Role of WW domain proteins WWOX in development, prognosis, and treatment response of glioma

Shin-Yi Liu¹, Ming-Fu Chiang^{2,3} and Yu-Jen Chen^{4,5}

¹Department of Medical Research, Mackay Memorial Hospital, Taipei 104, Taiwan; ²Department of Neurosurgery, Mackay Memorial Hospital, Taipei 104, Taiwan; ³Graduate Institute of Injury Prevention and Control, Taipei Medical University, Taipei 110, Taiwan;

⁴Department of Radiation Oncology, Mackay Memorial Hospital, Taipei 104, Taiwan; ⁵Graduate Institute of Pharmacology, Taipei Medical University, Taipei 110, Taiwan

Corresponding authors: Ming-Fu Chiang and Yu-Jen Chen. Emails: chiang66@gmail.com, chenmdphd@gmail.com

Abstract

Glioblastoma multiforme (GBM) is the most aggressive and malignant brain tumor. Delicate microenvironment and lineage heterogeneity of GBM cells including infiltration, hypoxia, angiogenesis, and stemness make them highly resistant to current conventional therapies, with an average life expectancy for GBM patients of less than 15 months. Poor response to cytotoxic agents of GBM cells remains the major challenge of GBM treatment. Resistance of GBM to clinical treatment is a result of genomic alteration and deregulated signaling pathways, such as p53 mutation and apoptosis signaling blockage, providing cancer cells more opportunities for survival rather than cell death. WW domain-containing oxidoreductase (WWOX) is a tumor suppressor gene, commonly downregulated in various types of tumors, including GBM. It has been found that the reintroduction of WWOX induced p53-mutant GBM cells to undergo apoptosis, but not in p53 wild-type GBM cells, indicating WWOX is likely to reopen apoptosis pathways in a p53-independent manner in GBM. Identifying the crucial target modulated by WWOX deficiency provides a potential therapeutic target for GBM treatment. Here, we have reviewed the literatures about the role of WWOX in development, signaling pathway, prognosis, and treatment response in malignant glioma.

Keywords: WW domain-containing oxidoreductase, glioma, p53, resistance

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Introduction

Glioblastoma multiforme (GBM) is the most progressive and malignant brain tumor. According to histological features and clinical outcomes, GBM is classified as a Grade IV central nervous system tumor by the World Health Organization.¹ As indicated in its name “multiforme,” GBM cells are characterized and composed of a heterogeneous cell population, which provides GBM with high degree of infiltration, angiogenesis, genomic instability, cancer stem cell production, and resistance to clinical therapies.^{2,3} Aggressive surgical resection and advanced radiation with chemotherapy have limited impact on the survival of GBM patients. The current standard treatment of GBM relies on the use of radiation combined with the alkylating agent temozolomide; however, the median life expectancy of GBM patients is still less than 15 months.^{4,5} The failure of a standard treatment for GBM is due to the incomplete eradication of invasive tissue by surgery and multiple aberrations of the signaling pathway in response to therapeutic agents. The deregulated signaling includes augmented survival pathways and defective apoptosis signaling machinery, allowing GBM cells to overcome

deleterious environments and increase opportunities of recurrence.^{6–9}

Hyperactive receptor tyrosine kinases in GBM, such as epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR), mediate survival signals via mitogen-activated protein kinase kinase (MEK) / extracellular-signal-regulated kinase (ERK) and phosphatidylinositol-3-kinase (PI3K) / v-Akt murine thymoma viral oncogene (Akt), whereas apoptosis resistance is associated with the upregulation of BCL-2 and BCL-XL and the downregulation of BAX in recurrent GBMs.^{10,11} The expression level of BCL-2 family proteins is controlled by several factors, including p53. Nuclear p53 promotes apoptosis via its transcriptional target, such as *BAX*, *BIM*, *PUMA*, and *NOXA*, and cytoplasmic p53 regulates mitochondrial membrane permeabilization, leading to the release of cytochrome c and the induction of apoptosis.¹² However, the *TP53* mutation is present in ~42% of GBM patients, and the p53 pathway alternation may be required for pathogenesis of GBM.¹³ Loss of function in p53 contributes to apoptosis resistance and increased malignancy in GBMs. Our previous study has demonstrated that the reintroduction of a tumor suppressor

protein, WWOX, induces apoptosis in U373MG cells harboring mutant p53 but not in U87MG cells expressing wild-type p53.¹⁴ This result indicates that WWOX is likely to play a crucial role in restoring apoptotic cell death abolished in GBM cells in a p53-independent manner.

WW domain-containing oxidoreductase

The WWOX gene is located on chromosome 16q.23.1, a common fragile site called FRA16D, frequently subjected to loss of heterozygosity (LOH) in breast cancers.¹⁵ Fragile sites are chromosomal regions susceptible to DNA instability with a high frequency of homozygous deletion and translocation when cells are exposed to intrinsic replication stress or external mutagens.¹⁶ Genome instability is a crucial step for malignant transformation and progression; thus, fragile sites are considered the principal targets for cancer development. The characterization of FRA16D by Bednarek *et al.* isolated a gene expanding within this region named WW domain-containing oxidoreductase (WWOX) or fragile site FRA16D oxidoreductase (FOR).¹⁵ Protein sequence analysis has found that WWOX/FOR is composed of two N-terminal WW domains and one short-chain dehydrogenase/reductase (SDR) domain at C-terminus¹⁷ (Figure 1). These two domains predict the function of WWOX in diverse signaling networks and in redox reactions, as WW and SDR motifs are responsible for protein-protein interaction and oxidoreductase activity, respectively. The WW domain contains conserved proline and tryptophan residues required by the specific proline-rich ligand (PPXY)^{18,19} for binding protein. Numerous reports have identified the target proteins of WWOX involved in different cellular pathway, including development, differentiation, apoptosis, and tumor suppression.^{20,21} In addition, the SDR motif can metabolize steroid hormones and other substrates, including alcohol, sugar, retinoid, and the keto- and hydroxyl-groups.²² There is a nuclear localization sequence between two WW domains and a mitochondrial targeting sequence within SDR domain (Figure 1). Although the localization of murine WWOX has been demonstrated in mitochondria,²³ Bednarek group provided another evidence showing that human WWOX resides in the Golgi apparatus.²⁴ This inconsistency in WWOX localization needs further clarification.

Figure 1 Human WWOX protein structure. Human WWOX gene encodes a 414 amino acid protein, which is composed of two WW domains and one SDR domain. A nuclear localization sequence (NLS) is located between the two WW domains, and a mitochondrial targeting sequence is mapping within the SDR domain. Phosphorylation of WWOX by Src at Tyr33 in the first WW domain enhances the binding to other proteins, and Ack1-mediated phosphorylation of WWOX at Tyr287 leads to polyubiquitination and degradation of WWOX. SDR domain: short-chain dehydrogenases/reductases domain

The WWOX protein is a 414-amino acid protein, encoded from a 1245 bp transcript composed of nine exons. The expression levels of WWOX are mostly reduced in various types of tumors, including breast, ovarian, prostate, gastric, lung, liver, colon, pancreatic, bladder, nasopharyngeal, renal, hematopoietic, squamous, meningioma, cholangiocarcinoma, osteosarcoma, and astrocytomas.²⁵ The expression of WWOX in neoplasms is silenced by the regulation of homozygous deletion, LOH, hypermethylation, ubiquitination, and microRNA. WWOX can be modified by activated Cdc42-associated tyrosine kinase 1 (Ack1) and E3 ubiquitin ligase (ITCH) through phosphorylation and polyubiquitination, respectively. The phosphorylation of WWOX by activated Ack1 at Tyr287 leads to the polyubiquitination and degradation of WWOX, while polyubiquitinated WWOX by ITCH triggers nuclear translocation and induced cell death.^{19,26} Activated Ack1 in prostate cancer cells enhances cell growth and tumorigenesis, and primary prostate cancer cells show higher activated Ack1 and lower WWOX expression. Moreover, WWOX can be targeted by microRNA-134, which is highly expressed in head and neck squamous cell carcinomas and is associated with metastasis and mortality.²⁷

WWOX in development and neoplasm

In normal tissues, WWOX is highly expressed in epithelial or hormonally regulated tissues, such as the mammary glands, testes, ovaries, and prostate, implying its regulation of hormone metabolism.^{15,28} During normal keratinocyte differentiation, phosphorylated WWOX has been found.²⁹ In murine fetuses, WWOX is present in the brainstem, spinal cord, and peripheral nerve bundles, but decreases expression after birth.³⁰ In addition, WWOX is expressed in neurons, ependymal cells, and astrocytes.³¹ These data indicate that the expression of WWOX is required for the development of gonads, keratinocytes, and neurons. In human family, it has been found that germline mutation in WWOX gene led to early onset neurodegenerative disease including epilepsy, mental retardation, cerebellar ataxia, and prominent spasticity.^{32,33} This mutation of WWOX at proline 47 abolishes the binding of WWOX to protein partners and the complete loss of WWOX function results in cerebellar dysfunction phenotypes reminiscent of the WWOX knock-out mice and *Ide* (lethal dwarfism and epilepsy) rat models.^{34–36} *Ide* rats present spontaneous mutation of WWOX and showed lethal dwarfism, ataxic gait, and audiogenic seizures. The *Ide* locus, close to the WWOX gene, shows a 13-bp deletion in exon 9 of the WWOX gene resulting in the frame shift mutation and undetectable expression of WWOX protein in the testes and hippocampi of *Ide* rats, implying that abnormal neuronal excitability in *Ide* rats may be due to a loss of function mutation of WWOX.

In cancer cells, WWOX is decreased or absent in most cases compared to a normal cell state, suggesting the functions of WWOX are hampered to permit the onset of malignant transformations. For GBM patients, a low expression of the WWOX protein is associated with the LOH and the promoter hypermethylation.³⁷ A reduced expression of the WWOX protein has been found in 50% (19/38) of astrocytomas and

correlates with patient age, tumor localization, symptoms severity, and shorter survival times.³⁸ The Polycomb group gene Bmi1, which allows gene silencing through modifications of chromatin organization,³⁹ is highly expressed in GBM and enriched in CD133-positive cells.^{40,41} WWOX is one of the target genes of Bmi1 and the expression of WWOX transcript is reduced in the presence of Bmi1 in small-cell lung cancer cells.⁴² The relationship between Bmi1 and WWOX level in GBM needs further clarification.

To validate the tumor-suppressor function of WWOX, several groups generated transgenic knockouts or heterozygous mice. Mice with WWOX knocked out develop spontaneous osteosarcomas, and the inactivation of one allele of WWOX results in lung papillary carcinomas.³⁴ The targeted deletion of WWOX in mice shows postnatal lethality, bone defects, impaired steroidogenesis, and gonadal abnormalities.⁴³ Other reports also support the findings that homozygous WWOX gene-trap mice have shorter lifespan, reduced fertility, and lymphoma formation.⁴⁴ The conditional inactivation of WWOX in the germline has shown postnatal lethality and decreased bone formation,⁴⁵ while defects have appeared with the conditional deletion of WWOX in mammary glands during mammary branching morphogenesis, but no increased premalignant lesions have been found.⁴⁶ Another conditional knockout mouse with the C3H mouse mammary tumor has found mammary carcinoma formation, suggesting the haploinsufficiency of WWOX is cancer predisposing.⁴⁷ These *in vivo* data indicate that the physiological functions of WWOX are involved in the development of bone, gonadal, and steroid-regulated tissues, and heterozygous WWOX mice are prone to trigger malignancies intrinsically or induced by carcinogens.

WWOX in cell signaling

To assess the signaling pathways involved by WWOX, several groups have manipulated the expression of WWOX and investigated the cellular behaviors in *in vitro* systems. For knockdown experiments, silencing WWOX in hepatocellular cancer cells increases proliferation and resistance to the JNK inhibitor.⁴⁸ The knockdown of WWOX in ovarian cancer cells increases adhesion to fibronectin, but only detached cells undergo apoptosis followed by a reduced expression of integrin- α .⁴⁹ The depletion of WWOX in breast cancer cells leads to resistance to tamoxifen and estrogen treatments, and enhanced cell survival associated with a dramatic increase of fibronectin.⁵⁰ Meanwhile, the knockdown of WWOX in normal breast cells affects cell proliferation, motility, and attachment.⁵¹ The knockdown of WWOX in cervical cancer cells promotes cell proliferation and inhibits apoptosis.⁵² On the other hand, overexpression experiments have been elucidated in breast,²⁴ lung,^{53–56} prostate,⁵⁷ pancreatic,⁵⁸ ovarian,^{49,59,60} hepatic,⁶¹ cervical,⁵² squamous,⁶² and gastric⁶³ cancer cells. Consistent results show the ectopic expression of WWOX inhibited cell growth and tumorigenicity, accompanied by the induction of cell cycle arrest and apoptosis in some cases. These observations conclude that a deficiency of WWOX leads to the increase in proliferation, adhesion,

survival, drug resistance, and tumorigenicity, along with a decrease in apoptosis.

Under stress stimuli, WWOX can be induced or activated by phosphorylation. Treatment with hyaluronidase induces the expression of murine WWOX and enhances tumor necrosis factor-induced cytotoxicity by the release of WWOX from the mitochondria to the nucleus.²³ In response to UV, anisomycin, etoposide, hypoxia, 17 β -estradiol (E2), and antrogen, murine WWOX are phosphorylated at Tyr33 and correlated with the progression of breast and prostate cancer.^{64,65} The exogenous complement C1q also activates WWOX phosphorylation and induces apoptosis after the ectopic expression of WWOX in human prostate cancer cells.⁶⁶ However, the opposite results show that treatment of UV or Benzo(a)pyrene diolepoxide downregulates WWOX expression followed by S-phase arrest.⁶⁷ Arsenite treatment also decreases WWOX level in uroepithelial cancer cells via ERK1/2 activation.⁶⁸ These data suggest WWOX is a stress-responsive gene, and activated WWOX by phosphorylation elevates the binding capacity of target proteins for tumor suppression.

Protein interactions with WWOX

To explore the detailed regulation of WWOX at the molecular level, many studies have looked for proteins interacting with WWOX. WWOX can bind to partners through WW domain-dependent or -independent mechanisms. WWOX can interact with partner proteins via its WW domain, including mothers against decapentaplegic homolog 3 (SMAD3),⁵¹ mammalian target of rapamycin (mTOR),⁶² activating enhancer binding protein 2 γ (AP-2 γ),⁶⁹ p73,⁷⁰ Δ Np63 α ,⁷¹ WBP1/2,⁷² SIMPLE,⁷² ezrin,⁷³ Dvl,⁷⁴ transmembrane protein 207 (TMEM207),⁷⁵ Gli1,⁷⁶ LMP2A,⁷⁷ ErbB4,⁷⁸ Zfra,⁷⁹ runt-related transcription factor 2 (RUNX2),⁸⁰ and MEK1.⁸¹ Proteins lacking a proline-rich motif, such as JNK1,⁸² p53,⁸³ Hyal-2,⁸⁴ cAMP response element-binding protein (CREB),⁸⁵ and c-Jun,⁸⁶ also interact with phosphorylated WWOX in WW domain-independent manner. Besides, Src-phosphorylated WWOX at Tyr33 further enhances interaction with the binding protein in a WW domain-dependent and WW domain-independent manner. Another exception, GSK3 β binds to WWOX through the SDR domain and the signaling of GSK3 β is suppressed by WWOX for Tau hyperphosphorylation, leading to microtubule stabilization and neurite outgrowth.⁸⁷

In most situations, WWOX executes antagonistic function through binding and sequestering the target proteins. Constriction of binding proteins by WWOX suppresses transcriptional ability of transcription factor or protein function, such as AP-2 γ , JNK1, p73, Δ Np63 α , Dvl, CREB, SMAD3, Gli1, ErbB4, c-Jun, Zfra, RUNX2, and GSK3 β . The formation of a WWOX/AP-2 γ complex suppresses the transactivation ability of AP-2 γ , resulting in growth inhibition in prostate cancer.⁶⁹ The association of WWOX and membrane ErbB4 correlates with a favorable outcome in breast cancer.⁸⁸ RUNX2 is the principal transcriptional regulator of the osteoblast differentiation and association of WWOX and RUNX2 suppresses its activity.⁸⁰ Quenching SMAD3 by WWOX explains the tumorigenic effect of TGF β signaling in breast cancer.⁵¹ Inhibiting WWOX activates Wnt/ β -catenin

signaling by dishevelled proteins (Dvl).⁷⁴ In damaged rat neurons, nuclear accumulation of WWOX, CREB, and NFκB correlated with apoptosis induction, indicating WWOX can suppress the neuroprotective function of CREB and enhance the apoptotic activity of NFκB.⁸⁵ Etoposide treatment activates WWOX and JNK simultaneously in human embryonic kidney HEK293 cells, suggesting WWOX is antagonistic to JNK.⁸⁹ WWOX blocks the tumorigenesis effect of viral oncoprotein Tax via recruiting IKKα for RelA phosphorylation.⁹⁰ Overexpression of Zfra sequesters WWOX, p53, p65, phosphorylated ERK in cytoplasm and counteracts the apoptotic function of WWOX.⁷⁹ Drosophila ortholog of WWOX is involved in aerobic metabolism and oxidative stress regulation, contributing to glycolysis being observed in cancer cells.⁹¹

In contrast, WWOX plays a synergistic role for p53, ezrin, Hya-2, TMEM207, mTOR, and LAMP2A proteins. Murine WWOX enhances p53-mediated apoptotic signaling after phosphorylation.⁸³ The translocation of WWOX into the nucleus is essential for p53-mediated apoptosis via the transactivation of p53, Bcl-2, and Bcl-XL through the SDR domain of WWOX. Transforming growth factor β (TGFβ) binds to hyaluronidase Hyal-2 and then recruits WWOX. The Hyal-2/WWOX complex translocates into the nucleus and triggers SMAD activation for cell death induction.⁸⁴ The interaction of WWOX and TMEM207, a protein expressed in gastric singlet-cell carcinoma cells, attenuates its function in cell invasion and metastasis.⁷⁵ In squamous cell carcinoma, methotrexate (MTX) treatment induces WWOX expression and mTOR phosphorylation in MTX-sensitive cells, contributing to autophagy inhibition and apoptosis activation.⁶² Latent membrane protein 2A, an oncoprotein of the Epstein-Barr virus, interacts with WWOX and induces a cancer promoting and invasion effect via activation of ERK1/2 and MMP9.⁷⁷

Therapeutic potential of WWOX in GBM

So far, the study of WWOX on GBM is at the beginning. Previous studies have revealed that the expressions of WWOX in human nervous system tumors are different, concluding that WWOX, NF3/merlin, and mutant p53 are intensely expressed in GBM specimens, but the expression of WWOX is reduced in metastatic carcinomas.⁹² The ectopic expression of WWOX in U251MG cells decreases FasL and TGFβ expression, resulting in inhibiting apoptosis and increasing the proliferation of co-cultured Jurkat T cells.⁹³ The loss of WWOX in GBM not only enhances carcinogenesis but also suppresses immune cell attack by apoptotic signaling. The overexpression of WWOX induces apoptosis in p53-mt U373MG cells via mitochondria-independent and caspase 3-independent pathways but not in p53-wt U87MG cells.¹⁴ The reconstitution of WWOX in GBM cells harboring mutant p53 induces apoptosis in a p53-independent manner, and one possible mediator, glioma-associated oncogene homolog 1 (Gli1), is worthy of subsequent investigation.

It has been demonstrated that WWOX formed a complex with Gli1 and impairs transcriptional activity of Gli1 via the inhibition of the hedgehog pathway in breast cancer.⁷⁶ The

hedgehog signaling pathway plays a crucial role in tumor progression and pathogenesis of GBM. This pathway is activated by the binding of sonic hedgehog (Shh) to its receptor patched, PTCH, triggering Gli1 activation. Twenty-two percent of GBM patients possess an amplified Gli1 locus and higher Gli-1 expression, which correlates with a poor prognosis.⁹⁴ The Gli1 signaling axis also controls the self-renewal of cancer stem cells, contributing to the resistance of GBM cells to therapeutic agents.⁹⁵ Truncated splicing variants of Gli1 have been found in GBM to regulate genes that are not modulated by Gli1, including CD24 and vascular endothelial growth factor A (VEGFA), resulting in angiogenesis vasculature.⁹⁶ In addition to Shh, Gli1 is also regulated by other proteins, such as Ras, Myc, Akt, p53, PTEN,⁹⁷ and WWOX.⁷⁶ Silencing Gli1 expression in chondrosarcoma activates apoptosis independent of caspase 3 and induces autophagy via mTOR signaling.⁹⁸ WWOX-mediated apoptosis in p53-mutated U373MG cells is also through the caspase-3-independent pathway; it is worth evaluating whether WWOX triggers apoptosis through the binding to Gli1 in GBM cells.

EGFR amplification and phosphatase and tensin homolog (PTEN) mutation are two common genetic alterations in GBM. The *EGFR* gene is amplified in 40–50% of GBMs and the most common mutant, *EGFRvIII*, is a constitutively active receptor that lacks a functional ligand-binding domain.^{99–101} Besides, the tumor suppressor gene *PTEN* is inactivated in 20–40% of GBMs.^{102,103} These alterations facilitate cell survival, proliferation, migration, invasion, and tumorigenesis. JNK activation in GBM is associated with EGFR amplification, inactivation of PTEN, or others,^{104–107} and survival signal from activated JNK1 counteracts apoptotic event mediated by WWOX. Another important factor for glioma initiation and progression is the canonical Wnt/β-catenin signaling pathway.^{108,109} Persistent activation of β-catenin has been implicated in self-renewal and differentiation of neural stem/progenitor cells.^{110,111} Besides, it was shown that Dishevelled-2 (Dvl-2) was overexpressed in GBM patients.¹¹² Increased expression of WWOX in GBM can sequester Dvl and block Wnt/β-catenin signaling pathway, reducing the population of cancer stem cell. Ionizing radiation induces TGFβ production in cancer cells *in vitro* and *in vivo*.^{113–115} TGFβ is involved in a range of effects on glioma microenvironment, including extracellular matrix deposition, angiogenesis, metastasis, and invasion.¹¹⁶ TGFβ2 is overexpressed in gliomas,¹¹⁷ while TGFβ1 has been found in anaplastic gliomas than in GBM.¹¹⁸ TGFβ pathways transmit signaling via SMAD2/3/4 complexes and induce expression of genes involved in proliferation, motility, metastasis, and survival of malignant GBM cells. Reconstitution of WWOX can confine SMAD3 in cytoplasm to suppress TGFβ-dependent effects such as radioresistance and stemness of glioma-initiating cells. Key factors modulated by WWOX in GBM are summarized in Figure 2.

Concluding remarks

The challenges of GBM treatment are due to the heterozygous and highly resistant nature of GBM cells in response to any therapeutic modality. It seems that GBM cells can

Figure 2 WWOX signaling in GBM. In the absence of WWOX, cancer-promoting signals are transmitted by aberrant activation of EGFR, WNT/ β -catenin, or TGF β pathways in GBMs. Restoring WWOX levels suppresses tumorigenesis of GBM in synergy with other mechanisms, such as loss of p53 through retaining target proteins in cytoplasm, including JNK1, Dvl, Smad3, p73, and Gli1. Upon stress stimulation, WWOX is phosphorylated and binds to p53, initiating apoptosis processes after translocation of WWOX/p53 complex to mitochondria and nucleus. Unfortunately, GBM cells usually show systematic defects in the apoptosis signaling machinery. In p53 wild-type GBM cells, p53 negatively controls the activity of Gli1, attenuating the function of Gli1 in stem cell self-renewal, cell proliferation, and tumor growth. Thus, overexpression of WWOX in p53-mutant GBM cells provides alternative origin of restricted activities of Gli1 and inhibition of Gli1 can trigger caspase 3-independent apoptosis and autophagy, which might shift the balance from apoptosis resistance to greater therapeutic benefits. Dvl: dishevelled; EGFR: epidermal growth factor receptor; Gli1: glioma-associated oncogene homolog 1; GSK3 β : glycogen synthase kinase 3 beta; LPR: lipoprotein receptor-related protein; NF κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; PTCH: patched; Shh: sonic hedgehog; SMAD3: SMAD family member 3; SMO: smoothened; TGF β : transforming growth factor beta; WWOX: WW domain containing oxidoreductase

tolerate lethal threats and escape from cell death via aberrant signaling pathways, such as constitutional activated oncogenic signaling and defect apoptosis regulation. WWOX has been found to be lost in various types of tumors and in GBM as well. A low expression of WWOX correlates with invasion, metastasis, and a poor response to treatment, including unfavorable prognoses and survival. This suggests the increased expression of WWOX offers an opportunity to develop a successful therapy for incurable malignancies.

The regulation of WWOX is a complex network as numerous proteins have been found to be associated with WWOX through WW domain-dependent or -independent interactions. The fate of cells controlled by WWOX can be interfered by several competitors. First, there are truncated forms of WWOX caused by the deletion of exon 5–8 produced in malignant tissue, but not in normal tissue.^{17,24,119,120} These truncated proteins of WWOX share WW domain and differ at their C-terminal the SDR motif,

displaying abnormal nuclear localization in contrast to the wild-type WWOX protein. The absence of an SDR motif in the truncated WWOX protein leads to the loss of oxidoreductase activity but remains capable of binding to target proteins. Second, other WW domain-containing proteins possess the same binding ability as WWOX-interacting proteins, such as the interaction of Yes-associated protein (YAP). YAP has been characterized as a transcriptional co-activator of p73, RUNX, and ErbB4.^{121–125} When co-expression of YAP, p73, and WWOX, WWOX was able to suppress p73 transactivation function indicating WWOX acts as a transcriptional co-repressor antagonizing the function of YAP. This suggests that in WWOX-negative cells, YAP-interacting complex can translocate to the nucleus and transactivate target genes regulating cell proliferation, differentiation, and cancer progression. Third, at least nine isoforms of p53 have been identified in tumor cells;¹²⁶ whether these isoforms of p53 impeded p53-mediated apoptosis awaits further investigation. It is worth

considering whether the aberrant expression of these competitors of WWOX can serve as a negative or feedback regulator of WWOX functions.

Complexities of WWOX-mediated regulation, including cellular growth, differentiation, development, apoptosis, transcriptional control, and tumor suppression,^{127,128} provide greater therapeutic benefits by targeting survival and apoptosis signaling simultaneously. Understanding WWOX functions in the premalignant stage and in the treatment response of GBM cells would shed light on discovering effective therapeutic strategies for GBM treatment.

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