w obu chorobach występuje ten sam proces neurodegeneracji górnych i dolnych neuronów ruchowych. Sugeruje to, że mogą one również mieć ten sam profil ekspresji miRNA, który można będzie uznać za wspólny biomarker.

Słowa kluczowe: *miRNA, biomarkery, choroby neuronu ruchowego, neurodegeneracja, choroba rzadka*

The Nature of Biomarkers

According to the definitions provided by the US Food and Drug Administration (FDA) and the National Institutes of Health (NIH), a biomarker is an objective indicator measurable outside the patient's body. It serves as a quantifiable measure of various biological or pathogenic processes, responses to interventions, and exposures.[1-6]

Biomarkers play a pivotal role in medical applications such as diagnosis, disease classification, and monitoring therapeutic responses.[7] Califf et al. further categorized biomarkers into groups based on their functions, including diagnostic, monitoring, pharmacodynamics/response, predictive, prognostic, safety, and susceptibility/risk.[3] This article specifically delves into three subtypes of biomarkers: diagnostic, predictive, and prognostic.[1,3] Diagnostic biomarkers are instrumental in confirming the presence of a disease or identifying specific disease subtypes, as outlined by the FDA-NIH definition.[1,4] Predictive biomarkers play a crucial role in identifying individuals likely to experience either favorable or unfavorable effects from exposure to medical products or environmental agents.[3,4] An illustrative example includes free-circulating miRNAs, which may signal impending events such as neurodegeneration.[1,3,4] Prognostic biomarkers are utilized to assess the likelihood of clinical events or disease progression in individuals with the condition (FDA-NIH definition). C-reactive protein (CRP) stands as an exemplar, serving as a prognostic biomarker for inflammation, as elucidated by different scientists.[5,6]

Several researchers[8-13] have focused their studies on neurodegenerative biomarkers, often derived from diverse sources such as peripheral blood, muscle biopsies, or cerebrospinal fluid (CSF). Whole blood, in particular, has emerged as an attractive source due to its ease of collection, relative noninvasiveness, and suitability for longitudinal studies, enabling the tracking of disease progression over time.[2,10,14] Among potential biomarkers, microRNAs (miRNAs) have garnered considerable attention, especially in neurodegenerative research. With promising characteristics, miRNAs have been employed to monitor different gene expressions associated with neurodegenerative diseases, as evidenced by numerous studies.[10,15-17]

MicroRNA Biogenesis and Role in Gene Expression

MicroRNAs (miRNAs) are endogenous, non-coding single-stranded RNA molecules that play important roles in eukaryotic gene expression through post-transcriptional regulation. They were first identified via classical forward genetics experiments.[18,19] miRNA mainly binds to the 3'-untranslated region (3'UTR) of the messenger RNA (mRNA) from target protein-coding genes, leading to gene silencing by mRNA cleavage, translational repression and deadenylation.[18] A miRNA particle is a part of ribonucleoprotein complexes that specifically block the mRNA translation. Unlike siRNAs, miRNAs are not characterized by complete sequence identity with the target mRNA and therefore can silence different genes. The miRNA biogenesis is a complex process, which can be generated through the classical canonical and non-canonical pathway depending on cell types and their environment.[20] It begins in the nucleus when 1-3 kilobase (kb) precursor miRNAs (pre-miRNAs) are generated either by the microprocessor complex (Drosha and DGCR8) RNaseIII enzyme processing of primary miRNA (pri-miRNAs) transcripts or by spliceosome processing of precursor mRNA (pre-mRNA) transcripts followed by the Lariat Debranching Enzyme (DBR1) processing of the resulting introns.[21]

The primary transcript (pri-miRNAs) of miRNA is specifically recognized by the microprocessor complex composed of nuclear ribonuclease III, Drosha, and is the binding partner of the DGCR8 protein, which recognizes the stem-loop structure in pri-miRNA. Drosha then cleaves the pri-miRNA into a 70 nt pre-miRNA, which is translocated to the cytoplasm by exportin 5 protein.[18] In the cytosol, pre-miRNAs are further processed into 19-23 nucleotide mature miRNA duplexes by a protein complex consisting of DICER (RNase) and the double-stranded RNA-binding domain proteins TRBP, PACT and Ago2.[20] One strand of the miRNA duplex is incorporated into the RNA-induced silencing complex (RISC) as a mature miRNA, whereas the other strand is degraded. miRNA can also be generated from hairpin introns, snoRNAs, tRNAs and endogenous shRNAs as a result of splicing, debranching and other complex processing mechanisms without RISC involvement.[18-22]

The intracellular concentration of miRNA may change in different physiological and pathological processes due to modifications in their transcription, maturation and stability. Despite their intracellular activity, miRNAs have been found in extracellular human body fluids such as blood plasma/serum, urine, saliva, semen, CSF, and milk. Therefore, some free-circulating miRNAs have been successfully assigned as biomarkers for several cancers, cardiovascular diseases, and brain or liver injuries. Such changes of miRNA levels in different brain areas are also indicative of many neurodegenerative diseases and other brain pathologies.[12,22-25]

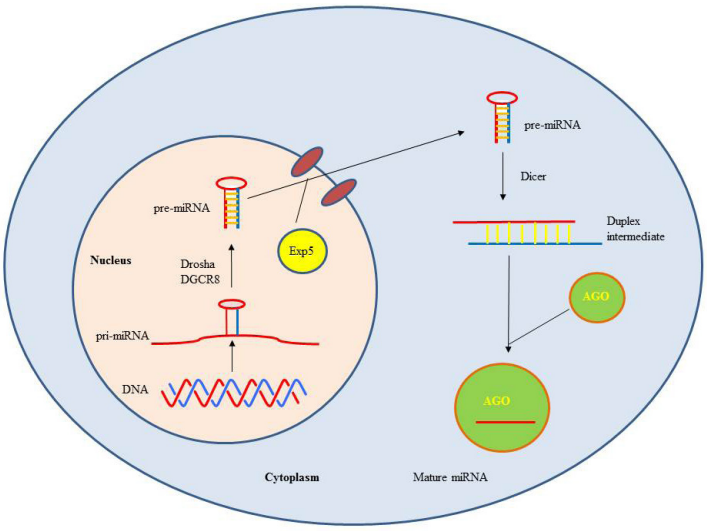

Figure 1. The miRNA biogenesis process

miRNAs are stable and resistant to RNase activity, which makes circulating miRNAs potentially novel sources of biomarkers for various diseases. Due to the inherent heterogeneity within a neurodegenerative disease miRNA can comprise both sporadic and familial genetic forms of alteration.[2] Serving as master regulators of gene expression, they can be used as diagnostic, predictive, and prognostic biomarkers across various disease states, as they are increasingly applied in the field of neurodegenerative disorders.[3,16]

Neurodegenerative Diseases and Progressive Changes in Cell Homeostasis

Neurodegenerative diseases are marked by the progressive loss of neural tissues, leading to irreversible degradation of neurons that cannot be regenerated after cell death or damage.[26] These disorders are characterized by a very broad and a diverse background, encompassing well-known conditions like Alzheimer's disease (AD) and Parkinson's disease (PD), as well as rarer ones often defined by genetic defects, such as Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and hereditary spastic paraplegia (HSP).[21] They also include various types of ataxias,[27] spinal and bulbar muscular atrophy (SBMA), and several other rare types.[28,29] Cellular pathologies and molecular dysfunctions in the neurodegenerative process generally lead to functional changes, including oxidative stress, protein oligomerization and aggregation, axonal transport deficiency, calcium deregulation, mitochondrial dysfunctions, neuroinflammation, neuron-glial interactions, aberrant RNA processing, and DNA (deoxynucleic acid) damage.[20] Figure 2 highlights the neurogenerative process.

The neurodegeneration process is initiated by metabolic disruptions in the neurons, such as protein overexpression or misfolding. These events activate microglial cells and the Blood Brain Barrier (BBB) by cytokines which induce neuroinflammation and lead to neuronal damage and finally neurodegeneration.

Mitochondrial dysfunction, prevalent in aging brains and neurodegenerative diseases, mainly involves molecular damage to mtDNA by intracellular events such as nucleases, reactive oxygen species (ROS), and spontaneous hydrolytic processes. Single-strand breaks in mtDNA can be generated either by ROS or from aberrations in replication processes. In an aging brain and in the neurodegenerative process the misrepair of double-strand breaks causes the mtDNA deletion seen in aged human tissues. This implies that aging affects normal replication, leading to the majority of deletions at mtDNA replication sites.[30] Figure 3 shows possible factors of neurodegeneration.

As mentioned above, neurodegenerative diseases are often characterized by the presence of inclusions of aggregated protein, for example amyloid β aggregation in AD, accumulated proteins forming Lewy bodies in PD, polyglutamine proteins in SCA, HTT in HD, or TDP-43 in ALS.[31,32]

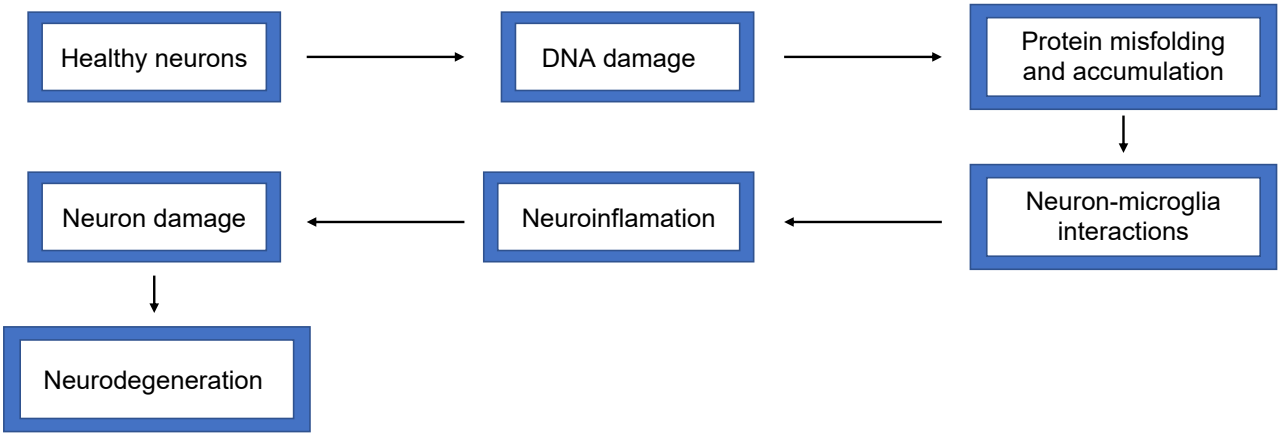

Figure 2. The neurodegeneration process

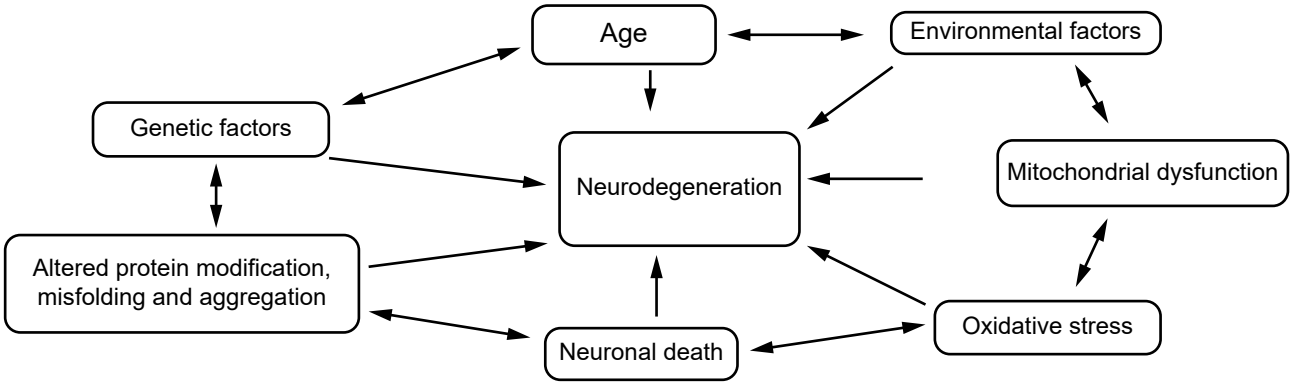

Figure 3. Various factors involved in progression of neurodegenerative disease

Recent studies indicate that aberrant gene expression caused by elevated miRNA levels is increasingly recognized as an important predictive tool in neurodegenerative disorders. Also, it has been reported that miRNA is involved in the development of the central nervous system and neurodegeneration.[20,32-35] Current research on the role of miRNA in neurodegeneration as biomarkers remains limited. Those that do exist, show potential changes in the process but do not yet define their significance in the general aspect of neurodegeneration. For a brief summary of miRNAs involved in neurodegenerative diseases see Table 1.

miRNAs play essential role in regulating various physiological processes, such as neuronal stem cell differentiation, neurogenesis, neuronal survival, and synapse formation is crucial. They have also been shown to be involved in neurodegeneration, which is why their importance and value as biomarkers for diagnosis and therapeutic strategies continues to grow.[12,20,22,32-34]

Several authors have demonstrated that miRNA can act as both neuroregulators and neuroprotectors, influencing the expression of specific genes. They reported that the level of miR-29 is reduced in AD and HD and in mouse models. Furthermore, miR29 plays a key role in amyloid beta formation, leading to AD pathology. Moreover, the neuroprotective miR-29-a-1 and miR-29-b-1 are downregulated, which promotes the progression of AD by regulating beta-secretase-1, increasing Ab formation.[20,32,53,54]

Huntington's disease is a neurodegenerative disorder characterized by cognitive and motor defects, caused by CAG repeat expansion in the huntingtin (*HTT*) gene. Pathologically, it involves progressive neuronal death in the cortex and striatum of HD patients.

Table 1. Brief summary of known miRNAs involved in neurodegenerative diseases

Neurodegenerative diseases	miRNAs	Target genes/proteins	Functional changes	Ref.
Alzheimer's disease	miR-9	BACE1	APP cleavage	[36]
	miR-29	Bim; Bmf, Hrk; Puma	Neuronal survival	[37]
	let-7b	TLR7(binding)	Immune response	[38]
	miR-106b	APP	APP signaling	[39]
Parkinson's disease	miR-133b	Pitx3	DA neuron differentiation and survival	[39]
	miR-107	Progranulin; CDK6	Neuronal survival	[40]
	miR-34	SIRT1; Bcl-2; CDK4	Neuronal survival	[39,41]
	miR-205	Lrrk2	Neuritis outgrowth	[42]
Huntington's disease	miR-9	REST/CoREST	Neurogenesis	[39]
	miR-22	HDAC4; Rcor1; Rgs2; p38; Tp53inp1	Neurogenesis and neuronal survival	[43]
	miR-128	HTT; HIP1	Neuronal differentiation	[39,44]
	miR-132	P250GAP; MeCP2	Neurogenesis	[39]
Amyotrophic Lateral Sclerosis	miR-206	HDAC4	Nerve reinnervation	
	miR-155	SOCS1; TGF-β	Immune response	[45-47]
Hereditary Spastic Paraplegia	miR-140	REEP1	Nucleation	[48]
	miR-182	SPAST	Nucleation	[49]
	miR-96		Neuronal survival	[50]
	miR-33		Neurogenesis	[51]
	miR-224	SPG7	Neuronal survival	[52]

The mutant HTT protein, which harbors an expanded polyglutamine tract, has been shown to be associated with Ago2 and P-bodies, cytoplasmic sites of RNA metabolism, and RNAi and miRNA activity. Thus, a change in miRNA activity has been proposed to be responsible for the HD defects. It has been shown that several miRNAs are indicative of the disease (miR-100, miR-139-3p, miR-196a, miR-133a, miR-330).[20,21,32,55,56]

miRNAs play a significant role in cell apoptosis and may activate dramatic changes in brain tissue. For example, miR-144 found in the aging human cerebellum and cortex can bind to the 3'UTR of programmed cell death protein 4, suggesting a possible role in apoptosis in aging brains.[20,32-34,57] Furthermore, a possible link exists between miRNA expression and cell apoptosis. miR-16, miR-128, miR-15, and miR-497 regulate B cell lymphoma 2 protein (bcl2), which induces apoptosis, also within neurons.[20] Certain studies have demonstrated that changes in miRNA expression during neurodegeneration (miR-548d, miR-224, miR-373, miR-198) show specific deregulation and altered expression in PD patients.[20,58] It appears likely that miRNAs can be used as accurate diagnostic and prognostic biomarkers for neurodegenerative diseases.

Amyotrophic Lateral Sclerosis (ALS) – Involvement of miRNA

Amyotrophic lateral sclerosis (ALS) is one of the most devastating neurodegenerative diseases; it progresses very rapidly and leads to death within 2-5 years of onset, usually beginning in the fifth decade of life. It is characterized by the neuronal death of upper and lower motor neurons, skeletal muscle atrophy, and eventually death, mainly due to the loss of respiratory muscles.[18,19,21,32] 10% of all ALS cases are understood to be hereditary. These mutations may be related to several genes, one of which is *SOD1*, coding for copper/zinc superoxide dismutase. This gene is involved in skeletal muscle atrophy, paralysis, and neuronal death. It used to appear as the best biomarker for familial ALS (fALS).[18,19,21,32-34] Another protein involved in ALS pathology, TAR DNA-binding protein (TDP-43), interacts with the Dicer and Drosha complexes responsible for miRNA biogenesis. It has been shown that mutations in this gene result in the differential expression of mature and functional miRNAs.[18,21,34] Mutations in the gene encoding fused in sarcoma/translocated in sarcoma (FUS/TLS) share a common mechanism with TDP-43, promoting the biogenesis of specific miRNAs via recruiting Drosha complexes to primary transcripts of miRNA. FUS/TLS interacts mainly with the mutant TDP-43, which dysregulates miRNA biogenesis and the overexpression of miR-132, miR-143, miR-558, and miR-9, which is identified with axonal growth.[18,21,33] In recessively inherited ALS2, a rare juvenile-onset disorder, mutations in the *ALS2* gene are responsible for the production of the abundant protein alsin, involved in neurite outgrowth and endosomal trafficking. The lack of alsin production leads to increased degradation, decreased signaling, and decreased turnover of membrane components, and these factors may underlie the disease pathology.[32,59] Neurofilament heavy peptide (NEFH) is a key protein in axonal transport and maintenance. Changes in its cross-linking properties may contribute to the aberrant neurofilamentous accumulation found in the proximal axons of ALS motor neurons directly, even in sporadic ALS.[60] A different type of mutation concerns the *C9orf72* locus containing hexanucleotide repeats of GGGCC (G_4C_2), and the expansion of the number of repeats is now considered to be the most frequent genetic cause of ALS.[18,19,21] Although the overexpression of C9orf72 is highlighted in neurodegeneration, the normal cellular function is still unknown. The protein is highly conserved and expressed in many tissues, including the cerebellum, cortex, and spinal cord.[18,19,21] Altogether, over 50 potentially causative disease-modifying genes have been identified, but for the purpose of this review, we have limited them to those with linked miRNA. For more details, see Table 2.

The lack of a common cause of ALS has resulted in difficulties with diagnosis and treatment, especially when the underlying molecular causes of pathology are unknown or unclear. A significant impact may be driven by miRNA in our understanding of their biogenesis and functions, as well as profiling their expression and their involvement in disease progression.[32-34]

In addition, studies on mouse models of ALS have shown the involvement of miRNA in the progression of neurodegeneration via the deletion of Dicer (a complex converting primary miRNA to mature miRNA). Several studies have shown that miR-206 was observed to be overexpressed in mouse model muscles of ALS, which carries mutation in *Sod1*. Another increase in the miR-338-3p level regulating the expression of several different miRNAs was observed in the leukocytes of ALS patients. Furthermore, recent studies have shown the expression of miR-23a, miR-29b, miR-455 in skeletal muscle tissue from ALS patients, which can cause deregulations in mitochondrial gene expression. miR-132, miR-134 and miR-9 were found to take place in synaptic plasticity and neuronal development.[18,21] For more details concerning ALS serum related miRNAs, see Table 2.

Table 2. Free-circulating miRNAs that regulate some ALS-related genes

Gene	Gene description	Related serum miRNA	miRNA description		Ref.
TDP-43	Approximately 5% of fALS, dysregulates miRNA biogenesis, splicing regulation, RNA transport	miR-132	Angiogenic role in oncogenesis, overexpressed in chronic lymphoblastic leukaemias	Expression is deregulated in TDP-43 deficient cells, neuritis outgrowth	[2,18,21, 32-34, 61-65]
		miR-143	Expression of miR-143 demonstrated significantly reduced levels of the miRNA in precancerous and neoplastic colorectal tissue		
		miR-558	Elevated miR-558 levels detected in gastric cancer cells		
		miR-374b-5p	Circulating miR-374b-5p negatively regulates osteoblast differentiation in the progression of osteoporosis	Decreased level in serum of ALS patients	
NFL	Play a role in intracellular transport to axons and dendrites, disorders are characterized by distinct neuropathies	miR-146a	miR-146a modulates innate immunity through regulation of Toll-like receptor (TLR) signaling and cytokine response	Directly regulate involvement in the selective suppression of NFL mRNA	[32-34,63, 64,66]
		miR-524-5p			
		miR582-3p			
MyHC	This gene is most highly expressed in muscle fibers, and encodes a protein found uniquely in striated muscle	miR-208b	Up-regulation in the skeletal muscle in ALS patients Increased levels of miR-208b and miR-499 were significantly associated with coronary artery disease (CAD) severity		[32-34,63, 67,68]
		miR-499			
SOD1	Autosomal dominant inheritance, approximately 15-20% of fALS, also detected in sporadic ALS, cytosolic antioxidant	miR-206	Elevated expression after denervation, miR-206 delays the onset and progression of ALS by promoting the regeneration of neuromuscular synapses MiR-206 has been shown to be markedly downregulated in many cancers, such as lung cancer, breast cancer, rhabdomyosarcoma and head and neck squamous cell carcinoma		[2,18,33, 34,63,69, 70-72]
		miR-23a	miR-23a was identified as over-expressed in the serum of various types of human cancer, including breast, gastric, pancreatic, and esophageal squamous cell carcinoma, as well as in malignant astrocytoma	Elevation in skeletal muscle tissue from ALS, dysregulation in mitochondrial gene expression	
		miR-29b	miR-29b exhibits low-level expression, with rapid degradation in several different diseases: cancer, kidney, cardiovascular diseases		
		miR-455	Promotes cell growth, invasion and migration in cancer		
		miR-134	Synaptic plasticity and neuronal development in ALS. Expression is associated with cell migration and invasion in different diseases		
		miR-155	Contributes to microglia-mediated immune response and neuroinflamation		
No specific gene		miR-9	Expression is elevated in induced stem cell derived neurons from ALS patients Hyper-methylation at miR-9 loci is correlated with the cancer metastasis observed in various malignancies, including breast, lung, colon, and head and neck cancers, as well as melanoma, and acute lymphoblastic leukemia		[18,32-34, 73,74]
		miR-106	Regeneration of neuromuscular junction. Abnormal expression presented in cancer		[18,32-34]
		miR-338-3p	Expression is elevated in ALS Downregulated in metastatic neuroblastoma, inhibits cell growth, invasion and migration		[18,32, 75-77]
		miR-451	Deregulated in immune cells. Downregulation in a variety of tumors including glioma, breast carcinoma, gastrointestinal carcinoma, non-small cell lung carcinoma, hepatoma, nasopharyngeal, esophageal, bladder, osteosarcoma, epithelial ovarian, renal and thyroid carcinomas		[18,32-34, 78]
		miR-218	Progressive motor neuron cell loss		[32-34,77]

Hereditary Spastic Paraplegias (HSPs)

Hereditary spastic paraplegias (HSPs) are a group of heterogeneous, genetically determined disorders resulting from neurodegeneration in the corticospinal tracts. The main clinical feature is progressive spasticity and weakness of the lower limbs. To date, 64 spastic paraplegia genes (SPG) have been identified within 79 different loci, and all modes of inheritance have been described among them. Due to the number of the HSP-causing genes, several cellular pathways implicated in its pathogenesis have been distinguished: membrane and axonal transport, endoplasmic reticulum membrane modeling and shaping, mitochondrial functions, DNA repair, fatty acids and phospholipid metabolisms, and myelination processes.[79] However, the epigenetic factors involved in the HSPs pathomechanism are barely known. There are only a few papers available in PubMed describing the possible microRNAs involvement in hereditary spastic paraplegia. Considering the known regulatory role of the miRNA molecules in neurodegeneration, a possible role of miRNA in SPG pathogenesis warrants thorough investigation. Even though there have been broad studies on the role of miRNA in neurogenetics, especially Alzheimer's (AD), Parkinson's (PD) and amyotrophic lateral sclerosis (ALS), the miRNA research on hereditary spastic paraplegia pathogenesis is limited. Some premises suggest that miRNAs are also involved in spastic paraplegia pathomechanism. The process of motoneuron degeneration shows the same pathway as observed in HSP and ALS. Thus, it is suggested that these two diseases may be linked together by shared miRNA. It is only a matter of time before both pathologies are compared at the miRNA level. One research study of this kind has shown that the regulation of *SPAST* gene (SPG4) expression is carried out by transcription factors (proteins NRF1 and SOX11) and miRNAs. The 3'UTR (UnTranslated Region) of the *SPAST* gene contains the complementary sequences for the miRNA-96 and miRNA-182 molecules, which negatively regulate the *SPAST* gene synthesis and influence the stability of the spastin mRNA level in the cell.[80] Recent reports have indicated that miRNA-33, located within the intron of sterol regulatory element binding protein (SREBP)-2, controls cholesterol homeostasis and can be a potential therapeutic target for the treatment of atherosclerosis. It had been shown that the *SPAST* gene, which encodes a microtubule-severing protein called spastin, is a novel target gene of miR-33 in humans. The miR-33 binding site in the *SPAST* 3'UTR is conserved, and it is possible to clarify the role of miR-33 on *SPAST.* It has been demonstrated that inhibition of miR-33a, a major form of miR-33 in human neurons via locked nucleic acid (LNA)-anti-miR ameliorated the pathological phenotype in HSP-SPG4 patients' induced pluripotent stem cell (iPSC)-derived cortical neurons. Thus, miR-33a may be a potential therapeutic target for novel treatment of HSP-SPG4.[51] A different study indicates that in the SPG7 type, miR-224 may target the 3'UTR of *SPG7* mRNA. This inhibits SPG7 expression by enhancing the ability to recruit a voltage-dependent anion channel (VDAC), resulting in the failing repression of SPG7 expression. *SPG7* is one of the targets of miR-224, and it is necessary for the mitochondrial permeability transition pore (MPTP) formation in multiple cell types that promote Ca^{2+}-induced MPTP opening. The miR-224 inhibitor prevents oxygen/glucose deprivation (OGD)-induced neuronal apoptosis. Downregulation of SPG7 suppresses mitochondrial signaling, which prevents VDAC from forming the MPTP involved in mitochondria-mediated apoptosis. It also inhibits OGD-induced mitochondrial membrane potential and higher mitochondrial calcium retention.[52] Another example is related to the single-nucleotide changes identified in the *REEP1* 3'UTR region in hereditary spastic paraplegia patients (SPG31). This condition is characterized by spasticity (muscle stiffness) and paraplegia (paralysis of the lower limbs) caused by degeneration of the neurons that trigger muscle movement. Degeneration of the upper motoneuron leads to a progressively spastic gait. The vast majority of individuals suffering from SPG31 show clinically pure HSP, i.e., with predominant pyramidal signs, and there is no evidence of lower-motoneuron involvement. Consistent with these clinical and genetic observations, endogenous *REEP1* has been detected in the brain and in cultured cortical neurons.[80-88]

The mutations of the *REEP1* gene are often present as insertion or deletion and result in a short, nonfunctional protein.[81,83,85,87] It is assumed that the 3'UTR variants consist of the complementary sites of miRNAs. miRNA-140 and miRNA- 691 binding sites may affect the post-transcriptional regulation of the *REEP1* gene expression.[82,84,88]. Table 3 sums up the data on serum-related miRNAs involved in HSP with related genes.

The receptor expression-enhancing protein 1 (REEP1) is found in neurons of the brain and spinal cord, accumulating in mitochondria and the endoplasmic reticulum, responsible for protein processing and transport. The REEP1 protein forms a network of tubules of the endoplasmic reticulum, regulating its size and determining protein processing. As part of its role in the endoplasmic reticulum, the REEP1 protein enhances the activity of certain other proteins called G protein-coupled receptors. These receptor proteins are eventually embedded within the outer membrane of cells, where they relay chemical signals from outside

the cell to its interior. The exact function of the REEP1 protein in the mitochondria is not fully known.[82,86]

It is unclear how *REEP1* gene mutations lead to the signs and symptoms of spastic paraplegia type 31. Researchers have shown that mitochondria in the cells of affected individuals are less able to produce energy, which may contribute to the death of neurons and lead to the progressive movement problems of spastic paraplegia type 31. However, the exact mechanism that causes this condition is unknown, and we cannot be certain that miRNAs are not involved in this process. So far, there is no evidence for this because of the limited amount of research conducted to date. But this does not change the fact that miRNA could play a significant role in *REEP1* gene mutations.[81,83-88]

Table 3. Free-circulating miRNAs that regulate some HSPs - related genes

Gene (protein)	Gene description	Related miRNA	miRNA description		Ref.
SPAST (spastin)	Microtubule-severing activity, early secretory pathway, BMP signaling, plays role in the cell activity, components and protein regulation, particularly in neurons	miR-182	Overexpressed in human metastatic melanoma cell lines and its expression increased with progression from primary to metastatic melanoma	Post-transcriptional regulation of endogenous SPAST mRNA and spastin protein level	[49-51, 80,89]
		miR-96	miR-96 promotes cell proliferation, migration and invasion by targeting PTPN9 in breast cancer		
		miR-33	Decreases the neurite length of cortical neurons derived from iPSCs through *SPAST* 3'UTR regulation		
REEP1	ER-shaping protein, ER-microtubule interaction, mitochondrial function, mutations have been linked to neurodegenerative disorders of upper and lower motor neurons	miR-140	MIR140 is almost exclusively expressed in chondrocytes and regulates gene expression mainly at the post-transcriptional level	No specific description in HSPs	[19,48, 82,89]
		miR-691	–		
SPG7	Encodes a mitochondrial metalloprotease protein that is a member of the AAA family. Members of this protein family share an ATPase domain and have roles in diverse cellular processes including membrane trafficking, intracellular motility, organelle biogenesis, protein folding, and proteolysis	miR-224	miR-224 inhibitor may suppress neuronal apoptosis by targeting SPG7 mRNA and thereby preventing MPTP formation.		[52]

Conclusion and Future Perspectives

Despite constantly evolving diagnostic methods and the significant development of research on miRNAs as biomarkers of neurodegeneration, the research into ALS and HSP is still at a very early stage. When it comes to miRNA research in ALS, the level of it is much higher than that into HSP, where, due to the high heterogeneity and rarity of the particular types of the disorder, research in terms of miRNAs is hardly carried out.

Another issue is the limitation of patients included in the studies. These disorders are rare, and as a rule, the study groups are small in number, making it difficult to compare the studies with each other, due to the number of respondents and the variety of research methods.

Furthermore, due to the limited number of ongoing scientific projects, it is difficult to characterize the individual miRNAs that bring together both diseases. Future research should include a large-scale sequencing panel of potential miRNAs that may be associated with neurodegenerative pathology.

It has been demonstrated that miRNAs are potential biomarkers for detection and differentiation and therapeutic targets in neurodegenerative disorders. miRNAs have been suggested as having both high diagnostic effectiveness and promising value as non-invasive biomarkers, and as a consequence, potentially promising therapeutic targets. Recent evidence suggests the existence of specific expression signatures in some brain pathologies, though the findings are diverse from study to study. Therefore, future research on circulating miRNAs to better define their potential for early diagnoses, and to monitor disease progression and response to therapies are required.[10,32,51,52,77,90-93]

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The authors declare no conflict of interest.

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