



Cleaning Up Epilepsy and Neurodegeneration: The Role of Autophagy in Epileptogenesis

Impaired Autophagy in Neurons After Disinhibition of Mammalian Target of Rapamycin and Its Contribution to Epileptogenesis.

McMahon J, Huang X, Yang J, Komatsu M, Yue Z, Qian J, Zhu X, Huang Y. *J Neurosci* 2012;32:15704–15714.

Certain mutations within the mammalian target of rapamycin (mTOR) pathway, most notably those affecting the tuberous sclerosis complex (TSC), lead to aberrant activation of mTOR and result in a high incidence of epilepsy in humans and animal models. Although hyperactivation of mTOR has been strongly linked to the development of epilepsy and, conversely, inhibition of mTOR by rapamycin treatment is protective against seizures in several models, the downstream epileptic mechanisms have remained elusive. Autophagy, a catabolic process that plays a vital role in cellular homeostasis by mediating the turnover of cytoplasmic constituents, is negatively regulated by mTOR. Here we demonstrate that autophagy is suppressed in brain tissues of forebrain-specific conditional TSC1 and phosphatase and tensin homolog knock-out mice, both of which display aberrant mTOR activation and seizures. In addition, we also discovered that autophagy is suppressed in the brains of human TSC patients. Moreover, conditional deletion of *Atg7*, an essential regulator of autophagy, in mouse forebrain neurons is sufficient to promote development of spontaneous seizures. Thus, our study suggests that impaired autophagy contributes to epileptogenesis, which may be of interest as a potential therapeutic target for epilepsy treatment and/or prevention.

Commentary

Autophagy, literally meaning “self-eating,” is an evolutionarily conserved housekeeping function of most cells. Autophagy involves sequestration, removal, and degradation of cellular components by lysosomes. Three subtypes of autophagy have been described—macroautophagy, microautophagy, and chaperone-mediated autophagy—which differ in their mechanisms of delivery of substrates to the lysosome for degradation. Macroautophagy (henceforth referred to as autophagy) represents the primary, best characterized form and involves several steps. First, an initial membrane apparatus forms within the cytoplasm as a site for assembly and collection of cellular debris. The structure then elongates and creates a double membrane, forming a vacuolar autophagosome, which is then transported by microtubules toward microtubule-organizing centers where lysosomes are located. Finally, fusion of the autophagosome and lysosome allows the contents to be degraded by lysosomal enzymes.

Autophagy plays a central role in many important physiological and pathological processes (1). First of all, autophagy helps remove old or dysfunctional cellular components, such as misfolded proteins or damaged organelles. Without autophagic clearance, the accumulation of intracellular debris could result in release of harmful reactive oxidation species into the cytoplasm

or have other toxic effects on cells. As autophagic mechanisms are closely coupled to related mechanisms of apoptosis, autophagy is integrally linked to decisions about cellular fate, survival, and death. Autophagy is also involved in the body’s response to environmental stressors, such as trauma, infection, inflammation, and starvation. For example, under catabolic states of nutrient deprivation, autophagy adopts a critical metabolic function in providing key nutrients, such as amino acids, derived from degradation of autophagic substrates. Recently, autophagy has also been implicated in basic brain development and function, such as synaptic transmission and plasticity that may relate to learning and memory (2, 3).

Dysfunction of autophagic mechanisms has been associated with a variety of disease states, including neurodegenerative disorders (4, 5). Several classic neurodegenerative diseases such as Huntington, Parkinson, and Alzheimer disease are characterized pathologically by accumulation of abnormal protein aggregates or other cytoplasmic inclusions. Although the pathophysiological significance of these protein aggregates is still debated, a prominent hypothesis is that these aggregates have toxic effects that cause neuronal degeneration. A defect in autophagic mechanisms could contribute to accumulation of protein aggregates in these neurodegenerative diseases. For example, in cellular and mouse models of Huntington disease, autophagic vacuoles demonstrate inefficient recognition and accumulation of targeted cargo, which might result in toxic aggregates of huntingtin protein (6). Conversely, from a therapeutic standpoint, induction of



autophagy with rapamycin has been shown to reduce toxicity of polyglutamine aggregates and improve behavioral motor deficits in mouse models of Huntington disease (7).

The potential role of autophagy in epilepsy has been relatively unexplored. One possible link to epilepsy is via an upstream signaling pathway that regulates autophagy. The mammalian target of rapamycin (mTOR) pathway is a ubiquitous signaling mechanism involved in regulating a variety of important cellular and physiological functions, such as cell growth, proliferation, metabolism, and protein synthesis. In addition, the mTOR pathway, particularly mTOR complex 1 (mTORC1), is a potent negative regulator of autophagy. Furthermore, the mTORC1 inhibitor, rapamycin, induces autophagy. Accumulating data, primarily from animal models, indicate that the mTOR pathway may be involved in mechanisms of epileptogenesis in different types of epilepsy (8). This relationship is most strongly implicated in the genetic epilepsy, tuberous sclerosis complex (TSC), but there is also some support for involvement of mTOR in other types of epilepsy, such as acquired temporal lobe epilepsy, infantile spasms, and absence seizures. However, the mechanisms downstream from mTOR that promote epileptogenesis are poorly understood.

The recent study by McMahon et al. starts to address a link between mTOR, epilepsy, and autophagy. First, they show in mice that genetic manipulations that cause disinhibition of mTOR by deleting upstream *Tsc1* or *Pten* genes in forebrain neurons lead to seizures and impaired autophagy activity. Similarly, brain tissue from human patients with TSC display markers of increased mTOR activation and decreased autophagy. While a causal role of autophagy in promoting epilepsy in these mouse models and human tissue is not definitively established, separate experiments show that genetic inactivation of *Atg7*, an essential promoter of autophagy, results in spontaneous seizures in mice, thus indicating that inactivation of autophagy is sufficient to cause epilepsy.

This study is significant in establishing a distinct role of autophagy in the inhibition of epileptogenesis. However, much more work needs to be done to identify the specific cellular and molecular mechanisms involved, as well as to explore potential therapeutic applications for epilepsy. There are a number of ways in which impaired autophagy may lead to epilepsy. Given an overlap between autophagic and apoptotic mechanisms, suppression of autophagy may promote apoptosis, and neuronal death may then cause hyperexcitable circuits that lead to seizures. However, the McMahon study did not find any overt evidence of neurodegeneration in the *Atg7* knock-out mice, indicating that cell death was not a causal factor in epileptogenesis in this case. As autophagic mechanisms regulate synaptic transmission and plasticity (2, 3), impaired autophagy could facilitate aberrant synaptic reorganization, such as mossy fiber sprouting, and formation of epileptic circuits. The effects of abnormal autophagy on synaptic function and neuronal excitability in the context of epileptogenesis need to be investigated in more detail.

Most intriguingly, the findings from this study raise the possibility that impaired autophagy could result in protein aggregates, which, in addition to being implicated in classic neurodegenerative diseases, might also play a novel role in epilepsy. While epilepsy has a variety of primary and secondary neurologic

etiologies, epilepsy per se is not traditionally viewed as a neurodegenerative disorder. However, Lafora body disease, a rare progressive myoclonic epilepsy, is characterized by a pathological hallmark of cytoplasmic inclusions (Lafora bodies) owing to mutation in laforin, a protein phosphatase. Laforin has been shown to stimulate autophagy in an mTOR-dependent fashion, suggesting that impaired autophagy resulting from laforin mutations accounts for the cytoplasmic aggregates and possibly epilepsy in this disorder (9). While Lafora body disease represents a true neurodegenerative disorder, the possibility of protein aggregates contributing to primary "idiopathic" epilepsies has recently also been proposed. In particular, in a rare familial case of generalized epilepsy with febrile seizures plus (GEFS+), a mutant GABA_A receptor subunit has been found to exhibit abnormally slow degradation and the formation of protein aggregates, similar to classic neurodegenerative disorders (10). Although the mechanism of this protein aggregation is not established, this mutant GABA_A receptor is at least partially degraded by lysosomes, suggesting the potential involvement of impaired autophagy. Regardless of whether defective autophagy is a primary cause of the protein aggregation and epilepsy in these cases, these findings still suggest a novel therapeutic approach of using autophagy inducers, such as rapamycin, to treat epilepsy.

by Michael Wong, MD, PhD

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