Review

MicroRNAs and neurodegeneration: role and impact

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Neurodegenerative diseases are typically late-onset, progressive disorders that affect neural function and integrity. Although most attention has been focused on the genetic underpinnings of familial disease, mechanisms are likely to be shared with more predominant sporadic forms, which can be influenced by age, environment, and genetic inputs. Previous work has largely addressed the roles of select protein-coding genes; however, disease pathogenesis is complicated and can be modulated through not just protein-coding genes, but also regulatory mechanisms mediated by the exploding world of small non-coding RNAs. Here, we focus on emerging roles of miRNAs in age-associated events impacting long-term brain integrity and neurodegenerative disease.

miRNAs and neurodegeneration

Neurodegenerative diseases are a group of typically late-onset, progressive disorders that lead to cognitive and/or movement disorders. Some of the most studied include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and polyglutamine (polyQ) disorders such as Huntington's disease (HD) and the spinocerebellar ataxias (SCAs) [1–5]. These diseases share features such as the abnormal accumulation of protein, which includes plaques and tangles in AD, Lewy bodies in PD, bunina bodies in ALS, and nuclear and cytoplasmic accumulations in polyQ disease. In these diseases, key proteins accumulate, the genes of which are ones in which familial mutations can be found. Mechanisms that affect disease pathogenesis involve multiple fundamental cellular pathways, including protein folding and clearance processes. Thus, understanding the pathogenic mechanisms requires studying a broad spectrum of basic cellular machineries.

miRNAs are small RNAs of approximately 20–24 nucleotides (nt) that regulate the translation or levels of target mRNA transcripts [6–9]. Hundreds of miRNAs have been discovered in plants and animals that impact various biological processes. miRNAs are generated through cleavage of a primary transcript in the nucleus (pri-miRNAs) by the Drosha/DGCR8 (Drosha/Pasha in *Drosophila*) microprocessor to generate a precursor miRNA (pre-miRNA) (Figure 1). The pre-miRNA is exported to the cytoplasm, where Dicer cleaves it to release the double-stranded miRNA duplex. One of these strands preferentially associates with the Ago complex to form miRNA-induced Silencing Complex (miRISC), while the other strand is usually degraded. In animals, miRNAs recognize their targets primarily through complementarity with the seed sequence at nucleotides 2–8 of the 5′ end of the miRNA. Hundreds of mRNA targets could exist per miRNA family and it is possible that most protein-coding genes are targets of miRNAs.

Three main approaches have been used to study the effects of miRNAs on long-term brain integrity and neurodegenerative disease. First is the disruption of proper miRNA biogenesis followed by examination of the effect on the brain over time. Second is the identification of individual miRNAs that target specific disease genes and their impact. Third is the examination of the impact of disease-associated proteins on the miRNA pathway, such as miRNA biogenesis or mRNA-silencing function (Figure 2). Here, we describe recent advances in each approach that reveal critical roles of miRNAs in brain integrity.

Disrupting the miRNA biogenesis pathway causes neurodegeneration

A range of approaches – including cloning of miRNAs, miRNA microarrays, and small RNA deep-sequencing analyses – have revealed expression of select miRNAs in the developing mammalian brain and primary neuronal cultures [10–13]. Analysis of the expression pattern of miRNAs using *in situ* hybridization with locked nucleic acid (LNA) probes in zebrafish identified their tissue-specific patterns [14]. Such patterns indicated a potential role of miRNAs in neuronal development and function. Subsequent studies revealed roles of specific miRNAs, such as miR-124, in these processes [15–17].

A functional link between miRNAs and neurodegeneration was discovered in studies of the effect of global disruption of miRNA biogenesis on neuronal development. Mutants of Dicer in the mouse die early, before neurulation, precluding the ability to assess function in the brain [18]. However, disruption of Dicer in zebrafish revealed an essential role in brain morphology and neural differentiation [19]. Injecting a miR-430 duplex rescued the defects in brain morphology, indicating the importance of this specific miRNA. Subsequently, conditional disruption of Dicer in different neuronal populations or cell lines revealed the effect of the miRNA pathway on proliferation, migration, and differentiation, as well as long-term neural integrity

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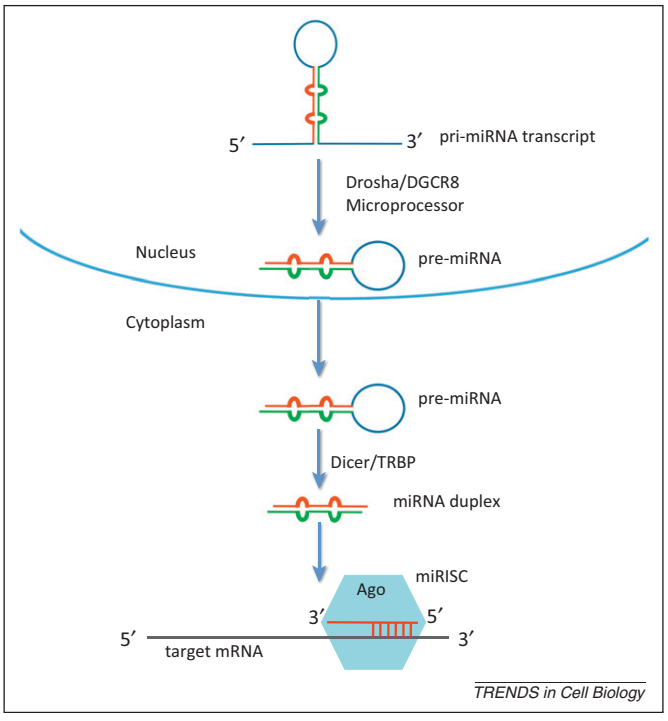

Figure 1. The miRNA biogenesis pathway. The biogenesis of a miRNA starts with the transcription of the primary transcript, the pri-miRNA, by RNA polymerase II. The pri-miRNA is cleaved by the microprocessor complex (Drosha/DGCR8 in vertebrates, Drosha/Pasha in *Drosophila*) to generate the precursor miRNA (pre-miRNA). The pre-miRNA is transported to the cytoplasm, then cleaved by the Dicer/TRBP (Dicer-1/Loqs-PB in *Drosophila*) complex to generate the miRNA duplex. After incorporation into miRNA-induced Silencing Complex (miRISC) and strand selection, the mature miRNA strand induces translational repression and/or mRNA cleavage, leading to reduction of the protein.

[20–25]. For example, depleting Dicer in embryonic stem (ES) cells reduces the ability of the cells to differentiate into midbrain dopaminergic neurons [24] – a major neural population compromised in PD. Transfecting the small RNA fraction from embryonic mouse midbrain cells rescues the defect, assigning the role to small RNAs. Consistent with this, deleting Dicer in midbrain dopaminergic neurons in the mouse causes progressive loss of the cells, concomitant with disruption of locomotion, reminiscent of PD [24].

In another example, loss of Dicer from mouse cerebellar Purkinje cells did not impair cellular morphology or function at young ages (8–10 weeks). However, by 13 weeks of age, Purkinje cells, which are the cell type compromised in many ataxias, had progressively degenerated. Intriguingly, the older mice also developed slight tremors and mild ataxia that worsened with age [20]. Disruption of Dicer in spinal motor neurons mimics clinical and pathological features of ALS, a disease associated with loss of motor neurons, indicating a possible impact of the miRNA pathway in the pathogenesis of this disease [26]. Interestingly, some key proteins associated with this disease have been shown to modulate miRNA biogenesis or function (see below). Deletion of Dicer from glial cells such as astrocytes and oligodendrocytes can cause neural degeneration in the mouse [27,28]. In addition, conditional loss of Dicer in Schwann cells in the mouse revealed its importance for axonal integrity [29]. In humans, Dicer protein levels have been found to be decreased in temporal lobe epilepsy patients with hippocampal cell loss (sclerosis), with about half of the miRNAs in the tissue reduced in levels [30].

In *Drosophila*, knockdown of Dicer-1 is also associated with dopaminergic neural loss and climbing defects [31]. Loss of Dicer-1 also enhances the toxicity of human pathogenic neurodegenerative disease proteins Ataxin-3 (associated with spinocerebellar ataxia type 3) and Tau (associated with AD and frontotemporal dementia [FTD]) [32]. Intriguingly, depleting Dicer from human HeLa cells also enhances the toxicity of disease-associated pathogenic Ataxin-3 protein and is rescued by adding back the small RNA fraction indicating a role for miRNAs. This study identified a specific miRNA, bantam, that modulates Ataxin-3 and Tau toxicity. Supporting the role of miRNAs in polyQ disease pathogenesis, a study of *Drosophila* miR-34 revealed a potent neuroprotective function in mitigating the toxicity of pathogenic forms of Ataxin-3 [33]. Beyond Dicer, haploinsufficiency of DGCR8, a component of the microprocessor complex that cleaves pri-miRNAs to generate pre-miRNAs, leads to neuronal dysfunction in the mouse [34–36].

These studies, which target disruption of components of the miRNA biogenesis pathway, strongly suggest that miRNA activity impacts long-term brain integrity. Note, however, that the identification of the individual miRNAs involved is a crucial component of such work. One reason for this is the potential effect of disrupting miRNA biogenesis on the proper expression of many related or unrelated proteins. Another reason is that disrupting the major components of miRNA biogenesis may cause dysfunction or degeneration independent of an effect on miRNAs. For example, Drosha, another component of the microprocessor, is reported to regulate neurogenesis by controlling Neurogenin 2 expression independent of its role in miRNA processing [37]. This function entails Drosha binding and cleavage of a hairpin structure in the 3′ untranslated region (UTR) of Neurogenin 2 mRNA. Recent studies have also shown that DGCR8 has a much broader impact on RNA processing beyond just miRNAs [38].

Individual miRNAs target disease genes

Efforts to profile miRNAs in tissue from patients with neurodegenerative disease has identified miRNAs that are misregulated in the brain, some of which have been shown directly to target transcripts of familial disease genes. Recent reviews discuss the roles of individual miRNAs on the common neurodegenerative diseases [39–41]. In general, discovering specific miRNAs that target the 3′UTR of key disease genes, then assessing the expression pattern and level of those miRNAs, can uncover the extent to which they may impact the level of the disease protein and thus pathogenesis. Here, we highlight a few examples to illustrate the effect of specific miRNAs on select diseases.

AD is the most common neurodegenerative disease and, although it is predominantly sporadic, analysis of familial situations has identified critical genes for its etiology [2]. The pathological features of AD are the deposition of intracellular neurofibrillary tangles containing Tau protein and extracellular plaques containing amyloid-beta (Aβ) peptides in the brain. Increased production and impaired clearance of Aβ is a likely cause of Aβ accumulation.