Nrf2 to the Rescue

Nrf2 Defense Pathway: Experimental Evidence for its Protective Role in Epilepsy.

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OBJECTIVE: Epigenetic mechanisms involved in transcriptional regulation of multiple molecular pathways are potentially attractive therapeutic interventions for epilepsy, because single target therapies are unlikely to provide both anticonvulsant and disease-modifying effects. METHODS: A selection of epilepsy-related gene expression data sets were retrieved using NextBio software and imported to Ingenuity Pathway Analysis for transcription factor enrichment analysis. Nuclear factor erythroid 2-related factor 2 (Nrf2)—a transcription factor that promotes the expression of numerous antioxidant, anti-inflammatory, and neuroprotective proteins—was identified as a candidate for confirmation of mRNA expression in hippocampal tissue from patients with temporal lobe epilepsy and in mice following pilocarpine-induced status epilepticus (SE). Human Nrf2 was overexpressed via an adeno-associated virus (AAV) vector after the onset of spontaneous recurrent seizures (SRS) in the animals. At the end of a 5-week continuous monitoring period for SRS, quantitative immunohistochemistry using neuronal (neuronal-specific nuclear protein), astrocytic (glial fibrillary acidic protein), and microglial (ionized calcium binding adaptor molecule 1) markers was performed. RESULTS: A significant increase in Nrf2 mRNA expression was observed in human epileptic hippocampal tissue. Nrf2 expression levels increased progressively in mice, reaching a peak at 72 hours after SE, and then declined. Similar expression patterns were observed for 3 Nrf2-regulated genes: HO-1, NQO1, and mGST. Remarkably, mice injected with AAV Nrf2 displayed significantly fewer generalized seizures, with profound reduction in microglia activation. Hippocampal neurons were preserved, whereas the number of astrocytes was unchanged. INTERPRETATION: These findings extend the potential of Nrf2-based therapies to epilepsy and add to the rapidly accumulating evidence from other neurodegenerative and inflammatory disease models.

Commentary

The nuclear factor erythroid 2-related factor 2 (Nrf2), a master transcriptional regulator of antioxidant and detoxification genes, has recently emerged as an important therapeutic target for various diseases, including neurologic disorders (1). Nrf2, a ubiquitous member of the cap'n'collar transcription factors, is activated by cellular stress and initiates transcription of a diverse set of genes, such as antioxidant defense, drug transporters, metabolic enzymes, and transcription factors, by binding to the antioxidant response elements (AREs) or electrophile response elements (2). Activation of the Nrf2 pathway has been shown to be neuroprotective in various animal models, which supports the concept of therapeutic targeting with Nrf2 activators as a viable pathway for neurologic disorders. This is exemplified by the recent introduction of dimethyl fumarate, a weak Nrf2 activator for the treatment of nonremitting multiple sclerosis (3). One reason for considering the Nrf2 pathway in epilepsy arises from the recognition that oxidative stress reflected by altered steady-state glutathione levels occurs with epileptogenesis (4). Moreover, the ketogenic diet profoundly alters redox processes, in part by increasing cellular glutathione levels via activation of the Nrf2 pathway (5, 6). Therefore, a clear rationale exists for validating the Nrf2 pathway as antiepileptic or antiepileptogenic therapies.

Mazzuferi et al. (7) honed in on the Nrf2 pathway as a result of their search to identify transcription factors that cause gene expression changes in different studies, including animal models of epilepsy and animals treated with a ketogenic diet. The authors applied transcription factor enrichment analysis using IPA software. They compared the list of differentially expressed genes in the 10 data sets with the list of experimentally confirmed Nrf2 targets and found that the expression of 3.5% of all differentially expressed genes was directly regulated by Nrf2. More than 75% of these genes showed consistent up- or downregulation, which strongly suggests regulation by the same transcription factor across the independent studies. An enrichment analysis on these genes identified signaling in xenobiotic metabolism, acute-phase response, glucocorticoid receptor, pancreatic adenocarcinoma, glioblastoma multiforme, and G1/S checkpoint regulation as the biological processes that are significantly linked to Nrf2 across the 10 data sets.

Next, they investigated Nrf2 and ARE-containing gene expression in human and mouse temporal lobe epilepsy. The Nrf2 mRNA quantification was performed using branched nucleic acid technology, which revealed a significant twofold

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increase in the hippocampal tissue of patients with temporal lobe epilepsy (TLE) compared with controls. However, in mice with TLE, Nrf2 expression increased progressively and peaked at 72 hours with a subsequent decline in the chronic phase of the model. Additionally, they examined the expression of three ARE-containing genes—HO1, NQO1, and m-GST—which also demonstrated temporal changes correlating with Nrf2 expression levels.

To probe the role of Nrf2 activation in acquired epilepsy, Mazzuferi et al. (7) overexpressed Nrf2 by adeno-associated viral (AAV) vectors in mice and determined its effect on seizures and hippocampal pathology. AAV was selected as it provides long-term gene expression in a variety of cell types in the brain and has shown minimal toxicity in animal models and clinical trials. Control or Nrf2 virus was administered 2 weeks after status epilepticus to mice having a comparable number of spontaneous recurrent seizures. During the 5-week monitoring period, the number and frequency of generalized seizures were significantly decreased in mice that were administered the AAV overexpressing Nrf2 compared with mice injected with control virus. Additionally, they confirmed the spread of transgene expression in the hilus of the dentate gyrus and in the CA3 region along the mossy fiber pathway by visualizing green fluorescent protein (GFP) expression of the control virus in both naive and epileptic mice. In the latter group, neuronal, astrocytic, and microglial cells showed GFP expression, whereas the naive mice predominantly displayed neuronal expression of the transgene.

In line with previous studies performed in pilocarpine-induced epilepsy, the authors observed substantial cellular reorganization in the hilus of the dentate gyrus of epileptic mice, which included significant decreases in the number of neurons and increases in the number of astrocytes and activated microglial cells. Interestingly, the number of neurons was significantly increased in the mice injected with Nrf2 virus as quantified by neuronal-specific nuclear protein–positive cells compared with control AAV-injected epileptic mice. In contrast, the total number of astrocytes, as determined by glial fibrillary acidic protein staining, did not change between groups, whereas the total number of activated microglia (ionized calcium-binding adaptor molecule 1 or Iba-1 positive) was decreased significantly in the Nrf2-injected mice. Finally, the ratio of astrocytes to neurons as well as the ratio of activated microglia to neurons were both significantly reduced in Nrf2-injected epileptic mice compared with control virus-injected epileptic mice.

Mazzuferi et al. (7) thus successfully demonstrated for the first time that Nrf2 plays a protective role in acquired epilepsy. Using bioinformatic analyses, they identified Nrf2 as one of the transcription factors responsible for expression changes in a number of BioSETs from epilepsy-related studies. Consistent with this initial finding, they also found elevated Nrf2 mRNA levels in human hippcampal tissue resected from TLE patients. Similarly, in mice treated with pilocarpine, they observed acute increases in Nrf2 mRNA expression and in its target genes. The primary finding of this article was that overexpressing Nrf2 by AAV vectors in epileptic mice had a significant beneficial effect on the number and frequency of spontaneous recurrent seizures as well as hippocampal pathology, including neuronal death and inflammatory processes associated with the pilocarpine mouse model of TLE. This is particularly interesting considering that transgene delivery was performed in chronically epileptic animals, highlighting the direct potential of such interventions in the treatment of epilepsy. However, two key issues raised by this study remain to be addressed. First, despite observing beneficial effects of Nrf2 overexpression on seizure parameters and pathology, the authors did not test the effect of Nrf2 AAV injection on the mRNA expression of Nrf2 or its target genes or proteins in the epileptic mice to confirm that the protective effects were indeed due to Nrf2 overexpression. Second, the downstream genes underlying Nrf2's antiepileptogenic effects were not examined. In sum, these results validate the overall Nrf2 pathway as a target for treatment of TLE and other related epilepsies. Further development of Nrf2 activators will need consideration of notable untoward effects, such as cancer-associated overactivation of this pathway. Nevertheless, this study strengthens the case for Nrf2 and its downstream gene products as promising therapeutic targets for epilepsy.

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