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# MicroRNAs: novel therapeutic targets in neurodegenerative diseases

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**The prevalence of neurodegenerative disorders is rising steadily as human life expectancy increases. However, limited knowledge of the molecular basis of disease pathogenesis is a major hurdle in the identification of drug targets and development of therapeutic strategies for these largely incurable disorders. Recently, differential expression of endogenous regulatory small RNAs, known as 'microRNAs' (miRNAs), in patients of Alzheimer's disease, Parkinson's disease and models of ataxia suggest that they might have key regulatory roles in neurodegeneration. miRNAs that can target known mediators of neurodegeneration offer potential therapeutic targets. Our bioinformatic analysis suggests novel miRNA–target interactions that could potentially influence neurodegeneration. The recent development of molecules that alter miRNA expression promises valuable tools that will enhance the therapeutic potential of miRNAs.**

## Neurodegenerative disorders

Neurodegenerative diseases collectively present one of the leading causes of morbidity and healthcare costs to society, as well as being a source of severe human suffering [1,2]. The increasing prevalence of these diseases, coupled with the rise in the proportion of the elderly in the population, emphasizes the necessity of studying novel modes of therapeutic intervention. Neurodegenerative disorders include a wide range of clinical conditions associated with a progressive loss of neurons in specific parts of the cognitive, motor or sensory areas of the nervous system. Prion diseases, although rare, are also associated with protein aggregation – a mutant form of a specific protein resulting in the conversion of a normal protein into the pathogenic form. Motor neuron diseases, which include amyotrophic lateral sclerosis, are associated with degeneration of motor neurons, resulting in muscle atrophy and muscle wasting. The widely prevalent amyloid diseases are marked by the presence of protein aggregates in postmortem brain tissues of patients [3]. This class includes Alzheimer's disease, which is marked by the presence of proteinacious amyloid plaques; Parkinson's disease, which is characterized by intracytoplasmic aggregates called Lewi bodies; Huntington's disease; and a range of less prevalent ataxias marked by the presence of intranuclear protein aggregates [4]. Years of research have not provided a final answer to the debate about whether the protein aggregates are the cause or a consequence of neurodegeneration. Our understanding of the molecular basis of neurodegeneration has improved rapidly in the past two decades, spurred by the discovery of the genes responsible for the familial forms of these diseases. Recently, several groups have reported early findings on differential expression of microRNAs (miRNAs) in patient samples and models of neurodegenerative disorders. In a few cases, miRNA-mediated regulation of genes implicated in neurodegenerative disorders has also been demonstrated. It is widely believed that a thorough understanding of the molecular processes in neuronal function and dysfunction will present novel therapeutic opportunities. Here, we present an overview of the current understanding of small-RNA-mediated functions in neural systems and its therapeutic implications.

## miRNAs: novel regulators in the neuronal system

Several hundred small RNA molecules have been discovered in the recent past in almost all eukaryotic organisms. New classes of small RNAs are being discovered, even as previously unknown functions are being attributed to other small RNAs [5]. The most studied of these small RNAs is a class of 19–23 nt naturally occurring RNA molecules – miRNAs – that contribute to post-transcriptional regulation of gene expression. These RNA molecules are products

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**BOX 1**

### Rules of miRNA–target interaction

miRNA–target interaction involves miRNA binding to imperfect complementary sites in the 3′ UTR of the target transcript. Typically, the 5′ end of the miRNA contains a stretch of 7–8 nt with uninterrupted complementarity to the target RNA site, followed by bulges and mismatches. Additional weak complementarity towards the 3′ end of the miRNA stabilizes the miRNA–target interaction. In addition to the usual Watson–Crick base pairing, G:U base pairing might be involved in miRNA–target interaction. Perfect complementarity between the miRNA and target might lead to target cleavage, whereas imperfect complementary targets might be targeted for translational repression. A typical example of miRNA–target interaction is depicted below


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of enzymatic processing of longer precursor RNA molecules transcribed largely by RNA polymerase II from intergenic noncoding transcripts or noncoding regions of protein-coding transcripts [6]. The precursors typically contain imperfect inverted repeats that fold back to generate stem–loop structures. The stem–loop structures are cleaved at their base by the microprocessor complex consisting of the enzyme Drosha and its interacting partner, DGCR8/Pasha, within the nucleus [7]. The stem–loop structures are actively exported to the cytoplasm [8] and further processed by the highly conserved Dicer enzyme to release double-stranded RNA molecules corresponding to the stem region [9]. In a process that, as yet, has not been fully deciphered, the strands are selected and eventually associate with Argonaute proteins, which are crucial components of multiprotein complexes known as miRNA-RiboNucleoProtein complex (miRNPs). Different organisms differ in their number of Argonaute proteins, and the process by which different miRNAs associate with the Argonaute proteins is being studied intensively [10]. The association of the miRNPs (Box 1) with protein-coding transcripts bearing target regions complementary to the miRNA leads to interference in the translation of the target. The repressive role might be mediated by a reduction in the rate of translation by interfering with translational initiation or elongation [11]. Alternatively, specific miRNAs have been shown to destabilize targets by recruiting deadenylating enzymes [12]. In a large percentage of cases, miRNAs can also lead to degradation of the target, owing to cleavage by certain members of the Argonaute protein family.

miRNAs have important roles in early development and differentiation. A single miRNA can target multiple transcripts, and a single transcript can be targeted by a group of miRNAs [13]. The few hundred miRNAs in the human genome are predicted to collectively regulate nearly one-third of the protein-coding genes [14]. Loss of expression of miRNAs causes early developmental defects in model organisms and aberrant growth and cell division in cultured cells [15]. miRNAs have been implicated in cancer, and extensive clinical studies on their potential use as biomarkers are being undertaken [16]. Several miRNAs show tissue-specific expression patterns and have crucial roles in the differentiation and function of the tissue marked by their presence. For instance, the establishment of left and right asymmetry in a pair of bilateral neurons depends on a miRNA in *Caenorhabditis elegans* [17].

miRNAs have been associated with neuronal differentiation in *C. elegans*, zebrafish and mice. In mice and humans, several miRNAs with high expression in neuronal cells have been found. The crucial role of these regulators came to light when the ectopic expression of a brain-specific miRNA, miR-124, in HeLa cells resulted in alteration of the mRNA expression profile of these non-neuronal cells to resemble that of neuronal cells [18]. Like miR-124, miR-125b and miR-132 have also been shown to induce neuronal differentiation in stem cells [19,20]. Since then, studies into the specific roles of some neuronal miRNAs have resulted in an appreciation of the extensive interactions between these miRNAs and their targets in determining neuronal fate. In spite of the growing evidence in support of a role for miRNAs in neuronal differentiation and function, reports of their involvement in neurodegeneration are just beginning to emerge. In the following sections, we present a summary of three lines of evidence implicating miRNAs in neurodegeneration. These lines of evidence include differential miRNA expression, the targeting of disease-causing genes by miRNAs and genetic evidence from model organisms.

## Differential miRNA expression in neurodegenerative diseases

Several groups have used miRNA expression profiling as an exploratory approach to the identification of differentially expressed miRNAs in neurodegenerative diseases. Perkins *et al.* [21] identified 16 differentially expressed miRNAs by comparing the expression of 264 human miRNAs from the prefrontal cortex of 13 schizophrenia patients with that of 21 unaffected individuals. Notably, in this study, all but one of the miRNAs were downregulated. Mice injected intracerebrally with a pathogenic *scrapie* strain were compared to normal mice using microarray-based miRNA expression profiling followed by validation using real-time PCR assays [22]. All but one of the miRNAs of the eight differentially expressed miRNAs were upregulated. In mouse models of Parkinson's disease, miR-133b was found to be downregulated [23]. Alzheimer's disease is the only neurodegenerative disease

for which several independent attempts to identify differentially expressed miRNAs have been made. There is, however, little overlap between miRNAs identified in these studies [24]. Lukiw *et al.* [25] found miR-9 and miR-138 upregulated in the brains of Alzheimer's patients, whereas miR-9, miR-29a/29b and miR-107 were found to be downregulated in sporadic Alzheimer's patients [26,27]. In spite of attempts by several groups, an anticorrelation in microarray data between miRNA and target mRNAs has been elusive.

The biggest challenges in identifying functional roles from differential expression of miRNAs are twofold. First, identification of targets for the differentially expressed miRNAs can be a daunting task. Results from different computational algorithms for the prediction of miRNA targets differ widely, necessitating the slow and tedious process of experimental validation of miRNA–target interactions [28]. Currently, synthetic miRNA mimics are used to transiently elevate the miRNA levels several-fold, and reporter-3′ UTR fusions, often carrying multiple copies of the target region, are used to assess the effect. Tools for controlled expression of the miRNA, as well as the target, in relevant *in vivo* models are under development. These will be greatly beneficial because the miRNA–target interaction is influenced by the concentrations of both interacting molecules, as well as by other local cellular factors. Second, the heterogeneity of cell types and the high frequency of alternative splicing and polyadenylation in the brain provide a wider repertoire for miRNA–target interactions, presumably absent in other tissues. It is necessary, therefore, to assess whether the apparent differential expression of miRNAs arises from changes in composition of cell types or changes in expression. It might be impossible to identify crucial changes in miRNA expression restricted to a subpopulation of cells because these effects will be diluted away at the tissue level. These limitations notwithstanding, we find that a core set of miRNAs – miR-133b, miR-9 and miR-29a/b – have been repeatedly found to be differentially expressed in independent studies into miRNA expression profiling during neurodegeneration. Table 1 provides an overview of differentially expressed miRNAs identified in more than one study on neurodegenerative disease.

Of these miRNAs, miR-9 is highly conserved in mice, humans and rats and expressed highly in neuronal cells, including the cortex and cerebellum [29]. However, miR-29a/b has a broad expression profile, covering several cell types of the immune system besides lung, heart, bone and brain tissue. It has also been implicated in diverse biological processes, including osteoblast formation, insulin resistance, lung development and antiviral defense [30–33]. In addition, it is known to activate p53 by targeting Cell division control protein 42 homolog (CDC42) and the regulatory subunit of PI3 kinase [34]. It will be interesting to understand the basis of the tissue-specific effects of miR-29a in the neuronal system. miR-133 is highly expressed in heart tissue and was implicated in cardiomyocyte differentiation before it was involved in dopaminergic neuron maturation in the brain [23].

## Targeting of disease-causing genes by miRNAs

The causative genes and mutations for several familial forms of neurodegenerative diseases are known [35]. For example, CAG repeat expansions in approximately ten genes are known to cause different forms of ataxias, including Huntington's disease. Reduction in the levels of the pathogenic protein is one of the proposed therapeutic approaches to reduce their toxicity. miRNAs that target genes involved in neurodegeneration are of potential therapeutic value because they can mediate translational repression of the detrimental protein. Fig. 1 provides an overview of the known instances of miRNAs targeting neurodegeneration-related genes. Lee *et al.* [36] showed recently that three cellular miRNAs – miR-19, miR-101 and miR-130 – can directly target the ATXN1 gene (Fig. 1a), CAG repeat expansions that are responsible for SCA1, a typical polyglutamine-mediated aggregation resulting in the loss of neurons in purkinje cells. miR-101 and miR-130 were expressed highly in Purkinje cells, the site of degeneration in spinocerebellar ataxia. Liberating the ATXN1 transcript from miRNA-mediated repression by mutating the target sites enhanced its cytotoxicity, implying that these miRNAs reduce cytotoxicity by reducing the level of the pathogenic protein.

A similar, direct interaction between miRNAs and genes in neurodegeneration involves miR-29a/b (Fig. 1b). The BACE1 gene encodes β-secretase, an enzyme that brings about the rate-limiting step of processing of amyloid β-protein from amyloid precursor protein (APP) peptides that form the insoluble aggregates characteristic of Alzheimer's patients. BACE1 levels are elevated in

**TABLE 1**

**Overview of differentially expressed miRNAs identified in various neurodegenerative diseases**

| *Disease* | Gene | Differentially expressed miRNA | Refs |
|---|---|---|---|
| **Huntington's disease** | HTT (Huntingtin) | *miR-29a, miR-29b-1, miR-132, miR-135b* | [38] |
| **SCA1** | Ataxin 1 | *miR-19a, miR-101, miR-130a* | [36] |
| **SCA3** | Ataxin 3 | Bantam | [44] |
| **Schizophrenia** | – | *miR-26b, miR-30b, miR-29b, miR-195, miR-92, miR-7, miR-24, miR-30e* | [21] |
| **Prion-induced degeneration** | – | *miR-342-3p, miR-320, let-7b, miR-328, miR-128, miR-139-5p, miR-146a, miR-338-3p, miR-337-3p* | [22] |
| **Alzheimer's disease** | – | *miR-9, miR-138, miR-125b, miR-107, miR-103, miR-23b, miR-29a, miR-29b, miR-210, miR-181c, miR-15a, miR-22, miR-101, miR-197, miR-511, let-7i, miR-19b, miR-320, miR-106b, miR-26b, miR-363, miR-93* | [25–27,37] |

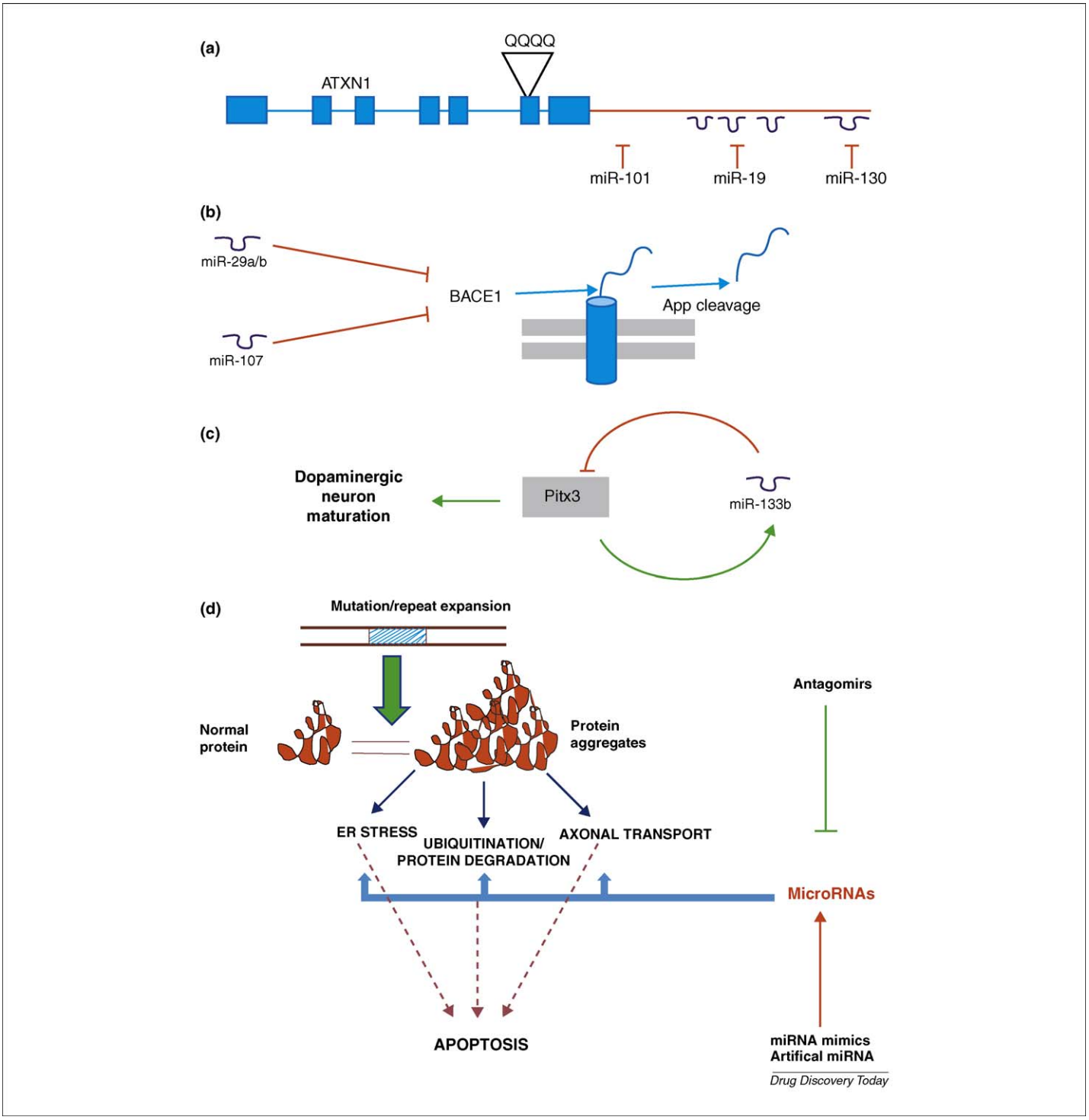


**FIGURE 1**

Examples of miRNA gene networks in neurodegeneration. **(a)** Three miRNAs target the ATXN1 gene polyglutamine expansion, which causes SCA1. **(b)** Two miRNAs, miR-29a/b and miR-107, downregulate BACE1, which encodes the rate-limiting β-secretase responsible for cleavage of APP, a component of amyloid aggregates in Alzheimer's disease. **(c)** miR-133b is under the regulation of the dopaminergic neuron transcription factor PITX3 but negatively regulates it, resulting in a negative feedback loop. **(d)** An overview of ways in which miRNAs might affect events in neurodegeneration and tools for modulating miRNA action.

sporadic Alzheimer's patients. Overexpression of miR-29a/b resulted in the reduction of BACE1 levels in cultured cells, and loss of miR-29a/b correlated with higher BACE1 levels in patients [27,37]. Furthermore, a direct miRNA–target relation was established by mutating the target site. BACE1 has also been regulated by miR-107 [26]. Interestingly, both 29a/b and miR-9 are expressed highly in several regions of the brain and are under the control of the transcription factor RE1 silencing transcription factor (REST). REST drives neuronal cell fate specification by inducing neuronal genes, besides miR-124 – a repressor of non-neuronal genes [38].

Another miRNA that is important in neuronal function is miR-133b. Expression of miR-133b is driven by the transcription factor

**TABLE 2**

**List of miRNAs predicted to target genes involved in various neurodegenerative diseases[a,b]**

| *Disease* | Gene | miRNAs |
|---|---|---|
| **Huntington's disease** | HTT | *miR-103, miR-107, miR-128, miR-146, miR-149, miR-15, miR-16, miR-181, miR-18, miR-195, miR-199, miR-204, miR-211, miR-214, miR-222, miR-27, miR-299, miR-30, miR-337, miR-363, miR-370, miR-381, miR-423, miR-424, miR-452, miR-485, miR-492, miR-512, miR-515, miR-518, miR-526, miR-556, miR-565, miR-575, miR-588, miR-596, miR-625, miR-631, miR-632, miR-634, miR-637, miR-646, miR-650, miR-765, miR-767* |
| **Dentatorubro-pallidoluysian atrophy (DRPLA)** | ATN1 or DRPLA | *miR-93, miR-768-5p, miR-668, miR-663, miR-659, miR-652, miR-650, miR-642, miR-639, miR-638, miR-637, miR-629, miR-612, miR-604, miR-523, miR-513, miR-485, miR-483, miR-346, miR-339, miR-328, miR-24, miR-197, miR-18b, miR-150, miR-122a, miR-107* |
| **SCA1** | ATXN1 (Ataxin 1) | *miR-101, miR-103, miR-106, miR-125, miR-130, miR-133, miR-139, miR-141, miR-148a, miR-149, miR-152, miR-17, miR-181, miR-188, miR-193a, miR-199, miR-200, miR-204, miR-29a, miR-29b, miR-211, miR-214, miR-221, miR-299, miR-302, miR-326,329, miR-339, miR-370, miR-448, miR-454-3p, miR-484, miR-486, miR-493, miR-509, miR-512-3p, miR-515, miR-518, miR-519, miR-520, miR-525, miR-527, miR-542-3p, miR-565, miR-588, miR-622, miR-625, miR-628, miR-630, miR-642, miR-650, miR-653, miR-7, miR-766* |
| **SCA2** | ATXN2 (Ataxin 2) | *miR-107, miR-147, miR-193b, miR-199a, miR-296, miR-299-3p, miR-302, miR-34a, miR-34b miR-363, miR-371, miR-422, miR-423, miR-432, miR-490, miR-492, miR-520, miR-524, miR-558, miR-560, miR-575, miR-601, miR-611, miR-615, miR-617, miR-623, miR-637, miR-767-5p, miR-768-5p* |
| **SCA6** | CACNA1A | *miR-502, miR-661* |
| **SCA17** | TBP (TATA-binding protein) | *miR-24, miR-149, miR-183, miR-324-5p, miR-370, miR-491, miR-500, miR-503, miR-519b, miR-523, miR-564, miR-612, miR-619, miR-622, miR-630, miR-638, miR-639, miR-654, miR-663* |

[a] Consensus predictions using miRNAMap [53] are derived from three computational tools: miRanda, RNAhybrid and Target Scan. To minimize false positives, only miRNA–target pairs predicted by at least two tools, with multiple targets of high accessibility within the same 3′ UTR, were used.
[b] ATXN3, ATXN7 and AR genes that affect SCA3, SCA7 and SBMA, respectively, did not show any consensus miRNA target sites, although individual prediction tools predicted a few sites.

PITX3. Using a frequently seen negative feedback regulatory motif (Fig. 1c), miR-133b downregulates PITX3. The miR-133b is also responsible for dopaminergic neuron maturation and survival. Expression of miR-133b is reduced in the midbrain of sporadic patients of Parkinson's disease [23], the second most frequently occurring adult neurodegeneration. Elevated expression of FGF20, a known risk factor of Parkinson's disease, has recently been associated with polymorphism in its 3′ UTR that relieves repression by miR-433 by disrupting its binding site [39]. The involvement of polymorphisms in miRNA–target interaction [40], collected in the dbSMR database [41], is likely to provide functional roles for single-nucleotide polymorphisms in noncoding regions of genes involved in neurodegenerative diseases.

We have seen earlier that consensus miRNA–target prediction derived from multiple miRNA–target prediction programs provides a short list of highly reliable targets suitable for experimental validation. We used this consensus target prediction approach to compile a list of miRNAs that have been computationally predicted to target neurodegeneration-related genes (Table 2).

## miRNAs in genetic models of neurodegeneration

A third line of evidence that implicates miRNAs in neurodegeneration comes from studies performed in fly, worm and mouse models. Polyglutamine expansion in Ataxin 3 causes SCA3, one of the polyglutamine-mediated neurodegenerative disorders characterized by the loss of cells in several regions of the brain stem and cerebellum [42]. Expression of the polyQ-expanded protein in the Drosophila eye results in cell death and easily recordable phenotypes, such as loss of pigmentation, collapse of the retina and reduction in the number of photoreceptors per ommatidium [43]. This powerful model of retinal neurodegeneration has allowed screens for genes that enhance or mitigate polyglutamine-mediated neurodegeneration. The drosophila gene, bantam (ban), which gives rise to a miRNA of the same name, was identified repeatedly in a screen for factors that mitigate the toxicity of the polyQ-expanded SCA3 allele [44]. Unlike the miRNAs targeting ATXN1, ban does not seem to act by reducing the pathogenic protein concentration. Ban is known to influence cell survival through targets in the apoptosis pathway [45]. Moreover, ban has been shown to mitigate the Tau-induced toxicity associated with Alzheimer's disease [43]. Therefore, instead of acting on the specific disease-causing protein, a more downstream role in protecting cells from programmed cell death owing to protein toxicity seems to be relevant. This has important therapeutic implications because it suggests that common modes of therapy enhancing cell survival through miRNAs are possible, even if the pathogenic proteins are not directly under the regulation of miRNAs. Notably, no mammalian homolog has been identified for bantam [44], although expression of the fly bantam miRNA in HeLa cells resulted in reduced aggregation of the polyglutamine-expanded form of ATXN3 [44]. The general role of miRNAs besides bantam in neurodegeneration is reinforced by the finding that Dicer mutants deficient in miRNA processing show severe neurodegeneration when the mutation is restricted to the drosophila eye. Similarly, in other model systems like the zebrafish, neuronal development is defective in Dicer mutants [46], reinforcing the role of miRNAs in neuronal function.

## The promise of miRNA therapeutics

Most neurodegenerative diseases do not have effective therapies or conventional druggable targets, partly because of the limited understanding of the molecular and cellular cascade of events

leading to neurodegeneration. Consequently, there is considerable optimism about RNAi-based therapeutics. There are several approaches for developing RNAi-based therapeutics [47]. Currently, siRNAs are being tested in animal models to study their potential effect on neurodegeneration [48,49]. Here, we discuss novel approaches to the development of miRNA-based therapeutics.

miRNA mimics, small RNA molecules that are presented in forms that resemble miRNA precursors, can be used to downregulate a specific target protein. The target protein can be any gene implicated in the progression of neurodegeneration, the gene harboring the pathogenic mutation or, more specifically, the mutant allele alone. In cases in which the disease gene does not have natural miRNAs, artificial miRNAs can be designed to target them. A striking feature of most neurodegenerative diseases is that the causative mutations are dominant. For example, polyglutamine-expanded proteins are believed to aggregate with the functional protein produced by the normal allele, and the mutant prion protein also converts the normal protein to the toxic form [36]. Therefore, reduction of the protein level itself is believed to offer a protective therapeutic strategy. In specific cases, it might even be possible to use the mutant allele to specifically develop allele-specific targeting strategies, although this approach might not be easily applicable for repeat expansion disorders. However, the disadvantages of using miRNA mimics include potential off-target effects and the possibility of overwhelming the RNAi processing machinery, interfering with the normal functioning of the cell. The half-lives of miRNA mimics in *in vivo* conditions have not been studied extensively. Presumably, repeated administration of the miRNA mimic will be required to create sustained effects.

A second approach to miRNA-based therapeutics is to downregulate miRNAs using anti-miRNA molecules, to augment natural cell survival pathways and to downregulate apoptotic pathways. This approach has the appeal of a generalized strategy, potentially useful in multiple degenerative diseases. Backbone modifications such as locked nucleic acid (LNA) modification of oligonucleotides have been used to enhance the specificity and reduce effective doses of the anti-miRNA molecules [50]. A striking feature of LNA-modified anti-miRNAs is that they not only hinder the miRNA–target binding but also reduce miRNA levels. Intravenous injection of LNA-modified anti-miRNAs targeting a liver-specific miRNA had long-lasting (five to seven weeks) but reversible effects on plasma cholesterol levels in nonhuman primates [51]. Such directed studies are required to assess specifically the dosage, period of activity and clearance of anti-miRNA molecules in *in vivo* models of neurodegeneration. Recently, LNA-based anti-miRNAs have been enhanced by the incorporation of an endo-nucleolytic DNAzyme motif [52]. These antagomiRzymes cause cleavage of the targeted miRNA, thus promising more effective clearance of the miRNA. These novel molecules, however, have yet to be tested in *in vivo* models.

The miRNA processing machinery is necessary for neuronal differentiation and function, as is evident from the defects of mutants lacking key components of the pathway. Although knockout of the miRNA-specific processing enzyme, Dicer 1, in drosophila causes neuronal cell death, the effect seems to be due to a general dysregulation of miRNAs [43]. It will be difficult to introduce specificity to any approach that targets the miRNA processing machinery because it influences several pathways. However, it cannot be completely ruled out; general approaches of transcriptional modulation, such as the use of chromatin-modifying agents, have earlier shown promise in countering neurodegeneration.

In summary, currently available evidence clearly points to a significant role for miRNAs in neurodegenerative diseases. Several leads for development of therapeutics against neurodegenerative disorders have emerged from studying differentially expressed miRNAs and their target (Fig. 1d). Development of tools for the delivery, stable expression and controlled modulation of miRNA levels and action *in vivo* will be valuable aids in enhancing the therapeutic value of miRNAs. Moreover, basic data on the pharmacological properties of miRNA mimics and anti-miRNA molecules have to be generated. In future, the increase in fundamental knowledge about the role of miRNAs in the neuronal system will be complemented with studies in other areas of drug development to assess and improve their therapeutic value.

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