The Role of MicroRNA-324-5p in Dendritic Spine Density and Morphology in Mouse Hippocampal Neurons

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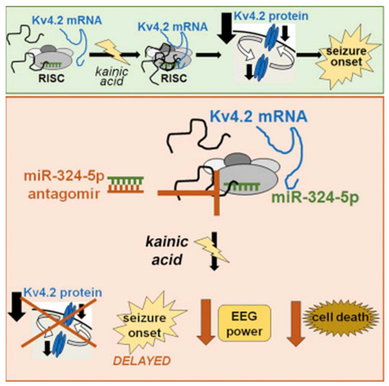
Introduction

Seizures are brief periods of excessive and uncontrolled bursts of electrical activity. Dendritic spines are small protrusions on dendrites and are sites of excitatory synapses. Dendritic spines are altered in epilepsy. Potassium channel Kv4.2 mediates transient A-type neuronal currents and has been shown by Tiwari et al. (2020) to down-regulate neuronal excitability. Kv4.2 may affect seizure susceptibility through altering dendritic spine density. In conditions such as epilepsy and autism, Kv4.2 expression is reduced. MicroRNA-324-5p (miR-324-5p) targets the complementary RNA sequence of Kv4.2, leading to its degradation by the RNA-induced silencing complex (RISC). Thus, a mechanism of altering Kv4.2 expression is through small noncoding RNAs called microRNA 324-5p.



[1]Figure from Gross *et al.* 2016

Expression of *miR-324* downregulates Kv4.2 leading to increased neuronal excitability and seizure susceptibility, but its affect on dendritic spine morphology and density is unknown.

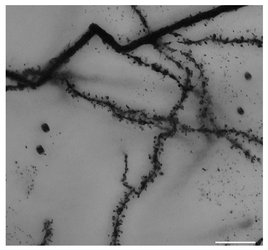
Hypothesis

Question: Does altering the expression of Kv4.2 by genetically reducing Kv4.2 or by knocking out the *miRNA-324* gene have a direct effect on dendritic spine density?
Hypothesis: We hypothesized that dendritic spine density is decreased in *miR-324* KO mice and morphology of Kv4.2 HET mice will be altered.

Methods

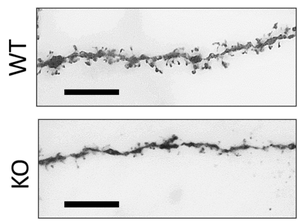
We assessed dendritic spine density in the hippocampal CA1 subregion of *miR324* knockout (KO), heterozygous (HET), and wild-type (WT) mice, and density and morphology in Kv4.2 HET and WT mice. Kv4.2 HET mice were used rather than full KO to better reflect human models.

miR-324 Mouse Models



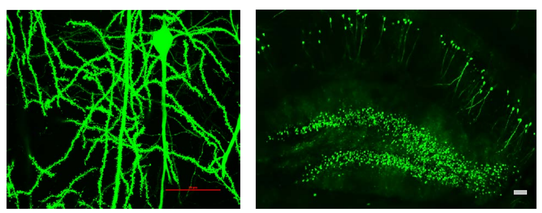
CRISPR-Cas9 was previously used to generate *miR-324* KO mice.

Established mouse models were generated by the CCHMC Transgenic Core, then images were previously obtained for this study.



Blinded data collection using ImageJ was performed by manually counting dendritic spines in images of Golgi-Cox stained neurons (shown above) from *miR324* KO, HET, and WT mice.

Kv4.2 Mouse Models



Previously, Thy1-EGFP mice were crossed with Kv4.2 HET mice, images were obtained with confocal microscopy, and 3D morphological measurements of dendritic spines were obtained using Neurolucida. Analysis of this data was performed with GraphPad Prism 8 for this study.

Results

Decreased Dendritic Spine Density of miR-324 KO CA1 Compared to miR-324 HET and WT Mice

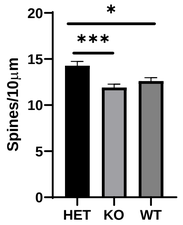
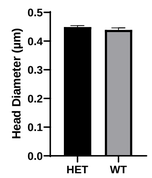


Figure 1. Hippocampal neurons from 4 *miR324* HET mice (44 dendrites), 3 KO mice (39 dendrites), and 2 WT mice (26 dendrites) were counted. One-way ANOVA shows a p= 0.0002. Post-hoc analysis for HET vs. KO, p*** = 0.0002; HET vs. WT, p*= 0.025. Low n number for *miR-324* WT mice skews the data.

No Difference in Average Head Diameter of Kv4.2 HET and Kv4.2 WT Mice

Figure 2. Average dendritic spine head diameters in Kv4.2 HET (n=4) and WT (n=3) mice show no significant difference when t-test is performed. Altered expression of Kv4.2 does not have an effect on this morphological measure.



Increased Average Dendritic Spine Volume in Kv4.2 HET Compared to Kv4.2 WT Mice

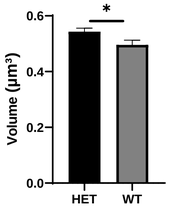
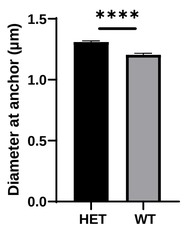


Figure 3. Unpaired t-test comparing Kv4.2 HET (n=5) vs. WT (n=3) dendritic spine volume shows significant difference (p*=0.043). Decreased expression of Kv4.2 causes increased dendritic spine volume.

Increased Dendritic Spine Diameter at Anchor of Kv4.2 HET Mice Compared to Kv4.2 WT Mice

Figure 4. Dendritic spine diameter at the anchor in Kv4.2 HET (n=4) is increased compared to Kv4.2 WT (n=3). Unpaired t-test shows significant difference (p****<0.0001). This suggests that reduced Kv4.2 expression effects dendritic spines at the anchor, but not the head (see Figure 2).



No Difference in Average Dendritic Spine Density in Kv4.2 HET vs. WT Mice

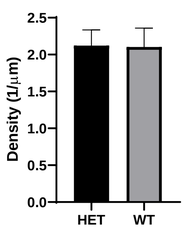


Figure 5. Dendritic spine density in Kv4.2 HET (n=5) compared to Kv4.2 WT (n=3) showed no significant difference when an unpaired t-test is performed.

Conclusion

Dendritic spine density was significantly decreased in *miR324* KO mice compared to *miR324* HET mice, though no difference was found between KO and WT. Spine morphology of Kv4.2 HET and Kv4.2 WT were analyzed for head diameter, spine volume, and spine diameter at the anchor. The difference in spine head diameter was insignificant, and spine density was unchanged. Kv4.2 HET mice showed significantly increased dendritic spine volume and spine diameter at anchor compared to Kv4.2 WT mice.

MiR324 KO results in decreased dendritic spine density. Decreased expression of Kv4.2 in Kv4.2 HET mice increases spine volume and diameter at anchor, but not head diameter.

Future experiments investigating how Kv4.2 expression influences cytoskeletal protein production may reveal the mechanism of observed morphological changes.

References

1. 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(1):37–45.
2. Tiwari et al. The potassium channel Kv4.2 regulates dendritic spine morphology, electroencephalographic characteristics and seizure susceptibility in mice. *Exp Neurology.* 2020;334:113437.

Acknowledgements

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