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Role of non-coding RNAs in non-aging-related neurological disorders

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Abstract

Protein coding sequences represent only 2% of the human genome. Recent advances have demonstrated that a significant portion of the genome is actively transcribed as non-coding RNA molecules. These non-coding RNAs are emerging as key players in the regulation of biological processes, and act as "fine-tuners" of gene expression. Neurological disorders are caused by a wide range of genetic mutations, epigenetic and environmental factors, and the exact pathophysiology of many of these conditions is still unknown. It is currently recognized that dysregulations in the expression of non-coding RNAs are present in many neurological disorders and may be relevant in the mechanisms leading to disease. In addition, circulating non-coding RNAs are emerging as potential biomarkers with great potential impact in clinical practice. In this review, we discuss mainly the role of microRNAs and long non-coding RNAs in several neurological disorders, such as epilepsy, Huntington disease, fragile X-associated ataxia, spinocerebellar ataxias, amyotrophic lateral sclerosis (ALS), and pain. In addition, we give information about the conditions where microRNAs have demonstrated to be potential biomarkers such as in epilepsy, pain, and ALS.

Key words: microRNA; Gene regulation; Molecular biomarkers

Introduction

Recent developments have indicated that numerous non-coding sequences present in the human genome are actively transcribed as non-coding RNA (ncRNA) molecules (1). These ncRNAs may be grouped into different classes and classified according to size and function. They have emerged as key players in the regulation of many biological processes and the fine-tune control of gene expression (2).

It is not surprising that the complexity of neurological disorders is determined by different molecular mechanisms, including genetic mutations and epigenetic factors. In this context, changes in ncRNA gene expression regulation have emerged as a putative mechanism in a variety of neurological disorders such as epilepsy, neurodegenerative disorders, and autoimmune conditions (3,4). Specific processes by which ncRNAs may influence disease vary widely and include quantitative changes in coding and ncRNA expression, induction of abnormal RNA species, and others (2,5). Furthermore, circulating ncRNAs may act as disease biomarkers, contributing to early disease diagnosis and treatment follow-up (6).

In this review, we discuss the classification, biogenesis, and mechanisms of action of ncRNAs. We also review key studies that show associations between microRNA (miRNA) and long non-coding RNA (lncRNA) dysregulation and different early and adult onset neurological disorders, as well as the use of circulating miRNAs as biomarkers and potential therapeutic strategies based on manipulating ncRNAs. The role of ncRNAs in aging-related neurological disorders, such as Alzheimer's or Parkinson's disease, are thoroughly reviewed elsewhere and are not the focus of the present review (7–9).

Structure, function, and classification of non-coding RNAs

ncRNAs are defined as RNA molecules transcribed from genomic DNA that are not translated into proteins (10). The earliest recognized members of this category of RNA molecules were transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) (10). More recently, an increasing number of other ncRNAs have been detected and characterized, leading to the discovery that at least two thirds of the mammalian genome is actively transcribed (1).

ncRNAs are, in a broader sense, classified as long or small RNAs. lncRNAs are molecules ranging from ~200 nucleotides (nt) to more than 20 kilobases. The major components of this category are rRNAs, tRNAs,

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X-chromosome inactivation RNAs (XIST RNAs) and regulatory lncRNAs (2). However, lncRNAs are an ever-increasing category, with more components than the four mentioned above (2). Small ncRNAs have lengths ranging from 20 to 200 nt, including small regulatory miRNAs, small nucleolar RNAs (snoRNAs), and piwi interacting RNAs (piRNAs) (11,12).

The molecular machinery responsible for miRNA biogenesis and interaction with mRNAs (Figure 1) is better elucidated than that underlying the activity of other ncRNAs. miRNA genes are transcribed by RNA polymerase II or III. This process generates a molecule, the pri-miR, that folds itself into a hairpin conformation and is 5′ capped and 3′ polyadenylated (13,14). The pri-miR molecule is recognized by the DROSHA RNAse III enzyme and cleaved, forming a 60- to 100-nt hairpin molecule, the pre-miR, that is exported from the nucleus to the cytoplasm (14,15). In the cytoplasm, the pre-miR is cleaved by the DICER enzyme, yielding a double-stranded ~22nt RNA molecule (16). One of the strands of the formed 22-nt miRNA molecule is loaded into an RNA-induced silencing complex (RISC) protein to serve as the template for target mRNA recognition (17).

Mature miRNA molecules loaded into RISCs have two mechanisms of action. Perfect or near-perfect base pairing of the entire miRNA molecule to a complementary region within an mRNA leads to mRNA degradation by RISC (18). Perfect base pairing of almost all 22 nt is an uncommon scenario in animals. The more common scenario involves imperfect pairing, or pairing of a 5–8 nt 'seed' region of the miRNA, which leads to reduced translation or destabilization of the target mRNA (19). A single miRNA molecule may regulate multiple genes that contain a sequence complementary to the miRNA seed, and a given mRNA may be regulated by different miRNAs (20). Notably, the administration of exogenous nucleic acid sequences can mimic miRNA action (mimic-miRs), and employ the endogenous cellular machinery for miRNA-mediated gene silencing (21). Another possibility is the administration of stabilized exogenous nucleic acid sequences that are complementary to endogenous miRNAs, such as antagomirs, resulting in the inhibition of target cellular miRNAs (22).

miRNAs are also present and enriched in the plasma and serum. Furthermore, these RNAs are especially

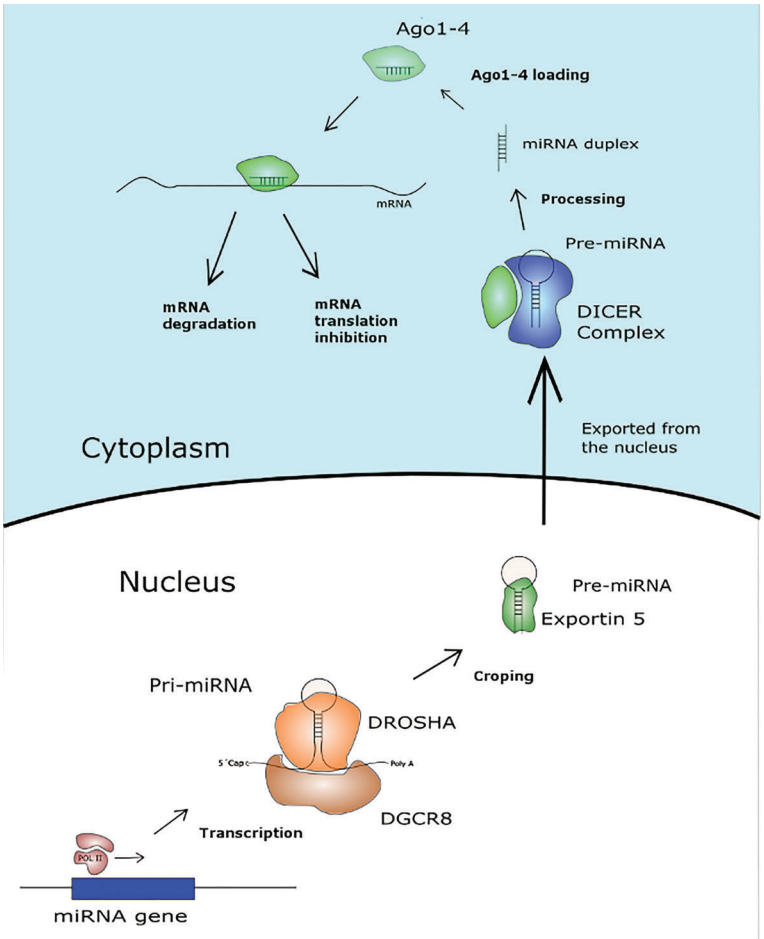

Figure 1. Main processes involved in the biogenesis and mechanism of action of microRNAs. DROSHA: Drosha ribonuclease III; DICER: Dicer 1; Ago1-4: Argonaute 1-4.

resistant to degradation (23). Blood circulating miRNAs are contained in microvesicles known as exosomes or are associated with Argonaute 2 complexes and, as a consequence, are protected from degradation (6,24). Because circulating miRNAs may originate from many different tissues throughout the body and may reflect normal function, changes in the circulating levels of these miRNAs may constitute a useful and easily accessible biomarker of many different pathological conditions. Moreover, it is feasible to quantify the levels of such circulating miRNAs by RT-PCR or even high throughput techniques such as micro-arrays or RNA-sequencing. The dysregulation of miRNA expression is well established in some tumors, and circulating miRNAs are indeed emerging as promising biomarkers in this field (23,25). The search for circulating miRNAs as biomarkers is also being applied to neurological disorders.

lncRNAs boast distinct and diverse molecular machinery involved in the regulation of gene expression (Figure 2). Most of these ncRNAs are RNA polymerase II products that lack open reading frames but are generally 5′ capped and 3′ polyadenylated (26,27). lncRNAs are numerous, with estimates in the range of thousands of lncRNA coding genes (28). Briefly, lncRNAs may act in *cis*, silencing or enhancing the expression of proximal genes on the same chromosome. For example, the lncRNA HOTTIP gene is present in the HOXA gene cluster, and its expression enhances the expression of other component genes in the same cluster (20). lncRNAs may also act in *trans*, silencing or enhancing the expression of genes on different chromosomes. One example of an lncRNA acting in *trans* is Six3OS. This lncRNA was shown to activate the targets of the retinal development involving the Six3 transcription factor (29). Another mechanism of action for lncRNAs is the regulation of other ncRNAs. lncRNA can act as a 'sponge' or decoy target. The lncRNA lincRNA-RoR mechanism of action illustrates this mechanism: this lncRNA has a binding site for miR-145, and the presence of lincRNA-RoR inhibits miR-145 action by interacting directly with lncRNA miRNA (30). The mechanisms of lncRNA-mediated regulation of protein-coding gene transcription are explored in more detail in the current literature (26,27).

Role of non-coding RNAs in disease

Table 1 presents a list of ncRNAs associated with mechanisms underlying selected neurological disorders.

Epilepsy. Epilepsy is a neurological condition with a high prevalence in the population (1.5–2%). A common feature of different epileptic conditions is the occurrence of seizures (31,32). The mechanism responsible for epileptogenesis (the process by which normal nervous tissue becomes epileptic) is complex and multifactorial (33). Evidence in the literature, as reviewed below, indicates that ncRNAs may have critical roles in the molecular mechanisms associated with epilepsy (34).

Hippocampal tissue from patients with mesial temporal lobe epilepsy (MTLE) who underwent temporal lobe resection for the control of seizures has been shown to have a reduction in the overall expression of miRNAs when compared with normal hippocampus from autopsy controls (35). Moreover, MTLE is associated with inflammation, and changes in the expression of miRNAs involved in the regulation of inflammation have been demonstrated in samples from MTLE patients (36,37). For example, miR-146-a, a miRNA involved in inflammation, is upregulated in resected hippocampus from MTLE patients (37).

In animal models of epilepsy, the dysregulation of miRNAs has been explored more extensively. miRNA expression studies were performed, using high-throughput platforms, in the animal model induced by lithium-pilocarpine, systemic kainic acid, and by intra-amygdalar kainic acid injection (38–40). Based on such studies, an extensive list of candidate miRNAs was found, but relatively few miRNAs were consistent among different studies. One example of replicable findings is mir-34a, which was found to be differentially expressed in two independent studies (38,41). mir-134 is another promising miRNA that may be involved in the molecular mechanisms of epilepsy. mir-134 was found to be differentially expressed in an epilepsy animal model, and the reduction in its expression by antagomir administration was shown to reduce cell death and seizure severity (42). In addition, downregulation of mir-132 in an animal model reduced seizure-induced neuronal death (40).

More recently, Jimenez-Mateos et al. (3) demonstrated that miR-22 downregulates the purinergic P2X7 receptor, a key component of the inflammatory response, in a mouse model of focal onset status-epilepticus. Furthermore, an increase in miR-22 activity by the administration of a Mir-22 mimic molecule reduced spontaneous seizures in these mice (3).

The role of lncRNAs has also been explored in the context of experimental animal models of epilepsy. Lee et al. (43) explored the expression of lncRNAs in two animal epilepsy models, pilocarpine- and kainic acid-induced seizures (43). These authors found hundreds of lnRNAs that were differentially expressed when comparing nervous tissue from controls with that of treated mice. Of these differentially expressed lncRNAs, 54 (for pilocarpine) and 14 (for kainic acid) were close to protein-coding genes and appear to induce significant changes in gene expression, thus indicating a possible *cis* effect of these lncRNAs (43).

The first evidence for the potential use of miRNAs as biomarkers in epilepsy also came from studies in experimental animal models. Liu et al. (44) demonstrated the differential regulation of several miRNAs isolated from the blood of rats that received the chemoconvulsant kainic acid. More recently, Roncon et al. (45) found 27 miRNAs to be differentially expressed in the plasma of rats treated with pilocarpine. In humans, Wang et al. (46), using RNA-sequencing and subsequent RT-PCR validation,

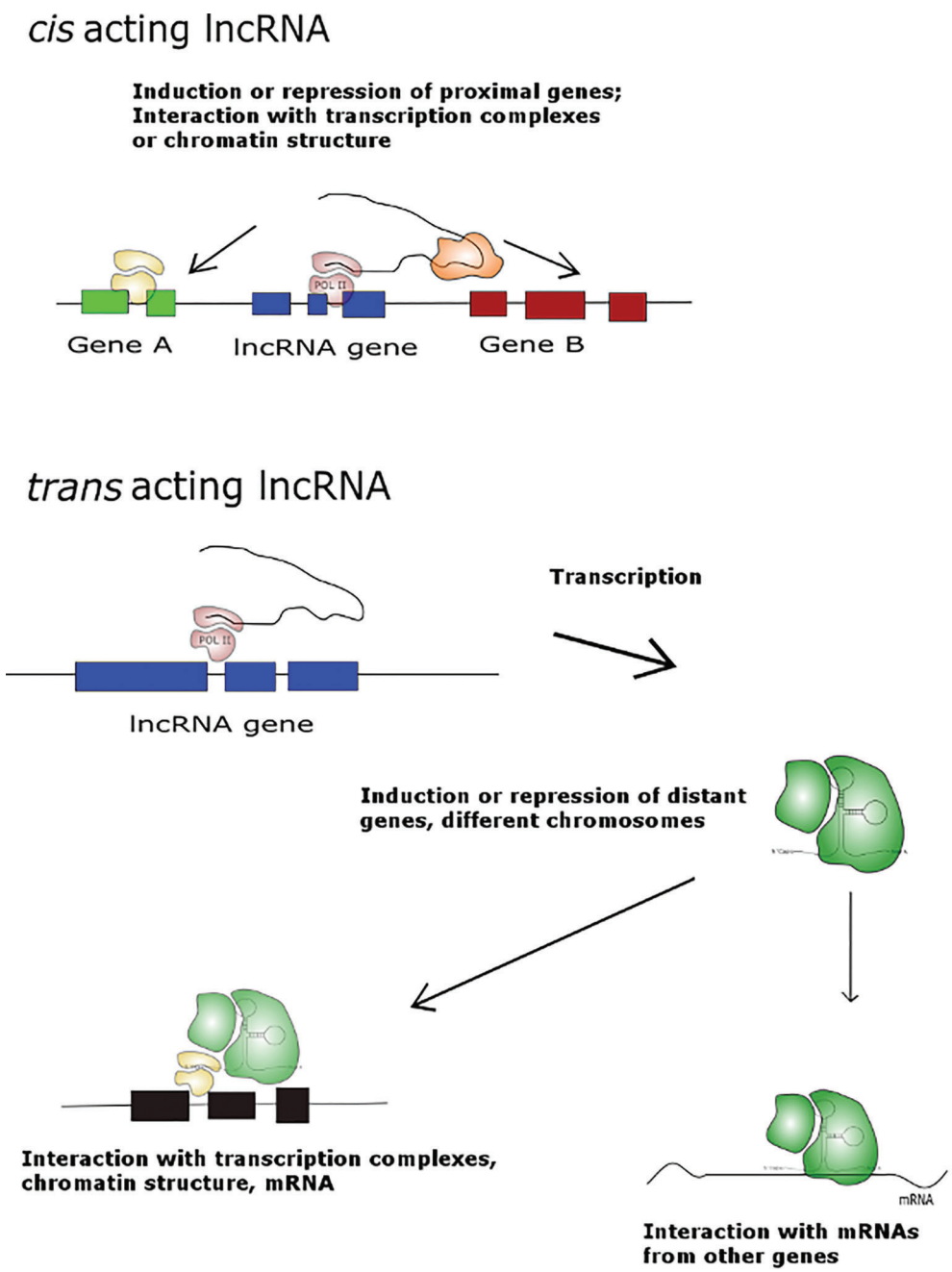

Figure 2. Mechanisms by which long non-coding RNAs (lncRNAs) can regulate gene expression.

found four upregulated and two downregulated blood circulating miRNAs when comparing epilepsy patients to healthy controls. Among the differentially expressed miRNAs, miR-106b-5p had the highest sensitivity and specificity (46). Furthermore, in a subsequent study, there were five circulating miRNAs identified as potential biomarkers of drug-resistant epilepsy, and miR-301a-3p had the highest sensitivity and specificity (47). We have identified that miR-134 is a circulating biomarker for patients with mesial temporal lobe epilepsy regardless of their response to treatment, which may help in the diagnosis of this type of epilepsy (48).

In focal cortical dysplasia, a cortical malformation frequently associated with refractory seizures, miR-4521 has been shown to be upregulated in the plasma of patients compared to control subjects (49).

Table 1. List of ncRNAs associated with different mechanisms underlying selected neurological disorders.

Disorder	Gene Affected	Proposed mechanisms associated with Noncoding RNAs	References
FXTAS	*FMR1; FMR4*	Sequestration of RNA binding protein; antisense transcript	Tassone et al. 2004 (58)
DM1	*DMPK*	Sequestration of RNA binding protein; antisense transcript	Rau et al. 2011 (66)
SCA1	*ATXN1*	Altered miRNA pathway	Galka-Marciniak et al. 2012 (56)
SCA3	*ATXN3*	An auxiliary toxic long CAG repeat RNA; altered miRNA pathway	Galka-Marciniak et al. 2012 (56)
SCA7	*ATXN7*	Antisense transcript repress sense ataxin-7	Tan et al. 2014 (63)
SCA8	*ATXN8OS; ATXN8*	Sequestration of RNA binding protein; antisense transcript	Daughters et al. 2009 (61); Moseley et al. 2006 (62)
HDL2	*JPH3*	Antisense transcript; polyQ toxicity	Wojciechowska and Krzyzosiak, 2011 (5)
MTLE	*P2X7*	Down-regulation by miR-22	Jimenez-Mateos et al. 2015 (3)
HD	*HTT*	An auxiliary toxic long CAG repeat RNA; altered miRNA pathway	Wojciechowska and Krzyzosiak, 2011 (5)
MTLE	Genes involved with inflammation	Up-regulation of miR-146a expression	Aronica et al. 2010 (37)
ALS	*SOD1* and others	An artificial microRNA may extend survival and delays paralysis; Up regulation of miR-206.	Stoica et al. 2016 (79); Takahashi et al. 2015 (81)
Cortical dysplasia	*Lis1*	Dysregulation of miR-139-5p	Huang et al. 2014 (90)
Pain	Inflammation, neural processing	Dysregulation of miR-1, -16, and -206	Kusuda et al. 2011 (86)

Neurodegenerative and neuromuscular disorders. Neurodegenerative disorders are associated with a wide range of genetic mutations and epigenetic and environmental factors. Among genetic mutations, trinucleotide repeat expansion is increasingly recognized as the cause of a large subset of these conditions. Trinucleotide repeat expansions account for more than 30 neurological and neuromuscular diseases that are categorized into coding and non-coding repeat expansion disorders, depending on the genetic location of their causative mutations (50–52). Disorders such as Huntington's disease (HD), spinocerebellar ataxia (SCA) types 1, 2, 3, 6, 7, 8, and 17, dentatorubral-pallidoluysian atrophy, and spinal and bulbar muscular atrophy are typically associated with a protein gain-of-function mechanism (53). In contrast, diseases such as myotonic dystrophy type 1 (DM1) (54,55), fragile X-associated tremor ataxia syndrome (FXTAS), myotonic dystrophy type 2 (DM2), SCA31, SCA10, SCA8, and, more recently, amyotrophic lateral sclerosis and frontotemporal sclerosis have been associated with an RNA gain-of-function mechanism in which the trinucleotide expansion leads to the formation of nuclear RNA foci that sequester specific RNA-binding proteins (5,56,57).

Studies of FXTAS have established that the sequestration of RNA-binding proteins due to the expression of pathogenic RNA with expanded repeats is involved in disease pathogenesis (58) (Figure 3). A recent study identified that the double-stranded RNA-binding protein DGCR8 binds to expanded CGG repeats, resulting in the partial sequestration of DGCR8 and its partner, DROSHA, within CGG RNA aggregates. Consequently, the processing of miRNAs is reduced, resulting in decreased levels of mature miRNAs in neuronal cells expressing expanded CGG repeats such as in brain tissue from patients with FXTAS (59).

SCA8 is a dominantly inherited, slowly progressive neurodegenerative disorder caused by a CTG CAG repeat expansion (60). In pathological samples from SCA8 patients, bidirectional (sense and antisense) expression of the SCA8 CTG · CAG expansion produces toxic non-coding CUG expansion in RNAs from the Ataxin 8 opposite strand (ATXN8OS) and a nearly pure polyglutamine expansion protein encoded by ATXN8 (61,62). In SCA7, the tissue-specific alterations caused by CAG repeat expression in the ATXN7 gene seems to be related to cross-talk between the lncRNA lnc-SCA7, the ATXN7 mRNA, and mir-124. Mutant ATXN7 disrupts this crosstalk and is itself upregulated, since it is not repressed by ncRNAs (63).

Recent studies have suggested that alterations in small regulatory ncRNAs, such as miRNAs, could contribute to the pathogenesis of several neurodevelopmental disorders. Some studies have found a relationship between miRNAs and DM1 (64). Alterations in the miRNA expression patterns have been observed in muscle-specific

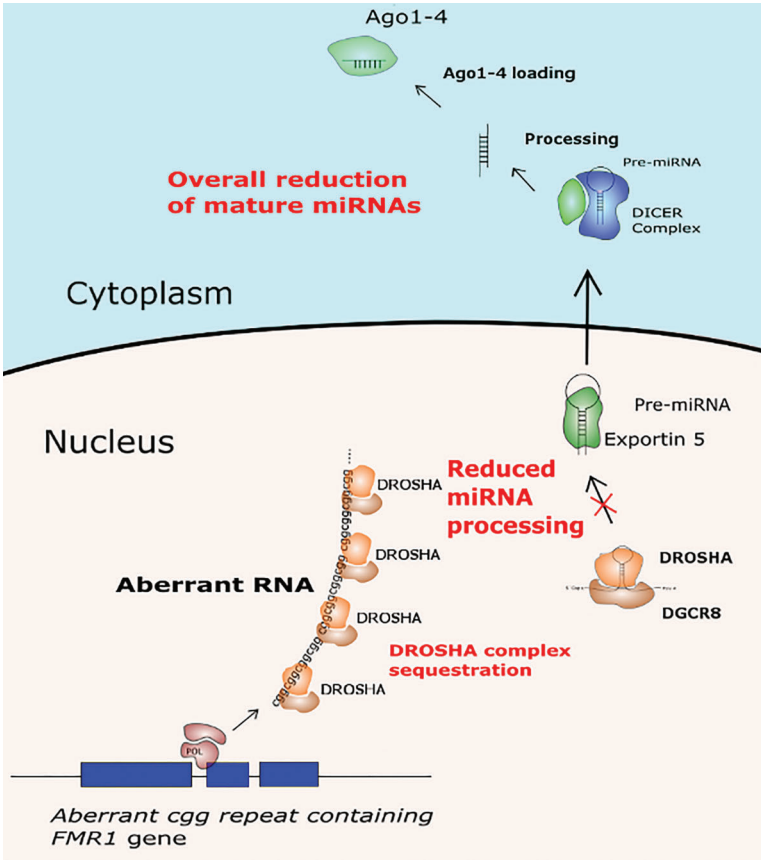

Figure 3. Mechanism involved in microRNA machinery sequestration by aberrant RNA species produced in a triplet repeat disease, fragile-X associated tremor ataxia syndrome (FXTAS). DICER: Dicer 1; DROSHA: Drosha ribonuclease III; Ago1-4: Argonaute 1-4.

miRNAs (myomiRs). Given the small distance between the seed binding sites of miR-206 and 148a in the DMPK 3′ UTR, Koscianska et al. (65) analyzed the binding mechanism of both miRNAs. They discovered cooperative binding; the joint binding of miRs 206 and 148a increased the negative regulation of DMPK mRNA. These findings provide mechanistic insights into the miRNA-mediated regulation of the DMPK transcript. In this regard, the dysregulation of DM1-associated miRNAs has also been linked to alterations in their predictive target expression, showing that miRNA dysregulation in DM1 is functionally relevant and may contribute to disease pathology (66,67). Furthermore, RNA toxicity has been confirmed in transgenic mice harboring long triplet repeats in the *dmpk* gene. Seznec et al. (68) showed that mice develop multi-system abnormalities mimicking the human DM phenotype, with predominant involvement of muscles and the central nervous system (CNS). Pathway and function analysis highlighted the involvement of the miRNA-dysregulated mRNAs in multiple aspects of DM2 pathophysiology as well (4,69).

Huntington's disease is characterized by widespread mRNA dysregulation, especially in the striatum and cortical regions and alterations in miRNA-mediated post-transcriptional regulation could be an important mechanism contributing to mRNA dysregulation in HD (70). In addition, there is evidence that abnormal neurodevelopment might also have a critical role in HD (71). These emerged from studies using mouse embryonic stem cells and patient-derived induced pluripotent stem cells (The HD iPSC Consortium, 2012) showing that chromatin modifications and DNA methylation status support the hypothesis that wild-type and mutant Huntingtin might affect key chromatin regulators such as DNA and histone methyltransferases, and demethylases (72–74). In fact, a growing body of evidence suggests that alterations of epigenetic modifications constitute a basic molecular mechanism caused by the HD mutation and are responsible for early features of the pathological process (75). Furthermore, a recent genome-wide screen of miRNAs in *post mortem* brains highlighted miRNAs that were differentially expressed in HD patients, especially miRNAs in the HOX family, which have been associated with early brain development (76). Indeed, there are several classes of lncRNAs that are potentially involved in developmental processes and that

were found to be dysregulated in brain tissue from patients with HD such as TUG1, NEAT1, MEG3, and DGCR5 (77).

Amyotrophic lateral sclerosis (ALS) is a widespread motor neuron disorder causing injury and death of lower and upper motor neurons. Familial ALS ($\sim$10% of all ALS cases) is inherited as a dominant trait, and 20% of these cases have mutations in the gene encoding Cu/Zn cytosolic superoxide dismutase 1 (*SOD1*) (78). A recent study demonstrated that an AAV9-delivered SOD1-specific artificial miRNA is an effective and translatable therapeutic approach to ALS (79). Another promising miRNA with a possible therapeutic use in ALS is mir-155. It was demonstrated that this inflammation-associated miRNA is upregulated in the mutant SOD1 mouse model and that reduction in the expression of mir-155 significantly extended the life span of this mouse (80).

In addition, expression levels of certain miRNAs, such as miR-4649-5p and hsa-miR-4299, were significantly correlated with disease progression and might be useful as prognostic biomarkers (81). Another potential biomarker was mir-206, found to be upregulated in the plasma of SOD1-G93A mice, an experimental ALS model, and in patients with confirmed ALS (82). In addition, there is evidence of dysregulation of miRNAs extracted from leukocytes from sporadic ALS patients (83). More recently, we have demonstrated that among 11 miRNAs identified as differently expressed in muscle of patients with ALS, only two, miR-214 and miR-424, correlated with clinical deterioration over time in these patients (84).

Pain. Conditions leading to chronic pain are related to multiple etiologic factors, ranging from maladaptive neuronal plasticity to diverse inflammatory pathways (85). Due to the complexity of chronic pain, some studies have explored the possible role of ncRNAs in different experimental pain models. Kusuda et al. (86) observed a change in the expression of three miRNAs, miRs 1, 16, and 206, in different pain conditions such as peripheral inflammation, nerve ligation, or axotomy. Other studies have employed low-density TaqMan arrays to profile the expression pattern of miRNAs after spinal nerve ligation in rats and found 63 altered miRNAs (87).

A possible role for lncRNAs has been explored in experimental models of neuropathic pain. A microarray analysis demonstrated hundreds of differentially expressed lncRNAs and mRNAs in the spinal cords of mice subjected to spinal nerve ligation. As demonstrated in other experiments, 35 differentially regulated lncRNAs were in genomic regions proximal to differentially regulated genes from the same dataset (88).

Non-coding RNAs as target treatments for neurologic disorders

The use of ncRNAs as therapeutic tools in human disorders is still in its early stages. To date, there is only one therapeutic use of human miRNA for the treatment of hepatitis C (HCV) that has passed phase IIa clinical trials (89). The clinical trial data showed the efficacy of the employed anti-miRNA in reducing viral load and showed good treatment tolerability, thus indicating the feasibility of similar strategies for other clinical uses such as in the case of neurological conditions.

Animal experiments already indicate some promising targets for the use of ncRNAs as therapeutic tools in disorders affecting the CNS. In epilepsy, the use of miR antagonists for miR-134 or mimic-miRs for miR-22 was capable of reducing neuronal death and seizure severity in animal models (3,42). These and other examples of pre-clinical uses of miRNAs for the treatment of neurological conditions need further study; however, due to the good tolerability already shown in the existing human clinical trial for HCV, there is optimism about the possible utility of ncRNAs in the treatment of neurological conditions in the future. However, several challenges remain for the efficient delivery of ncRNA molecules into the CNS, thus most of the pre-clinical studies still use invasive techniques for administering these molecules (4,44)

Conclusions

In conclusion, ncRNAs are emerging as key players in the field of neurological disorders. ncRNAs are involved in many conditions, either as part of the molecular mechanisms underlying disease or as biomarkers that may be used for improved diagnosis or assessment of disease progression. ncRNAs are also promising targets for new therapeutic strategies to be employed in the treatment of neurological conditions.

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