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

Giselle Scarano[1], Emma Parkins[1,2,3], Jeffrey Rymer[3], Yueh-Chiang Hu[4,5], Durgesh Tiwari[3,5], Christina Gross[2,3,5]

[1]University of Cincinnati College of Medicine; [2]University of Cincinnati Neuroscience Graduate Program; [3]Cincinnati Children's Hospital Medical Center, Division of Neurology; [4]Cincinnati Children's Hospital Medical Center, Division of Developmental Biology; [5]University of Cincinnati College of Medicine, Department of Pediatrics

Introduction: Dendritic spines are altered in epilepsy. Potassium channel Kv4.2 down-regulates neuronal excitability, and changes in its expression influence dendritic spine density and morphology. MicroRNA-324-5p represses Kv4.2 thereby regulating seizure susceptibility. However, the effect of *Mir324* (the gene encoding miR-324-5p) expression on dendritic spines is unknown. This prompts investigation into how altering expression of *Mir324* affects dendritic spine density and morphology.

Hypothesis: Based on preliminary data, we hypothesized that dendritic spine density is decreased in *Mir324* knockout (KO) mice, and that in mice with reduced Kv4.2 expression dendritic spine morphology is altered.

Methods: We assessed dendritic spine density in the hippocampal CA1 subregion of *Mir324* KO, heterozygous (HET), and wild-type (WT) mice, and density and morphology in Kv4.2 HET and WT mice. Blinded data collection using ImageJ was performed by manually counting dendritic spines in images of Golgi-Cox stained neurons from *Mir324* KO, HET, and WT mice. 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: Hippocampal neurons from 4 *Mir324* HET mice (44 dendrites), 3 KO mice (39 dendrites), and 2 WT mice (26 dendrites) were counted. Dendritic spine density was significantly decreased in KO mice and increased in HET mice, though no difference was found between KO and WT. Low n number for miR-324 WT mice skews the data. Spine morphology of Kv4.2 HET (4 mice) and Kv4.2 WT (3 mice) 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 insignificantly increased in Kv4.2 WT mice. Kv4.2 HET mice showed significantly increased dendritic spine volume and spine diameter at anchor compared to Kv4.2 WT mice.

Conclusions: *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.

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