REVIEW ARTICLE

MicroRNA Signatures in Neurological Disorders

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ABSTRACT: A class of small, non-coding transcripts called microRNAs (miRNAs) that play a major role in post-transcriptional gene regulation has recently emerged and become the focus of intense research. MicroRNAs are abundant in the nervous system, where they have key roles in development and are likely to be important mediators of plasticity. A highly conserved pathway of miRNA biogenesis is closely linked to the transport and translatability of mRNAs in neurons. MicroRNAs have been shown to modulate programmed cell death during development. Although there are nearly 750 known human miRNA sequences, each of only approximately 20-25 nucleotides in length that bind to multiple mRNA targets, the accurate prediction of miRNA targets seems to lie just beyond our grasp. Nevertheless, the identification of such targets promises to provide new insights into many facets of neuronal function. In this review, we briefly describe miRNA biogenesis and the principle approaches for studying the function of miRNAs and potential application of miRNAs as biomarkers, diagnostic targets, and potential therapeutic tools of human diseases in general and neurological disorders in particular.

RÉSUMÉ: Signatures de micro-ARN dans les maladies neurologiques. Une classe de petits transcrits non codants appelés micro-ARN (miARN), qui jouent un rôle majeur dans la régulation génique post-transcriptionnelle, sont connus depuis peu et font l'objet de recherches intensives. Les miARN sont abondants dans le système nerveux où ils jouent des rôles clés au cours du développement et sont vraisemblablement d'importants médiateurs de la plasticité. Une voie très hautement conservée de biogenèse des miARN est étroitement liée au transport et à la traductibilité des ARNm dans les neurones. Il a été démontré que les miARN modulent la mort cellulaire programmée pendant le développement. Bien qu'il y ait près de 750 séquences connues de miARN, chacune d'environ 20 à 25 nucléotides de long seulement et qui se lient à de multiples cibles d'ARNm, la prédiction exacte des cibles des miARN semble encore nous échapper. Néanmoins, l'identification de ces cibles fournira sans doute de nouvelles pistes de recherche sur de nombreux aspects de la fonction neuronale. Dans cette revue, nous décrivons brièvement la biogenèse des miARN et les principales approches pour l'étude de leur fonction et leurs applications potentielles comme biomarqueurs, cibles diagnostiques et outils thérapeutiques dans les maladies humaines en général et les maladies neurologiques en particulier.

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The development and function of the nervous system is orchestrated by a plethora of gene regulatory mechanisms. MicroRNAs (miRNAs), a novel class of small non-coding RNAs (Ribonucleic acid), are effective post-transcriptional regulators of gene expression[1]. Since the discovery of miRNAs, it has become evident that the genomic complexity of a cell is far greater than expected. MicroRNAs (miRNAs) are a new class of non-protein-coding, endogenous, small RNAs that seem to be of vital importance in the functioning of cells. MicroRNA regulates gene expression by translational repression, mRNA cleavage, and mRNA decay initiated by miRNA-guided rapid deadenylation. These processes are now being recognized as important post-transcriptional regulators of gene expression in the brain. MicroRNAs are an abundant class of endogenous small RNA molecules with a size varying from 20-25 nucleotides in length[1-4]. All pre-miRNAs have hairpin secondary structure[1-4]. Some miRNAs are highly conserved from species to species in animals and plants[5-6]. These miRNAs have been indicated to be of great importance in diverse biological process, including cell cycle regulation, apoptosis, cell differentiation, maintenance of stemness and imprinting[7]. The first miRNA (see Figure 1) was described in 1993, in which the *C. elegans* heterochronic gene

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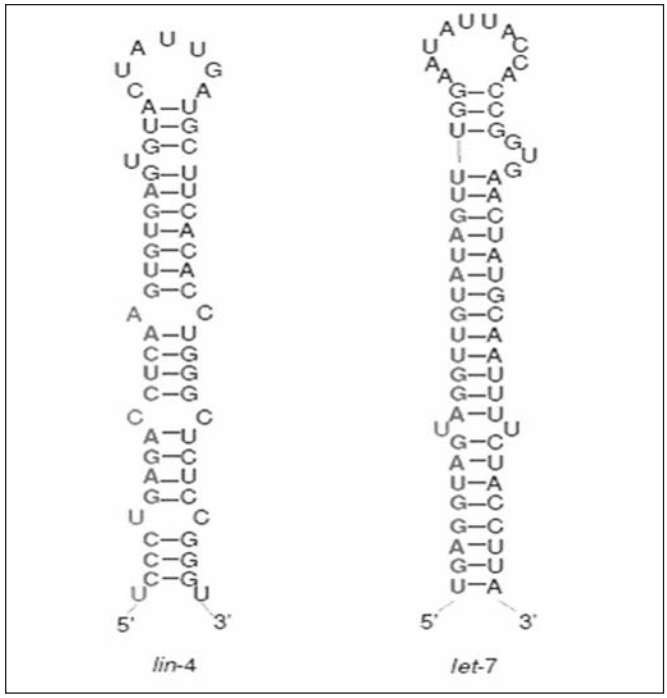

Figure 1: *The predicted stem loop structure of the two microRNA molecules first discovered; lin-4 (left) and let-7 (right). The sequence of mature miRNAs are shown in grey.*

lin-4 encoded small RNAs with antisense complementarity to *lin-14*[8]. It is estimated that vertebrate genomes encode more than 1,000 unique miRNAs[9], which are predicted to regulate expression of at least 50% of genes[10]. Though more than 735 miRNAs have been identified in humans, much remains to be understood about their precise cellular function and role in the development of diseases.

In recent years, the involvement of miRNA in the pathophysiology of neurological disorders has been assessed. Brain RNAs become pathologically altered in some neurological disorders. These changes have been reviewed elsewhere[11,12], but include aberrant RNA oxidation, RNA degradation, altered RNA splicing and ribosomal changes which cause mRNA translational frame-shifting abnormalities. MicroRNAs have been implicated in a number of pathological conditions of the central nervous system (CNS), ranging from cancer to neurodegenerative diseases such as Alzheimer's and Parkinson's disease[12]. However, a causal link between a specific miRNA and a disease has been established in just a few cases, and most of the mechanistic data originates from invertebrate model systems. MicroRNA expression profiling of human neurological disorders has led to the identification of signatures correlated with the diagnosis, staging, progression, prognosis and response to treatment. MicroRNA fingerprinting can therefore be added to the diagnostic and prognostic tools used by medical experts.

Recently, microarrays have been used to estimate the genome-wide variation in gene expression across different populations[12]. These studies have identified numerous genes that are expressed differently in different populations and suggest that natural variation in gene expression rather than structural changes in gene products probably account for a substantial part of phenotypic variation. However, the molecular basis of differences in gene expression is still unclear. Identification of causal genetic variants underlying complex traits is a major goal in molecular genetic studies. Polymorphisms (see Figure 2) in miRNA target sites represent a specific class of regulatory polymorphisms that may regulate posttranscriptional gene expression. Although each miRNA can control hundreds of target genes, identifying the accurate miRNA targets for brain research remains a huge challenge.

Biogenesis of MicroRNAs

Various biochemical approaches have provided a basic understanding of the molecular details of miRNA biogenesis[13]. A primary transcript RNA (pri-miRNA) transcribed from a miRNA gene by RNA polymerase II is first processed (as shown in Figure 3) into a stemloop structure of about 70-80 nucleotides known as precursor miRNA (pre-miRNA) by a microprocessor enzyme comprising of a double-strand (ds)-RNA-specific ribonuclease, *Drosha*, with the help of its binding partner DGCR8[14]. These pre-miRNAs are transported into the cytoplasm via an Exportin-5-RanGTP dependent mechanism[15-17]. In the cytoplasm, they are digested by a second, dsRNA-specific ribonuclease called *Dicer* with the help of TRBP and AGO2[18-20]. The 20-25 nucleotide long fragment released is the mature miRNA, which is bound by a complex called miRNA-associated RNA-induced silencing complex (miRISC). Two mechanisms for miRNA action are currently known: Cleavage and translational repression of mRNA without RNA cleavage. In animals, the complex-bound, single stranded miRNAs bind specific target mRNAs through a sequence that is significantly, though not completely complementary to the target mRNAs[21].

Figure 2: *miRNAs from the let-7 family with single nucleotide difference.*

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The bound mRNA remains untranslated, resulting in reduced expression of the corresponding genes by a mechanism that is not fully understood. A single miRNA could regulate multiple target genes, while a single gene could be targeted by multiple miRNAs, suggesting that the miRNAome and mRNAome interaction is a highly complicated network.

MicroRNAs in Neurological Disorders

The first hint that miRNAs might play a variety of roles within the nervous system came from cloning and expression analysis of miRNAs. A surprisingly high number of unique miRNAs were isolated from brain and neuronal cell lines[22]. A large number of these microRNAs were associated with polyribosomes[23-24], a hallmark of ongoing translation indicating that in the nervous system, like in other tissues, miRNAs might be involved in the regulation of translation.

Suppression of miRNA biogenesis by disrupting the zebrafish Dicer gene has provided the first evidence that miRNAs are necessary for the development of the nervous system[25]. It is unknown if Dicer plays a similar role during early neural development in mammals as Dicer deficient mice die at embryonic day 7.5 before neurulation occurs[26]. However, recent conditional gene targeting approaches have shed some light on the role of the miRNA pathway in the CNS in mammals. Similarly, progressive cell death was observed when Dicer was inactivated postnatally in the cerebellum[27] or in dopaminergic neurons in the forebrain[28].

In the mature nervous system, miRNAs are emerging as important regulators of synaptic plasticity, the dynamic functional and morphological modifications that occur at the synapse and are thought to underlie higher cognitive functions such as learning and memory[29].

MicroRNAs are involved in a variety of physiological and pathological processes in multi-cellular organisms, ranging from patterning to cancer development[30]. Since the CNS is a rich source of miRNAs that often display a brain specific expression pattern (discussed later), and since a single miRNA is able to target up to a few hundreds of different mRNAs[9], it is hardly surprising that the number of roles assigned to miRNAs during all stages of CNS development and function is rapidly expanding.

MicroRNAs have been implicated in a number of neurological disorders, ranging from cancer to neuro-degenerative diseases such as Alzheimer's and Parkinson's[11]. Analysis of single-nucleotide polymorphisms (SNPs) in humans revealed a strong negative selection against mutations in miRNA binding sites in the 3"UTR[31]. Thus linkage analysis of disease-associated SNPs is likely to reveal several links between miRNAs and neurological conditions. A first case of a deleterious polymorphism in a miRNA target mRNA has been identified in a candidate gene for Tourette's syndrome, SLIT and Trk-like 1(SLITRK1), whose protein product is necessary for the growth, guidance and branching of neuronal cells as well as other associated processes. Two identical mutations in the mir-189 binding site on the SLITRK1 3"UTR have been detected in unrelated afflicted individuals[32]. The intriguing reduction of mir-133 levels in the midbrain of patients with Parkinson's disease has been shown. Interestingly, neurodegeneration is also observed in mice that lack Dicer, specifically in the cerebellum.

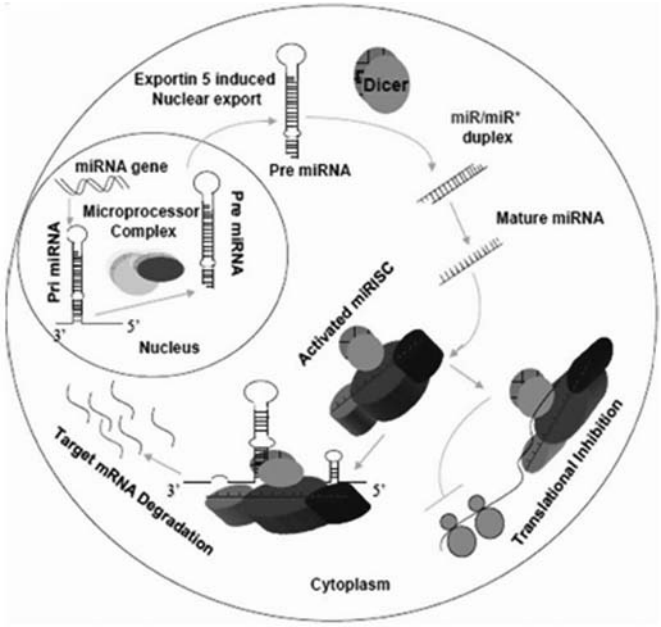

Figure 3: *MicroRNA: Processing and Activity; Depicts the formation of long primary micro RNA (pri-miRNA) in the nucleus which is processed by microprocessor complex (Drosha- an RNase III enzyme and Pasha- double stranded RNA binding protein) into pre-microRNA (70 nucleotide stem loop structure) and transported to cytoplasm by Exportin 5 mediated export, where Dicer, an RNase II enzyme cleaves it to 20 – 23 nucleotide mature micro RNA that integrates it into the microRNA Inducing Silencing Complex (miRISC), a complex of proteins that is responsible for regulation of gene expression either by translational inhibition or target RNA degradation.*

In these animals Purkinje neurons progressively degenerate and the animals develop ataxia[27]. Further, alterations of miRNA levels have also been detected in the prefrontal cortex of patients with schizophrenia[33], and SNP-genetic linkage analysis identified two miRNA loci associated with schizophrenia[34]. These correlations await further validation. However, they strongly suggest a connection between miRNAs and diseases that affect the dopamine system. A link between miRNAs and polyglutamine-induced neurodegeneration has recently been discovered. In this study, toxicity of the spinocerebellar ataxia type 3 protein was strongly enhanced by mutations of the drosophila Dicer homologue, and a genetic screen identified the miRNA Bantam as suppressor of spinocerebellar ataxia type 3 protein toxicity[35]. The role of the miRNA pathway in the development of several kinds of cancer is an area of intense research[36]. Related to the CNS a set of miRNAs whose expression is altered in glioblastomas has been identified by microarray analysis[37-38]. Intriguingly, inhibition of one of these miRNAs (mir-21) in a glioblastoma cell line has been shown to induce apoptosis[38], implicating miRNAs as potentially attractive targets for anti-cancer therapy. In a fetal mouse cerebral cortex culture model, the pro-apoptotic function of mir-21 was counteracted by mir-335, where the expression of both miRNAs was regulated by ethanol exposure. Toxic levels of ethanol

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suppressed mir-21 expression, while lower levels of ethanol induced mir-335 expression[39] suggesting antagonistic roles of miRNAs during ethanol induced neurotoxicity. MicroRNAs continue to be expressed in adult neurons, many at relatively high levels. A facet of neuronal function to which miRNA-mediated regulation seems particularly well suited is local translational control of plasticity[40].

Researchers have reported for the first time the involvement of miRNA regulation in brain pathogenesis associated with middle cerebral artery occlusion. They have also provided evidence that some of the miRNAs that are highly expressed in the ischemic brain can be detected in blood samples[41].

Although recent studies are beginning to unravel how miRNAs fit into signaling pathways that govern cell fate decisions, the factors that control miRNA expression and their precise roles during the acquisition of a specific cell phenotype are yet to be explicitly elucidated. During cardiogenesis, for example, serum response factor induces the expression of muscle-specific miRNA, miR-1, which in turn controls cardiomyocyte proliferation by down-regulating the Hand2 transcription factor[42]. Establishment of the left–right asymmetry of chemosensory neurons in *C. elegans* also involves a signaling network of transcription factors and miRNAs[43-44]. In the mammalian nervous system, where cellular diversity is extreme, the factors responsible for miRNA gene regulation are only just emerging[45].

MicroRNAs are also abundantly expressed in the adult brain and appear to regulate the maintenance of mature neural traits and synaptic plasticity[46-53]. Numerous studies suggest that miRNAs are intimately involved in synaptic function and input specificity during memory formation[40,23,53-56]. Moreover, transcripts encoding synapse-associated proteins also comprise the largest subgroup of predicted miRNA targets, including synapsin 1 and the fragile X mental retardation protein[57].

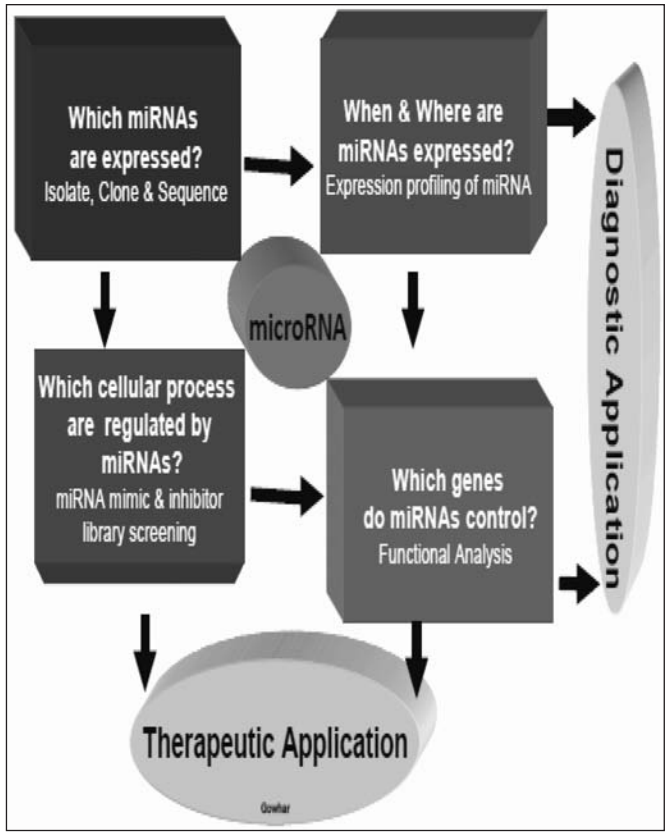

Figure 4: *Common questions researchers are faced with and the technical approaches that can be adopted in resolving these questions on miRNA.*

Methods Involved in MicroRNA Study

The small size of miRNAs poses a unique challenge in their detection, requiring a method that is both highly sensitive and discriminatory[58]. Most commercially available technologies for global miRNA expression profiling are based on DNA microarrays, in which the probes are spotted onto a glass surface. Such probes, however, are not optimal for miRNA detection because of cross-hybridization and lack of specificity; discrimination between single-nucleotide differences is not always possible[59].

In order to gain insight into these tiny regulators, researchers around the world are asking fundamental research questions (see Figure 4). Now that many miRNA sequences are known (cataloged in the miRBase Sequence Database at http://microrna.sanger.ac.uk), the next step would be analysis of miRNA expression levels between different tissues, developmental stages, or disease states. MicroRNA expression levels can be studied using several different methods: Microarray analysis, real-time polymerase chain reaction (PCR), Northern blots, in situ hybridization etc. Of these techniques, quantitative reverse transcription PCR (qRT-PCR) and Microarray are the most sensitive and accurate methods. A crucial requirement in the miRNA field is the development of precise methods for quantification. Characterization of miRNA targets is insufficient without understanding the stoichiometric relationship between a specific miRNA and the copy number of its mRNA target(s). This information will be particularly important in neurons, which are highly polarized.

Even though genomic technologies are advanced, scientists continue to build upon them to make these even better. Two cutting-edge technologies in modern biology, microarrays and Real Time PCR (Polymerase Chain Reaction) are in the lead to revolutionize biology and medicine. RNAi, a powerful new approach for achieving targeted gene silencing using siRNA/miRNA as the triggering agent, offers remarkable specificity, scalability, potency, and reproducibility. MicroRNAs can be a challenge to study because of their small size. They require specialized and dedicated tools for analysis. For qRT-PCR and microarray applications, the following are the requirements:

1. Effective method of miRNA isolation from samples
2. RT-PCR and Microarray reagents optimized for miRNA detection
3. Assays specific to the miRNAs of interest
4. Real-time and Microarray analytical instruments and reagents validated for miRNA detection protocols

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Sample Collection and Storage

Samples should be processed immediately after collection – tissue should be frozen, preferably in liquid nitrogen as small pieces, or placed in RNAlater (an Ambion product) solution for storage until RNA extraction is performed.

Total RNA Isolation and Enriching of MicroRNA

MicroRNA can be isolated from total RNA that includes the small RNA fraction from the samples of interest. However, not all isolation methods retain the small RNA fraction. Therefore, it is important to use RNA isolation methods specifically adapted to retain it. Several companies have developed isolating kits to retain these small RNA species either in a background of total RNA or as an enriched fraction of RNA species of 70-200 nucleotides in size or smaller. Briefly, sample is collected in RNA stabilization solution and treated with chloroform to separate aqueous, organic and interphase. RNA partitions to the upper, aqueous phase, while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is extracted, and ethanol is added to provide appropriate binding conditions for all RNA molecules from 18 nucleotides upwards. The sample is then applied to the RNeasy Mini spin column, where the total RNA binds to the membrane and phenol and other contaminants are efficiently washed away. High quality RNA is then eluted in RNase-free water. For qRT-PCR and microarray detection of miRNA, total RNA is usually sufficient, although enrichment of the small RNA fraction can increase sensitivity in many applications.

Quantity and Quality of RNA

Purity, quantity and quality are the factors important for successful gene expression analysis. RNA yield can be measured by looking at the A_{260} reading using a spectrophotometer. A reliable and inexpensive method to look at RNA quality is to run the samples on a polyacrylamide gel.

Microarray

Microarrays provide a snapshot of gene expression of all the genes in a cell. A microarray works by exploiting the ability of a given miRNA molecule to bind specifically to, or hybridize to, the DNA template from which it originated. By using an array containing many DNA samples, researchers can determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of RNA bound to each site on the array. With the aid of a computer using specific computational methods the amount of RNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell. Using bioinformatics and statistical tools, the data is interpreted for meaningful information. The process of microarray based microRNA profiling; initially developed by Liu et al[60] involves four main steps: target labeling, DNA-DNA hybridization, staining and signal detection. MicroRNA microarray is a high-throughput approach to study the expression of miRNAs in cultured cells or tissues. Unlike the traditional cDNA microarray expression profiling, RNA samples used for miRNA microarray hybridization need to be enriched for small RNAs. Usually, the first step of a miRNA microarray experiment is the isolation of total RNA and the enrichment or direct isolation of small RNA. The miRNAs are then labeled and cleaned-up, following with miRNA hybridization to arrays spotted with miRNA probes. After washes and scanning, the differential miRNAs are identified. Subsequent validation is recommended using Northern blot, quantitative reverse transcription-polymerase chain reaction (qRT-PCR), or other analytical methods.

The recent development of highly efficient labeling methods and novel microarray probe designs enable direct labeling of as low as 120 ng of total RNA using Cy3 or Cy5, without fractionation or amplification, to produce precise and accurate measurements that span a linear dynamic range from 0.2 amol to 2 fmol of input miRNA. The assay is also applicable for formalin-fixed paraffin-embedded samples[61].

Real-Time PCR Assay

A real-time PCR assay is a tool that can be used to quantify differential gene expression. This type of assay measures (quantifies) the amount of a nucleic acid target during each amplification cycle of the PCR. The target may be DNA, cDNA, or RNA. In the initial cycles of PCR, there is little change in fluorescence signal. This defines the baseline for the amplification plot. An increase in fluorescence above the baseline indicates the detection of accumulated PCR product. A fixed fluorescence threshold can be set above the baseline. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold.

Relative quantitation might be used to measure gene expression in response to a chemical (drug) as an example. The level of gene expression of a particular gene of interest in a chemically treated sample would be compared relative to the level of gene expression in an untreated sample. Another method of looking at gene expression using qRT-PCR is to look at absolute quantitation and standard curves made using a dilution curve.

Real-time PCR can also be employed to quantify miRNA expression profiles and study the potential function of miRNAs in cancer pathogenesis. Real-time PCR was recently employed to measure miRNA precursors and to study the expression of 23 miRNA precursors in six cell lines[62]. These PCR-based analyses quantify miRNA precursors and not the active mature miRNAs. The relationship between pri-miRNA and mature miRNA expression has not been thoroughly addressed. This relationship is critical in case real-time PCR analysis is carried out to study the function of miRNAs.

Perspective of miRNA Research

The genomic miRNA loci comprises of a host of completely unexplored genes, each with its specific background. Annotating these miRNAs with functional assignments, knockout or knockdown techniques, establishing target fields specific settings, and quantifying the miRNA interactome directing the implementation of its function, promises to sustain this field for some outcome.

Discovery of the role of miRNAs in various pathological processes has opened up possible applications in molecular diagnostics and prognostics. Some miRNAs are controlled by epigenetic alterations in cancer cells, including DNA

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methylation and histone modification. Using chromatin modifying drugs to activate disease specific miRNAs can regulate target genes, and it may lead to novel therapies in future. MicroRNAs can complement other genomic and proteomic biomarkers for diagnosis and prognosis. Although each miRNA can control hundreds of target genes, it remains a great challenge to identify the accurate miRNA targets for research.

MicroRNAs: From Bench to Bedside

MicroRNAs provide a particular layer of network for gene regulation of a broad spectrum of biological pathways through fine-tuning protein expression levels. Aberrant cellular miRNAs expression is causally associated with a variety of diseases including neurodegenerative diseases. This implies that miRNAs have great potential to be developed as a novel class of therapeutic targets. To this end, methods for altering the levels of miRNA expression have been adapted from existing gene therapy and RNAi technologies, where synthetic siRNAs molecules have been routinely used to inactivate gene expression in mammalian cells. Specific knockdown of the target miRNAs by anti-miRNA oligonucleotides, or overexpression of miRNA duplex by transfection of vectors encoding this structure has been conducted both *in vitro* and *in vivo*. Although research is presently at the bench level, miRNAs are promising tools to be translated into therapeutic agents in the future for tackling diseases at the cellular miRNA level. In addition, although siRNAs or shRNAs have been widely used as the gene-silencing molecules, and even a few siRNAs are beginning to enter clinical trials[63], the intrinsic drawbacks of siRNA methodology have been revealed, including the off-target effects, elicitation of the interferon response, and interference of the endogenous miRNA biogenesis[64-67], all of which greatly hamper its therapeutic use. However, compared to siRNA methodology, the unique biogenesis and mechanism of miRNA action allow it to be exempt from these problems. With the advantage of specificity and toxicity and the attractive feature of multiple targeting potential, miRNA can potentially become a great tool for gene intervention.

Modified anti-miRNA oligonucleotides (AMOs), also designated as "antagomirs", are currently the most readily available tools for miRNA inhibition[68-72]. As an approach firstly adopted by Boutla and colleagues[73], different types of modified AMOs complementary to mature miRNAs have inhibited specific endogenous miRNAs in various experimental models: cell culture[69,71-74], flies[72,74-75], and mice[72,76-77]. Computer program-assisted sequence predictions, in combination with empirical efforts and more complete knowledge of miRNA mechanisms, will promote the development of artificial miRNAs.

Conclusion

Neurodegenerative diseases are the result of deterioration of neurons, ultimately leading to disabilities and the possibility of death. There are dozens of identified neurodegenerative diseases, and research suggests that gene defects play a major role in disease pathogenesis. Many such genetic studies have been carried out in the Indian population, signifying the role genetic polymorphisms play in neurodegenerative diseases. Such genetic studies to date have provided valuable clues into the etiology and pathogenesis of many neurodegenerative diseases from a molecular perspective, but there is significant work to be done before a full picture can be obtained. Due to the lack of comprehensive information on these diseases, cases thought to be unique and devoid of inheritance, might eventually prove to originate from specific genetic mutations or genetic risk factors. Further, the possibility exists that there may be only a few common genetic and mechanistic factors contributing to neuronal cell death. Future studies examining the genetic basis of neurodegeneration will truly be the key to gleaning information for the design and development of early prediction, prevention, and treatment options for addressing these diseases. The study of miRNA is a novel approach to understand neurodegenerative diseases. Recent studies have hinted at miRNA-linked modulation of long-term neuron integrity in response to toxic human disease proteins with neurodegenerative diseases. Traditionally, miRNA expression has been tested using low-throughput techniques such as Northern-blot analysis and real-time PCR, but new developments in microarray technology now enable global profiling of all miRNA genes and their precursors in any sample type.

Once ignored completely or overlooked as cellular detritus, short snippets of RNA discovered only a little over a decade ago are turning out to be crucial regulators of cell growth, differentiation, and death. Rodriguez-Lebron and Paulson[78] reviewed allele-specific RNA interference for neurological diseases. It has been shown that suppression of toxic gene expression through RNAi can be used for treatment of human disease in general and the neurological disorders in particular. The strategies used to achieve allele-specific silencing in light of recent developments in the field of RNAi biology are being reviewed. Especially, new insights into siRNA and miRNA processing are discussed as a means to improve efficiency and specificity of RNAi therapy. In addition, steps that can be taken to maximize the therapeutic benefits of this powerful technology have also been discussed.

MicroRNAs regulate multiple pathways in multiple tissues at multiple stages. Emerging evidences suggest that miRNAs are essential for the normal development of almost all animal tissues[22], including stem cells[79-86], brain[87-94] embryo[95-98], heart[22,98-99], limb[100], liver and other tissues[22]. Specific miRNAs control specific tissue development at particular stages of development[22].

The concept of the expression signature of microRNAs as representing a distinct and well defined experimental state that can then be connected to an otherwise unrelated biological system opens the way for a myriad of applications to communicate and understand biological complexity. The ability of an expression signature to connect two states, where the expression signature is the intermediary, is exemplified in the recent studies of Lamb et al, which describe a 'Connectivity Map'[101]. For example, the connection between miR-34s and p53, has quickly shifted focus to answering one key question – Is miR-34 sufficient and/or necessary for any of the biological outcomes elicited by p53? However, two of the best studied p53 outputs; growth arrest and apoptosis have been proved[102]. The potential of miRNAs to recognize many targets through imperfect base pairing, the pleiotropic effects of putative

'guardian of microRNAs: miR-34s' may simply reflect the different spectrum of target mRNAs available in a given system, and this may represent a recurring theme within miRNA-mediated regulatory pathways.

Numerous reports have demonstrated the role of miRNAs in neural development. Evidence for a role in Parkinson's and Alzheimer's disease[11] comes from animal model studies published recently, showing that loss of miRNAs may be involved in the development and progession of the disease. In cell culture experiments, transfer of small RNA fragments partially preserved miRNA deficient nerve cells.

There are umpteen numbers of ways of exploiting this area of research for a variety of disorders in general and neuro-degenerative disorders in specific. A lot of research has gone into the impact of miRNAs in diseases like cancer, while it is still in the rudimentary stages as far as the molecule's role in the nervous system disorders is concerned. It can evolve into an effective diagnostic tool in identifying over-expression of disease specific miRNA species in a specific neuronal disorder, or conversely the under-expression of specific miRNAs involved in protection against neurodegenerative conditions. In addition, prognosis can be a promising option if researchers are able to identify an abnormal proliferation of certain miRNA molecules associated with pre-disease conditions. Although options can be innumerable with these incredibly tiny but significant micromolecules, a lot of research needs to be carried out by narrowing the plethora of specificities, while still having the broad perspective in mind.

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