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Epigenetic Therapeutic Intervention for a Rare Epilepsy Disorder

Histone Deacetylase Inhibitors Restore Normal Hippocampal Synaptic Plasticity and Seizure Threshold in a Mouse Model of Tuberous Sclerosis Complex

Basu T, O'Riordan KJ, Schoenike BA, et al. *Sci Rep*. 2019;9:5266. doi:10.1038/s41598-019-41744-7.

Abnormal synaptic plasticity has been implicated in several neurological disorders including epilepsy, dementia, and autism spectrum disorder. Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder that manifests with seizures, autism, and cognitive deficits. The abnormal intracellular signaling underlying TSC has been the focus of many studies. However, nothing is known about the role of histone modifications in contributing to the neurological manifestations in TSC. Dynamic regulation of chromatin structure via posttranslational modification of histone tails has been implicated in learning, memory, and synaptic plasticity. Histone acetylation and associated gene activation plays a key role in plasticity and so we asked whether histone acetylation might be dysregulated in TSC. In this study, we report a general reduction in hippocampal histone H3 acetylation levels in a mouse model of TSC2. Pharmacological inhibition of histone deacetylase (HDAC) activity restores histone H3 acetylation levels and ameliorates the aberrant plasticity in $TSC2^{+/-}$ mice. We describe a novel seizure phenotype in $TSC2^{+/-}$ mice that is also normalized with HDAC inhibitors. The results from this study suggest an unanticipated role for chromatin modification in TSC and may inform novel therapeutic strategies for TSC patients.

Commentary

The number of individuals affected with an epilepsy disorder worldwide increases to approximately 50 million when the disorder arises simultaneously in the presence of another disease or condition.[1] Tuberous sclerosis complex (TSC) is a multisystem, autosomal dominant disorder resulting from mutations in 1 of 2 genes, *TSC1* (encoding hamartin) or *TSC2* (encoding tuberin). Ultimately, a *TSC1* or *TSC2* mutation leads to formation of brain lesions during development, known as tubers, that are highly associated with epilepsy, cognitive disability, and autism spectrum disorder.[2,3] The proteins, hamartin and tuberin, act in a complex to suppress cellular growth by inhibiting the mechanistic target of rapamycin complex 1 (mTORC1).[4] To date, studies have shown that a mutation in *TSC1* or *TCS2* results in changes with mTORC1 signaling in conjunction with abnormal hippocampal synaptic plasticity, plus impairments in learning and memory.[5] Thus, prior studies have primarily focused on protein kinase signaling and activity of mTORC1 in TSC. The present study now adds to these previous studies by describing a novel role for epigenetic mechanisms in TSC.

Over the years, epigenetic factors have gained interest in the epilepsy field and offer great potential for new therapeutic targets for treatment of the disorder. The 3 main categories of epigenetic markers, DNA methylation, posttranslational histone modifications, and small noncoding RNAs, are considered as central regulators of synaptic plasticity and learning and memory.[6,7] Additionally, studies have shown that dysfunctional methyl binding of proteins influences seizure activity indicating another critical role for epigenetics.[8,9] In this new study, Basu et al take advantage of fundamental epigenetic techniques to characterize the novel role of histone modifications in a mouse model of TSC. Specifically, calling on their previous knowledge of epigenetics and epilepsy, the authors were able to take well-informed approaches to better understand the role of histone modifications in TSC using the heterozygous, inactivating, mutant TSC2 ($TSC2^{+/-}$) mouse model that presents with impairments in synaptic plasticity and a seizure phenotype. The authors initially hypothesized that $TSC2^{+/-}$ mice would have decreased histone deacetylase (HDAC) activity leading to an increase in histone acetylation levels. However, when the authors measured hippocampal histone acetylation levels in $TSC2^{+/-}$ mice, they discovered a global decrease in histone acetylation levels compared to wild-type (WT) controls, specifically at the lysine residue 9 (H3K9Ac) and lysine residue 27 (H3K27Ac) on histone H3. Both H3K9Ac and H3K27Ac have been previously found to be transcriptionally activating at gene promoters. The authors therefore postulated that this unexpected reduction in acetylation levels is due to HDACs working in

balance with histone acetyltransferases (HAT) and that this balance is dysregulated in this mouse model of $TSC2^{+/-}$.

To further understand the role of epigenetics in $TSC2^{+/-}$ mice, the authors explored hippocampal plasticity by electrophysiology. As previously observed, the authors were able to show that $TSC2^{+/-}$ mice exhibit a stable long-term potentiation in response to a single theta burst (1×TBS) while WT controls exhibit show short-term potentiation (STP). When the authors used pharmacological inhibition of HDACs by Trichostatin A (TSA) in $TSC2^{+/-}$ mice, 1×TBS resulted in STP resembling untreated WT controls. Another way the authors showed dysregulated synaptic plasticity was by measuring metabotropic glutamate receptor (mGluR)-mediated long-term depression (LTD). The authors had previously showed that mGluR-LTD magnitude is reduced in $TSC2^{+/-}$ juvenile (p21-p24) mice when compared to their littermate controls. In this study, they now challenged their previous observation by incubating slices with TSA showing that that HDAC inhibition is able to increase mGluR-LTD magnitude to the levels of untreated WT controls. As previously mentioned, the mTORC1 pathway has been studied as a key component in the TSC. The authors were therefore interested to see if mTORC1 can also be affected by HDAC inhibition. To test this they used rapamycin, an mTOR inhibitor. While $TSC2^{+/-}$ mice display abnormal rapamycin-insensitive (mTOR independent) mGluR-LTD, they were able to show that HDAC inhibition restores normal mTORC1 dependent mGluR-LTD in adult $TSC2^{+/-}$ as seen in WT controls.

To delve deeper into the comorbidities associated with TSC, Basu et al wanted to look at seizure threshold in $TSC2^{+/-}$ mice. Until this study, seizure threshold had not been characterized in $TSC2^{+/-}$ mice. The authors were able to uncover a very novel finding that juvenile $TSC2^{+/-}$ mice (p18-p21) exhibit 17% reduced seizure threshold compared to aged matched WT controls. This was determined using a volatile convulsant, flurothyl, to induce generalized tonic clonic seizures $TSC2^{+/-}$ mice. In order to explore the effect of HDAC inhibition on seizure threshold in TSC, the authors used a clinically available, blood–brain barrier permeable, Food and Drug Administration approved HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). Interestingly, $TSC2^{+/-}$ mice that underwent SAHA treatment were able to show a significant decrease in seizure threshold when compared to nontreated $TSC2^{+/-}$ mice. Altogether, using fundamental epigenetics techniques, the authors were able to show a clear role of histone acetylation in $TSC2^{+/-}$ mice.

With a growing epilepsy population all over the world and a large number of patients being intractable to current treatments, developing better therapeutic approaches is an important goal for public health. This study meets this challenge and does a great job of using well-established neurological techniques with epigenetic mechanisms to better understand an understudied epilepsy disorder. Tuberous sclerosis complex is not as prevalent as primary epilepsy disorders but with very similar comorbidities and for TSC patients at risk of developing epilepsy, a better understanding of TSC will certainly hold tremendous promise for the identification of novel therapeutic interventions for epilepsy disorders in general. Accordingly, several lines of evidence support further investigation of epigenetic mechanisms as an attractive area of research as they can be influenced by interventions, such as HDAC inhibitors. Notably, the study by Basu and colleagues highlights the importance of manipulating epigenetic mechanisms using the HDAC inhibitor, TSA in order to normalize seizure activity in $TSC2^{+/-}$ mice. However, it is important to note that while some epigenetic interventions can have beneficial effects in certain neurological disorders they could, at the same time, be detrimental in other conditions. Additionally, the authors examined LTD/synaptic plasticity mechanisms and seizure threshold; however, there is still a need for further examination in the context of behavioral studies to better understand the functional impact of HDAC inhibition on learning and memory deficits associated with TSC. Furthermore, the authors postulate that the dysregulated acetylation levels observed in their TSC model is due to an HDAC/HAT imbalance, but this requires further exploration with additional measurements to look at HAT activity. Therefore, future studies should consider investigation of the molecular mechanisms of action by which HDAC and HAT modifiers contribute to aberrant regulation of chromatin environments in TSC and associated comorbidities.

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