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Epileptic Neurons Know JAK/STAT3

Selective Neuronal Knockout of STAT3 Function Inhibits Epilepsy Progression, Improves Cognition, and Restores Dysregulated Gene Networks in a Temporal Lobe Epilepsy Model

Tipton AE, Del Angel YC, Hixson K, Carlsen J, Strode D, Busquet N, Mesches MH, Gonzalez MI, Napoli E, Russek SJ, Brooks-Kayal AR. *Ann Neurol*. 2023 Mar 19. doi:10.1002/ana.26644

Objective: Temporal lobe epilepsy (TLE) is a progressive disorder mediated by pathological changes in molecular cascades and hippocampal neural circuit remodeling that results in spontaneous seizures and cognitive dysfunction. Targeting these cascades may provide disease-modifying treatments for TLE patients. Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) inhibitors have emerged as potential disease-modifying therapies; a more detailed understanding of JAK/STAT participation in epileptogenic responses is required, however, to increase the therapeutic efficacy and reduce adverse effects associated with global inhibition. Methods: We developed a mouse line in which tamoxifen treatment conditionally abolishes STAT3 signaling from forebrain excitatory neurons (nSTAT3KO). Seizure frequency (continuous in vivo electroencephalography) and memory (contextual fear conditioning and motor learning) were analyzed in wild-type and nSTAT3KO mice after intrahippocampal kainate (IHKA) injection as a model of TLE. Hippocampal RNA was obtained 24 h after IHKA and subjected to deep sequencing. Results: Selective STAT3 knock-out in excitatory neurons reduced seizure progression and hippocampal memory deficits without reducing the extent of cell death or mossy fiber sprouting induced by IHKA injection. Gene expression was rescued in major networks associated with response to brain injury, neuronal plasticity, and learning and memory. We also provide the first evidence that neuronal STAT3 may directly influence brain inflammation. Interpretation: Inhibiting neuronal STAT3 signaling improved outcomes in an animal model of TLE, prevented progression of seizures and cognitive co-morbidities while rescuing pathogenic changes in gene expression of major networks associated with epileptogenesis. Specifically targeting neuronal STAT3 may be an effective disease-modifying strategy for TLE.

Commentary

The Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway is essential for regulating the expression of genes related to cellular survival and immune responses.[1] First identified 30 years ago, JAK/STAT signaling molecules are implicated in the regulation of cellular growth, differentiation and proliferation, apoptosis, hematopoiesis, immune cell differentiation, cytokine signaling, and inflammation.[1] Dysregulation of JAK/STAT activity is associated with tumor and cancer development, inflammatory and autoimmune disorders, and neurodegenerative disorders.[1,2] There is growing evidence suggesting that increased JAK/STAT activity also plays a role in epilepsy in both human and experimental settings (animal and cell/organotypic culture models).[3-6] However, the precise contribution of this activity to epileptogenesis is still being established. The study by Tipton et al investigated the specific role of neuronal JAK/STAT3 function in epileptogenic processes, unprovoked seizure progression, and cognitive comorbidities in a mouse model of acquired temporal lobe epilepsy (TLE) generated by administration of intrahippocampal kainic acid (IHKA).[3]

Brain injury linked to events of traumatic brain injury, ischemia, and status epilepticus (SE) can trigger hyperactivation of the JAK/STAT pathway.[4,6,7] In a pilocarpine rat model of SE and acquired TLE, this group previously reported that suppression of the JAK/STAT signaling cascade with WP1066, a small molecule inhibitor of STAT3 signaling, given at the onset of SE resulted in a long-lasting reduction of spontaneous recurrent seizures (SRS).[4] To further interrogate the specific role of neuronal JAK/STAT signaling in epileptogenesis, Tipton et al knocked out (KO) the function of STAT3 in excitatory forebrain neurons (nSTAT3KO) with the administration of tamoxifen to camk2a-cre/ERT2/Stat3$^{fl/fl}$ mice, and 2 weeks later induced SE with IHKA.[3] Then, the authors assessed occurrence of SRS, learning and memory, cell loss and mossy

fiber sprouting, and performed transcriptomic profiling in hippocampi of nSTAT3KO and wild-type (WT) mice treated with IHKA or saline.

The severity of SE induced by IHKA was similar in both WT mice and nSTAT3KO mice. Continuous EEG monitoring revealed that whereas both mouse groups developed SRS to a similar extent during the first 2 weeks, the frequency of SRS was significantly reduced in nSTAT3KO mice during the third and fourth weeks. Thus, it is likely that nSTAT3KO inactivation does not prevent epileptogenesis but instead promotes seizure control in existing hyperexcitable networks, suggesting that nSTAT3 signaling plays a significant role in the progression of epilepsy. Attractive approaches that can further support the potential anti-seizure effect of JAK/STAT3 inhibition in chronic epilepsy would require long-term monitoring of both frequency and duration of seizures at later time intervals spanning from 8 to 24 weeks in both nSTAT3KO and WT mice, and/or administering tamoxifen to inhibit neuronal JAK/STAT3 function in already epileptic animals. This evidence could strengthen the value of STAT3 as a potential druggable target for disease modification in cases with chronic epilepsy, in particular those characterized by drug-resistant seizures (e.g., TLE, focal cortical dysplasia).

A modifying role in the pathophysiology of epilepsy is also supported by the inactivation of the JAK/STAT3 signaling cascade in excitatory neurons which resulted in enhanced cognitive abilities in epileptic nSTAT3KO mice. Using rotarod to evaluate sensorimotor skill learning and contextual fear conditioning to test hippocampal-dependent learning and memory, the authors showed that IHKA promoted memory defects in WT mice but not in nSTAT3KO mice. It is possible that the reduced seizure frequency in nSTAT3KO mice contributes to the improved cognitive functions of these mice relative to epileptic WT mice. Interestingly, both the seizure reduction and the cognitive improvement achieved in epileptic nSTAT3KO mice did not parallel improvement in the neuropathology. Substantial hippocampal cell loss and mossy fiber sprouting which are typically found days (1-3 days) and weeks (>3 weeks) after IHKA, respectively, were not different between WT and nSTAT3KO mice that sustained SE.

Tipton et al also employed RNA sequencing to assess the immediate effects of IHKA-induced SE and JAK/STAT3 inactivation on gene expression patterns in hippocampi of both WT and nSTAT3KO mice. Twenty-four hours after SE in WT mice, the authors found differential expression in 3190 genes, with an enrichment of pathways associated with synaptic signaling, immune signaling/inflammation, and 216 STAT3 related genes. Interestingly, selective inactivation of STAT3 in excitatory neurons in IHKA-treated nSTAT3KO mice rescued the expression of 1609 genes. While some of the rescued genes have neuronal specific roles such as synaptogenesis and synaptic plasticity, others involved unexpected pathways related to immune and microglial functions such as inflammation, migration, and phagocytosis. Given the neuronal specificity of STAT3 inactivation, it is possible that STAT3 activity directly influences neuronal activity. Another possibility is that neuronal STAT3 signaling orchestrates cross talks between neurons and glia or immune cells, and indirectly modulates neuroinflammation and immune dysregulation, which in turn can contribute to the development and progression of SRS.

The author's suggestion that microglial migration and phagocytosis pathways may be potential targets of neuronal JAK/STAT3 modulation is indeed intriguing. Microglial migration/proliferation along with the expression of phagocytosis markers are prominent in the hippocampal CA1 region between 2 and 3 weeks after SE when SRS develop in the pilocarpine rat model of acquired TLE.[8,9] These temporal correlations align with the timeline of seizure rescue reported in the study conducted by Tipton et al suggesting a potential interplay between neuronal JAK/STAT3 signaling and microglia in exacerbating seizure progression during the later stages of epileptogenesis. However, it is important to consider a limitation in the interpretation of the role played by differentially expressed genes in relation to the rescue of seizure and cognitive pathology in nSTAT3KO mice. The transcriptional profiles were examined at 24 hours, while functional assessments were conducted at >1 to 4 weeks after SE. Conducting a time matched structure-function analysis would help establish a stronger connection that supports a mechanistic role for neuronal JAK/STAT3 inactivation in the corresponding functional outcomes.

Strengths of this study include a comprehensive analysis that identified differentially expressed genes and associated cellular pathways that rely on neuronal STAT3 function in the context of SE that can be targeted in future mechanistic studies. Additionally, Tipton et al confirmed and reproduced previous results demonstrating that suppressing JAK/STAT3 signaling during SE reduces SRS in the pilocarpine rat model of SE and acquired TLE.[4] These findings provide strong evidence for a potential role of JAK/STAT signaling in the pathophysiology of established epilepsy. However, it is important to acknowledge that our understanding of the involvement of the JAK/STAT pathway in epilepsy is still emerging and further research is needed to appreciate its contribution to epileptogenic processes and outcomes. It is noteworthy that JAK/STAT small molecule inhibitors are already being effectively used to treat various autoimmune and cancer disorders.[2] Therefore, as new discoveries continue to establish JAK/STAT signaling as a causal mechanism underlying epilepsy, these existing drugs could be repurposed for use in epilepsy treatment. This potential repurposing could offer new therapeutic avenues for managing epilepsy using already approved and well-studied medications.

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Declaration of Conflicting Interests

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