Regulation of cell signaling and apoptosis by tumor suppressor WWOX

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Abstract

Human fragile *WWOX* gene encodes a tumor suppressor WW domain-containing oxidoreductase (named WWOX, FOR, or WOX1). Functional suppression of WWOX prevents apoptotic cell death induced by a variety of stress stimuli, such as tumor necrosis factor, UV radiation, and chemotherapeutic drug treatment. Loss of *WWOX* gene expression due to gene deletions, loss of heterozygosity, chromosomal translocations, or epigenetic silencing is frequently observed in human malignant cancer cells. Acquisition of chemoresistance in squamous cell carcinoma, osteosarcoma, and breast cancer cells is associated with WWOX deficiency. WWOX protein physically interacts with many signaling molecules and exerts its regulatory effects on gene transcription and protein stability and subcellular localization to control cell survival, proliferation, differentiation, autophagy, and metabolism. In this review, we provide an overview of the recent advances in understanding the molecular mechanisms by which WWOX regulates cellular functions and stress responses. A potential scenario is that activation of WWOX by anticancer drugs is needed to overcome chemoresistance and trigger cancer cell death, suggesting that WWOX can be regarded as a prognostic marker and a candidate molecule for targeted cancer therapies.

Keywords: Tumor suppressor, apoptosis, signal transduction, transcription factor, chemoresistance

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Introduction

Human *WWOX* gene resides in a common fragile site *FRA16D* on chromosome 16q23.3-24.1. The *WWOX* gene contains nine exons spanning over one million bases of human chromosomal DNA and encodes a tumor suppressor WW domain-containing oxidoreductase (designated as WWOX, FOR, or WOX1).^{1–4} The full-length 46-kDa WWOX protein consists of two N-terminal WW domains, a nuclear localization sequence between the WW domains, and a C-terminal short-chain alcohol dehydrogenase/reductase (SDR) domain (Figure 1). The C-terminal region of WWOX protein encoded by exons 5–8 is frequently deleted or alternatively spliced in a variety of human cancer cells.^{4,5} In contrast, the WW domain region is rarely deleted.

WW domains are small protein modules that consist of ~40 amino acids with a pair of highly conserved tryptophan residues spaced 20–23 amino acids apart and show a triple-stranded antiparallel β -sheet conformation.⁶ Specific protein–protein interactions mediated through the binding of WW domains to proline-containing motifs have been found to be associated with various cellular functions, including

regulation of transcription factors (TCF), protein ubiquitination, and cell growth control.^{6,7} WW domains can be classified into four groups according to their binding specificity for peptide ligands.⁸ Proteins with group I WW domains, such as dystrophin, Yes-associated protein (YAP65), and Nedd4 E3 ubiquitin ligase, have been shown to interact with peptide motifs containing Pro-Pro-X-Tyr (PPXY, where X represents any amino acid) or Leu-Pro-X-Tyr (LPXY). Group II WW domains mediate binding to PPLP motifs and group III WW domains mediate binding to PPR motifs. Group IV WW domains recognize p(S/T)P motifs. Group I WW domains have been studied most intensively. The first WW domain of WWOX protein has been reported to interact with various PPXY motif-containing proteins, such as p73, activator protein 2 γ (AP2 γ), ErbB-4, ezrin, small membrane protein of the lysosome/late endosome (SIMPLE), and Dishevelled 1 (DVL1) (Figure 1).^{9–14} A recent study suggested that WWOX binds to LPXY motif-containing proteins, such as the E3 ubiquitin ligase ITCH, via its first WW domain (Figure 1).¹⁴ WW domain interactions with PPXY motif can be regulated by phosphorylation of tyrosine residues within the group I WW domains.⁸

Figure 1 WWOX binding proteins. The first WW domain of WWOX protein binds to PPXY or LPXY motif(s) in the indicated proteins. WWOX also interacts with many proteins which do not possess PPXY or LPXY motif via its first WW domain. These proteins include p53, Δ Np63 α , JNK1, Hyal-2, and HIF-1 α . The binding of Tau, GSK-3 β and DVL2 to the C-terminal SDR domain of WWOX is shown

Phosphorylation of Tyr33 in the first WW domain of WWOX has been demonstrated to be crucial for its binding to p53, c-Jun N-terminal kinase 1 (JNK1) and p73 (Figure 1).^{9,15,16} However, no PPXY or LPXY is identified in p53 and JNK1. Src phosphorylates WWOX at Tyr33 in the first WW domain.⁹ Structural analysis has revealed that Tyr33 within the first WW domain of WWOX is engaged in key intermolecular contact with the proline residues within PPXY motifs of target proteins.¹⁷ The second WW domain of WWOX has an atypical structure with the replacement of a signature tryptophan by tyrosine. A recent study has demonstrated that the second WW domain of WWOX serves as a chaperone in a context of tandem module to enhance binding of the first WW domain to the PPXY motifs within the intracellular domain of receptor tyrosine kinase ErbB-4.¹⁷

The C-terminal SDR domain has been shown to mediate the binding of WWOX with Tau for regulating Tau hyperphosphorylation (Figure 1).¹⁸ WWOX also binds to glycogen synthase kinase 3 β (GSK-3 β) via its SDR domain to inhibit GSK-3 β -mediated Tau phosphorylation (Figure 1).¹⁹ Bouteille et al.²⁰ demonstrated that both WW and SDR domains of WWOX protein interact with DVL2 (Figure 1). Many SDR family proteins catalyze reactions that regulate cellular metabolism and redox functions. Whether WWOX exerts its enzymatic function via binding

to its interacting proteins, such as Tau, GSK-3 β , and DVL2, for regulating steroid metabolism and redox balance is unclear and remains to be clearly characterized.

WWOX functions as a tumor suppressor

Chromosomal fragility has been suggested to be associated with cancer development. Frequent deletions, loss of heterozygosity, and translocations of human WWOX gene have been found in numerous types of cancers, including hepatoma (28.7%), breast tumors (81.8%), esophageal squamous cell carcinoma (SCC) (38.9%), non-small-cell lung cancer (37%), pancreatic adenocarcinoma (26.7%), and gastric carcinoma (30.8%).^{4,21} Mutations in the exons and non-coding regions of human WWOX gene have been identified in the SCC and non-small-cell lung cancer samples.^{4,22} Promoter hypermethylation of WWOX gene may cause the reduced expression of WWOX in cancer cells.^{23–25} Poor prognosis or unfavorable clinical outcome in patients is associated with low or absent expression of WWOX in cancer specimens.^{26–29} Aberrant WWOX transcripts have been noted in breast, ovarian, lung, pancreatic, and other cancers.^{4,30,31} High expression levels of two alternatively transcribed WWOX variants have been identified in human breast tumors, suggesting that these variant proteins may be involved in cancer progression.³²

However, the stability and function of these truncated proteins have yet to be determined.

Ablation of *Wwox* gene has been shown to cause higher tumor incidence in mice.³³ There was occurrence of spontaneous osteosarcomas in juvenile *Wwox*^{-/-} and lung papillary carcinomas in adult *Wwox*^{+/-} mice.³³ However, all whole-body *Wwox* gene knockout mice died by four weeks of age. Restoration of *Wwox* expression by the infection of a recombination adenovirus carrying *Wwox* cDNA has been demonstrated to suppress tumor growth in lung and breast cancer xenograft mouse models, suggesting that WWOX is a *bona fide* tumor suppressor.^{34,35}

WWOX regulates cell apoptosis in stress responses

WWOX has been reported to be involved in stress responses. UV light exposure significantly upregulates WWOX protein expression in hairless mouse epidermis.³⁶ Constant light-induced retinal degeneration is associated with nuclear and mitochondrial accumulation of Tyr33-phosphorylated *Wwox* protein in rodent photoreceptors.³⁷ Upon sciatic nerve transection in rats, significant upregulation of *Wwox* expression has been detected in the injured neurons.³⁸ Treatment of a dopaminergic neurotoxin 1-methyl-4-phenyl-pyridinium (MPP⁺) increases *Wwox* protein expression and phosphorylation at Tyr33 in rat brains.³⁹ Complement C1q treatment induces cluster formation of WWOX-containing microvilli on the cell membrane of prostate cancer cells.⁴⁰ Together, these studies have revealed that WWOX is activated via phosphorylation at Tyr33 and translocates to the cell membrane, mitochondria, and nucleus under stress conditions (Figure 2).

Substantial evidence suggests that WWOX plays an important role in the regulation of cell apoptosis. WWOX enhances the cytotoxic function of tumor necrosis factor (TNF) in L929 fibroblasts via its WW and SDR domains.³ The enhancement of TNF cytotoxicity by WWOX is due to its significant downregulation of the apoptosis inhibitors Bcl-2 and Bcl-xL but upregulation of the proapoptotic p53.³ WWOX physically interacts with p53 during stress responses and both proteins induce cell death synergistically (Figure 2).¹⁵ Phosphorylation of WWOX at Tyr33 is crucial for its enhancing effect on p53 protein stability during TNF- and staurosporine-mediated cell death.¹⁵ Ectopically overexpressed WWOX induces caspase activation and apoptosis in cancer cells, and inhibits the growth of lung, breast, prostate, and pancreatic tumors in nude mice.^{23,34,35,41} Suppression of WWOX expression by antisense mRNA and small interfering RNA (siRNA) protects cells from apoptosis induced by TNF, staurosporine, UV light, and ectopic p53 *in vitro*.^{3,15,36} The inhibition of cell apoptosis by dominant negative mutants and a short synthetic peptide that have been shown to suppress Tyr33 phosphorylation of WWOX also indicates a critical role of WWOX phosphorylation in the regulation of cell death.^{15,39,40}

WWOX controls the functional activities of p53 family proteins

WWOX has been demonstrated to regulate transcriptional factor activities for controlling a variety of cellular physiological functions. The first identified transcription factor was p53, a well-known proapoptotic mediator.³ p53 functions as a potent tumor suppressor that regulates a large number of target genes involved in apoptosis, cell cycle control, DNA repair, cellular senescence, angiogenesis, and metabolism. Upon binding to consensus p53-responsive elements present in target gene promoters, p53 activates gene transcription of many downstream targets including the cyclin-dependent kinase inhibitor p21^{Waf1/Cip1} and E3 ubiquitin protein ligase MDM2. p53 may also induce the expression of mediators that trigger apoptosis, such as BAX and PUMA. In response to DNA damages and stress signals, activation and stabilization of p53 protein are prerequisite for p53 functions. Treatment of cells with TNF- α , UV irradiation, staurosporine, anisomycin, chemotherapeutic drugs, hypoxia induces protein phosphorylation of WWOX at Tyr33.^{15,36,42} Tyr33-phosphorylated WWOX is essential for binding and stabilizing Ser46-phosphorylated p53 (Figure 2).¹⁵ The active WWOX-p53 complexes then translocate into the nucleus where they promote apoptosis (Figure 2).^{3,15} The environmental cue leading to protein phosphorylation is crucial for coordinating the functions of tumor suppressors p53 and WWOX. 17 β -estradiol has been shown to activate WWOX via binding to the conserved NSYK motif in SDR domain and trigger nuclear translocation of WWOX-p53 complexes in COS7 cells.⁴³ A recent report showed that WWOX overexpression increases the expression levels of p73, PUMA, and BAX in gallbladder cancer cells.⁴⁴ The presence of WWOX is inversely correlated with the mRNA expression levels of cyclins D1 and E1 in neuroblastoma.²⁵ Whether WWOX regulates the expression of genes involved in cell cycle regulation and apoptosis via p53 remains to be verified.

Tyr33-phosphorylated WWOX also binds p73, a p53 homolog, and this interaction induces apoptosis.⁹ WWOX interacts with the ⁴⁸⁴PPPPY⁴⁸⁸ motif of p73 via its first WW domain, and phosphorylation of WWOX at Tyr33 is important for their physical association (Figure 2).⁹ In contrast to stress-induced nuclear translocation of WWOX-p53 complexes, ectopic WWOX inhibits the transcriptional activity of p73 by preventing its nuclear translocation (Figure 2).⁹ Cytoplasmic p73 exerts its proapoptotic activity with WWOX in SAOS-2 cells.⁹ The E3 ligase ITCH mediates Lys63-linked ubiquitination of WWOX and induces nuclear relocation of WWOX for enhancing p73-induced cell death in HEK293 cells.¹⁴

p63 is another protein in the p53 family that binds WWOX.⁴⁵ The p53 family members share similar structures, but may have distinct biological functions. Striking defects have been observed in p63-null embryos, suggesting its crucial role in embryonic development. In addition, p63 is required for maintaining epithelial cell homeostasis and keratinocyte differentiation. The *TP63* gene encompasses two promoters that generate two protein isoforms.⁴⁶ The full-length TAp63 protein contains the N-terminal

Figure 2 WWOX regulates multiple signaling pathways. WWOX inhibits the transactivation function of p73, Δ Np63 α , AP2 γ , ErbB4, RUNX2, and c-Jun by sequestering them in the cytoplasm, thus preventing their binding to the promoter region of target genes. Stress-induced activation of WWOX involves Tyr33 phosphorylation and translocation to the mitochondria and nuclei. Tyrosine kinase Src phosphorylates WWOX. Phosphorylation of WWOX at Tyr33 is essential for its binding and stabilizing Ser46-phosphorylated p53. The active WWOX–p53 complexes then translocate into the nucleus where they promote apoptosis. JNK1 and Zfra counteract the apoptotic function of WWOX. Tau and GSK-3 β interact with WWOX via the C-terminal SDR domain. WWOX also regulates Hedgehog signaling, Wnt/ β -catenin axis, and glucose metabolism via interacting with GLI1, DVL1/DVL2 and HIF-1 α , respectively. Ack1 induces Tyr287 phosphorylation of WWOX for the degradation of WWOX via ubiquitin/proteasome pathway

transcriptional transactivation (TA) domain, a DNA-binding domain and an oligomerization domain. The Δ Np63 isoform is produced by the use of the second promoter located in intron 3. The resulting N-terminal truncated protein lacks the TA domain for transactivation.⁴⁶ TAp63 has been shown to inhibit tumorigenesis and cancer cell metastasis, suggesting its role as a tumor suppressor. Interestingly, the Δ Np63 α isoform may function as a dominant negative factor counteracting the tumor suppressor activities of p53 family proteins to promote cell survival and proliferation.⁴⁶ WWOX has been shown to interact with Δ Np63 α but not TAp63 α via its first WW domain (Figure 1).⁴⁵ However, no PPXY or LPXY motif is identified in the unique N-terminus of Δ Np63 α . Mutations in the PPXY motif within the C-terminal common region of TAp63 α and Δ Np63 α failed to disrupt the binding of WWOX to Δ Np63 α , suggesting that WWOX may interact with a different motif rather than the canonical proline-rich motifs (Figure 1).⁴⁵

The interaction of WWOX with Δ Np63 α has been demonstrated to sequester Δ Np63 α in the cytoplasm, thereby suppressing Δ Np63 α transactivation of target genes (Figure 2).⁴⁵ WWOX stabilizes Δ Np63 α by inhibiting ITCH-mediated polyubiquitination and degradation of Δ Np63 α .⁴⁵ Co-expression of WWOX and Δ Np63 α significantly increases cisplatin-induced apoptosis in human osteosarcoma SAOS-2 cells as compared with the cells expressing Δ Np63 α or WWOX alone.⁴⁵ Whether the binding of WWOX to Δ Np63 α blocks the dominant-negative effect of Δ Np63 α on proapoptotic TAp63 α is unclear.

WWOX has been linked to the regulation of many TCF

WWOX inhibits the transactivation function of AP2 γ by sequestering it in the cytoplasm, thus preventing the binding of AP2 γ to the *ERBB2* promoter region and downregulating AP2 γ /ErbB2-mediated prostate cancer cell growth (Figure 2).^{10,47} Tyr33-phosphorylated WWOX binds to the

⁵⁶PPPYFPPPY⁶⁴ peptide sequence of AP2 γ .¹⁰ WWOX binding to AP2 γ downregulates androgen receptor-mediated growth signaling in LNCaP cells.⁴⁷ In basal-like breast cancer cells, elevated nuclear AP2 γ expression may be partially due to the reduced WWOX expression, indicating that the formation of WWOX-AP2 γ complexes is involved in the regulation of basal-like breast cancer development.⁴⁸

UV irradiation induces phosphorylation of Wwox at Tyr33 in hairless mouse epidermis.³⁶ Following exposure to UV irradiation, WWOX interacts with the PPVY motif of phosphorylated c-Jun and attenuates MEKK1-stimulated binding of c-Jun to an AP-1 element in human HaCaT keratinocytes (Figure 2).⁴⁹ After sciatic nerve transection in rats, TCF NF- κ B, ATF3, and phosphorylated c-Jun, and CREB rapidly accumulate in the nuclei of injured neurons.³⁸ Wwox is also upregulated in the dorsal root ganglion (DRG) neurons within hours.³⁸ Phosphorylated Wwox protein blocks the prosurvival CREB- and AP-1-mediated promoter activation, indicating concurrent activation of Wwox with TCF for regulating apoptosis of small DRG neurons upon neuronal injury in rats.³⁸

WWOX has been reported to suppress retroviral oncoprotein Tax-mediated tumorigenesis.⁵⁰ Tax protein is encoded by human T-cell leukemia virus type I (HTLV-I). Persistent activation of both canonical and noncanonical NF- κ B pathways by HTLV-1 tax has been linked to the pathogenesis of adult T-cell leukemia. WWOX inhibits Tax-mediated recruitment of IKK- α to RelA and subsequent RelA phosphorylation at Ser536, thereby suppressing HTLV-1 Tax-induced activation of the canonical NF- κ B pathway (Figure 2).⁵⁰ Interestingly, Tax-induced activation of noncanonical NF- κ B pathway suppresses WWOX expression in lymphoma cells to promote tumorigenesis.⁵⁰

The receptor tyrosine kinase ErbB4 mediates the signals of neuregulins and other epidermal growth factor-like ligands, and regulates many cellular activities, including cell proliferation, differentiation, and migration. Upon ligand binding, proteolytically released intracellular domain of ErbB4 may translocate into the nucleus and regulate gene transcription. WWOX interacts with the PPXY motif in the intracellular domain of ErbB4 via its first WW domain, and this binding causes cytoplasmic retention of ErbB4 (Figure 2).¹¹ Substitution of Tyr33 with Arg33 in the first WW domain of WWOX abolishes the association of WWOX with ErbB4.¹¹ YAP is a WW domain-containing protein that also binds to the PPXY motif of ErbB4 and acts as a transcriptional co-activator. WWOX competes with YAP for interaction with ErbB4, and this competition modulates the transactivation function of ErbB4 (Figure 2).¹¹ The presence of WWOX protein in clinical breast cancer samples has been shown to be associated with localization of ErbB4 at the cell membrane and breast cancer survival.²⁷

Runt-related transcription factor 2 (RUNX2) is a key transcription factor associated with osteoblast differentiation and bone homeostasis. *Wwox* knockout mice display elevated RUNX2 levels in femurs, decreased trabecular bone density and abnormal bone metabolism.^{51,52} WWOX physically associates with RUNX2 and suppresses its transcriptional activity (Figure 2).⁵¹ Ectopic WWOX expression in osteosarcoma cells downregulates RUNX2 expression,

cell proliferation, and invasion.⁵² An inverse correlation between WWOX and RUNX2 expression in human osteosarcoma tissue samples suggests the physiological relevance and the potential prognostic value in patients with osteosarcoma.⁵²

Hedgehog signaling is an essential and highly conserved signaling pathway involved in many developmental processes. When sonic hedgehog ligand binds to Ptch receptor, the zinc-finger transcription factor GLI1 is activated by release from a large protein complex and translocates into the nucleus to regulate gene expression. GLI1 also plays important roles in cell cycle progression and cancer metastasis. Aberrant expression of GLI1 has been found in many human cancers. WWOX directly interacts with GLI1 protein and triggers redistribution of nuclear GLI1 to the cytoplasm in breast cancer cells (Figure 2).⁵³ In addition, ectopic WWOX downregulates mRNA levels of *GLI1*.⁵³ The molecular mechanism by which WWOX suppresses GLI1 expression and function for regulating breast cancer cell growth is largely unclear.

WWOX has been linked to the regulation of glucose metabolism.⁵⁴ Warburg effect is a hallmark of cancer cells manifested by high rates of aerobic glycolysis and high levels of lactate and pyruvate generation. WWOX physically interacts with hypoxia-inducible transcription factor 1 α (HIF-1 α) and suppresses HIF-1 α -mediated transcriptional activation of downstream targets such as glucose transporter 1 (GLUT1) (Figure 2).⁵⁴ The reduced expression of GLUT1 leads to the downregulation of glucose uptake and glycolysis in cells.⁵⁴ HIF-1 α upregulation induced by loss of WWOX expression is associated with tumorigenesis in mice.⁵⁴ How WWOX controls HIF-1 α expression is unclear. WWOX deficiency decreases mitochondrial respiration in mouse embryonic fibroblasts.⁵⁴ The mechanism by which WWOX regulates oxidative phosphorylation in the mitochondria remains to be further investigated.

WWOX participates in multiple signaling pathways

WWOX has been suggested to be involved in many signaling pathways that regulate cell proliferation, embryonic development, metabolism, and apoptosis.⁴ WWOX is a downstream effector in TNF signaling pathway and capable of enhancing TNF cytotoxicity.³ TNF and UV light induce physical association of a zinc finger-like protein Zfra with WWOX and their translocation to the mitochondria.⁵⁵ Zfra is a 31-amino-acid protein that belongs to the C2H2 zinc-finger protein family.⁵⁶ Zfra is ubiquitously expressed in many organs and tissues, including the brain, heart, intestine, liver, lung, spleen, and testis.⁵⁶ Zfra has been shown to participate in TNF signaling and modulate the apoptotic functions of death domain-containing proteins, such as TRADD (TNF receptor-associated death domain protein), FADD (Fas-associated death domain-containing protein) and RIP (receptor interacting protein).⁵⁷ Transiently overexpressed Zfra induces apoptosis, and Ser8 phosphorylation is essential for its apoptotic function.⁵⁷ In response to the treatment of TNF and UV light, cytosolic Zfra is Ser8-phosphorylated and translocates to

Figure 3 The potential role of WWOX in anticancer drug-induced cancer cell apoptosis. Right (red area): In susceptible cells, anticancer drugs upregulate WWOX expression and suppress autophagy, thereby sensitizing cancer cells to apoptosis. mTOR signaling is generally considered to promote cell growth. mTOR activity is transiently upregulated in anticancer drug-treated SCC cells. Whether the failure to sustain mTOR activation renders cells vulnerable to death remains to be determined. WWOX also interacts with $\Delta Np63\alpha$ and $AP2\gamma$ and sequesters them in the cytoplasm for increasing apoptosis. Left (green area): Basal autophagy is active in chemoresistant cancer cells, as evidenced by the presence of numerous autophagosomes in the cytoplasm. Following anticancer drug treatment, the expression levels of Beclin-1 and Atg12-Atg5 are upregulated and autophagy remains robust in these cells. The drug-resistant cells may utilize the autophagic pathway as a survival mechanism to evade the perturbations of cellular biosynthetic processes by antimetabolites and sustain cell viability upon metabolic stress. Failure to induce WWOX expression in cancer cells may lead to chemoresistance

the mitochondria. When co-overexpressed in cells, Zfra and WWOX nullified each other's apoptotic function (Figure 2).⁵⁵ This appears to be related with the Zfra/WWOX interaction and suppression of WWOX nuclear localization by Zfra (Figure 2).⁵⁵ Zfra also blocks WWOX-induced cytochrome *c* release from the mitochondria.⁵⁷ Similarly, TNF and UV light induce Zfra to rapidly self-associate and bind p53, and JNK1.⁵⁵ Ectopic Zfra inhibits phosphorylation and nuclear translocation of p53 and its apoptotic effects in certain cells. In contrast, a Ser8 mutant of Zfra has no blocking effect.⁵⁵

WWOX has been demonstrated to physically interact with TRADD.⁵⁵ The WWOX/TRADD interaction enhances the apoptotic pathway triggered by TNF.³ Moreover, inhibition of JNK by SP600129 enhances WWOX-induced apoptosis.⁵⁸ Indeed, JNK1 binds Tyr33-phosphorylated WWOX and inhibits WWOX-mediated apoptosis (Figure 2).¹⁶

Transforming growth factor beta (TGF- β) has been demonstrated to stimulate nuclear accumulation of Tyr33-phosphorylated WWOX and Smad4 in L929 fibroblasts.⁵⁹ Interestingly, TGF- β 1 induces nuclear translocation of Tyr33-phosphorylated WWOX in type II TGF- β receptor (T β RII)-deficient HCT116 cells, indicating that TGF- β binds to an alternative receptor and stimulates WWOX

activation.⁵⁹ The binding of TGF- β 1 to cell surface hyaluronidase Hyal-2 on microvilli results in recruitment of WWOX and formation of Hyal-2-WWOX complexes for relocation to the nucleus (Figure 2).⁵⁹ Substitution of Tyr33 in the first WW domain of WWOX with arginine abolishes Hyal-2-WWOX complex formation, suggesting that Tyr33 phosphorylation is essential for Hyal-2-WWOX interaction.⁵⁹ Previous studies have shown that, in response to TGF- β stimulation, activated Src is translocated to the cytoplasmic membrane to phosphorylate its substrates T β RII and focal adhesion proteins.^{60,61} Whether Src phosphorylates WWOX at Tyr33 after TGF- β 1 stimulation and regulates TGF- β 1-induced formation of Hyal-2-WWOX complexes remains to be determined. WWOX and Hyal-2 synergistically enhance the promoter activity driven by Smad, which subsequently leads to cell death.⁵⁹ Transient overexpression of WWOX and Hyal-2 increases TGF- β 1-induced apoptosis in L929 cells. WWOX-knockdown in normal epithelial cells prevents growth suppression and apoptosis caused by TGF- β 1, as well as by overexpressed Smad4. These findings suggest that TGF- β 1 invokes a novel signaling to regulate cell growth and apoptosis by binding to cell surface Hyal-2 and inducing Hyal-2-WWOX complex formation for activating Smad-driven promoter in the nucleus (Figure 2).

The canonical Wnt/ β -catenin axis is critical for development and tissue homeostasis. Dysregulation of Wnt/ β -catenin signaling leading to aberrant activation of target genes may cause many diseases, including developmental disorders and cancers. The DVL proteins are essential effectors of Wnt/ β -catenin signaling. Upon Wnt ligand stimulation, DVL proteins shuttle dynamically between the cytoplasm and nucleus and function as a hub in controlling the transcriptional activity of β -catenin-lymphoid enhancer-binding factor 1 (LEF1)/T cell-specific TCF complexes in the nucleus. WWOX interacts with DVL2 and sequesters DVL2 in the cytoplasmic compartment, thereby inhibiting the function of DVL2 in facilitating β -catenin-LEF1/TCF-mediated target gene expression (Figure 2).²⁰ WWOX has been suggested to promote SH-SY5Y neuronal differentiation via inhibiting GSK-3 β activity.¹⁹ Whether WWOX modulates GSK-3 β activity for controlling the activation of Wnt/ β -catenin signal pathway is unknown. Whether WWOX regulates the developmental processes and cancer growth via its inhibition of Wnt/ β -catenin signaling pathway remains to be determined.

Chemotherapeutic drug susceptibility is associated with WWOX-mediated cancer cell death

Verrucous carcinoma is a distinctive variant of SCC. Continuous intra-arterial infusion of a folate antagonist methotrexate (MTX) resulted in complete tumor regression in patients with verrucous carcinoma.⁴² MTX treatment upregulates WWOX expression along with caspase activation and apoptotic cell death in both SCC tumor biopsies and cancer cell lines.⁴² Upregulation of WWOX expression increases chemotherapeutic drug-induced apoptosis by dampening autophagy in MTX-sensitive SCC cancer cells.⁴² Autophagy, a conserved intracellular catabolic process, has been suggested to facilitate the survival of rapidly growing cancer cells that have outgrown their vascular supply and encounter oxygen shortage or metabolic stress. Autophagy may also help cancer cells fight off chemotherapeutic drug-induced stress responses. WWOX triggers autophagy-inhibitory mTOR signaling and downregulates the expression of autophagy-essential proteins Atg12-Atg5, Beclin-1, and LC3-II in MTX-sensitive SCC cells (Figure 3).⁴² In contrast, autophagy is highly active in MTX-resistant SCC cells (Figure 3). The failure to induce WWOX upregulation in these SCC cells has been demonstrated to be associated with chemoresistance.⁴²

WWOX interacts with Δ Np63 α and inhibits its function for sensitizing human osteosarcoma cell line SAOS2 to cisplatin-induced apoptosis (Figure 3).⁴⁵ Knockdown of WWOX expression in MCF7 cells leads to estrogen receptor- α downregulation and tamoxifen resistance, and facilitates anchorage-independent breast cancer cell growth.⁶² The reduced expression of WWOX protein and increased nuclear accumulation of AP2 γ are associated with tamoxifen resistance in patients with advanced breast cancer.⁶³

Collectively, these studies indicate that WWOX is associated with anticancer drug-induced cell apoptosis, and suggest important implications for the potential use of

tumor suppressor WWOX as a chemotherapeutic target for cancer treatment.

Conclusions and perspectives

Emerging evidence suggests that WWOX regulates cancer development and progression. As *Wwox* knockout mice die early in life from multiple physiological defects before the development of cancers, organ- or tissue-specific ablation of *Wwox* gene in mice will help unravel the subtleties of WWOX action in cancer pathogenesis. High WWOX expression in tissue specimens from cancer patients has been found to be associated with increased disease-free survival. The fragile WWOX gene can be considered as a potential prognostic biomarker of human cancers. WWOX is involved in many signaling pathways for regulating cell apoptosis, autophagy, differentiation, and metabolism. WWOX sensitizes cancer cells to chemotherapeutic drugs via suppressing autophagy and blocking prosurvival effector proteins. WWOX is apparently an attractive target for therapeutic intervention in cancers. Conceivably, delineation of the molecular mechanism by which WWOX regulates chemotherapeutic drug-induced cell apoptosis will have a great impact on our understanding of cancer treatment. Recent studies have suggested that WWOX acts as a regulator in metabolic processes. The regulatory roles of WWOX in these effects will be fascinating goals for future studies.

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REFERENCES

1. Reid K, Finnis M, Hobson L, Mangelsdorf M, Dayan S, Nancarrow JK, Woollatt E, Kremmidiotis G, Gardner A, Venter D, Baker E, Richards RI. Common chromosomal fragile site FRA16D sequence: identification of the FOR gene spanning FRA16D and homozygous deletions and translocation breakpoints in cancer cells. *Hum Mol Genet* 2000;9:1651-63
2. Bednarek AK, Laflin KJ, Daniel RL, Liao Q, Hawkins KA, Aldaz CM. WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. *Cancer Res* 2000;60:2140-5
3. Chang NS, Pratt N, Heath J, Schultz L, Slevin D, Carey GB, Zevotek N. Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity. *J Biol Chem* 2001;276:3361-70
4. Chang NS, Hsu LJ, Lin YS, Lai FJ, Sheu HM. WW domain-containing oxidoreductase: a candidate tumor suppressor. *Trends Mol Med* 2007;13:12-22
5. Paige AJ, Taylor KJ, Taylor C, Hillier SG, Farrington S, Scott D, Porteous DJ, Smyth JF, Gabra H, Watson JE. WWOX: a candidate tumor

- suppressor gene involved in multiple tumor types. *Proc Natl Acad Sci USA* 2001;**98**:11417–22
6. Bork P, Sudol M. The WW domain: a signalling site in dystrophin? *Trends Biochem Sci* 1994;**19**:531–3
 7. Sudol M, Chen HI, Bougeret C, Einbond A, Bork P. Characterization of a novel protein-binding module: the WW domain. *FEBS Lett* 1995;**369**:67–71
 8. Ilesley JL, Sudol M, Winder SJ. The WW domain: linking cell signaling to the membrane cytoskeleton. *Cell Signal* 2002;**14**:183–9
 9. Aqeilan RI, Pekarsky Y, Herrero JJ, Palamarchuk A, Letofsky J, Druck T, Trapasso F, Han SY, Melino G, Huebner K, Croce CM. Functional association between Wwox tumor suppressor protein and p73, a p53 homolog. *Proc Natl Acad Sci USA* 2004;**101**:4401–6
 10. Aqeilan RI, Palamarchuk A, Weigel RJ, Herrero JJ, Pekarsky Y, Croce CM. Physical and functional interactions between the Wwox tumor suppressor protein and the AP-2gamma transcription factor. *Cancer Res* 2004;**64**:8256–61
 11. Aqeilan RI, Donati V, Palamarchuk A, Trapasso F, Kaou M, Pekarsky Y, Sudol M, Croce CM. WW domain-containing proteins, WWOX and YAP, compete for interaction with ErbB-4 and modulate its transcriptional function. *Cancer Res* 2005;**65**:6764–72
 12. Jin C, Ge L, Ding X, Chen Y, Zhu H, Ward T, Wu F, Cao X, Wang Q, Yao Y. PKA-mediated protein phosphorylation regulates ezrin-WWOX interaction. *Biochem Biophys Res Commun* 2006;**341**:784–91
 13. Ludes-Meyers JH, Kil H, Bednarek AK, Drake J, Bedford MT, Aldaz CM. WWOX binds the specific proline-rich ligand PPXY: identification of candidate interacting proteins. *Oncogene* 2004;**23**:5049–55
 14. Abu-Odeh M, Bar-Mag T, Huang H, Kim T, Salah Z, Abdeen SK, Sudol M, Reichmann D, Sidhu S, Kim PM, Aqeilan RI. Characterizing WW domain interactions of tumor suppressor WWOX reveals its association with multiprotein networks. *J Biol Chem* 2014;**289**:8865–80
 15. Chang NS, Doherty J, Ensign A, Schultz L, Hsu LJ, Hong Q. WOX1 is essential for tumor necrosis factor-, UV light-, staurosporine-, and p53-mediated cell death, and its tyrosine 33-phosphorylated form binds and stabilizes serine 46-phosphorylated p53. *J Biol Chem* 2005;**280**:43100–8
 16. Chang NS, Doherty J, Ensign A. JNK1 physically interacts with WW domain-containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. *J Biol Chem* 2003;**278**:9195–202
 17. Schuchardt BJ, Bhat V, Mikles DC, McDonald CB, Sudol M, Farooq A. Molecular origin of the binding of WWOX tumor suppressor to ErbB4 receptor tyrosine kinase. *Biochemistry* 2013;**52**:9223–36
 18. Sze CI, Su M, Pugazhenth S, Jambal P, Hsu LJ, Heath J, Schultz L, Chang NS. Down-regulation of WW domain-containing oxidoreductase induces Tau phosphorylation in vitro: a potential role in Alzheimer's disease. *J Biol Chem* 2004;**279**:30498–506
 19. Chang HY, Juo LI, Lin YT, Hsiao M, Lin JT, Tsai CH, Tzeng YH, Chuang YC, Chang NS, Yang CN, Lu PJ. WW domain-containing oxidoreductase promotes neuronal differentiation via negative regulation of glycogen synthase kinase 3β. *Cell Death Differ* 2012;**19**:1049–59
 20. Bouteille N, Driouch K, Hage PE, Sin S, Formstecher E, Camonis J, Lidereau R, Lallemand F. Inhibition of the Wnt/β-catenin pathway by the WWOX tumor suppressor protein. *Oncogene* 2009;**28**:2569–80
 21. Lewandowska U, Zelazowski M, Seta K, Byczewska M, Pluciennik E, Bednarek AK. WWOX, the tumor suppressor gene affected in multiple cancers. *J Physiol Pharmacol* 2009;**60**(Suppl 1):47–56
 22. Baykara O, Demirkaya A, Kaynak K, Tanju S, Tokar A, Buyru N. WWOX gene may contribute to progression of non-small-cell lung cancer (NSCLC). *Tumor Biol* 2010;**31**:315–20
 23. Qin HR, Iliopoulos D, Semba S, Fabbri M, Druck T, Volinia S, Croce CM, Morrison CD, Klein RD, Huebner K. A role for the WWOX gene in prostate cancer. *Cancer Res* 2006;**66**:6477–81
 24. Cantor JP, Iliopoulos D, Rao AS, Druck T, Semba S, Han SY, McCorkell KA, Lakshman TV, Collins JE, Wachsberger P, Friedberg JS, Huebner K. Epigenetic modulation of endogenous tumor suppressor expression in lung cancer xenografts suppresses tumorigenicity. *Int J Cancer* 2007;**120**:24–31
 25. Pluciennik E, Nowakowska M, Wujcicka WI, Sitkiewicz A, Kazanowska B, Zielińska E, Bednarek AK. Genetic alterations of WWOX in Wilms' tumor are involved in its carcinogenesis. *Oncol Rep* 2012;**28**:1417–22
 26. Donati V, Fontanini G, Dell'Omodarme M, Prati MC, Nuti S, Lucchi M, Mussi A, Fabbri M, Basolo F, Croce CM, Aqeilan RI. WWOX expression in different histologic types and subtypes of non-small cell lung cancer. *Clin Cancer Res* 2007;**13**:884–91
 27. Aqeilan RI, Donati V, Gaudio E, Nicoloso MS, Sundvall M, Korhonen A, Lundin J, Isola J, Sudol M, Joensuu H, Croce CM, Elenius K. Association of Wwox with ErbB4 in breast cancer. *Cancer Res* 2007;**67**:9330–6
 28. Jenner MW, Leone PE, Walker BA, Ross FM, Johnson DC, Gonzalez D, Chiechio L, Dachs Cabanas E, Dagrada GP, Nightingale M, Protheroe RK, Stockley D, Else M, Dickens NJ, Cross NC, Davies FE, Morgan GJ. Gene mapping and expression analysis of 16q loss of heterozygosity identifies WWOX and CYLD as being important in determining clinical outcome in multiple myeloma. *Blood* 2007;**110**:3291–300
 29. Ramos D, Abba M, López-Guerrero JA, Rubio J, Solsona E, Almenar S, Llombart-Bosch A, Aldaz CM. Low levels of WWOX protein immunoreexpression correlate with tumour grade and a less favourable outcome in patients with urinary bladder tumours. *Histopathology* 2008;**52**:831–9
 30. Paige AJ, Taylor KJ, Taylor C, Hillier SG, Farrington S, Scott D, Porteous DJ, Smyth JF, Gabra H, Watson JE. WWOX: a candidate tumor suppressor gene involved in multiple tumor types. *Proc Natl Acad Sci USA* 2001;**98**:11417–22
 31. Pluciennik E, Kusińska R, Potemski P, Kubiak R, Kordek R, Bednarek AK. WWOX-the FRA16D cancer gene: expression correlation with breast cancer progression and prognosis. *Eur J Surg Oncol* 2006;**32**:153–7
 32. Driouch K, Prydz H, Monese R, Johansen H, Lidereau R, Frengen E. Alternative transcripts of the candidate tumor suppressor gene, WWOX, are expressed at high levels in human breast tumors. *Oncogene* 2002;**21**:1832–40
 33. Aqeilan RI, Trapasso F, Hussain S, Costinean S, Marshall D, Pekarsky Y, Hagan JP, Zanesi N, Kaou M, Stein GS, Lian JB, Croce CM. Targeted deletion of Wwox reveals a tumor suppressor function. *Proc Natl Acad Sci USA* 2007;**104**:3949–54
 34. Iliopoulos D, Fabbri M, Druck T, Qin HR, Han SY, Huebner K. Inhibition of breast cancer cell growth in vitro and in vivo: effect of restoration of Wwox expression. *Clin Cancer Res* 2007;**13**:268–74
 35. Fabbri M, Iliopoulos D, Trapasso F, Aqeilan RI, Cimmino A, Zanesi N, Yendamuri S, Han SY, Amadori D, Huebner K, Croce CM. WWOX gene restoration prevents lung cancer growth in vitro and in vivo. *Proc Natl Acad Sci USA* 2005;**102**:15611–6
 36. Lai FJ, Cheng CL, Chen ST, Wu CH, Hsu LJ, Lee JY, Chao SC, Sheen MC, Shen CL, Chang NS, Sheu HM. WOX1 is essential for UVB irradiation-induced apoptosis and down-regulated via translational blockade in UVB-induced cutaneous squamous cell carcinoma in vivo. *Clin Cancer Res* 2005;**11**:5769–77
 37. Chen ST, Chuang JI, Cheng CL, Hsu LJ, Chang NS. Light-induced retinal damage involves tyrosine 33 phosphorylation, mitochondrial and nuclear translocation of WW domain-containing oxidoreductase in vivo. *Neuroscience* 2005;**130**:397–407
 38. Li MY, Lai FJ, Hsu LJ, Lo CP, Cheng CL, Lin SR, Lee MH, Chang JY, Subhan D, Tsai MS, Sze CI, Pugazhenth S, Chang NS, Chen ST. Dramatic co-activation of WWOX/WOX1 with CREB and NF-κB in delayed loss of small dorsal root ganglion neurons upon sciatic nerve transection in rats. *PLoS ONE* 2009;**4**:e7820
 39. Lo CP, Hsu LJ, Li MY, Hsu SY, Chuang JI, Tsai MS, Lin SR, Chang NS, Chen ST. MPP⁺-induced neuronal death in rats involves tyrosine 33 phosphorylation of WW domain-containing oxidoreductase WOX1. *Eur J Neurosci* 2008;**27**:1634–46
 40. Hong Q, Sze CI, Lin SR, Lee MH, He RY, Schultz L, Chang JY, Chen SJ, Boackle RJ, Hsu LJ, Chang NS. Complement C1q activates tumor suppressor WWOX to induce apoptosis in prostate cancer cells. *PLoS ONE* 2009;**4**:e5755
 41. Nakayama S, Semba S, Maeda N, Aqeilan RI, Huebner K, Yokozaki H. Role of the WWOX gene, encompassing fragile region FRA16D, in suppression of pancreatic carcinoma cells. *Cancer Sci* 2008;**99**:1370–6

42. Tsai CW, Lai FJ, Sheu HM, Lin YS, Chang TH, Jan MS, Chen SM, Hsu PC, Huang TT, Huang TC, Sheen MC, Chen ST, Chang WC, Chang NS, Hsu LJ. WWOX suppresses autophagy for inducing apoptosis in methotrexate-treated human squamous cell carcinoma. *Cell Death Dis* 2013;**4**:e792
43. Chang NS, Schultz L, Hsu LJ, Lewis J, Su M, Sze CI. 17 β -Estradiol upregulates and activates WOX1/WWOXv1 and WOX2/WWOXv2 in vitro: potential role in cancerous progression of breast and prostate to a premetastatic state in vivo. *Oncogene* 2005;**24**:714–23
44. Wei D, Zhang X, Zou H, Wang L, Fu B, Wu X, Luo Z, Li X, Ge J, Li Y, Zhu H, Wang K, Wang T, Yang P, Hou Z, Wang W. WW domain-containing oxidoreductase induces apoptosis in gallbladder-derived malignant cell by upregulating expression of P73 and PUMA. *Tumour Biol* 2014;**35**:1539–50
45. Salah Z, Bar-mag T, Kohn Y, Pichiorri F, Palumbo T, Melino G, Aqeilan RI. Tumor suppressor WWOX binds to Δ Np63 α and sensitizes cancer cells to chemotherapy. *Cell Death Dis* 2013;**4**:e480
46. Yang A, Kaghad M, Caput D, McKeon F. On the shoulders of giants: p63, p73 and the rise of p53. *Trends Genet* 2002;**18**:90–5
47. Qin HR, Iliopoulos D, Nakamura T, Costinean S, Volinia S, Druck T, Sun J, Okumura H, Huebner K. Wwox suppresses prostate cancer cell growth through modulation of ErbB2-mediated androgen receptor signaling. *Mol Cancer Res* 2007;**5**:957–65
48. Guler G, Huebner K, Himmetoglu C, Jimenez RE, Costinean S, Volinia S, Pilarski RT, Hayran M, Shapiro CL. Fragile histidine triad protein, WW domain-containing oxidoreductase protein Wwox, and activator protein 2gamma expression levels correlate with basal phenotype in breast cancer. *Cancer* 2009;**115**:899–908
49. Gaudio E, Palamarchuk A, Palumbo T, Trapasso F, Pekarsky Y, Croce CM, Aqeilan RI. Physical association with WWOX suppresses c-Jun transcriptional activity. *Cancer Res* 2006;**66**:11585–9
50. Fu J, Qu Z, Yan P, Ishikawa C, Aqeilan RI, Rabson AB, Xiao G. The tumor suppressor gene WWOX links the canonical and noncanonical NF- κ B pathways in HTLV-I Tax-mediated tumorigenesis. *Blood* 2011;**117**:1652–61
51. Aqeilan RI, Hassan MQ, de Bruin A, Hagan JP, Volinia S, Palumbo T, Hussain S, Lee SH, Gaur T, Stein GS, Lian JB, Croce CM. The WWOX tumor suppressor is essential for postnatal survival and normal bone metabolism. *J Biol Chem* 2008;**283**:21629–39
52. Kurek KC, Del Mare S, Salah Z, Abdeen S, Sadiq H, Lee SH, Gaudio E, Zanesi N, Jones KB, DeYoung B, Amir G, Gebhardt M, Warman M, Stein GS, Stein JL, Lian JB, Aqeilan RI. Frequent attenuation of the WWOX tumor suppressor in osteosarcoma is associated with increased tumorigenicity and aberrant RUNX2 expression. *Cancer Res* 2010;**70**:5577–86
53. Xiong A, Wei L, Ying M, Wu H, Hua J, Wang Y. Wwox suppresses breast cancer cell growth through modulation of the hedgehog-GLI1 signaling pathway. *Biochem Biophys Res Commun* 2014;**443**:1200–5
54. Abu-Remaileh M, Aqeilan RI. Tumor suppressor WWOX regulates glucose metabolism via HIF1 α modulation. *Cell Death Differ* 2014;**21**:1805–14
55. Hong Q, Hsu LJ, Schultz L, Pratt N, Mattison J, Chang NS. Zfra affects TNF-mediated cell death by interacting with death domain protein TRADD and negatively regulates the activation of NF- κ B, JNK1, p53 and WOX1 during stress response. *BMC Mol Biol* 2007;**8**:50
56. Hsu LJ, Schultz L, Mattison J, Lin YS, Chang NS. Cloning and characterization of a small-size peptide Zfra that regulates the cytotoxic function of tumor necrosis factor by interacting with JNK1. *Biochem Biophys Res Commun* 2005;**327**:415–23
57. Hsu LJ, Hong Q, Schultz L, Kuo E, Lin SR, Lee MH, Lin YS, Chang NS. Zfra is an inhibitor of Bcl-2 expression and cytochrome c release from the mitochondria. *Cell Signal* 2008;**20**:1303–12
58. Aderca I, Moser CD, Veerasamy M, Bani-Hani AH, Bonilla-Guerrero R, Ahmed K, Shire A, Cazanave SC, Montoya DP, Mettler TA, Burgart LJ, Nagorney DM, Thibodeau SN, Cunningham JM, Lai JP, Roberts LR. The JNK inhibitor SP600129 enhances apoptosis of HCC cells induced by the tumor suppressor WWOX. *J Hepatol* 2008;**49**:373–83
59. Hsu LJ, Schultz L, Hong Q, Van Moer K, Heath J, Li MY, Lai FJ, Lin SR, Lee MH, Lo CP, Lin YS, Chen ST, Chang NS. Transforming growth factor beta1 signaling via interaction with cell surface Hyal-2 and recruitment of WWOX/WOX1. *J Biol Chem* 2009;**284**:16049–59
60. Galliher AJ, Schiemann WP. Src phosphorylates Tyr284 in TGF- β type II receptor and regulates TGF- β stimulation of p38 MAPK during breast cancer cell proliferation and invasion. *Cancer Res* 2007;**67**:3752–8
61. Park SS, Eom YW, Kim EH, Lee JH, Min DS, Kim S, Kim SJ, Choi KS. Involvement of c-Src kinase in the regulation of TGF- β 1-induced apoptosis. *Oncogene* 2004;**23**:6272–81
62. Abdeen SK, Salah Z, Maly B, Smith Y, Tufail R, Abu-Odeh M, Zanesi N, Croce CM, Nawaz Z, Aqeilan RI. Wwox inactivation enhances mammary tumorigenesis. *Oncogene* 2011;**30**:3900–6
63. Guler G, Iliopoulos D, Guler N, Himmetoglu C, Hayran M, Huebner K. Wwox and Ap2gamma expression levels predict tamoxifen response. *Clin Cancer Res* 2007;**13**:6115–21.