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Editorial

Downregulation of WWOX and Inhibitory GABAergic Interneurons Correlates with Brain Inflammation During Progression of Alzheimer's Disease

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Abstract

WWOX gene is one of the risk factors for Alzheimer's disease (AD). Two modes of WWOX action is suggested to account for its association with AD. First, downregulation of WWOX protein in the hippocampus in middle ages may initiate slow aggregation of a protein cascade that ultimately results in increased accumulation of extracellular amyloid beta plaques and intracellular tau tangles, along with reduction of inhibitory GABAergic interneurons, in the brain of patients greater than 70 years old. Second, when pro-apoptotic pY33-WWOX is converted to pS14-WWOX, pS14-WWOX promotes neuronal degeneration as evidenced by the increased accumulation of the Ser14-phosphorylated form in the brain lesions. Suppression of Ser14 phosphorylation by a small peptide Zfra leads to enhanced protein degradation, reduction in NF-kappaB -mediated inflammation, and restoration of memory loss in triple transgenic mice for AD. In light of the promising findings, Zfra peptide is now on the road to clinical trials.

Keywords: *WWOX; Neurodegeneration; Alzheimer's Disease; Risk Factor; Tumor Suppressor*

Potential role of WWOX and its global interacting networks in preventing neurodegeneration

On February 28, 2019, NIH announced 5 newly discovered risk factors for Alzheimer's disease (AD). The announcement is based on large genome-wide association meta-analysis of thousands of human genes (94,437 individuals) [1]. One of the new risk genes for AD is WWOX. WWOX was first discovered in 2000 [2] and its association with AD was shown in 2004 [3]. WWOX directly binds Tau via its *C*-terminal SDR (short-chain alcohol dehydrogenase/reductase) domain [3,4]. The SDR domain also physically binds GSK3β to suppress hyperphosphorylation of Tau [3,4]. Also, the first WW domain of WWOX binds JNK and ERK, thereby preventing Tau hyperphosphorylation [3,4]. Tau protein supports polymerization of tubulin monomers to assemble microtubules, which are needed for neurite outgrowth [5].

Many nice review articles have comprehensively addressed the issue of WWOX in cancer and neurodegeneration [2,4-7]. In brief, WW-domain containing oxidoreductase, known as WWOX, FOR and WOX1, was first identified as a tumor suppressor [1]. However, in a mouse *Wwox* gene knockout model, WWOX protein participates in numerous physiological events, rather than acts simply as a tumor suppressor. In newborns, genetic deficiency of *WWOX* gene may lead to epilepsy, mental retardation, growth retardation, neurodegeneration, metabolic disorders, and early death [7-10]. Clinically, known disorders due to WWOX deficiency include disorder of sex differentiation (DSD), spinocerebellar ataxia (SCA), early infantile epileptic encephalopathy (EIEE), and *WWOX*-related epileptic encephalopathy (WOREE syndrome) [3,7-11]. No drugs have been developed to cure the diseases thus far.

WWOX binds numerous intracellular proteins in the brain, liver, lung, small intestine and many other organs *In vivo* [12]. When the strength of the binding of WWOX with partner proteins is increased *In vivo*, mice become resistant to cancer growth [12]. That is, reduced binding of WWOX with target proteins enhances cancer progression [12]. Supporting evidence shows that the reduced binding may link to the development of neurodegeneration. For example, as a binding partner, p53 works together with WWOX in inducing apoptosis [2,4].

Nonetheless, binding of p53 with WWOX may indeed result in functional antagonism between these two proteins *In vivo* [13]. p53 is pro-inflammatory and WWOX anti-inflammatory [13]. p53 blocks WWOX-mediated inhibition of inflammatory immune response induced by cancer [13]. Under this condition, proteins associated with AD become aggregated in brain of tumor-growing mice [13].

Reduced GAB Gergic inhibitory interneurons under WWOX deficiency

Loss of WWOX in human and mice results in increased activation of GSK-3β and upregulation of inflammatory microglia cells and astrocytes in the brain cortex and hippocampus of *Wwox* knockout mice [6,7]. In contrast, GAB Gergic inhibitory interneurons are significantly reduced. The observations suggest occurrence of chronic inflammation in the brain and resulting memory loss in mice [6,7]. Loss of GAB Gergic inhibitory interneurons leads to increased epileptic seizures and impaired memory capability, and that exogenous neuronal progenitor cells may restore the memory loss and suppress seizures [14]. Notably, when WWOX protein is significantly downregulated or is dysfunctional, excessive protein aggregation, including TRAPPC6AΔ, TIAF1 and SH3GLB2, occurs in the brain cortex and hippocampus that ends up with neuronal degeneration [15-19]. Consequently, *Wwox* knockout mice die in 3 to 4 weeks after birth. In humans, WWOX-deficient patients live up to 2 - 3 years and even longer. A human WWOX deficiency registry has recently been established in WWOX Foundation and WWOX Gene Mutation Support Group [https://www.wwox.org].

Zfra suppression of Ser14 phosphorylation in WWOX and restoration of memory loss

When WWOX is phosphorylated at Tyr33, pY33-WWOX plays an important role in the homeostasis of mitochondria [2,20]. When overly expressed *In vivo* and *In vitro*, pY33-WWOX mediates apoptosis and tumor suppression [2-5]. A portion of pY33-WWOX is localized in the cell membrane [21]. In response to TGF-beta1 or hyaluronan, pY33-WWOX physically binds Hyal-2 and Smad4 to signal for cell survival or death from the nucleus [21]. Another survival signaling is IκBα/WWOX/ERK, in which WWOX phosphorylation is at Ser14 [22,23]. This signaling is needed for forced differentiation of leukemia T cells. Whether neuronal differentiation is involved in the IκBα/WWOX/ERK signaling is unknown. We have determined that during the progression of AD or cancer, pS14 -WWOX is significantly upregulated in the lesions of cancer and AD hippocampus and cortex [24,25]. Inhibition of WWOX phosphorylation at Ser14 by Zfra significantly abolishes cancer growth in mice [24], and restores memory loss in Alzheimer's disease triple-transgenic (3xTg) mice for AD [25].

Zfra-based prevention and therapy for AD

The full-length Zfra (zinc finger-like protein that regulates apoptosis) is only 31 amino acids in length [24,25]. Both synthetic full-length Zfra1-31 and truncated Zfra4-10 (RRSSSCK) peptides are potent in cancer suppression and restoring memory loss [24,25]. One of the mechanisms for memory restoration in mice is due to dramatic Zfra suppression of S14 phosphorylation in WWOX (> 90%) [12,24,25]. Mechanistically, Zfra restores memory deficits in 3xTg mice by blocking aggregation of TRAPPC6AΔ, SH3GLB2, tau and amyloid β, providing rapid clearance of protein aggregates, and suppressing inflammatory NF-κB activation [25].

For treatment, suppression of pS14-WWOX by micromolar levels of Zfra4-10 peptide significantly reduces cancer growth [24] and mitigates AD symptoms in mice [24,25]. The full-length Zfra peptide acts in a similar fashion. For prevention, mice pretreated with Zfra4-10 peptide confers resistance to cancer growth [24]. Whether Zfra prevents the age-related generation of AD-like symptoms in 3xTg mice is being established in our laboratory.

A novel action of Zfra is due to its activation of spleen non-T/non-B Z lymphocytes [12,24,25]. Both Zfra1-31 and Zfra4-10 peptides induce memory anticancer response by expanding Zfra-reactive spleen Z lymphocytes [12,24,25]. Activated Z cells confer cancer resistance in recipient mice [12,24,25]. Zfra binds the membrane Hyal-2, followed by recruiting WWOX and Smad4 for nuclear translocation and gene transcription. Without prior encountering with cancer antigens, Zfra-activated Z cells are able to recognize many cancer cell types and suppress their growth [12,24,25]. Naïve Z cells cannot attack and kill cancer cells. We believe that activated Z cells are able to block

AD progression. Taken together, Zfra1-31 and Zfra4-10 are of great potential in treating and preventing AD and cancer [24,25]. Currently, Zfra for preclinical testing is underway. Hopefully these peptides will be in clinical trials in the upcoming 2 to 3 years.

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Bibliography

1. Kunkle BW., *et al*. "Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing". *Nature Geneics* 51.3 (2019): 414-430.

2. Chang NS., *et al*. "WW domain-containing oxidoreductase: a candidate tumor suppressor". *Trends Molecular Medicine* 13.1 (2007): 12-22.

3. Sze CI., *et al*. "Down-regulation of WW domain-containing oxidoreductase induces tau phosphorylation *In vitro* a potential role in ALZHEIMER'S disease". *Journal of Biological Chemistry* 279.29 (2004): 30498-30506.

4. Liu CC., *et al*. "WWOX Phosphorylation, Signaling, and Role in Neurodegeneration". *Frontiers in Neuroscience* 12 (2018): 563.

5. Chen YA., *et al*. "WW domain-containing proteins YAP and TAZ in the hippo pathway as key regulators in stemness maintenance, tissue homeostasis, and tumorigenesis". *Frontiers in Oncology* 9 (2019): 60.

6. Chang HT., *et al*. "WW domain-containing oxidoreductase in neuronal injury and neurological diseases". *Oncotarget* 5.23 (2014): 11792-11799.

7. Aldaz CM., *et al*. "WWOX at the crossroads of cancer, metabolic syndrome related traits and CNS pathologies". *Biochimica et Biophysica Acta* 1846.1 (2014): 188-200.

8. Cheng YY., *et al*. "Wwox deficiency leads to neurodevelopmental and degenerative neuropathies and glycogen synthase kinase 3β-mediated epileptic seizure activity in mice". *Acta Neuropathologica Communication* 8.1 (2020): 1-16.

9. Hussain T., *et al*. "WWOX, the FRA16D gene: A target of and a contributor to genomic instability". *Genes Chromosomes Cancer* 58.5 (2019): 324-338.

10. Ehaideb SN., *et al*. "Novel homozygous mutation in the WWOX gene causes seizures and global developmental delay: Report and review". *Translational Neuroscience* 9 (2018): 203-208.

11. Elsaadany L., *et al*. "W44X mutation in the WWOX gene causes intractable seizures and developmental delay: a case report". *BMC Medical Genetics* 17.1 (2016): 1-6.

12. Su WP., *et al*. "Therapeutic Zfra4-10 or WWOX7-21 Peptide Induces Complex Formation of WWOX with Selective Protein Targets in Organs that Leads to Cancer Suppression and Spleen Cytotoxic Memory Z Cell Activation *In Vivo*". *Cancers (Basel)* 12.8 (2020): 2189.

13. Chou PY., *et al*. "A p53/TIAF1/WWOX triad exerts cancer suppression but may cause brain protein aggregation due to p53/WWOX functional antagonism". *Cell Communication and Signaling* 17.1 (2019): 76.

14. Upadhya D., *et al*. "Human induced pluripotent stem cell-derived MGE cell grafting after status epilepticus attenuates chronic epilepsy and comorbidities via synaptic integration". *Proceedings of the National Academy of Sciences of the United States of America* 116.1 (2019): 287-296.

15. Chang JY., *et al*. "Trafficking protein particle complex 6A delta (TRAPPC6AΔ) is an extracellular plaque-forming protein in the brain". *Oncotarget* 6.6 (2015): 3578.

16. Chang JY and Chang NS. "WWOX dysfunction induces sequential aggregation of TRAPPC6AΔ, TIAF1, tau and amyloid β, and causes apoptosis". *Cell Death Discovery* 1 (2015): 1-11.

17. Sze CI., *et al*. "A cascade of protein aggregation bombards mitochondria for neurodegeneration and apoptosis under WWOX deficiency". *Cell Death and Disease* 6.9 (2015): e1881.

18. Sacher M., *et al*. "TRAPPopathies: An emerging set of disorders linked to variations in the genes encoding transport protein particle (TRAPP)-associated proteins". *Traffic* 20.1 (2019): 5-26.

19. Lee MH., *et al*. "TGF-β induces TIAF1 self-aggregation via type II receptor-independent signaling that leads to generation of amyloid β plaques in Alzheimer's disease". *Cell Death Disease* 1.12 (2010): e110-e110.

20. Chang NS. "A potential role of p53 and WOX1 in mitochondrial apoptosis (review)". *International Journal of Molecular Medicine* 9.1 (2002): 19-24.

21. Hsu LJ., *et al*. "Hyaluronan activates Hyal-2/WWOX/Smad4 signaling and causes bubbling cell death when the signaling complex is overexpressed". *Oncotarget* 8.12 (2017): 19137-19155.

22. Huang SS., *et al*. "Role of WW Domain-containing Oxidoreductase WWOX in Driving T Cell Acute Lymphoblastic Leukemia Maturation". *Journal of Biological Chemistry* 291.33 (2016): 17319-17331.

23. Huang SS and Chang NS. "Phosphorylation/de-phosphorylation in specific sites of tumor suppressor WWOX and control of distinct biological events". *Experimental Biology Medicine (Maywood)* 243.2 (2018): 137-147.

24. Lee MH., *et al*. "Zfra activates memory Hyal-2+ CD3− CD19− spleen cells to block cancer growth, stemness, and metastasis *In vivo*". *Oncotarget* 6.6 (2015): 3737.

25. Lee MH., *et al*. "Zfra restores memory deficits in Alzheimer's disease triple-transgenic mice by blocking aggregation of TRAPPC6AΔ, SH3GLB2, tau, and amyloid β, and inflammatory NF-κB activation". *Alzheimer's and Dementia (N Y)* 3.2 (2017): 189-204.

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