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The role of ubiquitin/Nedd4-2 in the pathogenesis of mesial temporal lobe epilepsy

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HIGHLIGHTS

- We explored that ubiquitin and Nedd4-2 differentiated as expressed in MTLE rats.
- Ubiquitin and Nedd4-2 co-localized during epileptogenesis both in vivo and in vitro.
- Inhibition of UPS could aggravate the epileptogenesis of MTLE.
- Nedd4-2 was a critical E3 ligase involved in the epileptogenesis of MTLE.

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ABSTRACT

Although the pathogenesis and epileptogenesis of mesial temporal lobe epilepsy (MTLE) have been studied for years, many questions remain. The ubiquitin–proteasome system (UPS) is one factor that might regulate ion channels, inflammation and neuron excitability. Nedd4-2 is an E3 ubiquitin ligase linked with ion channels and synaptic vesicle recycling. Here, we explore the role of the UPS and its E3 ligase Nedd4-2 in the pathogenesis of MTLE. Our western blot results revealed that ubiquitin and Nedd4-2 were expressed differentially in different stages of MTLE. Co-immunoprecipitation and double immunostaining results indicated that Nedd4-2 was the substrate protein of ubiquitin both in vivo and in vitro. Inhibition of the UPS aggravated the epileptogenesis of MTLE, causing early and frequent spontaneous seizures, more obvious neuron loss and aberrant mossy fiber sprouting. Inhibition of ubiquitin also enhanced the activation of Nedd4-2, and switched ion channel α-ENaC downstream. Our study is the first to report that the UPS participates in the pathogenesis of MTLE, inhibition of UPS could aggravate the epileptogenesis, and that Nedd4-2 is a critical E3 ligase involved in this process.

1. Introduction

Mesial temporal lobe epilepsy (MTLE) is a common, medically intractable syndrome. It arises from limbic structures, most notably the hippocampus, that is highly sensitive to the effects of stress. Hippocampal sclerosis with prominent neuronal loss and gliosis, as well as increased hyperexcitability and recurrent excitation associated with aberrant mossy fiber sprouting (MFS) in the hippocampus, are the most common pathologies in MTLE [1]. Although the pathogenesis and epileptogenesis of MTLE have been studied for years, many questions remain. Existing research has focused on genetics, ion channels, neurodevelopmental factors, endocytosis and exocytosis, autoimmunity and inflammation, hypoxia ischemic injury, stress, and so on [2,3]. The ubiquitin–proteasome system (UPS) is one factor that could regulate ion channels, inflammation and neuron excitability.

The UPS is the main stay of protein quality control in eukaryotic cells. It governs a wide variety of regulatory pathways, from cell-cycle control and transcription to development. The timely and selective degradation of surplus and/or aberrant proteins by the UPS is essential for normal cellular physiology. Any disturbance, delay or exaggeration in the process of selection, sequestration, labeling for degradation and degradation of target proteins by the UPS will compromise cellular and tissue homeostasis [4]. Previous studies have shown that alterations in the ubiquitin–proteasome pathway (UPP) can contribute to the development and progression of various human diseases, such as Parkinson's disease, Alzheimer's disease, Angelman syndrome, ischemic heart disease, and other neurodegenerative, autoimmune, and inflammatory diseases [5]. Also, studies about UPS involvement in long-term synaptic plasticity and epileptic diseases such as Lafora disease have recently emerged [6]. It is important to note that, although there is ample evidence implicating the UPS in neurological diseases, the molecular identity of the ligases and proteins associated with it remains largely unknown.

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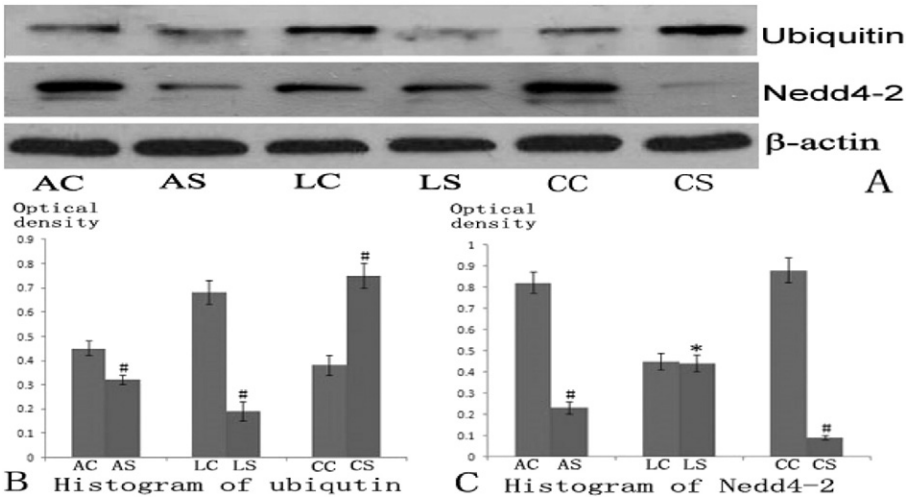

Fig. 1. Dynamic expression of ubiquitin and Nedd4-2 by western blot analysis in MTLE rats and controls. A: Gray value rate of ubiquitin, Nedd4-2 and β-actin expression in the three stages of MTLE development compared with control groups. B and C: ubiquitin significantly down-regulated in the acute stage (AS) and latent stage (LS) of MTLE rats, and up-regulated in the chronic stage (CS). Nedd4-2 significantly down-regulated in AS and CS, and showed no statistical significance in the latent stage, compared to the control groups ($^{\#}p < 0.05$, MTLE vs control; $^{*}p > 0.05$, LC vs LS).

The UPS is made up of six components: ubiquitin (Ub), the Ub-activating enzyme (E1), a group of Ub-conjugating enzymes (E2s), a larger group of Ub ligases (E3), the proteasome and the deubiquitinases (DUBs) [7]. During conventional ubiquitination, Ub is covalently conjugated to any target protein. Besides regulating the target protein turnover or clearance by the proteasomes, ubiquitination of some proteins regulates non-proteolytic processes such as endocytosis, protein localization/targeting, complex assembly and regulation of the duration and intensity of signaling by effector molecules. In our previous study [1,8], we found that ubiquitin was differentially expressed in the hippocampus of MTLE rats, suggesting that the UPS affects the pathogenesis of MTLE.

Nedd4-2 is an E3 ubiquitin ligase previously shown to regulate ion transport by controlling cellular trafficking/endocytosis and lysosomal degradation of ion channels and transporters [9]. It was recently shown to decrease in rat dorsal root ganglion in the spared nerve injury model of traumatic nerve injury-induced neuropathic pain [10]. NEDD4-2 can negatively regulate the epithelial Na^{+} channel (ENaC) and voltage-gated sodium channels (Navs) [11]. As we know, the electrical excitability of neurons is mediated primarily

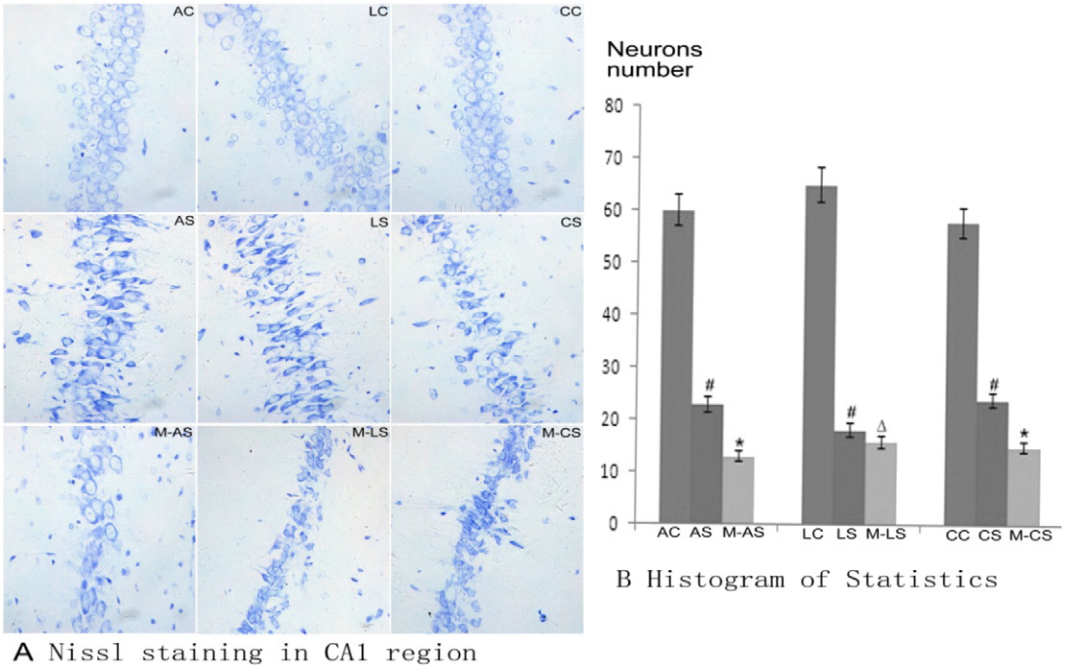

Fig. 2. Cytoarchitecture visualization and neuron density in the CA1 region. A: Nissl staining in the CA1 region. AS, LS, and CS show the neurons in the hippocampal regions of MTLE rats in the acute, latent and chronic stages. M-AS, M-LS, and M-CS show the same areas from MTLE rats after MG-132 pre-treatment. AC, LC, and CC show the same areas from control rats. B: The histogram shows neuron density in the CA1 region at different stages. Neuron loss was significant in MTLE rats compared with control rats. In the acute and chronic stages of MTLE rats pre-treated with MG-132, neuron loss and decreased nissl bodies were more obvious compared with MTLE rats. In the latent stage, no statistical differences were observed between MTLE rats and MG-132 pre-treated MTLE rats ($^{\#}p < 0.05$, MTLE vs control; $^{*}p < 0.05$, MTLE vs MG-132 pre-treatment MTLE; $^{\Delta}p > 0.05$, LS vs M-LS).