Emerging small-molecule therapeutic approaches for Alzheimer's disease and Parkinson's disease based on targeting microRNAs

Somaya A. Abdelrahman, Moustafa T. Gabr*

The role of microRNAs in the progression of neurodegenerative diseases: MicroRNAs (miRNAs) are endogenous, non-coding and short RNA nucleotides that regulate gene expression through base pairing with the targeted messenger RNA (mRNA) at the 3′untranslated region (3′ UTR). Thus, miRNAs play fundamental role in the inhibition or degradation of messenger RNAs (Gebert and MacRae, 2019). Two ribonuclease (RNase) III enzymes control the biogenesis of miRNAs; Drosha and Dicer. The first step in the biogenesis includes the cleavage of primary miRNAs (pri-miRNAs) in cell nucleus by Drosha, which leads to the formation of short stem-loop pre-miRNAs. Subsequently, these pre-miRNAs are transported to the cytoplasm and further processed by Dicer resulting in mature miRNAs (O'Brien et al., 2018).

Numerous diseases display abnormal expression of miRNAs, such as breast cancer, leukemia, hepatocellular carcinoma, cardiovascular and neurodegenerative diseases. More than 2000 miRNAs have been identified to have key roles in differentiating neurons, which highlights the involvement of miRNAs in different neuronal processes. Literature reveals that miRNAs are implicated in different pathways that are directly involved in the progression of neurodegenerative diseases (**Figure 1A**). α-Synuclein, a presynaptic protein implicated in neurological disorders, is an intrinsically disordered protein. The aggregation of α-synuclein to form insoluble fibrils and Lewy bodies is a key pathological hallmark for the diagnosis of neurological disorders, such as Parkinson's disease and Alzheimer's disease. The aggregation of α-synuclein in human neuroblastoma cells is promoted by miR-16-1, which is attributed to the ability of miR-16-1 to downregulate heat shock protein 70. Recently, the ability of miR-153 to negatively regulate α-synuclein has been demonstrated in parkinsonian mice (Cressatti et al., 2019). Moreover, the inhibition of miR-34b in patients with Parkinson's disease enhanced the expression of α-synuclein in human brain. Tau, a microtubule associated protein, is expressed in mature human brain in six isoforms. The abnormal hyperphosphorylation and aggregation of tau into mature fibrils is observed in Alzheimer's disease as well as other neurological disorders, called tauopathies. Downregulation of miR-212 and miR-132 was detected in neurally derived plasma exosomes of patients of Alzheimer's disease (Cha et al., 2019). Based on these findings, the assessment of the levels of miR-212 and miR-132 in extracellular vesicles is considered a promising diagnostic tool for Alzheimer's disease.

Numerous reports have demonstrated the association between neurodegenerative diseases and dysfunction in mitochondrial dynamics, such as variation in mitochondria size, altered shape, fission-fusion, and mitochondria movement). In this context, miR-27a has been reported to suppress the expression of PTEN-induced putative kinase 1, a mitochondrial serine/threonine kinase, which is a key player in mitophagy. Thus, miR-27a plays a key role in controlling the autophagic clearance of damaged mitochondria. Mutations in PTEN-induced putative kinase 1 are considered major contributor to autosomal recessive Parkinson's disease. Along these lines, miR-494 induces mitochondrial damage and oxidative stress-induced neuronal damage via decreasing the expression of Parkinson disease protein 7, an inhibitor of the aggregation of α-synuclein (Catanesi et al., 2020). In addition, novel miR-455-3p demonstrated protective activity against amyloid aggregation through controlling the expression of mitochondrial fission proteins and regulating mitochondrial dynamics (Kumar et al., 2019).

Over the past years, α-secretase 1 (BACE-1) has received increasing attention as a critical therapeutic target for Alzheimer's disease. BACE-1, also known as β-site amyloid precursor protein cleaving enzyme 1, is the major secretase involved in the production of amyloid-β peptides in human brain. Subsequent aggregation and fibrillization of these peptides to form amyloid plaques are key diagnostic features of Alzheimer's disease. Additionally, multiple lines of evidence have demonstrated the neurotoxicity of small oligomers of amyloid-β peptides. MiR-298 has been recently identified as a repressor of BACE-1 in human neuronal cell culture model revealing the therapeutic potential of miR-298 for neurodegenerative diseases (Chopra et al., 2020). Moreover, the expression of BACE-1 is directly regulated by miR-15b, which functions through targeting BACE1 mRNA 3′-UTR (Gong et al., 2017). These findings illustrate that miRNAs are key players in controlling the function of BACE-1 and its role in the pathogenesis of neurodegenerative diseases.

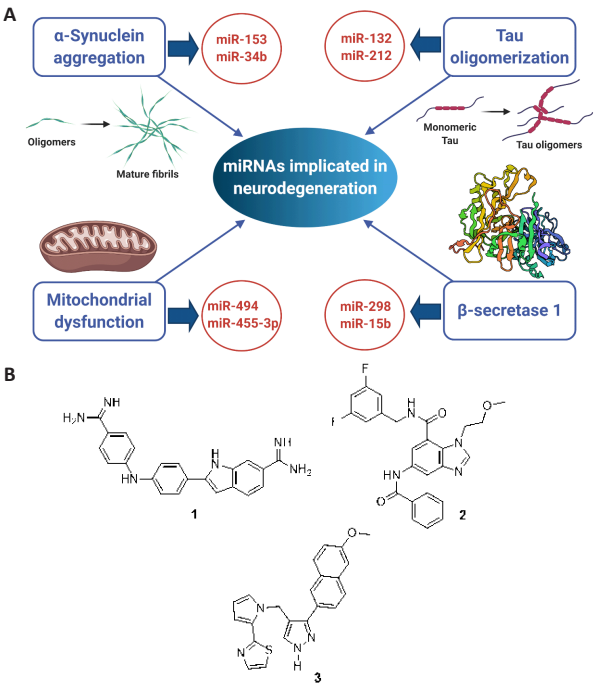

Figure 1 (A) The implication of miRNAs in different pathways for the progression of neurodegenerative diseases. (B) Chemical structures of compounds 1–3.

The development of small molecules for neurodegenerative diseases based on targeting miRNAs: Targeting disease-causing miRNAs with small molecules have revealed promising outcome in the *in vitro* as well as *in vivo* screening. Thus, considerable research efforts have been directed towards the development of new strategies for the identification and the design of small molecules with potential miRNAs binding capability. However, the short sequences of miRNAs have impeded the identification of potent small-molecule binders and shifted the focus towards targeting pre-miRNAs with longer sequences.

Inforna and Inforna 2.0 are tools developed to identify small molecules targeting structured RNAs. These approaches are based on mining motifs (secondary structures) of the RNAs based on their sequences. These motifs are then compared to a database of known RNA motif-small molecule binding partners. Inforna then reports the motif in the database as well as the target RNA. Inforna has been utilized recently in the identification of compound 1 (**Figure 1B**) as an inhibitor of α-synuclein expression (Zhang et al., 2020). Compound 1 targets the α-synuclein mRNA 5′ UTR and decreases the levels of a-synuclein via decreasing the amount of SNCA mRNA loaded into polysomes.

Recently, RIBOTACs (ribonuclease targeting chimeras) have evolved as a new class of small molecules that can target diverse RNA classes, including miRNAs and mRNAs (Dey and Jaffrey, 2019). The design of RIBOTACs is based on linking RNA-binding molecules to a small molecule that activates RNA L, a latent ribonuclease, which results in the degradation of the target RNA.

Multitargeted directed ligands (MTDLs) for neurodegenerative diseases focused on miRNAs: MTDLs are ligands that are designed to interfere with multiple pathways involved in the progression of neurological disorders because of their multifaceted feature. The remarkable success of MTLDs in the identification of small molecules with *in vitro* and *in vivo* efficacy in models of neurodegenerative diseases has resulted in continued efforts in the development of new tools for the design of MTDLs. In this context, a novel strategy for the identification of MTDLs for neurodegenerative diseases was recently reported, which is based on dual inhibition of acetylcholinesterase and the biogenesis of miRNAs (Gabr and Brogi, 2020). Compound 2 (**Figure 1B**), a dual inhibitor of acetylcholinesterase and biogenesis of miR-15b, was more efficient than donepezil and anti-miR-15 in protecting SH-SY5Y neuroblastoma cells from amyloid-beta induced cytotoxicity.

Moreover, dual inhibition of tau oligomerization and biogenesis of miRNAs implicated in the progression of neurological disorders (e.g. miRNA-146a) has been reported as a promising strategy for the design of MTDLs (Gabr and Barbault, 2020). The outcome of this approach furnished compound 3 (**Figure 1B**) which featured superior neuroprotective activity to miRNA-146a antagomir and MK-886, an inhibitor of tau aggregation. These studies further illustrate that the design of MTDLs focused on inhibition of the biogenesis of miRNAs has shown preferential therapeutic profile in comparison to monotargeted therapeutics. Therefore, further screening of the outcome of these approaches using *in vivo* models of neurodegenerative diseases will be critical for future optimization.

Future directions: Development of efficient strategies to identify small molecules that can selectively bind miRNAs will have a remarkable effect on the ability to control the expression of proteins implicated in neurodegenerative diseases. Small molecule inhibitors of Drosha and Dicer have been extensively studied as tools to inhibit the biogenesis of miRNAs. However, numerous inhibitors of Dicer and Drosha exhibit attenuated miRNA binding affinity in cellular environment as well as inability to block miRNA maturation. These results are attributed to promiscuous targeting of these small molecules as well as their competition with other macromolecules in binding miRNAs. Therefore, establishment of new approaches for selective binding of miRNAs is highly desirable. Kinetic target-guided synthesis (KTGS) is a fragment-based drug discovery approach in which biological target selects its bidentate ligands through assembling them from a pool of small reactive fragments. In KTGS, the biological target accelerates covalent bond formation between building blocks with complementary reactive functional groups via enhanced resonance time. After dissociation from the target, KTGS hits can be detected in the media by Liquid chromatography-mass spectrometry. A potential pathway for the identification of selective binders of miRNAs would be to use KTGS to identify small molecule binders of target miRNA.

Somaya A. Abdelrahman, Moustafa T. Gabr*

Department of Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt (Abdelrahman SA)

Department of Radiology, Stanford University School of Medicine, Stanford, CA, USA (Gabr MT)

***Correspondence to:** Moustafa T. Gabr, PhD, gabr2003@gmail.com.

https://orcid.org/0000-0001-9074-3331 (Moustafa T. Gabr)

Date of submission: December 17, 2020

Date of decision: January 19, 2021

Date of acceptance: March 3, 2021

Date of web publication: July 8, 2021

https://doi.org/10.4103/1673-5374.317977

How to cite this article: *Abdelrahman SA, Gabr MT (2022) Emerging small-molecule therapeutic approaches for Alzheimer's disease and Parkinson's disease based on targeting microRNAs. Neural Regen Res 17(2):336-337.*

Open peer reviewer: *Hong Xu, University of Pennsylvania, USA.*

Additional file: *Open peer review report 1.*

References

Catanesi M, d'Angelo M, Tupone MG, Benedetti E, Giordano A, Castelli V, Cimini A (2020) MicroRNAs dysregulation and mitochondrial dysfunction in neurodegenerative diseases. Int J Mol Sci 21:5986.

Cha DJ, Mengel D, Mustapic M, Liu W, Selkoe DJ, Kapogiannis D, Galasko D, Rissman RA, Bennett DA, Walsh DM (2019) miR-212 and miR-132 are downregulated in neurally derived plasma exosomes of Alzheimer's patients. Front Neurosci 13:1208.

Chopra N, Wang R, Maloney B, Nho K, Beck JS, Pourshafie N, Niculescu A, Saykin AJ, Rinaldi C, Counts SE, Lahiri DK (2020) MicroRNA-298 reduces levels of human amyloid-β precursor protein (APP), β-site APP converting enzyme 1 (BACE1) and specific tau protein moieties. Mol Psychiatry doi:10.1038/s41380-019-0610-2.

Cha D, Mengel D, Mustapic M, Liu W, Selkoe D, Kapogiannis D, Galasko D, Rissman R, Gong G, An F, Wang Y, Bian M, Yu LJ, Wei C (2017) miR-15b represses BACE1 expression in sporadic Alzheimer's disease. Oncotarget 8:91551-91557.

Cressatti M, Song W, Turk AZ, Garabed LR, Benchaya JA, Galindez C, Liberman A, Schipper HM (2019) Glial HMOX1 expression promotes central and peripheral α-synuclein dyregulation and pathogenicity in parkinsonian mice. Glia 67:1730-1744.

Dey SK, Jaffrey SR (2019) RIBOTACs: Small molecule target RNA for degradation. Cell Chem Biol 26:1047-1049.

Kumar S, Reddy AP, Yin X, Reddy PH (2019) Novel microRNA-455-3p and its protective effect against abnormal APP processing and amyloid beta toxicity in Alzheimer's disease. Biochem Biophys Acta Mol Basis Dis 1865:2428-2440.

O'Brien J, Hayder H, Zayed Y, Peng C (2018) Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol 9:402.

Gabr MT, Barbault F (2020) First dual binder of microRNA-146a and monomeric tau: A novel approach for multitargeted therapeutics for neurodegenerative diseases. Chem Commun 56:9695-9698.

Gabr MT, Brogi S (2020) MicroRNA-based multitarget approach for Alzheimer's disease: Discovery of the first-in-class dual inhibitor of acetylcholinesterase and micro-RNA-15b biogenesis. J Med Chem 63:9695-9704.

Gebert LFR, MacRae IJ (2019) Regulation of microRNA function in animals. Nat Rev Mol Cell Biol 20:21-37.

Zhang P, Park HJ, Zhang J, Junn E, Andrews RJ, Velagapudi SP, Abegg D, Vishnu K, Costales MG, Childs-Disney JL, Adibekian A, Moss WN, Mouradian MM, Disney MD (2020) Translation of the intrinsically disordered protein a-synuclein is inhibited by a small molecule targeting its structured RNA. Proc Natl Acad Sci U S A 117:1457-1467.

P-Reviewer: Xu H; C-Editors: Zhao M, Qiu Y; T-Editor: Jia Y