Construction and Initial Characterization of the Densin Knockout Mouse

Thesis by
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Abstract

Densin-180 is a core protein of postsynaptic densities (PSDs) in excitatory neurons. Densin is known to interact with Maguin-1 and PSD-95, suggesting that it plays a role in the NMDA receptor complex. Densin also interacts with δ-Catenin and N-Cadherin, an adhesion complex known to play a role in spine morphology. A ternary complex of Densin, CaMKII, and alpha-actinin suggests that Densin plays a key role in cytoskeleton dynamics. Finally, Densin can directly bind to shank, a core scaffolding molecule of the postsynaptic density. The association of Densin with such diverse complexes of proteins suggests that it acts as an integrator of numerous signaling cascades. Here I describe the construction and initial characterization of a Densin knockout mouse. Mice homozygous for the Densin deletion are prone to seizures induced by barbiturates. Also, Densin$^{-/-}$ animals have altered spine morphologies and show changes in the expression levels of other core PSD proteins. Densin$^{-/-}$ neurons in culture exhibit an overall decrease in their dendritic complexity. Furthermore, we show that in the absence of the NMDA receptor, Densin can act to bind CaMKII in the PSD. A new high-throughput method for studying changes in gene transcription, RNA seq, was also used to study the effect of the Densin deletion on the forebrain and the hippocampus. This work represents the first time RNA seq has been used to study an animal with a knockout mutation. Two candidate genes that may mediate the seizure sensitivity, Npas4 and $GABA_A\alpha2$, were identified by this method. Npas4 is known to directly affect the number of inhibitory synapses formed by neurons, and $GABA_A\alpha2$ is a major GABA receptor subunit that mediates the effects of Nembutal. These results suggest that Densin may play a role in maintaining the balance between inhibitory and excitatory networks. Together, our results demonstrate that Densin is important for dendritic arbor formation, spine morphology, CaMKII localization in the PSD, and seizure susceptibility.

Table of Contents

Chapter 3: Analysis of Dendritic Arborization and Spine Morphology

Chapter 4: The Role of Densin in the Postsynaptic Density and Docking of CaMKII

Chapter 5: Global Analysis of the Effect of Densin Knockout on Gene Transcription in the Brain