



MiR-129-5p: a novel therapeutic target for amyotrophic lateral sclerosis?

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Amyotrophic lateral sclerosis (ALS) is a debilitating neurodegenerative disease that is defined by the progressive loss of motor function due to degeneration of upper and lower motor neurons in the brain and spinal cord tissue, respectively, with an average life-expectancy of 2–5 years post-diagnosis (1). Riluzole and edaravone are currently the only two FDA approved drugs for patients with ALS, but these drugs have minimal effect, extending life only for a matter of months (2).

In 1993, the first causative mutations of ALS were identified in the *SOD1* gene (3). Today, mutations in *SOD1* is the second most common cause of genetic ALS (~5–6% of all ALS cases), only falling behind the hexanucleotide repeat expansions observed in *C9ORF72* (~12–14% of all ALS cases). However, the vast majority of ALS cases (~80%) have no known genetic background giving no clear indication on the causative factor that results in the disease development (1). In the last decade, miRNAs have been increasingly implicated in the pathogenesis of ALS due to the mass dysregulation of these molecules observed in ALS spinal cord and motor neurons (4–8).

MiRNAs are small RNA molecules (~22–25 nucleotides) that are primarily responsible for post-transcriptional gene-silencing generally through interactions with the 3' untranslated region (UTR) of messenger RNA (mRNA) (9). Their interactions are highly dependent on environmental cues. Therefore, cell development, cell type, cell stress, and aging all have an impact on mRNA post-transcriptional

regulation via miRNAs. This is an especially true for neurons which have spatiotemporal needs within the cell where post-transcriptional regulation via miRNAs differs depending on soma, dendritic and axonal needs (9,10). Further, miRNAs are also regulated by other non-coding RNAs (i.e., long non-coding RNAs, circular RNAs, etc.) which work together to control the degree of expression of a gene (11), highlighting the sophistication of these non-coding RNA networks (*Figure 1*). Not surprisingly, because of miRNAs dynamic nature in these RNA networks, it has become increasingly of interest to determine the contribution of miRNAs in disease.

As mentioned, miRNAs have become increasingly implicated in ALS pathogenesis. This has mainly stemmed from observations that in sporadic ALS (sALS) spinal cord and motor neurons there are pools of miRNAs that are reduced (4–6). In contrast, mutant *SOD1* (mtSOD1) ALS cases and rodent models have observed that miRNAs are generally upregulated, suggesting inhibition of these miRNAs may have therapeutic effects (7,12,13). In particular, high levels of miR-155 in mtSOD1 rodent models has been reported to promote neuroinflammation. Interestingly, inhibition of this miRNA via antisense oligonucleotides (ASOs) has been shown to decrease neuroinflammation and alleviate ALS-like phenotypes in rodent models (7). Others have used miRNAs to reduce *SOD1* expression, and in turn reduce mtSOD1s toxic effects, by intrathecally delivering artificial miRNAs that

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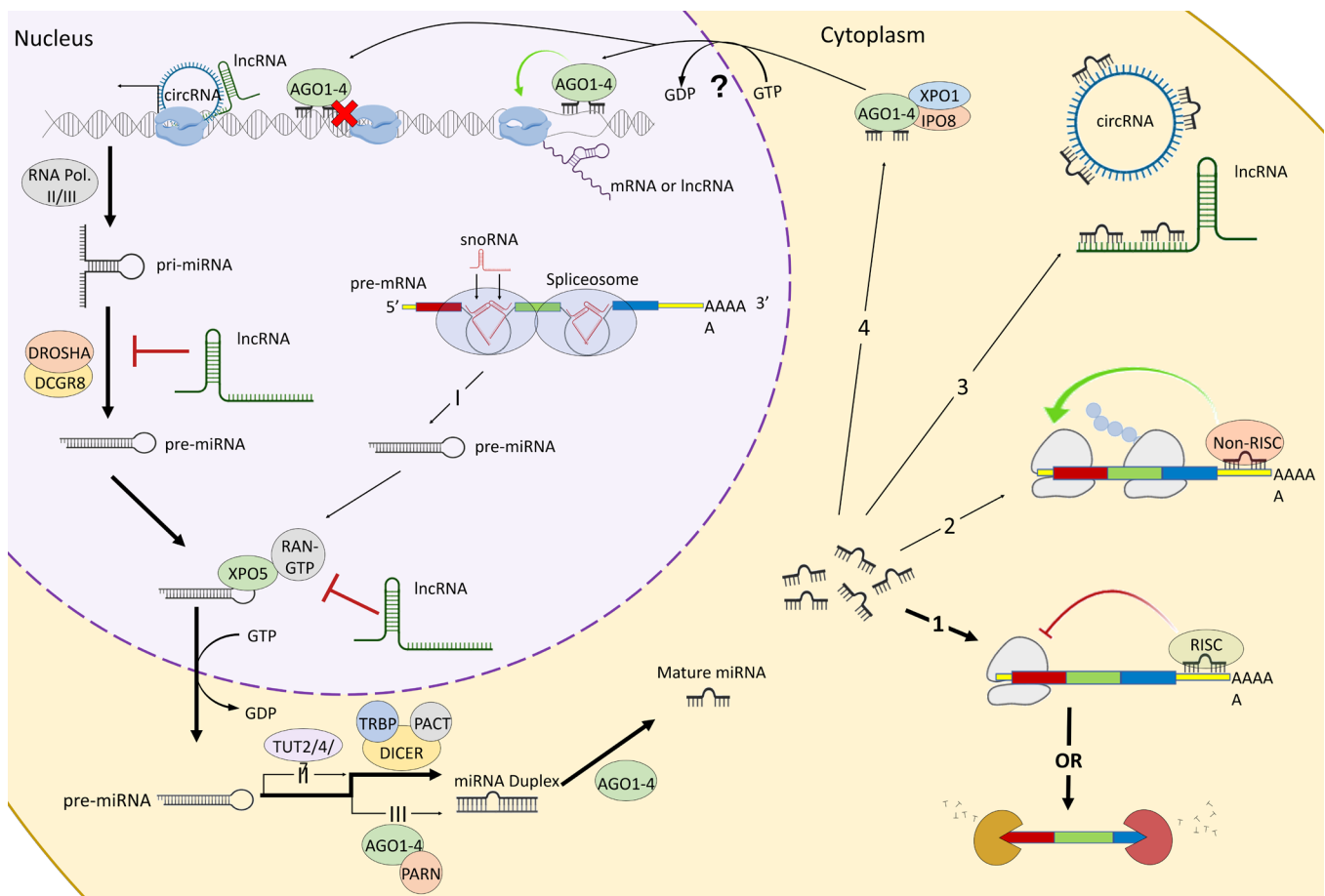


Figure 1 MiRNA biogenesis and regulation. The *canonical biogenesis pathway* (represented by bold arrows): MiRNAs are transcribed from the genome via RNA polymerase (RNA pol.) II/III producing a primary miRNA (pri-miRNA). Interestingly, both circular and long non-coding RNAs (circRNAs and lncRNAs, respectively) are known to assist in RNA pol. II transcription indicating these class of non-coding RNAs may assist in miRNA transcription. Further, these pri-miRNAs can be transcribed into small nucleolar RNA (snoRNA) and transfer RNA (tRNA) transcripts. Once produced, pri-miRNAs are processed by the DROSHA/DCGR8 complex into a pre-miRNA which is shuttled from the nucleus to the cytoplasm via XPO5 and RAN-GTP. Both DROSHA processing and pre-miRNA nuclear export have been shown to be inhibited by lncRNAs. Cytoplasmic pre-miRNAs are then processed by DICER/TRBP/PACT complex to produce a miRNA duplex which is unwound via AGO1-4 creating a mature miRNA. *Non-canonical biogenesis pathway*: There are 3 major non-canonical pathways – I) pre-miRNAs are produced directly from the spliceosome after pre-mRNA processing skipping DROSHA/DCGR8 processing; II) cytoplasmic pre-miRNAs need to get mono-uridylated by TUT2/4/7 proteins before being processed by DICER/TRBP/PACT; or III) cytoplasmic pre-miRNAs get processed by AGO1-4/PARN rather than DICER/TRBP/PACT to produce the miRNA duplex. *MiRNA regulation*: Once produced miRNA are: (I) incorporated into the RNA-induced silencing complex (RISC) and generally target the 3'UTR of mRNA to suppress translation OR induce mRNA degradation. This represents the primary function of miRNAs; (II) bind to the 3'UTR of mRNA to promote translation and mRNA stability usually with proteins not associated with the RISC complex (non-RISC). However, this is far less common than gene silencing; (III) targeted by circRNAs and lncRNAs that act as sponges to prevent miRNAs from reaching their mRNA targets; (IV) MiRNAs can be incorporated into the AGO complex and transported back into the nucleus via IPO8 and XPO1 to either promote or suppress transcription by binding to promoter/enhancer regions of the genome. While presumed that GTP converts to GDP via IPO8 to allow entry of the miRNA in the nucleus, this has yet to be shown in the literature. This figure highlights this highly sophisticated RNA network between circRNAs/lncRNAs/snoRNAs/ mRNAs that are responsible for regulating miRNA biogenesis and function ensuring gene expression is tightly controlled.

were cloned into an Adeno-associated virus (AAV) and target the *SOD1* transcript. Use of *SOD1* targeting artificial miRNAs has been shown to have therapeutic effects in both rodent and macaque models (14,15). However, the effectiveness of these therapeutics in humans is still unknown. This previous research provides evidence of the utility of miRNAs as potential therapeutic targets, and further, highlights the use of either ASOs or AAV-miRNAs as ways to develop therapeutics that will potentially treat neurodegeneration.

Recent work published by Loffreda *et al.*, 2020 in *Progress in Neurobiology* highlights miR-129-5p as another upregulated miRNA in rodent models of mtSOD1 ALS which, if inhibited using an ASO, could have therapeutic effects for patients with ALS (16). The research group looked at mtSOD1 (*SOD1* G93A) *in vitro* neuronal cell models in addition to *in vivo* mouse models and determined that mtSOD1 causes an increase in miR-129-5p levels. Further, they observed increased levels of miR-129-5p in peripheral blood mononuclear cell (PBMC) samples of sALS patients, suggesting that this phenomenon may be related to ALS more broadly rather than just those who contain mutations in *SOD1*. These researchers reported that increased levels of miR-129-5p in both their *in vitro* and *in vivo* models correlates with decreased levels of *ELAVL4*, otherwise known as HuD—a protein critical for neuronal cell identity, maturation, axogenesis, dendrite growth, plasticity and survival (17). Through robust experimental analyses, the authors were able to show that increased levels of miR-129-5p were directly responsible for the reduced levels of HuD, identifying the biological mechanism by which high levels of miR-129-5p may contribute to neurodegeneration. Having made this observation, the authors then examined the ability of an ASO targeting miR-129-5p to reduce its levels in mtSOD1 rodent models. Administration of the ASO via intracerebroventricular injection at an early symptomatic stage (post-natal 80 days) led to increased survival, improved motor capabilities, and increased neuromuscular junction connections. However, it only had a mild effect on preventing motor neuron degeneration which was not statistically significant. The authors concluded that while reducing levels of miR-129-5p may have some therapeutic effects, the most optimal approach may be to pair their ASO with another miRNA-based therapeutic currently in development—an ASO targeting miR-155 (7,18).

ASO-based therapeutics have become popular as an

approach to treat neurodegenerative diseases due to the success in treating Spinal Muscular Atrophy (SMA)—a juvenile neurodegenerative disease caused by loss of lower motor neurons—using an ASO targeting *SMN2* (19). Several modifications to the nucleotide backbone of an ASO can be done to increase its affinity to its target and make it resistant to nucleases. For example, morpholino modifications were used to develop the *SMN2* ASO and the miR-129-5p ASO (16,19). Morpholino modifications alter the sugar-phosphate backbone by changing the sugar ring from a five-membered to a six-membered ring and the sugar rings are linked to phosphoramidates groups rather than phosphate groups. These modifications have been proven to be beneficial to develop ASOs that either knockdown expression of a specific gene, as seen in Loffreda *et al.*, 2020, or alter mRNA splicing as seen for the ASO that targets *SMN2* (16,19). Moreover, the ASO produced in Loffreda *et al.*, 2020 is called an antagomir—an ASO that targets a miRNA—and they have been increasingly shown to be effective therapeutics. For example, the drug miravirsin, which has completed phase II clinical trials, is an antagomir targeting miR-122 and has been shown to prevent propagation of hepatitis C virus (HCV) RNA in the liver highlighting the potential use of antagomirs in clinical practice (20). Despite these developments, ASOs still have their limitations which include their potential off-target effects, their expense to develop, and their inability to cross the blood-brain barrier; making them difficult to administer for neurological diseases (21). However, the current success of ASO treatments thus far has sparked more innovative research to determine whether further modifications to these oligos will allow us to overcome these limitations, offering new opportunities for therapeutic development.

Loffreda *et al.*, 2020 provides clear evidence that increased levels of miR-129-5p is caused by mtSOD1 and that lowering its levels in mtSOD1 mice has some therapeutic effects; however, it is unclear whether reduction of miR-129-5p would be a good ALS therapeutic more broadly (16). The authors identified increased levels of miR-129-5p in sALS patient PBMCs, but not in human spinal cord or motor neurons (16). As mentioned previously, the environmental context matters when discussing miRNA regulation (9). Further, in the several studies that have examined miRNA expression in the spinal cord and motor neurons of sALS cases, upregulation of miR-129-5p has not been observed (4-6). Thus, it is unclear whether this observation of increased levels of miR-129-5p in PBMCs

is relevant to the dysfunction of motor neurons, or more broadly, the central nervous system of the sALS patient group within their study.

Further, the use of an ASO to suppress miR-129-5p expression should be approached with caution. In the central nervous system, miR-129-5p has been shown to suppress apoptosis and alleviate neuroinflammation (22). Low levels of miR-129-5p have been reported in the degenerating brain of patients with Alzheimer's Disease, and lack of this miRNA creates an inflammatory response that can be neurotoxic (23). Thus, too much or too little of miR-129-5p could be damaging to the central nervous system. This is likely because miRNAs can target several transcripts to regulate a network of genes, which can make cells sensitive to fluctuations in miRNA expression. In this case, the authors showed that miR-129-5p regulates HuD levels, but miR-129-5p has also been shown to regulate critical neuronal genes involved in synaptic plasticity (FMR1) and neuroinflammation (HMGB1) (22,24). All this suggests that further work needs to be done to ensure reduction of this miRNA will not have long-term effects that may be more damaging than therapeutic.

Loffreda *et al.*, 2020 proposed that while their ASO had some beneficial effects in their mtSOD1 models, matching it with an ASO that inhibits miR-155 may have the greatest overall therapeutic effect (16). This points to the idea that simply targeting one miRNA may not be enough to ameliorate ALS. In fact, as mentioned previously, several miRNAs have been shown to be upregulated in mtSOD1 models (7,12,13). Further, the article provides data indicating that mtSOD1 increased the levels of DICER, a critical protein in miRNA biogenesis, which may explain why miRNAs are upregulated in mtSOD1 models (16). Therefore, there seems to be a systemic issue with miRNA production in the presence mtSOD1. Thus, lowering levels of DICER might seem to be a better target to slow the production and reduce the toxicity of these upregulated miRNAs in mtSOD1 ALS cases. However, knockout of DICER in mouse motor neurons has been shown to lead to motor neuron degeneration, and thus, reducing levels of DICER should be approached with caution as well (25).

Overall, while this latest work by Loffreda *et al.*, 2020 provides another interesting avenue for miRNA-based therapeutics for ALS, this work still has far to go before we can think about administering it to patients. Large-scale preclinical studies still need to be done to determine if inhibition of miR-129-5p prevents motor

neurodegeneration, helps alleviate ALS-like phenotypes in several models of ALS or just SOD1-related models, and finally, determine whether downregulation of this miRNA has any potential negative long-term outcomes (i.e., chronic neuroinflammation). Further, we still do not know whether miR-129-5p is upregulated within the spinal cord or motor neurons of mtSOD1 ALS cases, which will be necessary to address to ensure development of this ASO as a therapeutic is not in vain. All this taken together, miR-129-5p could be a potential therapeutic for ALS, but in agreeance with the authors, it is unlikely that targeting this miRNA alone will be enough to completely halt the disease progression (16). However, the mere identification of this miRNA, its biological network and its potential relevance to disease will be incredibly important to our understanding of mechanisms associated with ALS progression, opening more avenues for potential therapeutics.

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