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 GABAergic 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, GABAergic 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 GABAergic 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 TRAPPC6Δ, 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 overexpressed *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 IkBa/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 IkBa/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 (RRSSCK) 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 TRAPPC6Δ, SH3GLB2, tau and amyloid β, providing rapid clearance of protein aggregates, and suppressing inflammatory NF-kB 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 antitumor 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.

Acknowledgement

This research was supported to NS Chang by grants from the Ministry of Science and Technology, Taiwan, the National Health Research Institutes, Taiwan, and the Department of Defense, USA.

Bibliography

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


Volume 13 Issue 1 January 2021
©All rights reserved by Nan-Shan Chang.