As a tumor suppressor, WWOX is a recently defined risk factor for Alzheimer's disease (AD). WWOX limits AD progression due, in part, to its suppression of tau tangle formation by direct binding to tau and beta-amyloid genes. Deficiency of WWOX leads to severe neurodegenerative, including epileptic encephalopathy, microcephaly, retinal dystrophy, severe psychomotor delay and intractable epileptic seizures. Recent evidence reveals that the p54-WWOX is shown to accumulate in the brain hippocampus and cortex. Suppression of p54-WWOX by a short synthetic peptide Zfra (zinc finger-like protein that regulates apoptosis) leads to up-regulation of tau tangle and amyloid plaques, and restoration of memory loss in triple transgenic mice for AD. Here, we determined that when Wwox wild type and heterozygous mice were pre-injected with a Zfra–4–10 peptide via tail veins, these mice resisted pentylenetetrazol (PTZ)-induced seizure. Mechanistically, Zfra significantly suppressed p54-WWOX in the auditory cortex and hippocampal CA1, CA3, or DG areas. Zfra blocked PTZ-induced activation of inflammatory microglia and astrocytes in the mouse hippocampus. Also, Zfra significantly blocked the expression of RE1-silencing transcription factor (REST) (>90%), which is a regulator of ion channels and neurotransmitter receptors associated with epilepsy. Together, our observations suggest that Zfra peptide is a potential agent in mitigating epileptic seizure caused by WWOX deficiency.

**Abstract**

As a tumor suppressor, WWOX is a recently defined risk factor for Alzheimer’s disease (AD). WWOX limits AD progression due, in part, to its suppression of tau tangle formation by direct binding to tau and beta-amyloid genes. Deficiency of WWOX leads to severe neurodegenerative, including epileptic encephalopathy, microcephaly, retinal dystrophy, severe psychomotor delay and intractable epileptic seizures. Recent evidence reveals that the p54-WWOX is shown to accumulate in the brain hippocampus and cortex. Suppression of p54-WWOX by a short synthetic peptide Zfra (zinc finger-like protein that regulates apoptosis) leads to up-regulation of tau tangle and amyloid plaques, and restoration of memory loss in triple transgenic mice for AD. Here, we determined that when Wwox wild type and heterozygous mice were pre-injected with a Zfra–4–10 peptide via tail veins, these mice resisted pentylenetetrazol (PTZ)-induced seizure. Mechanistically, Zfra significantly suppressed p54-WWOX in the auditory cortex and hippocampal CA1, CA3, or DG areas. Zfra blocked PTZ-induced activation of inflammatory microglia and astrocytes in the mouse hippocampus. Also, Zfra significantly blocked the expression of RE1-silencing transcription factor (REST) (>90%), which is a regulator of ion channels and neurotransmitter receptors associated with epilepsy. Together, our observations suggest that Zfra peptide is a potential agent in mitigating epileptic seizure caused by WWOX deficiency.

**Introduction**

**Results**

**Summary**

These results suggest that Zfra may play a pivotal role in mitigating WWOX-regulated epileptic seizure. Future directions for progression of WWOX-regulated epileptic seizure treatment will be better understood.

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**Figure 1** Clinical and genetic characterization of the consanguineous Egyptian family. Evaluated members of a consanguineous Egyptian affected by a novel syndrome with nervous growth retardation, microcephaly, retinal dystrophy, severe psychomotor delay and intractable epileptic seizures. (Modified from Abdel Salam et al. Orphanage Journal of Rare Diseases 2014, 9:12)

**Figure 2** | GABA Transporter: Effects on Synaptic Efficacy. Inhibitory synapse. The presynaptic neuron releases a neurotransmitter, which activates receptors on the postsynaptic cell. GABA binding to its postsynaptic GABAA receptors allows influx of Cl ions, which hyperpolarizes the postsynaptic neuron.

**Figure 3** | Function and structure of pentylenetetrazol (PTZ). Pentylenetetrazol (PTZ) is a tetrazol derivative (Storne, 1970) that has been shown to have convulsant action in mice, rats, cats, and primates, presumably by impairing GABA-mediated inhibition by an action at the GABA receptor (Olson, 1981; Ramanajunyulu and Tokui, 1984).

**Figure 4** | Zfra reduces resistance to PTZ-induced seizure in mice. (A) Mice pretreated with Zfra, followed by treating with PTZ, exhibited resistance to seizure. Zfra wild type and heterozygous mice were received three consecutive weekly injections of Zfra–4–10 solution (2 mM in phosphate buffered saline [PBS], 100 mL each injection) from the tail veins. One month later, animals were injected with a single dose of pentylenetetrazol (PTZ) (10 mg/kg) for acute seizure. (B) Zfra reduced seizure scores following PTZ induction. The differences were assessed for statistical significance by student’s test with p value (0.005, *p < 0.01, **p < 0.001).

**Figure 5** | Effect of Zfra on cytoarchitecture in parietal area and hippocampal CA3 of mice with epileptic seizure (Nissi staining). (A) Heterozygous mice developed neuronal heterotopia in parietal area. Aberrant neuronal migration to the allocortex was shown in the heterozygous mice. (B) Hippocampal CA3 neurons were loose and absent; the cytoplasm was stained in different shades; nuclei were missing or indistinct (arrow).

**Figure 6** | Zfra alleviated the expression levels of p64-kilobase in the auditory cortex and hippocampal CA1, CA3 or DG area. Additional evidence reveals that the p54-WWOX is involved in facilitating neuroregeneration.

**Figure 7** | Zfra suppressed the expression of inflammatory microglia cells and astrocytes. Zfra reduced PTZ-induced activation of astrocytes (GFAP positive) and microglia (IBA1 positive) in hippocampus in Zfra wild type and heterozygous mice.

**Figure 8** | Zfra suppressed the expression of REST. (A) Mechanistic roles of the REST in epileptogenesis. (B) Gene expression correlation analysis from TCGA and GTEX expression data. (C) Immunohistochemistry staining in cortex regions and hippocampus in Zfra wild type and heterozygous mice. (D) REST negatively regulates the expression of many neuronal genes, including type A GABA (GABAA) receptor (G3 subunit [GABRB3]), glutamate receptor (GRIA2).