Epilepsy Currents
2024, Vol. 24(1) 50-52

DOI: 10.1177/15357597231212373
journals.sagepub.com/home/epi
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MicroRNA 335-5p: The Sodium Channel Silencer

MicroRNA-335-5p Suppresses Voltage-Gated Sodium Channel Expression and May Be a Target For Seizure Control

Heiland M, Connolly NMC, Mamad O, Nguyen NT, Kesavan JC, Langa E, Fanning K, Sanfeliu A, Yan Y, Su J, Veno MT, Costard LS, Neubert V, Engel T, Hill TDM, Freiman TM, Mahesh A, Tiwari VK, Rosenow F, Bauer S, Kjems J, Morris G, Henshall DC. *Proc Natl Acad Sci USA*. 2023;120(30):e2216658120. doi:10.1073/pnas.2216658120. PMID: 37463203; PMCID: PMC10372546

There remains an urgent need for new therapies for treatment-resistant epilepsy. Sodium channel blockers are effective for seizure control in common forms of epilepsy, but loss of sodium channel function underlies some genetic forms of epilepsy. Approaches that provide bidirectional control of sodium channel expression are needed. MicroRNAs (miRNA) are small noncoding RNAs which negatively regulate gene expression. Here we show that genome-wide miRNA screening of hippocampal tissue from a rat epilepsy model, mice treated with the antiseizure medicine cannabidiol, and plasma from patients with treatment-resistant epilepsy, converge on a single target—miR 335-5p. Pathway analysis on predicted and validated miR-335-5p targets identified multiple voltage-gated sodium channels (VGSCs). Intracerebroventricular injection of antisense oligonucleotides against miR-335-5p resulted in upregulation of Scn1a, Scn2a, and Scn3a in the mouse brain and an increased action potential rising phase and greater excitability of hippocampal pyramidal neurons in brain slice recordings, consistent with VGSCs as functional targets of miR-335-5p. Blocking miR-335-5p also increased voltage-gated sodium currents and SCN1A, SCN2A, and SCN3A expression in human induced pluripotent stem cell-derived neurons. Inhibition of miR-335-5p increased susceptibility to tonic-clonic seizures in the pentylenetetrazol seizure model, whereas adeno-associated virus 9-mediated overexpression of miR-335-5p reduced seizure severity and improved survival. These studies suggest modulation of miR-335-5p may be a means to regulate VGSCs and affect neuronal excitability and seizures. Changes to miR-335-5p may reflect compensatory mechanisms to control excitability and could provide biomarker or therapeutic strategies for different types of treatment-resistant epilepsy.

Commentary

MicroRNAs (miRNAs) are small non-coding RNAs ($\sim$22 nucleotides) that negatively regulate gene expression by binding complementary sites in the 3′ untranslated region of target messenger RNAs (mRNAs). Through this interaction with miRNAs, mRNAs are recruited to the RNA-induced silencing complex where they are either translationally repressed or degraded. MicroRNAs can modulate the expression of multiple genes simultaneously, which might provide a therapeutic opportunity for complex disorders such as epilepsy where numerous mechanisms can influence neuronal excitability. Furthermore, there is evidence demonstrating that miRNAs can target and modulate ion channel activity in a bidirectional fashion.[1] For example, Sosanya et al., showed that miR-129-5p reduced the expression of the voltage-gated potassium channel $K_v1.1$ in an mTORC1 activity-dependent manner.[2] Sosanya et al also found increased miR-129-5p levels and reduced levels of $K_v1.1$ three weeks after kainic acid (KA)-induced status epilepticus in a rat model.[3] Gross et al., showed that miR-324-5p reduced $K_v4.2$ protein expression,[4] and antagonism of miR-324-5p resulted in delayed seizure onset and reduced total EEG power following intra-amygdala KA administration.[4] Similarly, miRNAs have been shown to regulate voltage-gated sodium channels (VGSCs) as Hu et al., found that miRNA-132 decreased protein expression of $Na_v1.1$ and $Na_v1.2$ whereas antagonism of miRNA-132 increased $Na_v1.1$ and $Na_v1.2$ expression.[5] While many studies have identified miRNAs as potential therapeutic targets for epilepsy, it remains to be established whether there are miRNAs that are more broadly beneficial across multiple types or models of epilepsy.

The current study by Heiland and colleagues identified miRNA-335-5p as a potential target for modulating VGSCs in a combination of animal and human epilepsy models.[6] Previous reports that aimed to identify miRNAs that were altered

in treatment-resistant forms of epilepsy were based on datasets from only rodent models or a single tissue type, which might limit clinical relevance. In contrast, Heiland et al., explored miRNAs that emerged from multiple datasets, including both rodent tissue and human biofluids, to increase the potential of identifying miRNAs that might be common to multiple forms of treatment-resistant epilepsy.[6] The expression datasets that were used were derived from a perforant path stimulation rat model of temporal lobe epilepsy, blood samples from patients with epilepsy, and mouse brain following treatment with cannabidiol (CBD). Out of over 200 candidates, a single miRNA, miR-335-5p, was found to be altered in all 3 datasets, and importantly, the sequence of miR-335-5p is conserved between rodents and humans, highlighting the potential translational relevance of miR-335-5p. The VGSCs *SCN1A, SCN2A*, and *SCN3A* were identified as targets of miR-335-5p, and miR-335-5p was found to be predominantly expressed in excitatory and inhibitory neurons.[6]

In validating that miR-335-5p could indeed modulate VGSCs, when human induced pluripotent stem cell (iPSC)-derived neurons were treated with Ant-335, an antagomir against miR-335-5p, the authors observed increased expression of *SCN1A*, *SCN2A*, and *SCN3A* while *SCN8A* expression was not affected.[6] Intracerebroventricular administration of Ant-335 in adult C57BL/6 mice resulted in higher levels of *Scn1a*, *Scn2a*, and *Scn3a* transcripts in the mouse hippocampus when examined 48 hours after Ant-335 administration. Furthermore, Ant-335 treatment resulted in a larger action potential amplitude and increased firing frequency in hippocampal CA1 neurons. To determine whether Ant-335 altered seizure susceptibility, mice were treated with either Ant-335 or a scrambled antagomir followed by administration of the proconvulsant pentylenetetrazole (PTZ). The authors found that Ant-335 reduced the latency to PTZ-induced seizures compared to control mice that received a scrambled antagomir. Consistent with these observations, Ant-335 also reduced the latency to seizures induced by intra-amygdala KA administration. Furthermore, the authors found that Ant-335 treatment partially blocked the protective effects of CBD. To determine whether overexpression of miR-335-5p might be seizure protective, the authors generated an adeno-associated virus (AAV) to overexpress miR-335 (AAV9-miR-335). When bilaterally injected into the ventral hippocampus, AAV9-miR-335 resulted in a ~10-16-fold upregulation of miR-335-5p and reduced the expression of *Scn1a*, *Scn2a*, and *Scn3a* transcripts. The authors found that AAV9-miR-335-treated mice exhibited lower seizure severity and greater survival following PTZ administration compared to mice treated with a scrambled AAV9 construct; however, AAV9-miR-335 treatment had no effect on intra-amygdala KA-induced seizures. While Heiland and colleagues found that lower levels of miR-335-5p were associated with increased seizure vulnerability, modest seizure protection was observed with overexpression of miR-335-5p.[6]

In addition to seizure phenotypes, Heiland et al., investigated the effect of overexpression of miR-335-5p on behavior. The authors observed comparable behavior in the open field paradigm, Y maze, marble burying, and nest building in wild-type mice treated with either AAV9-miR-335 or the scrambled AAV9 construct.[6] These observations are surprising given that AAV9-miR-335 reduced expression of VGSC transcripts, with the greatest reduction in *Scn1a* transcripts. Loss-of-function mutations in *SCN1A, SCN2A*, and *SCN3A* are associated with a range of epilepsy subtypes and numerous behavioral comorbidities in patients and rodent models. In contrast to the current study, Capitano et al., noted that spatial learning resulted in the downregulation of hippocampal miR-335-5p and that overexpression of miR-335-5p (using a miR-335-5p mimic) significantly impaired long-term spatial memory.[7] Long-term potentiation was also impaired with overexpression of miR-335-5p. Thus, additional studies on the modulation of miR-335-5p and cognitive behavior are warranted. Moreover, it would be worthwhile to examine the effect of Ant-335 on behavior as there are multiple epilepsy subtypes and behavioral comorbidities that are due to gain-of-function mutations in VGSCs.

The current study provides evidence that modulating miR-335-5p can regulate the expression of several VGSCs and might be beneficial in treatment-resistant forms of epilepsy; however, several factors should be considered with respect to miRNAs as a potential treatment target. First, one advantage of miRNAs is that they have multiple gene targets; however, this could also constitute a disadvantage. For example, in genetic forms of epilepsy like Dravet syndrome which is primarily caused by loss-of-function mutations in the VGSC *SCN1A*, reducing miR-335-5p could potentially increase *SCN1A* expression, but increased expression of other VGSCs could result in unwanted side effects. In contrast, as demonstrated by Heiland et al., overexpressing miR-335-5p results in decreased *SCN1A* expression which would be contraindicated in Dravet syndrome. Therefore, investigating the effect of Ant-335 and AAV9-miR-335 in genetic models of epilepsy would be valuable. The current study found that miR-335-5p was expressed in excitatory and inhibitory neurons; thus, utilizing strategies to restrict miRNA targeting to either excitatory or inhibitory neurons might reduce unwanted side effects. Second, it is possible that modulation of a single miRNA may not be as seizure protective as current anti-seizure medications. In the current study, Ant-335 partially blocked the seizure protective effects of CBD treatment, and AAV9-miR-335 increased resistance against PTZ-induced but not KA-induced seizures.[6] Third, while the current study focused on the role of miR-335-5p and modulation of neuronal excitability, miR-335-5p has also been implicated in other neurological disorders, including amyotrophic lateral sclerosis and multiple forms of cancer. Thus, other neuronal dysfunction could arise with miRNA modulation and caution should be exerted when considering miRNAs as a potential treatment for neurological disorders. Finally, in the current study, Heiland et al., focused on the interaction between miR-335-5p and several VGSCs; however, it is also important to note that many other mRNA targets were also identified, including glutamate receptors and potassium channels. Li and colleagues observed a downregulation of

miR-335 in blood samples of patients with major depressive disorder and showed that miR-335 can directly target the glutamate receptor, metabotropic 4.[8] Therefore, a greater understanding of how miR-335-5p and other miRNAs can broadly affect ion channel activity is necessary to determine the therapeutic potential given that ion channel dysfunction is an underlying cause of epilepsy and other neurological disorders.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Gross C, Tiwari D. Regulation of ion channels by microRNAs and the implication for epilepsy. *Curr Neurol Neurosci Rep*. 2018;18:60.
2. Sosanya NM, Huang PP, Cacheaux LP, et al. Degradation of high affinity HuD targets releases Kv1.1 mRNA from miR-129 repression by mTORC1. *J Cell Biol*. 2013;202:53-69.
3. Sosanya NM, Brager DH, Wolfe S, Niere F, Raab-Graham KF. Rapamycin reveals an mTOR-independent repression of Kv1.1 expression during epileptogenesis. *Neurobiol Dis*. 2015;73:96-105.
4. Gross C, Yao X, Engel T, et al. MicroRNA-mediated downregulation of the potassium channel Kv4.2 contributes to seizure onset. *Cell Rep*. 2016;17:37-45.
5. Hu XL, Wang XX, Zhu YM, et al. MicroRNA-132 regulates total protein of Nav1.1 and Nav1.2 in the hippocampus and cortex of rat with chronic cerebral hypoperfusion. *Behav Brain Res*. 2019;366:118-125.
6. Heiland M, Connolly NMC, Mamad O, et al. MicroRNA-335-5p suppresses voltage-gated sodium channel expression and may be a target for seizure control. *Proc Natl Acad Sci USA*. 2023;120:e2216658120. doi:10.1073/pnas.2216658120
7. Capitano F, Camon J, Licursi V, et al. MicroRNA-335-5p modulates spatial memory and hippocampal synaptic plasticity. *Neurobiol Learn Mem*. 2017;139:63-68.
8. Li J, Meng H, Cao W, Qiu T. MiR-335 is involved in major depression disorder and antidepressant treatment through targeting GRM4. *Neurosci Lett*. 2015;606:167-172.