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Journal of Movement Disorders 2011;4:55-59
pISSN 2005-940X / eISSN 2093-4939

REVIEW ARTICLE

MicroRNAs in Experimental Models of Movement Disorders

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MicroRNAs (miRNAs) are small RNAs comprised of 20-25 nucleotides that regulates gene expression by inducing translational repression or degradation of target mRNA. The importance of miRNAs as a mediator of disease pathogenesis and therapeutic targets is rapidly emerging in neuroscience, as well as oncology, immunology, and cardiovascular diseases. In Parkinson's disease and related disorders, multiple studies have identified the implications of specific miRNAs and the polymorphisms of miRNA target genes during the disease pathogenesis. With a focus on Parkinson's disease, spinocerebellar ataxia, hereditary spastic paraplegia, and Huntington's disease, this review summarizes and interprets the observations, and proposes future research topics in this field. **Journal of Movement Disorders 2011;4:55-59**

Key Words: MicroRNA, Parkinson's disease, Spinocerebellar ataxia, Hereditary spastic paraplegia, Huntington's disease.

What is a microRNA?

MicroRNAs (miRNAs) are small, non-coding RNAs comprised of 20-25 nucleotides that regulate expression by blocking translation or direct degradation of target messenger RNAs (mRNAs). miRNAs target genes through sequence-specific hybridization to the 3' untranslated region (UTR) of mRNAs. miRNAs were first discovered in *Caenorhabitis elegans* by Dr. Victor Ambros and colleagues in 1993,[1] and have now been identified in almost every species, including humans.

During the last decade, a number of notable studies have identified the biogenesis and regulatory pathway of miRNA.[2,3] In brief, miRNA genes are transcribed by RNA polymerase II into double-stranded primary miRNA (pri-miRNA), which forms a hairpin-like structure and a double-stranded RNA-specific nuclease cleavage signal. This pri-miRNA is processed by the Dorsha/DGCR8 ribonuclease complex to form a pre-miRNA. The pre-miRNA is exported into the cytoplasm by a nuclear export protein (Exportin 5). Then, the pre-miRNA is cleaved by a dicer enzyme into 20-25 nucleotide double-stranded mature miRNA. The double strands are composed of a guide (the effective strand that binds to the target mRNA) and a passenger strand. The guide single-strand miRNA assembles into a protein-RNA complex [RNA-induced silencing complex (RISC)], and targets mRNA by base pairing. At the 5' region of miRNA, 7-8 sequences make up the "seed" sequence, which is critical for base pairing between miRNA and target mRNA. The seed sequences are annealed with a 3' UTR of target mRNA. Although seed-target binding needs uninterrupted complementarities, additional weak binding between the 3' end of miRNA and the target sequences do not require perfect complementarities, which is why one miRNA can regulates hundreds of mRNAs.[4]

MiRNAs play an integral role in most physiologic phenomena and diseases, such as the immune response, cell-cycle control, metabolism, viral replication, stem cell differentiation, and development.[2] Recent studies are providing new evidence that miRNA also plays an important role in the central nervous system (CNS); indeed, the expression and functions of miRNAs are altered in various CNS diseases. For instance, the loss of Dicer, a key regulator of miRNA biogenesis, induces neurodegeneration of the striatum and cerebellum,[5,6] suggesting roles in neurodegenerative diseases. Accordingly, miRNA-based pathogenesis and drug

Received January 26, 2011
Accepted August 31, 2011

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· The authors have no financial conflicts of interest.

development highlighted the opening of a new field in biomedical and neuroscience. In addition, analysis of miRNA-related molecules can provide novel clinical insight. For example, low levels of Dicer and Drosha mRNA in ovarian cancer cells have associations with poor outcomes in patients with ovarian cancer.[7]

In this review, we have focused on the role and possible research topics of selective miRNAs in Parkinson's disease (PD), and related disorders.

MiRNAs Implicated in PD and Related Disorders

PD

MiRNA function is important for the maintenance of midbrain dopaminergic neurons. For example, the deletion of Dicer, the key enzyme during miRNA biogenesis, leads to the progressive loss of midbrain dopaminergic neurons.[8] PD brains have different subsets and profiles of miRNA expression compared to other neurodegenerative diseases, such as Alzheimer's disease.[9,10] One of the key miRNAs regulated in the dopaminergic cells is miR-133b, which was reported to be expressed in the midbrain dopaminergic neurons and deficient in midbrain tissues of PD patients, although only 5 controls and 3 PD brains were examined.[8] A transcription factor, Pitx3 mRNA, is suppressed by miR-133b (Pitx3 protein enhances the transcription of miR-133b), showing a reciprocal feedback between Pitx3 and miR-133b (Figure 1A). Identifying single nucleotide polymorphisms (SNPs) in miR-133b and Pitx3 genes[11] has failed to observe any risk alleles in the genes associated with PD. Accordingly, the acquired transcriptional changes in miR-133b expression might be more important than the genetic variability in the pathogenesis of PD.

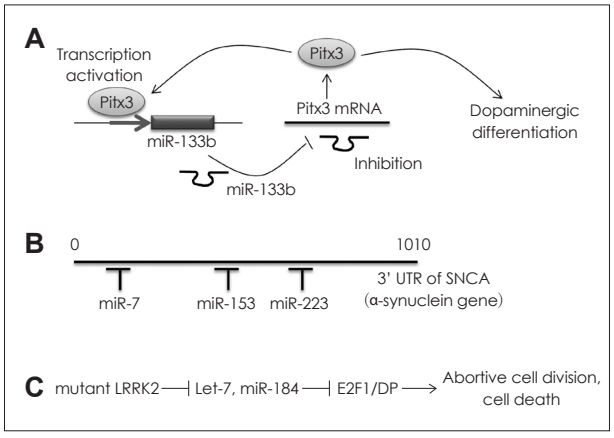

Figure 1. Key discoveries regarding miRNA-based pathogenesis in PD. A: miR-133b suppresses the translation of Pitx3, and Pitx3 activates the transcription of miR-133b, providing negative reciprocal interaction. Pitx3 regulates dopaminergic cell differentiation. B: The 3' UTR of SNCA, the α-synuclein gene, contains three putative loci predicted by TargetScan program (miR-7, miR-153, and miR-223 binding sites). C: Mutant LRRK2 reduces Let-7 and miR-184, which increases the E2F1 and DP1 transcription complex and induces abortive cell divisions and cell death of dopaminergic neurons. LRRK2: leucine-rich repeat kinase 2, mRNA: messenger RNAs, miR: microRNA. SNCA: synuclein alpha gene. UTR: untranslated region.

The expression of α-synuclein is repressed by miR-7 by targeting 3' UTR of the α-synuclein mRNA *in vitro*.[12] The down-regulation of α-synuclein by miR-7 protects cells from oxidative damage, and 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridinium (MPTP)-induced Parkinson mice have reduced miR-7 levels.[12] In addition, miR-153 also reduces α-synuclein translation in neuronal cultures.[13] Figure 1B shows the possible interaction between the α-synuclein gene (SNCA) and miRNAs predicted by the TargetScan program.[14]

Mutation of leucine-rich repeat kinase 2 (LRRK2) causes familial and sporadic PD, and accounts for several PD cases.[15] According to a recent discovery, mutation of LRRK2 is associated with dysfunctional machinery of miRNA-mediated translational repression (Figure 1C). Mutant LRRK2 antagonizes let-7 and miR-184 miRNAs, leading to the overproduction of the targets (E2F1/DP).[16] E2F1 and DP are components of the transcriptional regulator complex, the overproduction of which may lead to abortive cell division and death of dopaminergic neurons.[16]

Another genetic issue involves SNPs found in a miRNA binding site. Polymorphisms of fibroblast growth factor 20 (FGF20) have been associated with the increased risk of PD,[17] and FGF20 is highly expressed in substantia nigra pars compacta.[18] In the 3' UTR of the FGF20 gene, one SNP (rs-12720208, C→T) disrupts a binding site of miR-433, increasing the translation of FGF.[19] The increased FGF20 leads to α-synuclein overexpression, which corresponds to the pathologic process of PD.[19] Indeed, T allele carriers of SNP rs-12720208 are at increased risk of PD.[19] In addition, T allele carriers of SNP rs12720208 have diminished verbal episodic memory and a steeper decrease in hippocampal volume observed in MRI.[20] However, another study did not reproduce the influence of FGF20 rs12720208 SNP and miR-433 variation on PD in a separate population.[21] Although further studies are necessary, SNP information from genome-wide analysis might provide insights regarding disease risks, prognoses, and drug responses.

Spinocerebellar ataxia

A few studies have investigated the role of miRNAs in spinocerebellar ataxia (SCA). Although each patient with SCA has responsible mutant genes and miRNAs may not be the cause of the disease, miRNAs can be a mediator of the pathogenesis. For example, miR-19, miR-101, and miR-130 regulate ataxin1 levels, and inhibition of the miRNAs increases the neurotoxicity of polyglutamine-expanded ataxin1 *in vitro*.[22] It is also known that miR-144 represses ataxin1 *in vitro* and is reduced in the cerebellum of SCA1 patients.[23] In *Drosophila*,

miRNA-bantam and additional miRNAs are involved in mitigating polyQ-induced neurodegeneration in a SCA3 model.[24] In *Drosophila*, miR-8 targets atrophin-1, which is responsible for dentatorubral and pallidoluysian atrophy (DRPLA), preventing neurodegeneration.[25] Research involving miRNAs in SCA and cerebellar degeneration are preliminary and await further investigation.

Hereditary spastic paraplegia

Hereditary spastic paraplegia (HSP) is characterized by degenerative corticospinal tract axons that lead to progressive lower limb spastic paralysis. HSP is genetically heterogeneous, including autosomal dominant, autosomal recessive, and X-liked forms. The autosomal dominant form is the most frequent form, and mutations in spastin (SPG4) and atlastin (SPG3A) genes account for approximately 50% of the cases.[26] Recently, novel mutations in the REEP1 gene have been reported in autosomal dominant HSP patients.[27] Interestingly, three point mutations in 3' UTR of REEP1, which is likely to be a binding site of miR-140 or miR-691, have been observed.[27,28] The incidence of these mutations is very low; only 2 cases with mutations in 3' UTR of REEP1 gene were observed in 535 unrelated cases.[28] Nevertheless, these studies suggest that mutations in the miRNA binding sites can be a responsible locus of genetic disorders and the hidden mutations can cause disease phenotypes.

Huntington's disease

Huntington's disease (HD) is caused by the abnormal expansion of CAG repeats in *huntingtin*, resulting in the loss of medium spiny neurons in the striatum, involuntary choreiform movements, progressive cognitive impairment, and neuropsychiatric symptoms.[29] Although the responsible gene is defined, a number of miRNAs participate in the pathogenesis of abnormal CAG repeats. HD transgenic mice have altered expression of miRNA biogenesis enzymes, including drosha and dicer.[30] Importantly, dicer-knockout mice have behavioral and neuroanatomic phenotypes of striatal degeneration and Huntington's disease.[5]

A number of alterations in miRNA expression have been detected in HD human brains and transgenic mice.[30,31] In HD, enhanced interaction between RE1 transcriptional regulatory regions and RE1 silencing transcription factor (REST) causes transcriptional dysfunction.[32] Normal wild-type huntingtin inhibits the silencing activity of REST, and mutant huntingtin loses its control, resulting in increased binding of REST/ neuron restrictive silencer factor to RE1/ neuron restrictive silencer element, which causes persistent transcriptional dysfunction.[27,33] In brain tissues of HD patients, miR-9/9*, miR-29b, miR-124a, and miR-132 are downregulated by uncontrolled enhanced action of REST.[31,32] Because miR-9 and -9* repress REST and CoREST,[31] and miR-9 and -9* are controlled by the REST-RE1 interaction,[31] they form a double feedback loop. In addition, miR-124 is expressed at a low level in the brains of HD patients and transgenic mice.[30,31] Adult neurogenesis in the subventricular zone (SVZ) is enhanced by miR-124 in normal mice,[34] and the dynamics of SVZ neurogenesis are altered in HD mice.[35] Although we know the abnormal gene in HD, future research in miRNA might add to our knowledge about what mediates the neurotoxicity of abnormal CAG expansion.

Future Research Topics

Modulation of miRNA

Key miRNAs involved in the pathogenesis can be therapeutic targets. Thus, up-regulated miRNAs can be inhibited by antisense neutralizing oligonucleotides (*antagomir*), and down-regulated miRNAs can be delivered to the tissue and organ by using viral vectors or miRNAs themselves. Among these two options, the former is emerging as a hot topic in the drug development. The antagomirs can be generated by 2'O-methylated- or locked nucleic acid-based antisense oligonucleotides.[36] These antagomirs are resistant to RNase-induced degradation, reach target tissues, and penetrates the cell membrane when delivered systemically.[37] Meanwhile, naked miRNAs are vulnerable to RNase-induced degradation and a single miRNA can repress the production of hundreds of proteins.[4] Antagomirs targeting the upregulated, abnormally-altered, and correponding miRNAs in neurodegenerative diseases have high specificity for target binding, and thus warrant active research in the field.

SNP study

We have discussed that SNP or point mutations at 3' UTR of miRNA-binding sites might increase disease risks. Another interesting loci that needs screening for SNPs or point mutations are promoter sequences of miRNA genes. The mechanism which governs the expression of miRNAs has not been fully understood. Although mature miRNAs are generated by a series of enzymatic processing and regulatory factors for the enzymes have been suggested, transcription, degradation, and epigenetic factors influence the final level of each miRNA.[38] Accordingly, analysis of respective promoter regions of a selected miRNA might reveal an important locus of responsible genes for the neurodegenerative diseases. A more detailed discussion on this topic has been the subject of a recent review.[38]

Development of biomarkers based on miRNA

MicroRNAs can be used as biomarker of CNS disorders. Enhanced miRNAs in the brain can be released into the circulating blood and detected. The circulating miRNAs are substantially stable from degradation and have been reported as promising biomarkers in various disease conditions.[39] For example, the concentration of a brain-specific miRNA (miR-124)

is increased in the plasma of rats with acute stroke.[40] The plasma level of miR-134 is associated with the severity of manic symptoms of patients with bipolar disorder.[41] Patients with traumatic brain injury have altered plasma miRNA levels.[42] The method for precise measurement of miRNAs in circulating blood has been developed.[43] In addition, miRNAs in cerebrospinal fluid (CSF) can provide putative biomarkers for diagnostic uses in neurodegenerative diseases.[44] Accordingly, the measurement of circulating or CSF miRNAs in patients with movement disorders might be useful for diagnosis, prediction of prognosis, and estimation of drug responses.

Conclusion

Active miRNA research emerged less than a decade ago. Moreover, findings on the role of miRNA in neurodegenerative diseases and movement disorders have been reported since a few years ago. Although RNA-based approaches have not completely elucidated the pathogenesis of the diseases, it is clear that miRNA research has considerable appeal, providing new ideas on pathogenesis, candidate genetic markers, and therapeutic modalities. It is now up to physicians and neurobiologists to determine the clinical implications of miRNAs.

Acknowledgments

This work was supported by grants from the Korea Health 21 R&D Project, Ministry of Health & Welfare (A092058), and WCU Neurocytomics, South Korea. S-T.L. was supported by a grant of the Korea Healthcare technology R&D Project (A100548).

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