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Emerging Roles of Non-Coding RNA in Neuronal Function and Dysfunction

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Abstract

Advancements in RNA sequencing technologies in recent years have contributed greatly to our understanding of the transcriptome and the now widely recognized multifaceted functions of RNA. The discovery and functional analysis of an increasing number of novel small non-coding RNAs (ncRNAs) has highlighted their importance as critical regulators of gene expression and brain function. In particular, two diverse classes of ncRNAs, microRNAs (miRNAs) and tRNA-derived small RNAs (tsRNAs), are especially abundant in the nervous system and play roles in regulation of gene expression and protein translation, cellular stress responses and complex underlying pathophysiology of neurological diseases. This chapter will discuss the most recent findings highlighting the dysregulation, functions and regulatory roles of ncRNAs in the pathophysiological mechanisms of neurological disorders and their relevance as novel biomarkers of injury and therapeutic agents.

Keywords: non-coding RNA (ncRNA), microRNA (miRNA), tRNA-derived small RNAs (tsRNA), tRNA-derived stress-induced RNA (tiRNA), tRNA fragments (tRFs), epigenetics, molecular biology, neurological disorders

1. Introduction

Normal neuronal function and development is reliant on tightly controlled regulation of gene expression at many levels. Advancements in transcriptomics and functional validation has elucidated key biological roles for non-coding RNAs (ncRNAs), transcripts do not encode proteins, in the regulation of a wide range of neuronal functions and pathophysiological processes. Over the past two decades large international collaborative research efforts such as the Human Genome Project and the ENCODE (Encyclopedia of DNA Elements) project have estimated that approximately 80% of the mammalian genome transcribes ncRNA and that 97% of RNA transcripts in the cell are non-coding [1–3]. This remarkable and unexplored area of molecular biology has since yielded many more types of ncRNA that have been shown to play a crucial role in a variety of biological processes.

ncRNA are classified either by their length or by functionality (**Table 1**). Small ncRNA are considered transcripts <200 nucleotides (nts) in length and long ncRNA are those >200 nts. Housekeeping ncRNA are constitutively expressed and are involved in mechanisms of cellular activity that are vital for cell viability. These include rRNA, tRNA and the more recently identified small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and telomerase RNA (TERC). Regulatory ncRNA

Class	Abbreviation	Full name	Size (nts)
Housekeeping RNAs	rRNA	Ribosomal RNA	120–4500
	snoRNA	Small nucleolar RNA	60–400
	snRNA	Small nuclear RNA	100–300
	tRNA	Transfer RNA	76–90
	TERC	Telomerase RNA	—
Regulatory RNAs	circRNA	Circular RNA	100–10,000
	eRNA	Enhancer RNA	50–2000
	lncRNA	Long non-coding RNA	>200
	tsRNA	tRNA-derived small RNA	16–50
	piRNA	piwi-interacting RNA	26–32
	siRNA	Small interfering RNA	20–25
	miRNA	microRNA	21–23
	Y RNA	—	—

Table 1.
Classification of ncRNAs.

regulate gene expression through epigenetic, transcriptional and post-transcriptional mechanisms, and include microRNA (miRNA), tRNA-derived small RNA (tsRNA), piwi-interacting RNA (piRNA), long non-coding RNA (lncRNA) and circular RNA (circRNA) [4].

This chapter will focus on two classes of ncRNA, miRNA and tsRNA, which are highly enriched in the central nervous system (CNS) with important roles in neuronal function and dysfunction. The central roles played by these classes of ncRNAs and their dysregulation in disease, particularly their ability to regulate multiple genes, place them as promising biomarkers and therapeutic targets, entering many clinical trials.

2. microRNA

2.1 miRNA biogenesis and mechanism of action

miRNAs are transcribed by RNA polymerase II/III from either independent miRNA genes (monocistronic), as clusters of up to a few hundred miRNA (polycistronic) or from the introns of protein-coding genes (intronic). Approximately half of miRNAs are considered intronic, however a functional relationship between miRNAs and host genes is rarely found. Long primary miRNA (pri-miRNA) transcripts are processed in the nucleus by a microprocessor complex containing ribonuclease III, Drosha, and RNA-binding protein subunit DGCR8 (DiGeorge syndrome critical region 8). Cleavage of the pri-miRNA by Drosha results in a 2 nt 3' overhang and the characteristic 'hairpin' structure of the 65 nt precursor miRNA (pre-miRNA). The pre-miRNA is then exported to the cytosol by the exportin-5 (XPO5)/RanGTP complex, where it is further processed by the endonuclease Dicer, removing the terminal loop resulting in a double stranded miRNA containing the mature miRNA guide strand and passenger strand, typically 21 – 23 nts in length (**Figure 1A**).

The RNA-induced silencing complex (RISC) is a heterogeneous multi-protein complex that uses one miRNA strand as a template to target complimentary mRNAs

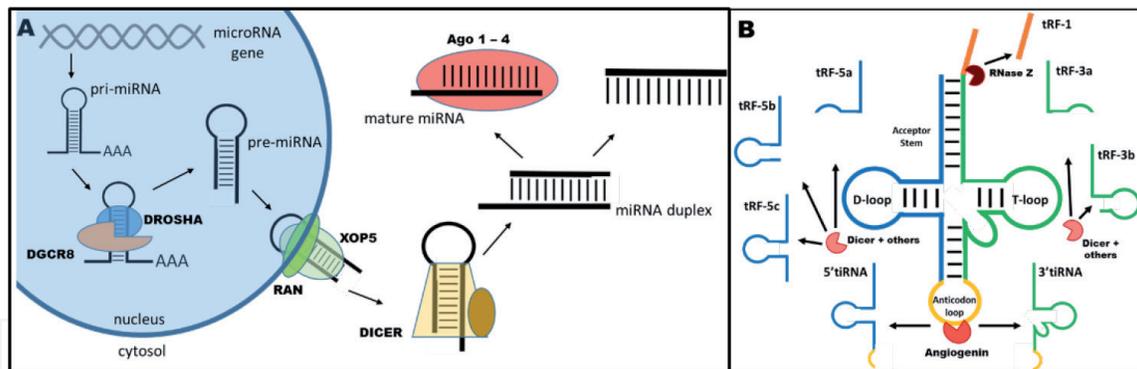


Figure 1.
Illustration of the biogenesis of (A) miRNA and (B) tsRNA.

for degradation or translational repression, post-transcriptionally regulating gene expression. The double-stranded miRNA duplex is loaded into a binding pocket within an Argonaute family (Ago1-4) protein, which constitutes the principal component of RISC, mediated by Hsc70/Hsp90. The miRNA is unwound to single-stranded miRNAs and one 'guide strand' is anchored into the Ago protein, determining the specificity of the RISC, while the passenger strand is subject to degradation. The directionality of the mature miRNA guide strand originating from the 5' or 3' arm of the pre-miRNA duplex determines the miRNA-5p and -3p species. While typically one strand is preferentially loaded, for some miRNA duplexes both arms can give rise to functional mature miRNAs that can be loaded into Ago proteins and used to guide the RISC to mRNA transcripts.

Recognition of target mRNA occurs by complementary base pairing between the miRNA seed region (2–8 nt) of the 5' end of the guide strand and the mRNA transcript, typically within the 3' UTR; however miRNA can also bind within mRNA promoter regions, the coding sequence, and 5' UTR. The Ago protein present and the degree of complementarity between the guide and target strand determines the mechanism of gene silencing, triggering target degradation or translational repression. Importantly, the short seed sequence requirement for mRNA targeting confers ability for individual miRNAs to target multiple genes across several different pathways. Similarly, an individual mRNA may contain target sites for multiple miRNAs, placing miRNAs in a powerful position in the regulation and modulation of the transcriptomic landscape. Dysregulation of miRNAs, therefore, has significant implications and consequences for biological functions in physiological and pathological conditions.

2.2 miRNA functions

2.2.1 Neuronal development and function

An extensive catalog of work has demonstrated the involvement of miRNAs across the development, function and maintenance of the CNS. The cell-specific deletion of Dicer inhibits the maturation of miRNA and has been shown to delay embryonic CNS development, alter dendritic and spine morphology and lead to early postnatal death [5–7]. Specific miRNAs have been identified with central roles in regulation of adult neural stem cell proliferation [8–10] and the differentiation of cells into specific neuronal sub-types [11–14]. Post-transcriptional regulation of N-cadherin expression by the miR-379-410 cluster mediates neuronal migration [15] and miR-132 is involved in the activity-dependent integration of neurons into the adult dentate gyrus [16].

The controlled extension of neuronal processes as well as the generation of adaptable synapses are key in the development of functional neural networks in the CNS. A number of miRNAs have been closely associated with the regulation of axonal and dendritic morphology, and synaptic plasticity. Neurite outgrowth is highly dependent on extracellular trophic cues that stimulate cAMP response element binding protein (CREB) transcription factor, a target of which is miR-132. In axons, miR-132 downregulates the activity of the GTPase-activating protein p250 GAP resulting in axonal sprouting [17]. A number of counteracting miRNAs tightly regulate axonal length. The miR-17/92 cluster downregulates *PTEN* resulting in activation of the mTOR pathway and axonal extension [18], whereas miR-9 has been shown to locally repress *Map1b* expression and inhibit axonal growth [19]. Conversely, miR-9 promotes dendritic development and its loss results in reduced dendritic length and complexity [20]. Similar to axonal extension, miR-132 has been shown to positively regulate dendritic length, arborization and spine density in dendritic extensions in an activity-dependent manner [21, 22]. miR-132-mediated regulation of spine density has been attributed to its direct association with matrix metalloproteinase-9 [23] and miR-132-mediated repression of p250GAP in dendritic spines has been associated with Leptin-induced synaptogenesis [24]. In *Drosophila melanogaster* miR-284 has been shown to affect the expression of the glutamate receptors GluRIIA and GluRIIB indicating a role in the regulation of synaptic strength [25] and in higher order animals the inhibition of miR-132 and miR-219 have been associated with disturbed circadian rhythm and the impairment of memory acquisition [26].

2.2.2 Inflammation

Inflammation in the CNS is an important process for the alleviation of infection or the resolution of cerebral damage; however, aberrant or chronic inflammation has been implicated in a number of neurological disorders [27]. Microglial cells, the resident immune cells of the CNS, are enriched in a number of miRNAs [28] and expression of these is altered in response to inflammatory stimuli [29]. Specific miRNAs have been associated with the development of a pro- or anti-inflammatory phenotype. miR-155 is a well-studied pro-inflammatory mediator in macrophages and microglia, targeting a number of anti-inflammatory regulators for degradation induced in response to NF- κ B dependent TLR signaling. Furthermore, p53-mediated induction of miR-155 is known to target anti-inflammatory transcription factor *c-Maf*, resulting in a pro-inflammatory reaction [30]. miR-124 and miR-146a are both widely reported negative regulators of CNS inflammation, down-regulating inflammatory mediators. miR-146a is also induced through TLR/NF- κ B-dependent signaling in response to various immune mediators, and subsequently reduces NF- κ B transcriptional activity. miR-146a expression is inversely correlated with inflammatory-related proteins [31]. Similarly miR-124, a highly abundant neuronal and immune cell miRNA, has been reported to negatively regulate TLR signaling [32] promote microglial quiescence, and reduce microglial MHC-II, TNF- α and ROS production [33].

2.2.3 Apoptosis

Neuronal cell death is a key feature in neurodegenerative diseases and has been shown to involve a number of miRNAs. In models of spinal cord injury, activation of miR-21-5p and miR-494 as well as the inhibition of miR-29b, reduced apoptosis through stimulation of the AKT/mTOR signaling pathway [34–36]. Specific miRNAs have been shown to have a more direct effect on the apoptotic cascade. Indeed, the inhibition of miR-24, miR-497, miR-15a/16-1, miR-181a and miR-106b-5p

increases expression of anti-apoptotic proteins Bcl-w, Bcl-2 and Bcl-xl resulting in attenuation of neuronal apoptosis [37–41].

3. tsRNA

Previously thought of as simple degradation products, tsRNA are cleaved fragments of full tRNA transcripts. In eukaryotes, tRNA genes are transcribed by polymerase III and the 5' leader sequence and 3' trailer sequences are removed from the pre-tRNA sequence by the endonucleases RNase P and RNase Z, respectively [42–44]. The mature tRNA is generated by the addition of a CCA tail by CCCase [45, 46]. Mature tRNAs are 73–90 nt long with a classic 'cloverleaf' secondary structure consisting of an anti-codon loop that recognizes mRNA codons, an acceptor stem that binds amino acids, a dihydrouridine (D) loop, a thymidine (T) loop and a variable (V) loop [47]. tRNA are a highly modified species with over 170 independent modifications reported to date [48]. These modifications are largely localized on the anticodon loop, affecting the speed and accuracy of decoding, or the structural core of the molecule affecting stability and degradation pathways [49, 50]. tsRNA are generated from the cleavage of tRNA by endonucleases and are classified by their cleavage site and length as either tRNA-derived stress-induced RNA (tiRNA) or tRNA fragments (tRFs).

3.1 Biogenesis and structure of tsRNA

3.1.1 tiRNA

The generation of tiRNA occurs when the stress-induced RNase angiogenin (Ang) cleaves mature tRNA at the anticodon loop [51]. This produces transcripts 31–40 nts long that are defined as either 5' or 3' tiRNA depending of the presence of a 3' or 5' end at the anticodon loop respectively (**Figure 1B**). The production of Ang is mediated by the transcription factor hypoxia-inducible factor-1 α (HIF-1 α) and thus tiRNA generation is closely linked with cellular stress [52]. Accumulation of tiRNA is known to occur following oxidative stress, heat shock, UV radiation, hypoxia and starvation [53–56].

3.1.2 tRF

tRFs are shorter transcripts of 14–30 nts that are produced by cleavage of tRNA at the D-loop, T-loop or stem region by Ang, Dicer and another yet to be identified member of the RNase superfamily [57]. Cleavage of tRNA at the D-loop generates fragments of three different lengths—tRF-5a (14–16 nts), tRF-5b (22–24 nts) or tRF-5c (28–30 nts). Similarly, cleavage at the T-loop produces tRF-3a (18 nt) or tRF-3b (22 nt). The cleavage of pre-tRNA at the 3' end results in the generation of tRF-1 (**Figure 1B**) [57, 58].

3.2 tsRNA mechanism of action

3.2.1 Gene silencing

Similar to miRNAs, tRFs have been associated with the epigenetic regulator RISC, however mechanistic details on the role of tsRNA in the RISC remain to be elucidated. A recent meta-analysis of short RNA libraries from HEK293 cells demonstrated that both tRF-3 and tRF-5 associate with Ago proteins; however, a

preference for Ago1, 3 and 4 over Ago2 was identified [59]. Interestingly a subsequent study in *D. melanogaster* revealed an age-related shift in tRF-Ago binding demonstrating a preference for Ago2 binding over Ago1 with increasing age [60].

3.2.2 Regulation of protein translation

The synthesis of protein is a central activity in all cells that consumes a high level of energy and is dynamic in response to metabolic conditions and external stimuli. The regulation of protein translation therefore is a vital process in the maintenance of cell viability and the stress response. Canonical cap-dependent translation begins with the formation of the eukaryotic initiation factor (eIF) 4F complex containing eIF4A, a DEAD-box helicase, eIF4E and eIF4G. The eIF4E subunit binds to the 5' m⁷GTP cap on target mRNA and the eIF4G subunit is a scaffold protein that mediates the recruitment of other proteins including eIF3 and poly(A) binding protein (PABP). eIF4F binding to the 5' m⁷GTP cap and the 3' poly(A) tail circularizes the target mRNA and allows the 48S pre-initiation complex, containing the 40S small ribosomal subunit, Met-tRNA_i^{met} and eIF2, to scan the 5' untranslated region and find the AUG start codon [61].

The dynamic regulation of protein translation in response to cellular stress and metabolic conditions is vital to cell survival. Stress-induced Ang-generated 5'tiRNA have been shown to halt the initiation of protein translation and facilitate the packaging of stalled translational complexes into stress granules [54]. Stress granules are cytoplasmic RNA-protein complexes that rapidly assemble and disassemble in response to cellular stress. This sequestration allows for the utilization of energy stores elsewhere and the recommencement of protein translation under optimum conditions [62]. Specific 5'tiRNA that contain a terminal oligoguanine (TOG) motif form stable G-quadruplex (G4) structures that directly bind the HEAT domain of eIF4G displacing eIF4A and inhibiting scanning of the mRNA target. Furthermore, 5'tiRNA with a 5' monophosphate modification have been shown to bind the RNA binding protein YB-1 via the cold shock domain to precipitate the formation of stress granules [63].

Current knowledge on the effect of tRFs on protein translation is less advanced. Research in prokaryotic cells has demonstrated that tRF-5c of Val-GAC can bind the small ribosomal subunit and interfere with peptidyl transferase activity thereby inhibiting protein translation [64, 65]. In eukaryotic cells the tRF-3b of Gly-GCC reduced the level of specific protein with no concomitant reduction on mRNA levels indicating regulation at the translational level [66].

3.3 tsRNA functions

3.3.1 tsRNA and apoptosis

Disruption to the tsRNA system has been associated with increased cell death. Hypo-methylation of tRNA that arises from the inhibition of NSun2 increases cleavage by Ang and the accumulation of 5'tiRNA. The subsequent sustained depression in protein translation results in neuronal shrinkage, impaired synapse formation, cell death and is associated with neurodevelopmental deficiencies [67]. Loss-of-function mutations in the RNA kinase CLP1 has been shown to increase the level of tyrosine pre-tRNA fragments resulting in exaggerated p53 activation and vulnerability to cell death in cells exposed to oxidative stress [68]. Conversely, Ang has been shown to reduce cell death in neurons exposed to hyperosmotic stress in a tiRNA-mediated fashion. Specific Ang-generated tiRNA interact with cytochrome c and form a ribonucleoprotein complex that limits the formation of apoptosomes and reduces caspase-3 activation [53].

3.3.2 tsRNA and inflammation

Little research has been carried out on the involvement of tsRNA in the immune system; however, the expression of 5'tiRNA and tRF5 has been reported in mouse leukocytes and human monocytes respectively [69]. It is possible that tsRNAs play a regulatory role in the cellular response to inflammatory signals. In human chondrocytes, the pro-inflammatory cytokine IL-1 β was shown to increase the expression of specific tRF-3s [70]. These fragments downregulated the cytokine signaling molecule JAK3 in an Ago-dependent manner. Furthermore, tsRNA may possess the ability to stimulate the immune response with reports demonstrating that tsRNA bind directly to Toll-like receptors on T-helper 1 and cytotoxic T cells [71].

4. ncRNA in neurological disease

4.1 Parkinson's disease

Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic neurons resulting in the deterioration of motor function. Altered expression of miR-133b has been reported in the midbrain of PD patients. This is notable as the transcription factor *Pitx3* is a target of miR-133b and is involved in the maturation and function of dopaminergic neurons [12]. Gain-of-function mutations to the leucine-rich repeat kinase 2 (LRRK2) has been closely associated with both sporadic and inherited forms of PD [72]. A reduction in miR-205 has been observed in sporadic PD patients with increased LRRK2 protein expression. Furthermore, *in vitro* studies revealed that miR-205 reduces LRRK2 expression and alleviates its neurodegenerative effect [73]. Conversely, LRRK2 has been shown to disrupt miR-187* and let-7-mediated regulation of protein translation resulting in a pathogenic overproduction of E2F1/DP [74]. Another PD-related gene *SNCA* has been reported as a potential target of miR-7, miR-153 and miR-433 [75, 76]. miR-124 has been reported to play protective roles in dopaminergic neuronal apoptosis and autophagy in PD by regulating the AMPK/mTOR pathway. Suppression of miR-124 was shown to regulate AMPK/mTOR signaling, significantly increasing p-AMPK activity and autophagy-associated Beclin 1 and LC3 II/LC3 I ratio [77].

In two independent studies, variations to the tsRNA-generating enzyme Ang have been reported in a subset of PD patients [78, 79]. Altered expression of tsRNA have also been reported in the amygdala [80], prefrontal cortex, cerebral spinal fluid and serum of PD patients [81]. Further work is required to elucidate the involvement of tsRNA in the pathogenesis of PD.

4.2 Alzheimer's disease

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by the progressive loss of cognition and memory due to severe neuronal cell loss. Hallmarks of the disease include the formation of extracellular amyloid plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau. Given the high degree of cell death and chronic inflammation in the CNS, it is unsurprising that a large number of miRNAs are differentially expressed in AD [82–85], however, a number of miRNAs have also been shown to affect the pathogenic mechanisms of the disease.

Alternative splicing of amyloid precursor protein (APP), the parent molecule of pathogenic A β , is regulated by miR-124. Indeed, miR-124 is downregulated in the AD brain and its expression was shown to inhibit polypyrimidine tract binding

protein 1 (PTBP1) resulting in increased APP with exon 7 and 8 inclusion [86]. Alterations of this kind have been associated with increased A β production [87]. Furthermore, miR-98 reduces the expression of insulin-like growth factor 1 (IGF-1) which is involved in the processing of APP. Overexpression of miR-98 downregulates IGF-1 resulting in increased A β production and tau phosphorylation [88]. The expression of tau is affected by the levels of miR-34a and miR-26b [89, 90]. Overexpression of miR-26b also leads to aberrant cell cycle entry that involves the nuclear export and activation of cyclin-dependent kinase 5 (CDK5), a major kinase involved in tau phosphorylation [89]. Finally, pro-inflammatory NF κ B-associated miRNAs such as miR-7, miR-9, miR-34a, miR-125b, miR-46a and miR-155 are all upregulated in AD [85]. Presenilin 2 (PS2) mutations have been implicated in the development of autosomal dominant AD, and microglial knockout of PS2 reduces miR-146 expression and results in an increased pro-inflammatory response [91]. The level of inflammation in the CNS is a strong determining factor for disease progression in AD [92].

Limited work has been carried out to date on the involvement of tsRNA in AD; however, similar to PD, mutation of tsRNA-generating enzyme Ang has been identified. In an Italian cohort of AD patients nonsense mutations in *ANG* were identified with 0.2% frequency resulting in a 51 amino acid shortening in the protein [93].

4.3 Stroke

Stroke remains one of the leading causes of death and disability worldwide, conferring a high morbidity, disability, and mortality. Cerebral ischaemia triggers a complex cascade of physiological, biochemical and gene expression changes primarily resulting from impaired cellular energetics and the collapse of ion gradients. In particular, ischaemia-mediated glutamate elevation and subsequent over-activation of glutamate N-methyl-D-aspartate (NMDA) receptors is central to excitotoxic neuronal injury and cell death during ischaemic stroke [94, 95].

miR-107 has been shown to play a key role in the regulation of excitotoxicity in ischaemic neuronal injury, associated with increased glutamate accumulation both *in vivo* and in ischaemic stroke patients [96]. Increased miR-107 following ischaemic stroke inhibits GLT-1 expression, an abundant glutamate transporter, resulting in the accumulation of glutamate. Hypoxamir miR-210 has been widely reported as a miRNA ubiquitously expressed in ischaemic cells and tissues, with a central role in adaptation to low-oxygen environments such as tumourigenesis and ischaemia [97]. Robust induction of miR-210-3p following ischaemic stroke *in vivo* has been associated with modulation of PI3K-p70S6K signaling in response to AMPK activation and NMDA receptor-mediated glutamate excitotoxicity [98]. A number of other miRNAs have also been reported to play roles in the regulation of glutamate neurotransmission and excitotoxicity in ischaemic stroke, including miR-223, miR-181, miR-125a, miR-125b, miR-1000, miR-132 and miR-124a [99].

miR-223 has been shown to regulate the functional expression of glutamate receptor AMPAR subunit GluR2 and NMDAR subunit NR2B, which control neuronal excitability in response to glutamate, reducing neuronal excitability and cell death by inhibition of NMDA-induced calcium influx in hippocampal neurons [100]. One of the most abundantly expressed neuronal miRNAs, dysregulation of miR-124 has been implicated in many CNS disorders and has been shown to be downregulated following ischaemic stroke [101]. Downregulation of miR-124 *in vivo* following ischaemic stroke has been associated with upregulation of death-associated protein kinase 1 (DAPK1), identified as a direct target of miR-124, caspase-3, and cleaved caspase-3, while over-expression of miR-124 was shown to significantly decrease DAPK1, caspase-3, cleaved caspase-3 levels and reduce

NMDA- and oxygen-glucose deprivation (OGD)-induced neuronal death *in vivo* [102]. Moreover, the neuroprotective role of miR-124 has been associated with decreased expression of pro-apoptotic protein Bax and increased expression of anti-apoptotic Bcl-2 and Bcl-xl [103].

In the context of inflammation associated with cerebral ischaemia, miR-181c has been shown to inhibit prominent pro-inflammatory cytokine TNF- α in response to OGD, reducing microglial activation and neuronal cell death [104]. Furthermore, miR-216a, miR-3437b and miR-126-3p or -5p have also been associated with regulation of TNF following cerebral ischaemia.

Recent studies have shown tiRNAs to be upregulated following ischaemia in models of OGD *in vitro* and following ischaemic-reperfusion injury *in vivo*. Rapid and response-specific increases in tiRNA levels have been shown to correlate with degree of tissue damage, highlighting the potential role of tiRNA detection as a stress biomarker of injury [55, 56, 105, 106]. Furthermore, upregulation of 5' tiRNA fragments has been shown to inhibit endothelial angiogenesis following ischaemic stroke, indicating a role in modulating cerebral responses to ischaemic injury [106].

4.4 Amyolateral sclerosis

Amyolateral sclerosis (ALS) is the third most common neurodegenerative disease and is characterized by the rapid degeneration of cortical and spinal motor neurons leading to paralysis and death within 3–5 years of diagnosis [107, 108]. Approximately 90% of cases are sporadic, however a number of genetic mutations have been identified that account for 11% of sporadic and 70% of familial ALS [107, 109]. Mutations involving superoxide dismutase (SOD1), fused in sarcoma (FUS), TAR DNA-binding protein 43 (TDP43) and a hexanucleotide repeat expansion on chromosome 9 in open reading frame 72 (C9ORF72) have all been associated with ALS pathology [109].

Deregulation of miR-142-3p has been identified in both SOD1 and TDP-43 mutant mice, as well as in serum from ALS patients. Subsequent bioinformatic analysis identified TDP-43 and C9orf72 as targets of miR-142-3p, further implicating this miRNA in ALS pathology [110]. The skeletal muscle-specific miRNA, miR-206, regulates myogenesis, promotes the formation of neuromuscular junctions and is upregulated in ALS [111, 112]. This protective response occurs early in disease progression and plateaus [111], and higher levels of miR-206 are found in spinal ALS which is associated with lower atrophy rates [113]. Upregulation of miR-155 has been identified in both sporadic and familial ALS, and its inhibition in SOD1 mutant mice resulted in increased survival [114]. Finally, a number of miRNA associated with regulation of oxidative stress are altered in ALS. X-linked inhibitor of apoptosis (XIAP) and the Nrf2-ARE pathway have been closely associated with neuronal dysfunction in ALS and are regulated by miR-34a and miR-27a [115, 116].

As seen with other neurodegenerative diseases, mutations to *ANG* have been identified in ALS and repeatedly validated in independent cohorts [79, 117–119]. Characterization of these mutations determined a reduction in ribonuclease activity and nuclear translocation of Ang [117]. Interestingly, the Ang-generated tiRNA 5' ValCAC is increased in SOD1^{G93A} mice at symptom onset and correlate with Ang expression and slower disease progression. Furthermore, increased 5' ValCAC in ALS patient serum samples is correlated with slower disease progression [120].

4.5 Epilepsy

Epilepsy is a heterogeneous group of disorders characterized by spontaneous and recurrent seizures that affects approximately 50 million people worldwide [121].

In the majority of instances, seizures can be controlled, however approximately 30% of cases are treatment resistant. Seizures arise from abnormal synchronous activity in hyperexcitable neuronal networks and while this can be attributed to altered electrophysiological properties of ion channels and neurotransmitter systems, converging lines of research have also indicated a central role for the regulation of protein translation [122, 123].

As described in Section 2.2.1, miRNAs play a key role in neuronal excitability and connectivity making them prime targets in epilepsy research. The growth, spine density and arborization of dendrites are directly regulated by miR-132, miR-134 and miR-9 [20–24, 124, 125]. miR-132 is significantly increased in the hippocampus of experimental mice undergoing seizure and its inhibition has been shown to increase neuronal survival and reduce seizure frequency [126, 127]. Upregulation of miR-134 has been identified in resected hippocampal and neocortical tissue of patients with treatment-resistant temporal-lobe epilepsy [128]. This was also observed in a number of animal models where inhibition of miR-134 was shown to reduce seizure occurrence and increase spine volume in hippocampal neurons [128–130]. Neuronal potassium channel expression is regulated by miR-92a and miR-324. miR-92a has been shown to be increased in temporal lobe epilepsy patients, and in animal models of epilepsy inhibition of miR-324 delays the onset of spontaneous seizures [131, 132]. Finally, the Ca²⁺ extruding pump ATP2B4 and the sodium-potassium-chloride transporter NKCC1 are regulated by miR-129 and miR-101a respectively. miR-129 is increased in temporal lobe epilepsy patients and inhibition of miR-129 and miR-101a have been shown to reduce hyperexcitability in animal models of epilepsy [133, 134].

Recently, serum from two independent cohorts of temporal-lobe epilepsy patients have revealed increased levels of three 5'tRFs, 5'AlaTGC, 5'GluCTC and 5'GlyGCC. These tRFs were detected in resected hippocampal and cortical tissue and were not associated with any disease related lesions. Furthermore, these fragments were detected in primary mouse hippocampal neurons and their expression was shown to be activity-related [135].

5. ncRNA as a biomarker for disease

5.1 ncRNA biomarkers in Parkinson's disease

A number of candidate miRNAs have been identified in plasma from PD patients by microarray, and validation in an independent cohort revealed the expression of miR-1826/miR-450b-3p, miR-626 and miR-505 were significantly different between control and PD subjects [136]. In a larger study, miRNAs known to be expressed in the CNS and involved in neuronal regulation were identified in the plasma of PD patients. The expression of miR-137 was increases and expression of miR-124 was decreased in PD patients compared with controls; however, there was no relation between these alterations and the severity of disease [137]. Using sequencing technologies a number of studies have identified miRNA candidates as biomarkers for PD. Subsequent validation by RT-PCR determined that miR-195 was increased and miR-185, miR-15b, miR-221, miR-181a, miR-141, miR-214, miR-146b-5p, miR-193-3p, miR-29c, miR-146a, miR-214 and miR-221 were decreased in PD patients [138–140]. Finally, one study identified differential expression of miR-1-3p, miR-22-5p and miR-29a-3p in the whole blood of PD patients using PCR [141]. It is important to note here that no miRNA has yet been identified as a biomarker for PD in two independent studies.

A number of independent studies have identified *Ang* variants in PD [78, 79], however the investigation of tsRNA as a biomarker for the disease is in its infancy. Using deep sequencing analysis of postmortem tissue Pantano et al. identified that tsRNA clusters can accurately differentiate between control, PD patients at premotor and motor stages of the disease [80]. Furthermore, in a small study sex-specific tsRNA differences were reported in the prefrontal cortex, cerebrospinal fluid and serum of PD patients [81].

5.2 miRNA biomarkers in Alzheimer's disease

In 2014, two independent studies reported a downregulation of miR-125b in the serum of AD patients and Tan et al. correlated this with cognitive decline [142, 143]. Interestingly, a number of studies have also identified multiple miRNA panels that demonstrate diagnostic value. In a small cohort of patients a group of 7 miRNAs were shown to be differentially expressed in the plasma of AD patients [144]. In a larger study, next-generation sequencing identified 140 differentially expressed miRNAs. Subsequent validation studies using RT-PCR in a cohort of 202 patient samples demonstrated a 12-miRNA signature to differentiate between AD and control samples to a high degree of sensitivity [145]. Finally, next-generation sequencing of exosomes extracted from the blood revealed a 16-miRNA signature differentially expressed in AD patients. Validated by RT-PCR and combined with known risk factors such as age, sex and apolipoprotein ϵ 4 allele status provided prognosis with high sensitivity [146].

5.3 miRNA biomarkers in stroke

The multi-targeting potential of miRNAs places them in a powerful position in the diagnosis and prognosis of heterogeneous conditions such as stroke, where early diagnosis has significant implications for prognosis. A number of miRNAs have been shown to demonstrate diagnostic and prognostic value in acute stroke, and a recent systemic review and bioinformatic analysis has highlighted and identified the most promising candidates [147]. miR-16 has been identified as significantly upregulated in the plasma of acute ischemic stroke (AIS) patients, and upregulation of miR-16 is associated with poorer prognosis (mRS 3–6) [148, 149]. Independent studies have identified the downregulation of miR-126 in plasma from AIS patients as a biomarker of disease severity. Circulating levels of miR-126 negatively correlated with pro-inflammatory cytokine levels and National Institute of Health Stroke Scale (NIHSS) scores [150–152]. Similarly, downregulation of circulating miR-355 has also been reported as having high sensitivity as a biomarker of acute ischaemic stroke and to correlate negatively with NIHSS scores in AIS patients [153].

Upregulation of miR-130a has been reported as a potential biomarker in the diagnosis of brain oedema and prognosis in haemorrhagic stroke, positively correlating with NIHSS and mRS scores [154]. Moreover, antagonism of miR-130a expression in *in vivo* and *in vitro* models of ischaemia demonstrated attenuation of brain oedema and reduced blood-brain barrier permeability.

5.4 ncRNA biomarkers in amyotrophic lateral sclerosis

In a series of studies Freischmidt et al. identified a number of miRNAs differentially expressed in the serum of familial (miR-143-5p/3p, miR-132-5p/3p and miR-574-5p/3p) and sporadic (miR-1234-3p and miR-1825) ALS, noting that miRNA targets in familial ALS were TDP-43 binding RNAs and that the miRNA signature in sporadic ALS was highly heterogeneous [155, 156]. A subsequent study determined

increased miR-374b-5p, and decreased miR-206 and miR-143-3p in sporadic ALS patient serum [157]. Finally, increased expression of miR-424 and miR-206 in sporadic ALS patient plasma has been shown to correlate with clinical deterioration over time [111].

Recent work has identified 5'tiRNA^{Val-CAC} as a potential biomarker for ALS. This tiRNA was found to be increased in the spinal cord of SOD1^{G93A} mice and is significantly increased in the serum of patients with slow progressing ALS [120].

5.5 ncRNA biomarkers in epilepsy

Circulating miRNAs have been found to be dysregulated in the serum of epilepsy patients compared with healthy controls. Validation experiments identified an upregulation of let-7d-5p, miR-106b-5p, miR-130a-3p and miR-146a-5p, and a downregulation of miR-15a-5p and miR-194-5p. The highest diagnostic value was found in the upregulated miR-106b-5p [158]. Further studies also revealed miRNAs differentially expressed in treatment-resistant compared to treatment-responsive and control samples. The expression of miR-194-5p, miR-301a-3p, miR-30b-5p, miR-342-5p and miR-4446-3p were altered in drug-resistant epilepsy serum samples, with miR-301a-3p showing the highest sensitivity [159]. Finally, sequencing analysis and RT-qPCR validation identified miR-27a-3p, miR-328-3p and miR-654-3p as differentially expressed in the plasma of epilepsy patients compared to control. Importantly, these miRNAs were detected using a prototype point-of-care device that would greatly improve diagnostic capability in-clinic [160].

Recent sequencing analysis has identified three circulating tRFs that are increased in the plasma of epilepsy patients. The differential expression of 5'AlaTGC, 5'GluCTC and 5'GlyGCC was validated by RT-qPCR in an independent cohort and detected in resected hippocampal and cortical tissue indicating a possible source. Finally, the generation and release of these tRFs was shown to be activity-related in mouse hippocampal neuronal cultures [135].

6. ncRNA as a therapeutic target

The direct involvement of miRNA and tsRNA in normal cellular activity, their dysregulation during disease pathogenesis and ability to target multiple genes within a particular pathway have made ncRNA an attractive and viable therapeutic target for the treatment of many neurological diseases. Therapeutic intervention strategies include the inhibition of overexpressed ncRNA and the restoration of repressed ncRNA. Small interfering RNA (siRNA) and antisense oligonucleotides (ASO) are the most common methods of miRNA inhibition. siRNA are short (20–25 nts) double-stranded RNA molecules that use the RNA interference RISC pathway to degrade target RNA. ASOs, also known as antimiRs or antagomiRs, are short single stranded oligonucleotides that hybridize with the target RNA and sterically interfere with its functionality. Recent advancements include the development of locked nucleic acid technology that increases the stability of ASO and siRNA [161]. The restoration miRNA expression suppressed in a given pathology through the delivery of synthetic double-stranded miRNA mimics, designed to mimic endogenous miRNAs, so far has primarily been used in gain-of-function studies to elucidate miRNA functions and mechanisms [98].

While a considerable amount of progress has been made with a number of miRNAs entering clinical trials, the development of RNA-based therapeutics has not been without issue. Double- and single-stranded RNA are recognized by the immune system, particularly the Toll-like receptors. To combat this, 2'O-methylation

and the neutralization of RNA molecules significantly reduces the immunogenicity of RNA-based therapeutics [162–164]. Delivery systems to aid passage across the cell membrane and the targeting of specific organs and cells types have also been developed. Lipid and metal-based nanoparticles as well as polymer vectors such as polyethylene imine, polylactic-co-glycolic acid and poly-amidoamine have improved the delivery of RNA-based therapeutics [165]. Furthermore, artificial manipulation of miRNAs with the delivery of miRNA mimics *in vivo* is associated with difficult to predict off-target non-specific and unintended alterations in gene expression, and toxicity, off-setting potential for therapeutic efficacy [166].

To date the Federal Drug Administration and the European Medicines Agency have approved a number of RNA-based therapeutics [167]. Notably the 18-mer ASO Nusinersen is an intrathecal administered therapeutic for the treatment of spinal muscular atrophy. Phase II and III clinical trials are also ongoing for RNA-based therapeutics for the treatment of Huntington's disease [165].

7. Conclusions

Over the past two decades research into miRNA and, more recently, tsRNA has demonstrated the integral role that these ncRNA play in cellular function and dysfunction. This has been particularly apparent in diseases of the central nervous system. Advancements in sequencing technologies and other RNA detection methods have highlighted their utility as biomarkers and the potential for disease stratification. RNA-based therapeutic intervention has shown great promise in areas with limited treatment options. Rapid improvements in the delivery and immunoreactivity of these treatments and the increasing number of clinical trials involving RNA-based therapeutics is encouraging.

Conflict of interest

The authors declare no conflict of interest.

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