SYMPOSIUM: MicroRNAs: Biology and Roles in Neurodegeneration and Brain Tumors

MicroRNAs (miRNAs) in Neurodegenerative Diseases

Peter T. Nelson[1,2]*; Wang-Xia Wang[1,2]*; Bernard W. Rajeev[1,2]*

[1] Sanders-Brown Center on Aging, and [2] Department of Pathology, University of Kentucky, Lexington, Ky.

Corresponding author:

Dr. Peter T. Nelson, MD, PhD, 311 Sanders-Brown Center on Aging, 800 S Limestone, University of Kentucky, Lexington, KY 40536-0230 (E-mail: *pnels2@email.uky.edu*)

* These authors contributed equally to the preparation of this manuscript.

Abstract

Aging-related neurodegenerative diseases (NDs) are the culmination of many different genetic and environmental influences. Prior studies have shown that RNAs are pathologically altered during the inexorable course of some NDs. Recent evidence suggests that microRNAs (miRNAs) may be a contributing factor in neurodegeneration. miRNAs are brain-enriched, small (~22 nucleotides) non-coding RNAs that participate in mRNA translational regulation. Although discovered in the framework of worm development, miRNAs are now appreciated to play a dynamic role in many mammalian brain-related biochemical pathways, including neuroplasticity and stress responses. Research about miRNAs in the context of neurodegeneration is accumulating rapidly, and the goal of this review is to provide perspective for these new data that may be helpful to specialists in either field. An overview is provided about the normal functions for miRNAs, including some of the newer concepts related to the human brain. Recently published studies pertaining to the roles of miRNAs in NDs—including Alzheimer's disease, Parkinson's disease and triplet repeat disorders—are described. Finally, a discussion is included with theoretical syntheses and possible future directions in exploring the nexus between miRNA and ND research.

INTRODUCTION: PERTINENT GENERAL ASPECTS OF NEURODEGENERATIVE DISEASES

"Neurodegeneration" is a term that has been used to refer to differing topics. Abundant nerve cell death occurs in the course of normal brain development, and many pediatric neurological diseases are characterized by pathological degeneration of neurons and/or muscles. Phenomena involving nerve cell death also occur in animal models and cells in culture. This review is focused upon the human-specific neurodegenerative diseases (NDs) that afflict mainly the elderly, particularly trinucleotide repeat diseases, Alzheimer's disease (AD) and the synucleinopathies, such as Parkinson's disease (PD).

In the course of NDs, neurons lose their connections and die prematurely. Although highly relevant to "physiological" cell death, the role of apoptosis in most NDs has not been conclusively proven, and will not be discussed here in great detail. Each nosological ND entity has its own set of pathological processes, some of which are presumably unique. However, there are six general ideas that are relevant at least circumstantially to how microRNA (miRNA) biochemistry may interact with the pathogenesis of NDs.

(i) Most NDs are not inherited in patterns that reflect simple Mendelian genetics

Most prevalent subtypes of NDs, such as AD and PD, are inherited in a manner termed "sporadic" (as opposed to "familial"), influenced by alleles with limited genetic penetrance, or are caused by genetic and/or environmental influences as yet uncharacterized (28, 102). These facts may shift the focus of ND genetic studies away from "traditional" genes which constitute only ~1%–2% of human DNA, and towards the other ~50% of transcribed DNA about which we are mostly ignorant (48, 96).

(ii) Brains of human ND patients show evidence of chronic stress

ND brains show evidence of considerable cellular stress (77, 105). Although this may be expected in disease processes that last for long periods and involve abundant cell death, this response may prove contributory because the stress response itself could play a role in the "cascade" effect (see below) that result ultimately in neurological deficits.

(iii) NDs may be exacerbated in the clinicobiological sense by the aberrant stimuli of developmental pathways

Some biochemical pathways that are up-regulated during normal brain development, but down-regulated in normal adulthood, are then aberrantly up-regulated again in the course of NDs. These include pathways involved in cell–cell signaling, cell division, neuroplasticity and apoptosis (see reviews 3, 17, 91). In the case of the microtubule-associated protein tau (MAPT), there is an mRNA splicing variant that is more highly represented both in early development and in some neurodegenerative diseases (57, 88). Furthermore, the developmentally up-regulated phosphorylation of MAPT is also up-regulated in AD neurofibrillary pathology (39, 119). These are only a few examples of how the up-regulation of

developmental pathways in the adult milieu may contribute to the pathological progression of some NDs.

(iv) RNA is pathologically altered in NDs

RNA biochemists are well aware that RNA is labile in even tightly controlled circumstances. In the course of some NDs, brain RNAs becomes pathologically altered. These changes have been reviewed elsewhere (81, 88, 118), but include aberrant RNA oxidation, RNA degradation, altered RNA splicing and ribosomal changes which cause mRNA translational frame-shifting abnormalities.

(v) Human NDs are chronic conditions that last for decades rather than just for several years

Recent studies have established the surprising chronicity of the processes underlying NDs. Using AD as an example, the time from patients' clinical diagnosis to death is typically ~8 years (126). However, the underlying pathological processes of AD occur over many decades. Persons at risk for developing AD in their seventh or eighth decade already show brain metabolic abnormalities by PET scan even in their third decade of life (103, 112). These are important data because they show that ND pathology involves a long-evolving shift in cellular equilibrium, which could be "tipped" even by subtle genetic and/or environmental influences.

(vi) Pathogenetic mechanisms of ND involve multiple distinct steps

Most NDs are probably not just a "one-hit" phenomenon, but rather they probably involve a sequential progression of pathological processes that result collectively in neuronal death and/or compromised connectivity. In the case of AD, the most prevalent hypothesis is the "amyloid cascade hypothesis" (43, 44), and both neuritic amyloid plaques and neurofibrillary tangles are apparently required to produce the advanced clinical stages of the disease (as described in 129).

These six features of NDs dovetail on some of the known characteristics of miRNA biochemistry in the brain. As described below, miRNAs derive from "non-traditional genes"; are related to pathways of cellular stress and neurodevelopment; may be altered by the changes seen in ND brains such as oxidation; and may ultimately contribute to step(s) that culminate in chronic brain diseases.

INTRODUCTION: GENERAL ASPECTS ABOUT TRANSCRIPTIONAL REGULATION AND miRNAs

In mammalian cells, mRNA levels and protein levels tend to correlate quite poorly (88). This may be because translation (RNA→Protein) is even more important than transcription (DNA→RNA) as a nidus for gene expression regulation. More than 95% of human cellular RNAs are "non-coding" RNAs, that is, other than mRNA, and most of these molecules participate in translational regulation (48, 96). These and other data have led researchers to seek a better understanding of mRNA translation.

miRNAs are small non-coding RNAs with potent biological activities involved in mRNA translational regulation (see Figure 1). Derived from genes that reside between or within protein-coding genes, miRNAs may be transcribed by RNA polymerase II (usually) or by RNA polymerase III (16, 19, 66). The precursor RNAs are processed endonucleolytically and exported to the cytoplasm where they are further trimmed by dicer protein to their mature form (89). These "mature", short (~22 nucleotides in length), single-stranded

miRNAs are bound intimately to argonaute proteins, which apparently mediate miRNAs' ultimate functions (20, 42). Argonaute proteins and miRNA biochemical machinery are universal among eukaryotes, but great diversity is evident in the particular characteristics of miRNA functions *in vivo*. The canonical function of argonaute-bound miRNAs is the suppression of target mRNAs. In other words, an mRNA that is "targeted" by miRNAs may be present by a cDNA microarray experiment, reverse transcriptase PCR, Northern blot or other "expression profiling" technique; however, there would be little or no polypeptide deriving from that mRNA.

Biochemical mechanisms by which miRNAs work are probably numerous depending on the availability of local regulatory factors (see for example 53, 60, 67, 95, 97). miRNAs are a relatively recent discovery, having been first recognized in animals in the context of worm development. In the *Caenorhabditis elegans* heterochronic pathway, miRNAs participate in the sequential process of cell fate determination by decreasing the production of certain proteins despite high levels of their cognate mRNAs (36, 65, 104). This system provides one contextual paradigm for miRNA action, in which a "one miRNA, one/few mRNA target(s)" rule seems to apply in a set developmental staging schema. However, the characteristics of miRNA-related biochemical pathways have provided many surprises, and more are no doubt yet to come.

One unexpected characteristic of some human miRNAs is their very high RNA copy number per cell. Particular miRNAs can be represented by 1000–30 000 copies per cell or more (1, 72). By comparison, the large majority of mRNAs typically number less than 100 copies per cell (21, 115).

If miRNAs participate in gene expression regulation and are represented in high numbers per cell, how extensive is the cellular impact of miRNAs? Numerous studies have now established that miRNAs can have a profound cellular impact by dint of sheer biochemical promiscuity: each miRNA can regulate hundreds, thousands or more mRNA targets. This was first indicated by predictive bioinformatics, which showed that evolutionarily conserved "miRNA recognition elements" are present in the 3′ untranslated region (3′UTR) of very many mRNAs per miRNA (7, 38, 56, 59, 69, 70, 83). Further studies have exploited the fact that in some circumstances, miRNAs actually cause down-regulation of target mRNA, in addition to protein, so that cDNA microarrays could be compared between functionally miRNA-transfected cells and controls (41, 73, 75, 124). These studies have shown convincingly that each miRNA can regulate thousands of different mRNA targets. Hence, these small non-coding RNAs, which are present in very high gene copy numbers and which work through a powerful translational regulation paradigm, can modify an exceptionally large number of genes.

Recent studies have revealed intriguing novel aspects of miRNA function (see Figure 2):

• Some miRNAs are edited post-transcriptionally, and thus their targets can be profoundly altered (15, 76, 93).

• Some human miRNAs are targeted to the nucleus for as yet unknown reasons (49).

• miRNAs are shown to alter mRNA splicing and/or DNA methylation in *trans* (14, 18, 34, 80).

• miRNAs (like siRNAs) may recognize mRNAs outside of the 3′UTR, opening up new possibilities for complex gene expression regulation (84).
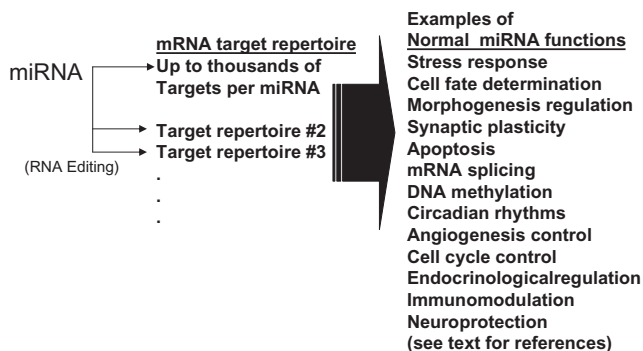
Figure 1. In the mammalian brain as elsewhere, miRNAs subserve multiple fundamental biological roles. Many miRNAs are thought to interact with thousands of different targets. Post-transcriptional RNA editing may change mRNA target specifity. References for the different hypothesized functions of miRNAs are given in the text.

• Viral-derived miRNAs/siRNAs can play important roles in host cell biochemistry (23, 30, 111).

• Under some circumstances, miRNAs can *increase* translation of targeted mRNAs (10, 11, 47). See note added in proof at end of article.

• Numerous cell- and organism-level functions as diverse as immunomodulation (26, 27, 108), endocrine function (29, 101, 109), circadian rhythms (24, 92), metabolic pathways (125), limb morphogenesis (45) and angiogenesis (63, 100, 120, 128) have been hypothesized to be regulated by miRNAs.

miRNAs IN CELLULAR STRESS

As mentioned above, cellular stress is a conspicuous feature of ND brains. A detailed catalog of the many roles now thought to be played by miRNAs in stress is beyond the scope of this review. However, miRNAs' expressions have been shown to change dramatically in response to cold and heat stress, hypoxia, oxidative

stressors, arsenic, irradiation, nutrient deprivation and other stresses (2, 25, 32, 51, 79, 82, 107, 110, 121). Typically, in stress conditions, a subset of miRNAs are increased, and a subset are decreased. The subset of miRNAs that are altered in one stress condition and in one cell type are not necessarily related to the miRNAs which change expression upon a different stressful stimulus. To illustrate the point, miR-93 is up-regulated in titanium-induced stress in a human osteoblast cell line and in the liver of chronically tamoxifen-fed rats, but down-regulated in hypoxic human trophoblasts; however, miR-93 showed no evident expression change in folate deficiency or arsenic exposure in a lymphoblastic cell line (31, 82, 94, 99).

The biochemical effects of miRNAs during cellular stress can change dramatically. For example, stress may alter a miRNA : mRNA interaction to produce translational up-regulation of target mRNAs (10, 11, 47), which is the opposite of "canonical" miRNA activity. This has prompted speculation that miRNAs may constitute a "safeguard against turmoil", by altering globally the parameters of translational machinery in the cells during stress (68). Plant miRNAs also participate in the stress response in part through a ubiquitin pathway (5, 25); this is intriguing partly because plant miRNA paradigms have been fundamentally important in understanding the anciently evolved miRNA pathways. In summary, during any conditions that are attended by cellular stress, such as NDs, it can be expected that miRNA expression patterns will change, which would be expected in turn to alter the translation of a large number of different cellular mRNAs.

miRNAs IN THE MAMMALIAN BRAIN

miRNAs are enriched in human and rodents brain: relative to other organs, more miRNAs are expressed in brain and at somewhat higher levels (4, 6, 9, 114, but see 37, 122). Very high-throughput sequencing experiments have been interpreted to suggest that the number of miRNAs expressed in human brain is over 1000, although currently only ~550 have been annotated in all humans (8). It has been speculated that miRNAs and other non-coding
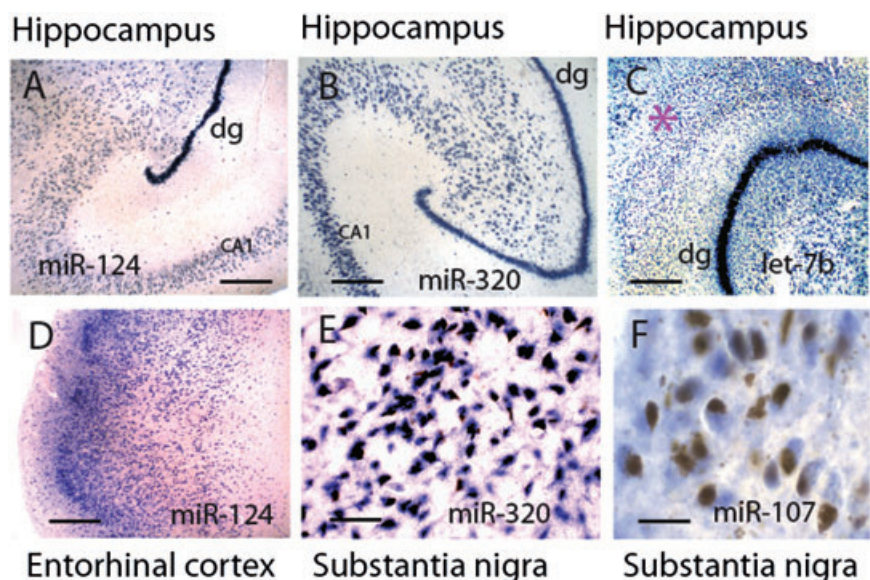

Figure 2. *In situ hybridization (ISH) from normal human brains for miRNAs gives an idea of how miRNAs are expressed in neuroanatomical areas that are vulnerable to NDs.* **A–C** show hippocampus. **A,B** shows miRNAs (miR-124 and miR-320) that are expressed predominantly in neurons. By contrast, let-7b is expressed in both neurons and glia, so cells outside the neuronal layers (such as the molecular layer, pink star, of the dentate granules, dg) are stained. **D** shows that cells of the superficial layer of entorhinal cortex are also stained avidly using a miR-124 probe. In the substantia nigra, miR-320 labels the pigment-containing cells (**E**), but miR-107 only labels them barely if at all (**F**). Scale bars: **A–D** = 500 µM; **E** = 125 µM; **F** = 62.5 µM. ISH was performed according to the protocol previously described (89) except fixed brain was cut with a freezing microtome.

Figure 3. Not only does a given miRNA recognize many mRNA targets, but the 3′ untranslated region (3′UTR) of many mRNAs are also the potential targets for many different miRNAs. Here are two examples that are relevant to NDs, the beta-amyloid precursor protein (APP) and alpha-synuclein (SNCA). miRNA target predictions are from the miRBASE registry (http://microrna.sanger.ac.uk/targets/v4/), and have not been validated. APP is predicted to be recognized by the products of 41 different miRNA genes, and SNCA is predicted to be recognized by that of 12.
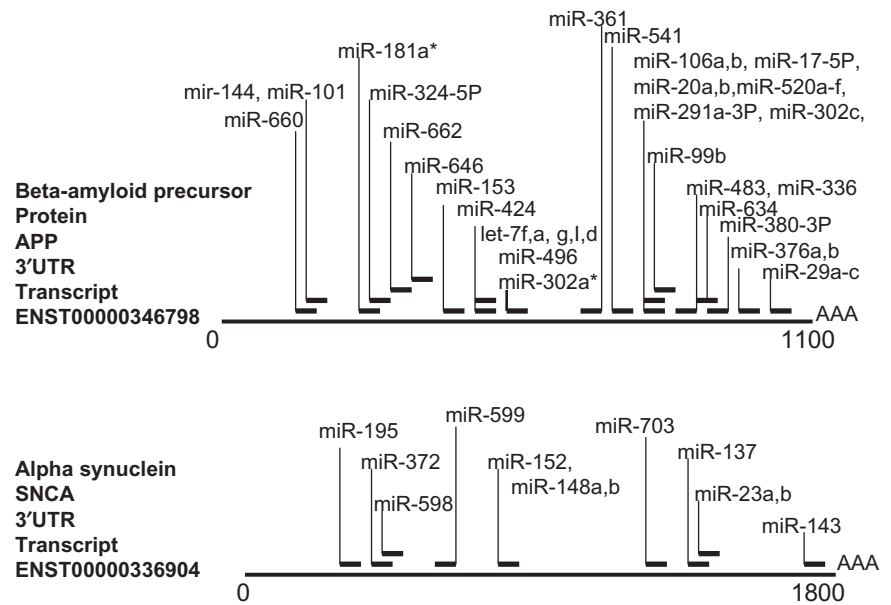
RNAs may serve to act as a "complexity multiplier" to help translate the ~25 000 human protein-coding genes into the human cerebral cortex with its ~10^{13} neurons, 10^{18} synapses and the staggeringly complex processes of neurodevelopment and neurophysiological network integration (35, 88).

The expression of brain miRNAs alters in the course of brain development. Accordingly, some miRNAs are relatively enriched during early development in the mammalian brain, and some are relatively enriched during later development (85, 90). These changes may relate to biochemical cues in cell fate determination, apoptosis and/or cell division programming (116). Some miRNAs are also relatively enriched in different neuronal nuclei, and/or different cell populations, including neurons or glia (117; see Figure 2). For example, it was recently shown that some rat miRNAs are relatively specific to the hippocampus relative to the rest of rat brain, and another miRNA may be enriched in the substantia nigra (46, 58). Furthermore, some miRNAs are relatively enriched in some neuronal cell compartments (somatodendritic vs. axonal) (64).

The function of brain miRNAs are evidently not related only to cell fate determination along developmental lineages. miRNAs may also play important roles in neuroplasticity and in many other neurobiological functions. These are beyond the scope of this review, and well discussed previously (61, 62). However, it bears repeating that probably most of the functions for miRNAs in the brain as elsewhere have yet to be discovered. The miRNA system is adaptable and researchers should be as open-minded as possible in developing testable hypotheses about physiologically relevant miRNA actions.

miRNAs IN NEURODEGENERATION—AN EXPANDING RESEARCH CORPUS

The current data that explicitly pertain to miRNAs and neurodegeneration must be characterized as preliminary, reflecting that our understanding of miRNAs is still in its infancy. However, recent studies have begun providing important glimpses into the functions of miRNAs in neuroprotection and neurodegeneration. These studies have been performed across a variety of cells and organisms. A table of some studies that include animal models is provided (Table 1).

Table 1.

Experimental system	Study relating miRNAs and ND	Notes	References
Fly eye	Dicer knock-down potentiates neurodegeneration in response to SCA3 and MAPT toxicity	Implicates miRNAs in neuroprotection from ND-related neurotoxicity	(12, 13)
Mouse cerebellum	Dicer knock-down leads to cerebellar neurodegeneration and ataxia	Analagies are drawn to spinocerebellar ataxia	(113)
Mouse and human substantia nigra	miR-133b is down-regulated in Parkinson's patients and may play a role in dopaminergic neuronal development	An important connection between novel new roles for miRNA function as well as ND-related dysfunction	(58)
Human brain and primary human neural cultures	A subset of miRNAs is increased in AD hippocampus and also in cell culture due to reactive oxygen species such as aluminum and iron sulfate	Draws a first direct link between oxidative stress, miRNAs, and NDs	(78, 79)

Abbreviations: AD = Alzheimer's disease; miRNA = microRNA; ND = neurodegenerative diseases; SCA3 = spinocerebellar ataxia type 3.

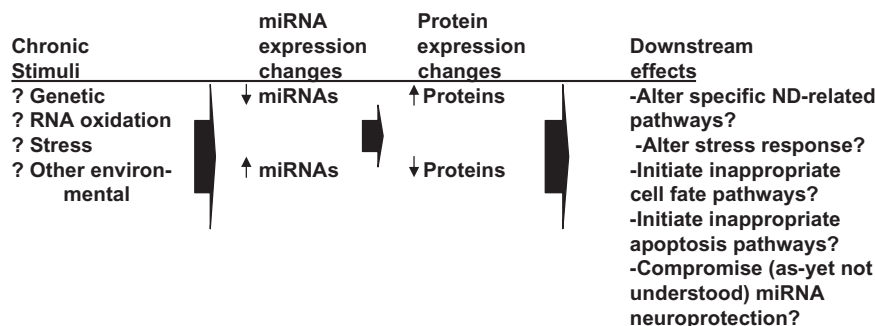| Chronic Stimuli	miRNA expression changes	Protein expression changes	Downstream effects
? Genetic ? RNA oxidation ? Stress ? Other environ- mental	↓ miRNAs ↑ miRNAs	↑ Proteins ↓ Proteins	-Alter specific ND-related pathways? -Alter stress response? -Initiate inappropriate cell fate pathways? -Initiate inappropriate apoptosis pathways? -Compromise (as-yet not understood) miRNA neuroprotection?

Figure 4. A schematic diagram about some roles miRNAs may play in NDs. The brains of persons at risk for NDs are subjected to chronic stimuli that can perturb specific and/or global miRNA expression. Either the aberrant stimulation or inhibition of miRNA expression may be associated with pathways involved in NDs or neuroprotection.

With regard to the functions of miRNAs in neuroprotection and NDs, experiments in invertebrates have provided important data. Research from Nancy Bonini's laboratory have shown that miRNAs can play an important role in modulating the nerve cell toxicity of pathologically altered SCA3 (spinocerebellar ataxia type 3) protein (12, 13). Increased number of polyglutamine repeats in SCA3 is what mediates the human clinical phenotype (106), and also correlates with cellular toxicity in the fly eye (13). SCA3 polyglutamine toxicity is greatly potentiated when miRNA pathways are inhibited. A particular miRNA (ban) is a factor of neuroprotection both in SCA3- and MAPT-related neurodegeneration in flies (12, 13). Also in flies, the miRNA miR-7 has been shown to potentiate stable EGFR signaling in photoreceptor cells (71). Still another fly miRNA (miR-14) also serves to help enable appropriate homeostasis in *Drosophila* eye cells, and particularly to play a role in adapting to stress (127). Taken together, these studies from different laboratories in flies demonstrate that animal miRNAs play a neuroprotective role in stress- and ND-relevant neuroprotection.

In addition to the studies in invertebrates, recent mammalian studies have also suggested a role for miRNAs in NDs and neuroprotection. Workers in Paul Greengard's laboratory used transgenic mice to give support to the hypothesis that miRNA function is critical for mammalian neuronal survival (and conversely, compromising miRNAs causes neurodegeneration) (113). In this study, the cerebellar Purkinje cell-specific Pcp2 promoter was used to drive a Cre recombinase causing the dicer gene (and the production of most mature miRNAs) to be knocked down beginning in the second week of life with Pcp2 gene activation. Loss of cerebellar expression of both dicer and most mature miRNAs is followed by Purkinje cell death and dendritic withering neuropathologically, and an ataxia phenotype develops in the mice. The authors hypothesize that as dicer down-regulation causes neuronal cell death, then some human neurodegenerative diseases may be caused by loss of small regulatory RNAs. As dicer is knocked out in the cerebellum, the authors conclude that their work may be relevant to spinocerebellar ataxias.

In another important work concerning miRNAs in NDs, research from Asa Abeliovich's laboratory indicates that a particular miRNA may play a significant role in PD (58). The authors show experimentally that miR-133b is relatively highly expressed at the tissue level in midbrain under normal conditions, but not during PD. Furhthermore, knocking out miRNAs generally *in vivo*, or miR-133b by itself in culture, dramatically decreases tyrosine hydroxylase and dopamine transporter levels in dopaminergic neurons. The data also support the hypothesis that miR-133b and the paired-like homeodomain transcription factor PITX3 regulate each other's expression. This study is important for a number of reasons. First, it indicates a discrete role for a particular miRNA in dopaminergic function. Second, previously, no particular miRNA–mRNA pair had been strongly implicated in a prevalent neurodegenerative disease. In other words, these investigators have provided plausible molecular neurobiological breakthroughs for both miRNA function and dysfunction. This study demonstrates also how miRNAs can be an important component of a "downward spiral" during NDs.

Two other excellent recent papers (79, 78) came from Walter Lukiw's laboratory. DNA arrays were analysed to evaluate the expression of a subset of 12 miRNAs in the AD hippocampus in comparison with non-demented controls and fetal brain. The results of the expression profiling was that miR-9 is elevated in both fetal hippocampus and AD hippocampus, miR-128a is elevated in AD but neither fetus nor non-demented controls, and miR-125b shows a trend to be increased in AD that is not statistically significant (78). In a second study from the same group (79), cultured human fetal brain-derived primary neural (HN) cells, which included both astrocytes and neurons, were treated with metal salts, such as aluminum and iron sulfates that stimulate reactive oxygen species (ROS). The RNA from HN cells was then compared using the same DNA miRNA array that was used in the study of AD brains. Remarkably, the HN cells treated using conditions that activate ROS also had essentially the same changes (increased expression of miR-9, miR-128 and, to a lesser extent, miR-125b) vs. controls, as were seen in the AD brain in comparison with non-demented brains. The authors make several inferences from these data. First, these data provide evidence in support of the hypothesis that ROS influence AD brain through pathways specifically mediated by miRNAs. Second, miR-125b, which trends higher in both AD brain and in ROS-treated HN cells, is predicted to target the mRNA of synapsin I and synapsin II, and thus down-regulation of synapsins in AD may be partially explained by miR-125b up-regulation (78, 79).

Some genes have been associated most strongly with NDs, and it would be interesting to see how strongly these genes are in turn regulated by miRNAs. Two examples are presented (Figure 3): beta-amyloid precursor protein and alpha-synuclein, which are important in the pathogenesis of AD and PD, respectively. An important characteristic of both of these genes is that studies have shown that the disease phenotypes in humans can be caused solely by increasing the expression of the genes (50, 87). As shown in Figure 3, each of these genes is also predicted to be the target of

multiple miRNAs. These predictions are not validated; however, they derive from only a single miRNA prediction database and still more miRNAs could easily target each of these (as well as many other) mRNAs.

Aside from the associations between miRNA changes and "classic" NDs, it is also notable that miRNAs may play a role in other degenerative brain diseases, including the fragile X mental retardation syndrome. Fragile X-associated tremor/ataxia syndrome (FXTAS) affects generally male carriers of expanded triplet CGG repeats on the fragile X mental retardation 1 (FMR1) gene. The polypeptide product of FMR1 is the protein FMRP, which is effectively silenced during the disease (36, 123). Neuropathologically, FXTAS syndrome is characterized by intranuclear inclusions, white matter changes and atypical appearing astrocytes profiles (40). Numerous lines of evidence suggest that FMRP interacts with the miRNA machinery, including miRNAs themselves (22, 33, 52, 55, 98). One hypothesis for the etiology of FMR is that a miRNA derived from the triplet repeat expansion in FMR1's 3′UTR mediates a methylation event that inhibits profoundly the transcription of FMR1 (54, 98). This hypothesis has been supported experimentally (74).

miRNAs IN NEURODEGENERATION—THEORETICAL ASPECTS AND QUESTIONS FOR THE FUTURE

Most of the details of how miRNAs participate in neurodegeneration have yet to be worked out. Hence, at this point, one may consider that the current research corpus is mostly a platform for future gains. Figure 4 shows some of the possibilities related to the interactions between miRNAs and NDs. Here are questions that may merit some reflection:

(i) Neurons are distinguished geometrically by their exceptionally asymmetric shape, with complicated functions requiring protein translation long distances—in some places, many centimeters—from the cell nucleus. Do miRNAs play a role in localized control of mRNA translation, and if so, does perturbation of that localized translational control contribute to NDs?

(ii) If RNAs are altered in NDs, are miRNAs altered also, and does ND-related miRNA alterations (eg, oxidation) alter biochemical function?

(iii) miRNAs are implicated in cell fate determination, apoptosis, cell division and stress, all of which are pathways thought to be aberrantly up-regulated in ND brains. Could the miRNA-related pathways relevant to earlier development be pathogenetic in the adult brain milieu?

(iv) Do risk factors for NDs (eg, the Apolipoprotein epsilon 4 allele) affect miRNA brain expression?

(v) To what extent are genes that are allelic for NDs, and for increased or decreased risk for NDs, target for miRNA regulation?

(vi) Is RNA editing of miRNAs changed in NDs?

(vii) What are the prospects for using miRNA-related biochemistry for ND therapeutics?

CONCLUSIONS

The regulation of human genomic expression is tremendously complex—it has to be in order to provide an information template for the human brain. We have only begun to understand the function of non-coding RNAs, such as miRNAs, in providing a complexity multiplier for the genome in the human CNS. It is hoped that research into the normal biochemical brain processes will dovetail with a better understanding of the pathological processes that these same molecules and pathways play. Tantalizing preliminary evidence suggests that miRNAs may play an important role in NDs.

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Note added in proof

129. Nelson PT, Jicha GA, Schmitt FA, Liu HL, Davis DG *et al*. Clinicopathological Correlations in a Large Alzheimer's Disease Center Autopsy Cohort: Neuritic Plaques and Neurofibrillary Tangles "Do Count" When Staging Disease Severity. *J Neuropathol Exp Neurol*, in press.

After submission of the manuscript, excellent additional work of miRNAs up-regulated targeted mRNAs was published by the laboratory of Joan Steitz.