Minireview

The *WWOX* gene in brain development and pathology

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Impact statement

WW domain-containing oxidoreductase encoded by the *WWOX* gene is a transcription regulator and a key player in a number of cellular and biological processes such as tumor suppression, cell proliferation, apoptosis induction, steroid metabolism, and central nervous system development. This review provides a comprehensive summary of currently known roles and discusses the importance of *WWOX* gene for CNS development and functioning.

Abstract

Shortly after its discovery in 2000, *WWOX* was hailed as a tumor suppressor gene. In subsequent years of research, this function was confirmed indisputably. Majority of tumors show high rate of loss of heterozygosity and decreased expression of *WWOX*. Nevertheless, over the years, the range of its known functions, at the cellular, organ and system levels, has expanded to include metabolism and endocrine system control and CNS differentiation and functioning. Despite of its function as a tumor suppressor gene, WWOX genetic alternations were found in a number of metabolic and neural diseases. A lack of WWOX protein as a consequence of germline mutations results in brain development disturbances and malfunctions.

Keywords: WWOX, WW domain-containing oxidoreductase, neurodegeneration, infantile encephalopathy, neurogenesis, neurodifferentiation, glioblastoma

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Introduction

The human WW domain-containing oxidoreductase gene locus is positioned on chromosome 16 in a common fragile site FRA16D (16q23.1-q23.2). The gene, spanning over 1.1 Mbp, is one of the longest in the human genome. The WWOX protein itself is highly conserved, with very similar orthologues being present in *Drosophila melanogaster*, and human and mouse WWOX proteins sharing 93.9% identity (Homologene DB). The encoded 414 aa protein belongs to a short-chain dehydrogenase/reductase family. Additionally, it possesses two WW interaction domains, which interact with proline-rich motifs of other proteins: the first WW domain recognizes the PPxY motif, whereas the second strengthens the resulting bond.[1,2] The observed role of WWOX in aerobic metabolism and ROS generation has been attributed to the presence of an SDR domain, which contains a catalytic site and has been found to bind NADPH.[3,4]

After cloning and identification, the *WWOX* gene was quickly classified as a tumor suppressor. Indeed, *WWOX* genomic alternations and expression changes have been shown in many types of cancer[5–7] with loss of heterozygosity (LOH) ranging from 70% in breast cancer to below 20% in glioblastoma.[7,8] The gene malfunctions present in various tumors are associated with progression, resistance to treatment, and worse prognosis for cancer patients, including those with breast, ovarian, or prostate cancer.[2,6,9–13] Rescue experiments with ectopic expression of *WWOX* in tumor cell lines restore a more physiological phenotype of breast cancer, ovarian cancer, or glioblastoma cells, among others.[14–19]

Notwithstanding, *WWOX* is not a classic tumor suppressor gene. First, it is not subject to Knudson's two-hit hypothesis: an inactivation of both gene alleles is rare but the loss of one of its alleles predisposes the bearing cells to cancerogenesis due to the insufficient amount of protein produced by the intact allele (haploinsufficiency). It is also impossible to functionally qualify *WWOX* as a single type of tumor suppressor, as its action is not limited to direct control of the cell cycle or genome integrity, but exerts a more global impact on cell that is determined by influence of WWOX on its partner proteins function.

Even 20 years since the discovery of the *WWOX* gene, its new functions are still being discovered at the cellular level, as well as in the development and functioning of the

individual organs and systems of the body. WWOX should be regarded as a global transcription modulator affecting a number of cellular signal transduction pathways.

WWOX can interact with a wide range of proteins through its WW domains, and over 200 potential interactions have been proposed.[2] Especially interesting is that WWOX binds a number of transcription factors, such as Jun, AP2gamma, NFkappaB, and ErbB4, and signaling pathway messengers like Dvl2, Gli1, SMAD3, SMAD4.[16,20–22] It has been found to regulate gene transcription by sequestering transcription factors in the cytoplasm, thus limiting their access to the cell nucleus and target genes,[23,24] and there is also one report indicating that WWOX may interact with transcription factor complex directly in nucleus.[25]

A recent study employing a tandem affinity purification-mass spectrometry (TAP-MS) allowed for identification of 216 WWOX-interacting proteins. Among them, beside the previously well-known binding partners, were key players in the functioning of endoplasmic reticulum (ER), Golgi apparatus, endosomes, lysosomes and protein transport, what suggests that WWOX might be responsible for proper functioning of perinuclear protein complexes participating in protein trafficking. Pathway analysis of identified WWOX binding proteins also confirmed its important role in metabolic pathways, setting it as one of the main regulators of glycolysis, fatty acid degradation, TCA cycle, *de novo* synthesis of lipids and amino acids.[26]

Up to date, disturbances in *WWOX* expression have been associated with cholesterol level disorders,[27,28] type 2 diabetes,[29] spinal-cerebellar ataxia and childhood encephalopathy,[30] and various types of cancer.[5,13,31–36]

WWOX has been found to play a crucial role in the differentiation of the skeletal system, mammary gland, and testicles.[37–40] Growing evidence suggests that it has also a considerable influence on the development of the central nervous system (CNS).[30] To date, WWOX has been shown to contribute to the signaling pathways regulating CNS development and neural differentiation such as WNT, Hedgehog, TGFβ, and Hippo.[16,20,21,41,42] Noteworthy, Dvl1 and Dvl2, core components of WNT pathway, were amongst the highest confidence protein interactors identified in TAP-MS WWOX protein interactome study.[26]

The aim of this review is to summarize the current knowledge on the functions of *WWOX* gene in the central nervous system (Figure 1). It provides a brief overview of current findings on its physiological role in brain development and pathology.

Encephalopathy

It has been observed that congenital mutations of the *WWOX* gene resulting in loss of WWOX protein functionality cause severe neurological defects.[43,44] The severity of neuropathological symptoms varies, depending on whether the mutation deprives the protein of some of its functionality or completely prevents its formation. All known patients harboring such biallelic mutation of *WWOX* suffered from epilepsy. Two neuropathological phenotypes of *WWOX* gene mutation have been distinguished.

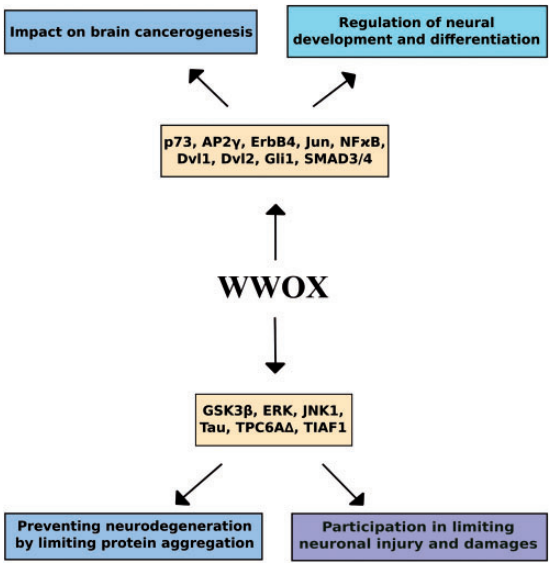

Figure 1. Pleiotropic role of WWOX in the development and function of the central nervous system, with an indication of interactions with known protein partners. (A color version of this figure is available in the online journal.)

The first form is characterized by the presence of hypomorphic sense mutations in the *WWOX* gene exon, causing partial loss of function of the encoded protein, and resulting in the development of SCAR12 spinocerebellar ataxia-12 (MIM 614322). The condition manifests as cerebellar ataxia, tonic-clonic epilepsy, intellectual impairment, and significant spasticity.[30,45] In the second form, the presence of premature STOP codons in two alleles results in a complete lack of WWOX expression and the development of WOREE WWOX-related epileptic encephalopathy (MIM 616211). This syndrome is characterized by severe developmental delay, intense and drug-resistant epilepsy with variable attack types (tonic, clonic, tonic-clonic, myoclonic, etc.), retinal and optic nerve dystrophy, corpus callosum hypoplasia, and premature death: life expectancy is about three years. The onset of epilepsy is earlier in WOREE (three months) than in SCAR12 syndrome.[30,44]

A detailed review of clinical and molecular data in patients with WWOX-related encephalopathies has recently been published by Serin *et al.*[30]

The molecular background of the phenotypes observed in *WWOX*-depleted individuals and rodent models is currently the subject of intense research. So far it is known that the presence of incorrect WWOX protein levels results in myelination retardation, as well as disorders in neurotransmitter management.[46,47]

A recent study on lde/lde rats, a model of WOREE syndrome, found defects in CNS development that concern both dendrite outgrowth and oligodendrocyte myelination.[47]

Studies on a *WWOX* knockout mouse model found the number of hippocampal GABA-ergic interneurons to be reduced accompanied by reduced GABA synthesis and the appearance of neuroinflammation markers.[46] This finding was confirmed in our previous research on human neural progenitor cells (hNPC), in which major changes in the transcription of genes related to neurotransmitter synthesis and management were observed after *WWOX*

silencing. WWOX also demonstrated a profound influence on adhesion, cytoskeleton organization, and cellular signaling in hNPC, thus probably contributing to proper neurodifferentiation and neuron migration. Consistently, neuronal progenitor cells with silenced *WWOX* showed enhanced adhesion to extracellular matrix proteins, down-regulation of MMP2/9 expression, and impaired 3D growth.[48] Recently, it has been reported that *WWOX* knock out in mice indeed cause a disruption of neuronal migration in developing cerebral cortex, hippocampus, and cerebellum. Neuronal migration disorders in KO mice result in foliation impairment and brain malformation. Furthermore, Purkinje cell loss and granular cell apoptosis in the cerebellum and severe hypomyelination were found in the examined animals.[49]

Neurodegeneration

All known neurodegenerative diseases are chronic and progressive and all demonstrate the hallmarks of protein aggregates, inclusion bodies, and tangled fibrous proteins located in the neurons, glial cells, and brain matrix.[50,51] Alzheimer's disease, the most common cause of a dementia, is distinguished by the presence of extracellular senile plaques made of fibrillar beta amyloid (Aβ) and intracellular neurofibrillary tangles (NFTs) made of aggregated Tau protein, the presence of which results in progressive loss of neurons and synaptic connections.[52,53] Neuronal death and blockade of neurogenesis are manifested as progressive learning and memory decline. WWOX is believed to influence the progress of Alzheimer's disease by modulating the activity of the GSK3β, EKR, JNK kinases responsible for the hyperphosphorylation of Tau protein.[54–57] The hyperphosphorylated Tau molecules aggregate and form NTFs, thus restricting neurite outgrowth and preventing neuronal differentiation.[58] In addition, WWOX can also physically bind Tau protein by its SDR domain and stabilize it.[54] This regulation of Tau phosphorylation is important not only in the context of neurodegeneration, but also neuronal differentiation, where the Tau protein takes part in microtubule assembly and neurite outgrowth. It has been hypothesized that WWOX may act as a chaperone that stabilizes Tau from misfolding and aggregation.[59] Furthermore, WWOX deficiency, or its loss of function, has been found to lead to the activation of a protein aggregation cascade. This cascade is led by TPC6AΔ and TIAF1, whose aggregation initiates caspase activation and contributes to amyloid precursor protein (APP) degradation, causing the formation of Aβ. It has been found that WWOX prevents TPC6AΔ and TIAF1 aggregation by physically interacting with them.[58] *In vitro* stimulation with TGFβ results in the dissociation of WWOX from the complex and the initiation of the protein aggregation cascade. TPC6AΔ and Tau plaques were found in the brain cortex of WWOX knockout mice as early as in the first three weeks of life, and WWOX-depleted mouse embryonic fibroblasts (MEF) contain aggregates of TPC6AΔ, TIAF1, JNK1, and Tau tangles.[59]

In addition, heterozygous $WWOX^{+/-}$ animals have been found to demonstrate a higher rate of memory decline,[60] and the level of *WWOX* expression is strongly down-regulated in the hippocampi of AD patients.[54] These findings strongly suggest that WWOX protein acts as an important agent protecting from neurodegeneration.

WWOX may also play a role in the pathogenesis of Parkinson's disease. It has been found that treatment with dopaminergic neurotoxin 1-methyl-4-phenyl-pyridinium (MPP+) induces Parkinson-like symptoms in rats. The damaged neurons display elevated levels of WWOX protein, with the protein itself being phosphorylated at Tyr33. WWOX in this activated form has been found to induce apoptosis in the SK-N-SH neuroblastoma cell line. It appears that the phosphorylated form of WWOX plays an important role in the course of neuronal damage in PD and that its proapoptotic function may be blocked by JNK1.[61]

Chang *et al.*[62] suggested that WWOX may function as a potential cytosolic or membrane receptor of steroid hormones. The SDR domain of the protein contains a NSYK motif capable of binding to estrogen and androgen. Depending on the choice of investigated cell line, estrogen and androgen are able to induce WWOX phosphorylation, at Tyr33, its translocation to the nucleus and apoptosis induction.

Brain cancers

Due to the early recognition of its effects on tumor development, a number of studies have examined the relationship between WWOX and carcinogenesis. It has since been shown to regulate such cellular aspects as proliferation, adhesion, motility, DNA repair, and aerobic metabolism.

Considering that *WWOX* is located in FRA16D (a genomic instability hot spot prone to breakage causing germline and somatic copy number variations),[63] this gene is a frequent target for deletions in cancer. Esophageal,[64] colon,[65] stomach,[66] bladder,[35] and uterine cancers[34] are commonly affected by WWOX deep focal deletions, whose occurrence significantly correlates with various pathological and clinical features.[67] Despite the knowledge of *WWOX* gene function in many cancers, data on brain tumors are limited. Although most studies on *WWOX* in brain tumorigenesis concern glioblastoma, its involvement has also been confirmed in other types of brain cancer.

Astrocytomas

One of the most common CNS neoplasms is astrocytomas, in which the predominant cell type is derived from astrocytes, i.e. star-shaped glia cells.[68] The WHO (2016) classifies astrocytomas into four histopathological grades: Grade I tumors are slow growing and considered as benign, they allow for long-term survival; Grade II are usually slow growing, although they can progress to more malignant forms; Grade III includes anaplastic astrocytoma; Grade IV, glioblastoma, is the most malignant form.[69]

Recent research has sought to broaden our understanding of the relationship between the *WWOX* gene and the development of astrocytoma. Its expression was determined in astrocytoma tumor samples of various grades from 38 patients by immunohistochemical staining. Interestingly, in all cases, *WWOX* gene expression was reduced by a high

(50% of cases), moderate (36.8% of cases), or mild (13.2% of cases) degree compared to normal controls. Furthermore, an inverse correlation was observed between patient survival and loss of *WWOX* gene expression, which was also related to supra-tentorial localization, age, or severity of symptoms. This lack of association between tumor grade and loss of *WWOX* gene expression, i.e. equal loss of expression was observed in both GII and GIII, may indicate that it is involved in the early stages of astrocytoma progression.[70]

Glioblastomas

The most progressive and malignant form of brain tumor is glioblastoma (GBM), which is classified as a grade IV astrocytoma.[71]

Clinically, primary GBMs arise *de novo*, while secondary form progresses and transforms from lower grade astrocytomas.[72]

Histologically, the subtypes cannot be distinguished.[73] The high heterogeneity of the cell population, the presence of cancer stem cells, and their strong infiltration potential entail enormous difficulties in the development of effective therapy and result in severely limited survival by GBM patients.[74–76]

A study of tumor samples obtained from 67 GBM patients revealed that the observed decrease in *WWOX* expression might be caused by a combination of loss of heterozygosity and promoter methylation. What is more, *WWOX* expression level has been found to positively correlate with *Bcl2* and *Ki67* level and to have a significant influence on the ErbB4 signaling pathway.[7]

U373MG cells with mutated *p53* have been found to enter apoptosis in a manner independent of mitochondria and caspase 3 level following *WWOX* overexpression. No such phenomenon was observed in U87MG cells expressing wild-type *p53*. It has been proposed that the interaction between the gain-of-function of p53 and WWOX may be relevant for glioblastoma cell survival.[77]

Furthermore, overexpression of the *WWOX* gene in glioblastoma cells is associated with increased radiosensitivity. Interestingly, this effect only occurs on lines with fully functional p53.[78]

The level of WWOX expression is thought to be regulated by the circular RNA CircMTO1: CircMTO1 upregulated WWOX production inhibits proliferation in the U251 glioblastoma cell line.[78]

It has also been found that glioblastoma cells demonstrating low levels of *WWOX* expression show greater capacity for invasive migration.[79]

Cancer cells often demonstrate a phenomenon called the Warburg effect, in which glycolysis takes place despite the availability of oxygen.[80] The WWOX protein has been found to be an important regulator of oxygen metabolism and oxidative stress in Drosophila, which probably takes place via interaction with isocitrate dehydrogenase (Idh) and Cu-Zn superoxide dismutase. It has been suggested that WWOX may inhibit the growth and invasion of cancer cells, regardless of their high glucose consumption, by inducing ROS production.[79] Hypoxia-inducible transcription factor (HIF1α) is a master transactivator of glycolytic genes encoding glucose transporter GLUT1, hexokinase, lactate dehydrogenase, pyruvate dehydrogenase kinase 1, among others. WWOX has been found to physically interact with HIF1α destabilizing this protein and inhibiting its transcriptional activity against aerobic glycolytic genes under physiological conditions. WWOX inhibits aerobic glycolysis and potentiate mitochondrial TCA cycle. Thus, WWOX deficiency may contribute to Warburg effect directly by stabilization of HIF1α transcription factor and, in consequence, increase of glucose intake and glycolysis while inhibiting TCA cycle.[81,82]

Studies of a T98G cell line with exogenously increased *WWOX* expression identified a reduction of cell malignancy. The transcriptome analysis revealed that increasing WWOX levels modified the expression of nearly 3000 genes. Of these, the Wnt, TGFβ, Notch, and Hedgehog pathways were highly modulated. Cells with increased WWOX showed significantly reduced proliferation and reduced adhesion to ECM proteins. Their ability to create 3D cultures was also impaired.[19]

Neuroblastomas

Neuroblastoma is the most frequent extracranial tumor found in children. It originates from the autonomic nervous system.[83] The tumor typically locates itself in the adrenal medulla, but can also be found in the chest, neck, and pelvis.[84] The incidence of neuroblastoma is rare in adults, with most cases concerning patients below the age of 10.[85]

A study of neuroblastoma tissue samples showed downregulation of *WWOX* gene expression in poorly differentiated samples as well as those classified as having

Table 1. The role of *WWOX* gene in various types of brain tumors.

Tumor	Findings	References
Glioblastoma	- *WWOX* is downregulated in GBM - LOH and hypermethylation of *WWOX* promoter - Ectopic *WWOX* is capable of inducing apoptosis in p53-mutant GBM cell lines - WWOX influences adhesion, proliferation rate, and 3D growth of T98G GBM cell line	7,75,85,86
Astrocytoma	- Highly reduced WWOX protein expression in comparison to the controls - Decreased WWOX protein expression associated with clinical variables such as patient age, supra-tentorial localization of the tumor, and severity of the symptoms	68
Neuroblastoma	- LOH in *WWOX* locus - Correlation of *WWOX* expression decrease with worse clinical status - Potential *WWOX* role in neuroblastoma cell differentiation	84

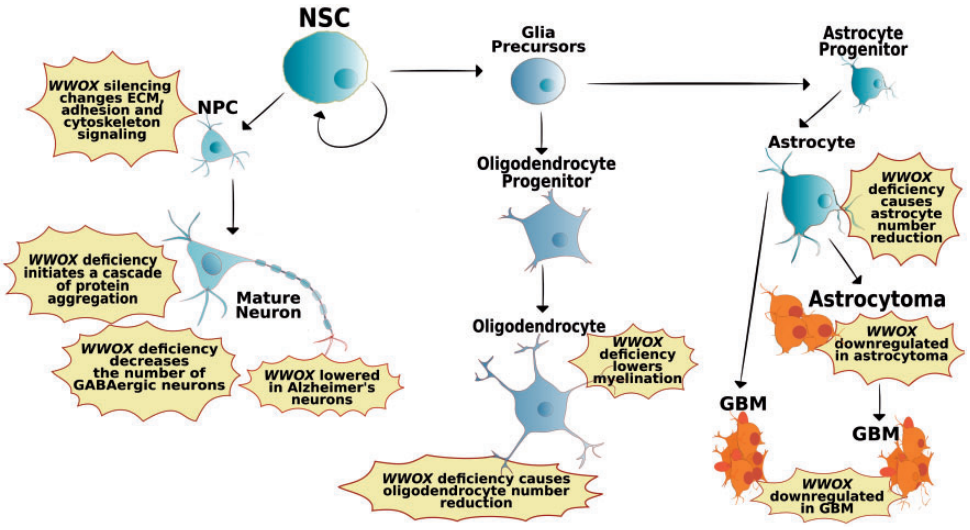

Figure 2. The *WWOX* involvement in neuronal cells differentiation and maintenance. (A color version of this figure is available in the online journal.)

an unfavorable prognosis.[86] The results suggest that WWOX protein might be involved in neuroblastoma cell differentiation.

Current findings related to *WWOX* participation in brain carcinogenesis are summarized in Table 1.

Conclusion

Molecular role of WWOX depends on WW domains, binding number of partner proteins including signal messengers and transcription factors. Therefore, WWOX deficiency effect is associated with distractions in a modulation of partner proteins function. Those WWOX partners are involved in different metabolic pathways and that may differentiate WWOX function depending on tissue type and metabolic stage. There is a large discrepancy between *WWOX* somatic and germline genomic alternations. Somatic *WWOX* aberrations are associated with tumorigenesis and tumor progression. Though loss of heterozygosity in brain tumors is not as high as in breast cancer, it still contributes in 20% of gliomas. On the other hand, germline mutations are observed very rare and in great majority may be lethal in embryonic development. The limited number of reported WWOX germline mutations in humans shows no increased rate of cancer but are associated with severe developmental disorders. In the CNS context, the *WWOX* gene has been found to be pleiotropic, playing a pivotal role in embryonic neural development,[87] neuronal injury, and damage,[61,88,89] and in preventing neurodegeneration by limiting pathological protein aggregation.[59] Germline genetic alternations in *WWOX* may be responsible for several developmental disorders and brain malfunctions, while somatic alternations and transcription deficiency contribute to brain cancerogenesis based on a tumor suppressor gene silencing model (summary in Figure 2).

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DECLARATION OF CONFLICTING INTERESTS

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