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Reigning in Excitatory Signaling in CDKL5 Deficiency

AMPA Receptor Dysregulation and Therapeutic Interventions in a Mouse Model of CDKL5 Deficiency Disorder

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Pathogenic mutations in cyclin-dependent kinase-like 5 (CDKL5) result in CDKL5 deficiency disorder (CDD), a rare disease marked by early-life seizures, autistic behaviors, and intellectual disability. Although mouse models of CDD exhibit dendritic instability and alterations in synaptic scaffolding proteins, studies of glutamate receptor levels and function are limited. Here, we used a novel mouse model of CDD, the Cdkl5R59X knock-in mouse (R59X), to investigate changes in synaptic glutamate receptor subunits and functional consequences. Male mice were used for all experiments to avoid the confounding effects of X-inactivation that would be present in female heterozygous mice. We showed that adult male R59X mice recapitulated the behavioral outcomes observed in other mouse models of CDD, including social deficits and memory and learning impairments, and exhibited decreased latency to seizure upon pentylenetetrazol (PTZ) administration. Furthermore, we observed a specific increase in GluA2-lacking AMPA receptors (AMPARs) in the adultR59X hippocampus, which is accompanied electrophysiologically by increased rectification ratio of AMPAR excitatory postsynaptic currents and elevated early-phase long-term potentiation (LTP). Finally, we showed that acute treatment with the GluA2-lacking AMPAR blocker IEM-1460 decreased AMPAR currents and rescued social deficits, working memory impairments, and seizure behavior latency in R59X mice.

Altered NMDAR Signaling Underlies Autistic-Like Features in Mouse Models of CDKL5 Deficiency Disorder

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Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is characterized by epilepsy, intellectual disability, and autistic features, and CDKL5-deficient mice exhibit a constellation of behavioral phenotypes reminiscent of the human disorder. We previously found that CDKL5 dysfunction in forebrain glutamatergic neurons results in deficits in learning and memory. However, the pathogenic origin of the autistic features of CDD remains unknown. Here, we find that selective loss of CDKL5 in GABAergic neurons leads to autistic-like phenotypes in mice accompanied by excessive glutamatergic transmission, hyperexcitability, and increased levels of postsynaptic N-methyl-D-aspartate (NMDA) receptors. Acute, low-dose inhibition of NMDAR signaling ameliorates autistic-like behaviors in GABAergic knockout mice, as well as a novel mouse model bearing a CDD-associated nonsense mutation, CDKL5 R59X, implicating the translational potential of this mechanism. Together, our findings suggest that enhanced NMDAR signaling and circuit hyperexcitability underlie autistic-like features in mouse models of CDD and provide a new therapeutic avenue to treat CDD-related symptoms.

Commentary

Cyclin-dependent kinase-like 5 (CDKL5) deletion produces an epileptic encephalopathy (CDKL5 deficiency disorder [CDD]) characterized by treatment refractory seizures, social and communication deficits, and severe intellectual and developmental delays.[1] Cyclin-dependent kinase-like 5, a serine-threonine kinase, is abundant in the brain and has been localized to dendrites where it interacts with synaptic scaffolding proteins such as postsynaptic density protein 95 (PSD-95).[2] Pathogenic mutations in *Cdkl5* result in decreased dendritic spine density,[2] and conditional deletion of *Cdkl5* in mice results in decreased dendritic arborization.[3]

Several strains of *Cdkl5* constitutive knockout mice[4,5] and several conditional knockout lines[3,6] have been generated which display, on the whole, deficits in social behavior, learning and memory, and altered synaptic transmission. Notably, no strains have been reported to have spontaneous seizures. Although the duration of video electroencephalogram

recording performed has differed across studies (ranging from hours to 10 days), the lack of spontaneous seizures has been consistent.[5] The vast majority of children with CDD have daily seizures,[1] and thus, even if seizures are present at a low frequency in one or more of these knockout strains and have been missed due to insufficient recording length, the frequency would certainly fall short of mirroring the human condition.

A couple of critical questions have remained: What synaptic alterations following CDKL5 deletion lead to behavioral deficits, and secondarily, do missense mutations in CDKL5 (as compared to complete deletion) recapitulate the behavioral phenotypes? Two recent studies from the University of Pennsylvania have addressed these questions and come to slightly different conclusions. Both studies employed the arginine 59 (R59X) missense mutation model, and one study (Tang et al) also examined a conditional deletion model involving forebrain-specific knockout of CDKL5 in GABAergic neurons (Dlx-cKO). Both studies highlight a role for excessive glutamatergic neurotransmission in the pathology of CDD, but differentially attribute these defects to AMPA or N-methyl-D-aspartate (NMDA) receptors.

In R59X mice, Yennewar et al reported hyperactivity, increased anxiety-like behavior, impaired motor learning, and decreased social interactions. Tang et al, using the same strain, found decreased spontaneous alternation (potentially reflecting impaired memory or exploratory drive), increased stereotypies (repetitive grooming), and decreased social interaction. The behavioral abnormalities in the R59X mice are similar to those observed in Dlx-cKO mice, are largely consistent across models of CDD and mirror the deficits seen in patient populations.

In addition to behavioral analysis, both groups examined glutamatergic neurotransmission and receptor subunit expression. Tang et al found that Dlx-cKO mice display a selective upregulation of the GluN1 and GluN2B subunits of the NMDA receptor in hippocampus, in the absence of alterations in AMPA receptor (AMPAR) subunits. This profile was also observed in R59X mice in the Tang's study, but not in the Yennewar's study. Further supporting these findings, increased GluN2B has also been previously reported in a different CDKL5 knockout strain.[6] Paralleling the upregulation of these subunits, excitatory postsynaptic current frequency was increased in Dlx-cKO mice; this effect, which occurred in the absence of alterations in inhibitory postsynaptic currents, would thus be expected to lead to a net hyperexcitable phenotype. Consistent with this, Dlx-cKO mice displayed enhanced paired-pulse facilitation in hippocampus.

By contrast, Yennewar et al reported selective decreases in the GluA2 subunit of the AMPAR in R95X mice in the absence of changes in NMDA receptor subunits. The observed decrease in GluA2 expression was confirmed functionally in these mice; AMPA-mediated currents displayed increased inward rectification and sensitivity to blockade by antagonists that block GluA2-lacking receptors. In these animals, enhanced hippocampal long-term potentiation, without a change in paired-pulse ratio, was observed. Moreover, these animals displayed a decreased latency to seizures following administration of pentylenetetrazol. The loss of GluA2 renders AMPARs permeable to calcium, and in turn, enhanced calcium entry may lead to a hyperexcitable state.

Several methodological differences may account for this apparent discrepancy across these studies: first, the antibodies used varied between the studies—both the Tang study and a prior study that reported increased GluN2B[6] used the same antibody, which differed from that used by Yennewar. Moreover, both studies that detected increased GluN2B did so when probing postsynaptic density fractions, whereas the Yennewar study found no changes in GluN, but decreased GluA2 using crude hippocampal lysates. Although GABAergic neuron-specific deletion of *Cdkl5* in the Tang et al's report uncovered cell-type specific functions of this protein, it does not recapitulate the clinical phenomenon, in which mutations or deletions are present in all cells. Although this may be seen as a limitation, it is not likely to account for the differences across these studies, as global deletion *Cdkl5* produced a similar increase in GluN2B expression.[6]

Both of these studies aim toward translation: Yennewar et al, through the use of IEM-1460, an AMPAR antagonist that selectively blocks GluA2-lacking receptors, and Tang et al, through the use of memantine, an NMDA receptor antagonist. IEM-1460 normalized social behavior in R59X mice and partially rescued learning and memory function. Memantine likewise rescued social behavior in R59X mice, but not learning and memory. These studies, together, clearly extend our knowledge of the synaptic mechanisms underlying behavioral deficits in CDD and suggest that excessive glutamatergic neurotransmission may underlie neurobehavioral deficits associated with deletion or truncation of CDKL5.

Is it AMPA? Or is it NMDA? In the end, both may be viable targets for intervention and both may have important caveats. First, only acute treatment was used in both studies—it remains an open question if efficacy would be maintained with chronic treatment. Second, both acute and chronic treatment with drugs that alter excitatory/inhibitory balance in the developing brain can have long-term effects on synaptic function and behavior.[7,8] However, the risk–benefit balance is likely different in a condition such as CDD, given the severity of the deficits present. Third, are there critical early-life periods where interventions might be employed to normalize developmental trajectories? Fourth, and perhaps most importantly, will these findings extend to a situation marked by recurrent seizures?

An all too common challenge faced in preclinical epilepsy research is that the seizure phenotype in models of genetic epilepsies often fails to mirror the clinical case. This is certainly true for CDD, where none of the animal models currently available display spontaneous seizures. However, it is nearly incontrovertible that repeated seizures during critical periods of brain development have lasting effects on brain structure and function.[9,10] The importance of the interaction between seizures and underlying neuropathology in determining the end phenotype thus cannot be underestimated.

Although work remains, these studies mark an impressive level of progress. The first cases linking CDKL5 to epileptic

encephalopathy were published just over 15 years ago.[11,12] In the intervening years, almost 200 papers have been published, and several foundations have been launched to support investigations of this disorder. Identification of druggable targets for CDD represents an important step forward.

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