Minireview

Strategies of oncogenic microbes to deal with WW domain-containing oxidoreductase

Yao Chang[1,2], Yu-Yan Lan[1,2], Jenn-Ren Hsiao[3] and Nan-Shan Chang[4]

[1]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Tainan 70456, Taiwan; [2]Graduate Institute of Basic Medical Science, Medical College, National Cheng Kung University, Tainan 70101, Taiwan; [3]Department of Otolaryngology, Medical College and Hospital, National Cheng Kung University, Tainan 70101, Taiwan; [4]Institute of Molecular Medicine, Medical College, National Cheng Kung University, Tainan 70101, Taiwan
Corresponding author: Yao Chang. Email: yaochang@nhri.org.tw

Abstract

WW domain-containing oxidoreductase (WWOX) is a well-documented tumor suppressor protein that controls growth, survival, and metastasis of malignant cells. To counteract WWOX's suppressive effects, cancer cells have developed many strategies either to downregulate WWOX expression or to functionally inactivate WWOX. Relatively unknown is, in the context of those cancers associated with certain viruses or bacteria, how the oncogenic pathogens deal with WWOX. Here we review recent studies showing different strategies utilized by three cancer-associated pathogens. *Helicobactor pylori* reduces WWOX expression through promoter hypermethylation, an epigenetic mechanism also occurring in many other cancer cells. WWOX has a potential to block canonical NF-κB activation and tumorigenesis induced by Tax, an oncoprotein of human T-cell leukemia virus. Tax successfully overcomes the blockage by inhibiting WWOX expression through activation of the non-canonical NF-κB pathway. On the other hand, latent membrane protein 2A of Epstein–Barr virus physically interacts with WWOX and redirects its function to trigger a signaling pathway that upregulates matrix metalloproteinase 9 and cancer cell invasion. These reports may be just ''the tip of the iceberg'' regarding multiple interactions between WWOX and oncogenic microbes. Further studies in this direction should expand our understanding of infection-driven oncogenesis.

Keywords: WW domain-containing oxidoreductase, infection-associated cancers, *Helicobactor pylori*, human T-cell leukemia virus, Epstein–Barr virus

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Introduction

Human WW domain-containing oxidoreductase, namely WWOX, WOX1 or FOR, participates in a broad spectrum of biologic functions, including control of neoplasia, stress response, metabolism, hematopoiesis, and cell differentiation.[1–5] WWOX is expressed in many tissues/organs (e.g. skin, bones, nerve systems, reproductive/endocrine systems, immune systems, and various cancers) and has been involved in homeostatic or pathogenic states therein. Its versatile nature is attributed to the broad subcellular localizations of WWOX and a wide variety of WWOX-interacting proteins.[2,3] Depending on cell types and assay conditions, WWOX has been detected in nuclei, perinuclear areas, mitochondria, Golgi, endoplasmic reticulum, and plasma membrane.[1,3] WWOX's interactions with partner proteins, as well as most of its known functions, predominantly depend on the first WW domain at the N-terminus of WWOX.[1–3] This domain, categorized as the group I WW domain, preferentially binds to a consensus PPXY motif present in many WWOX-targeting proteins.[6] A recent study reveals another consensus LPXY motif as a novel WW domain/WWOX-binding site, extending the spectrum of potential WWOX-interacting partners.[7] In addition, the C-terminus of WWOX contains a short-chain alcohol dehydrogenase–reductase domain, which is not only a putative steroid hormone-binding domain but also involved in some critical cases of WWOX's protein–protein interactions.[8,9]

Tumor suppression is a well-documented function of WWOX, and this function is majorly achieved by multiple interactions between WWOX and its specific partners.[1,2] Unsurprisingly, accumulating studies indicate that cancer cells utilize many approaches to alter WWOX expression or to withstand the anticancer effects of WWOX. On the other hand, relatively unknown is what happens to WWOX in those cancers associated with certain viruses or bacteria. Being effective initiators or promoters of

malignancy, these oncogenic microbes should have evolved several ways to deal with WWOX and incorporated them into their cancer-promoting mechanisms. Three recent studies support this idea, showing that some cancer-associated microbes may either adapt anti-WWOX approaches similar with those used by tumor cells, or even develop a novel mechanism to redirect WWOX's role in cancer progression.[10–12] Here we start from an overview of WWOX-mediated anticancer effects and their underlying mechanisms, followed by a summary of the anti-WWOX approaches used by cancer cells. Subsequently, we will focus on current knowledge about how oncogenic microbes affect WWOX's expression or function, and discuss potential directions to explore more interactions between WWOX and infectious pathogens.

WWOX as a tumor suppressor

The association between WWOX and cancers has been noticed since the gene encoding WWOX was initially identified within a common fragile site *FRA16D*, a chromosomal region frequently affected in many human cancers.[13,14] Although homozygous deletion or mutation of this gene is rare, downregulation of WWOX at the mRNA and protein levels is frequently observed in clinical specimens and cell lines of many human cancers. For example, compared with control normal tissues, tumor tissues showing complete loss or reduced expression of WWOX protein are detected in about 30–60% of breast cancers, 30% of ovarian carcinomas, 60% of osteosarcomas, 70% of cutaneous squamous cell carcinomas, and 80% of prostate cancers.[15–21] In addition, the clinical impacts of WWOX reduction in tumor tissues have been noticed. For breast cancers, absent or decreased WWOX expression is associated with clinical markers of poor prognosis, with poor overall survival, with high risk of recurrence, and with the poor response to tamoxifen treatment.[16,17,22–24] For ovarian carcinomas and renal cell carcinomas, reduced WWOX expression in tumor tissues is detected only in some specific histotypes but the expression reduction is also correlated with the unfavorable clinical outcome.[18,25] For breast cancers and osteosarcomas, WWOX expression in metastatic tumors is further reduced or even completely lost, suggesting that WWOX potentially hampers tumor metastasis.[19,23,26] Collectively these clinical observations shed light on WWOX's roles in tumor control.

The tumor-suppressive activity of WWOX is further supported by several experiments manipulating WWOX expression in cancer cell lines. For WWOX-null cell lines of lung cancers, prostate cancers, pancreatic cancers, breast cancers, osteosarcomas, and glioblastomas, ectopically forced expression of WWOX induces apoptosis and inhibits cell growth, thus suppressing the tumorigenicity of the cancer cells in immunocompromised mice.[19,21,27–30] Overexpression of WWOX sensitizes, while knockdown of WWOX attenuates, the cell death triggered by various apoptosis inducers including tumor necrosis factor α (TNFα), UV light, anticancer reagents, complement C1q, and transforming growth factor β1 (TGF-β1), further substantiating the critical function of WWOX in regulation of cell death.[20,31–35] For cell lines of hetapocellular carcinomas, gastric signet-ring cell carcinomas, osteosarcomas, and ovarian cancers, ectopic expression of WWOX suppresses cell adhesion to or cell invasion through extracellular matrix, while knockdown of WWOX enhances the matrigel invasion activity, suggesting that WWOX executes a function in control of cancer metastasis.[19,36–38]

The murine Wwox protein is 93.2% identical to human WWOX in the amino acid sequence, implying their functional conservation.[32] To get more insight into the biologic roles of WWOX, several mouse models with genetic manipulations of the *Wwox* gene have been generated, and these models corroborate the anticancer functions of murine Wwox. Although the homozygous *Wwox*-deficient ($Wwox^{-/-}$) mice with targeted disruption of exons 2–4 of the *Wwox* gene show severe metabolic disorders and die postnatally within 2–3 weeks, spontaneous occurrence of osteosarcomas in the juvenile is detected.[39,40] The mice with heterozygous *Wwox* deficiency ($Wwox^{+/-}$) develop lung papillary carcinomas spontaneously in adult, and upon treatment with a chemical mutagen, $Wwox^{+/-}$ mice develop more lung tumors and lymphomas than the wild-type mice.[39] In the *Wwox*-hypomorphic mice generated by a gene-trap strategy, the insufficient Wwox expression results in a higher incidence of spontaneous B-cell lymphomas in female.[41] In addition, the female $Wwox^{+/-}$ mice with the C3H mammary tumor-susceptible genetic background develop dramatically more mammary carcinomas than the control $Wwox^{+/+}$ mice with the same genetic background.[42] As is an exception, the *Wwox*-knockout mice with targeted disruption of the first exon of the *Wwox* gene show severe metabolic and hematopoietic defects without evidence of spontaneous neoplasia.[43] Generally these animal studies provide additional evidence of Wwox-dependent tumor suppression.

Molecular mechanisms underlying WWOX-mediated anticancer effects

As is mentioned, functions of WWOX, including those involved in tumor suppression, are majorly determined by protein–protein interactions between WWOX and specific partners. These interactions regulate downstream signaling pathways and transcriptional programs, thus affecting multiple biologic events. Here we summarize the WWOX–partner interactions and some downstream molecular mechanisms, with focus on those contributing to anticancer effects of WWOX (Figure 1).

Cellular tumor suppressor proteins are considered as the first group of WWOX-binding partners involved in the anticancer activity. Two well-recognized examples of this group are p53 and p73. Although p53 has no consensus PPXY motif, its N-terminal proline-rich domain (amino acid residuals 66–100) along with phosphorylation at the adjacent serine 46 is responsible for the physical interaction with the first WW domain of WWOX.[32] Phosphorylation of tyrosine 33 (Tyr33) in the first WW domain of WWOX activates WWOX and enhances its association with p53, resulting in stabilization of serine 46-phosphorylated p53.[35] The WWOX–p53 complex is translocated into the nucleus and

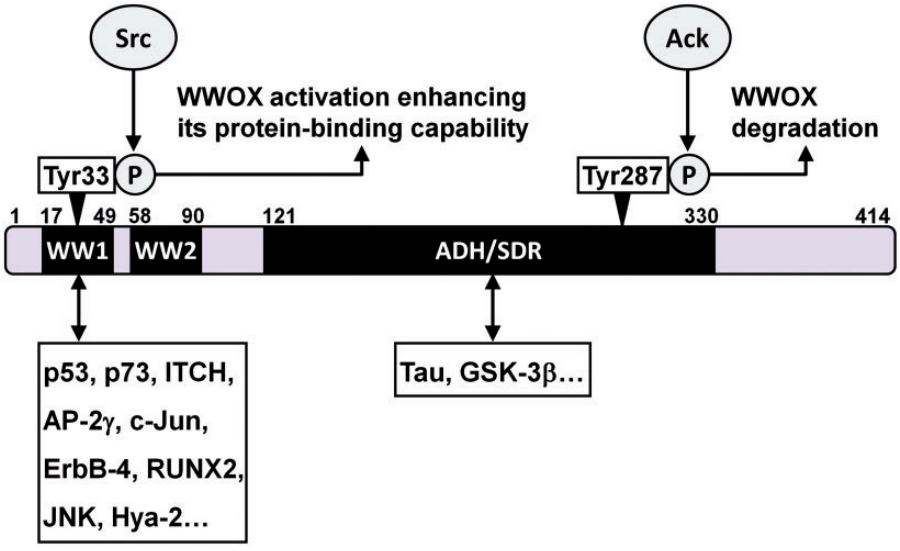

Figure 1 WWOX contains domains for protein–protein interaction. WWOX is a 414-amino-acid protein with two N-terminal WW (WW1 and WW2) domains and a C-terminal short-chain alcohol dehydrogenase–reductase (ADH/SDR) domain. A tyrosine kinase Src phosphorylates WWOX at Tyr33, which activates WWOX and enhances its interaction with partner proteins. Another tyrosine kinase Ack1 phosphorylates WWOX at Tyr287, which accelerates WWOX degradation. The first WW domain of WWOX is responsible for interaction with most of known WWOX-binding proteins, while the ADH/SDR domain mediates the interaction with Tau and GSK-3β

mediates the cell death triggered by TNF, UV light, and other apoptosis inducers.[32,35] Steroid hormone 17β-estradiol induces WWOX activation and nuclear translocation of the WWOX–p53 complex in the cells expressing no estrogen receptor, but fails to do so in estrogen receptor-positive cells, suggesting that certain factors regulate the WWOX–p53 pathway.[26] On the other hand, p73 contains a PPPPY motif serving as a binding site for the Tyr33-phosphorylated WW domain of WWOX.[44] The Src kinase is responsible for the Tyr33 phosphorylation, and similar to that observed in WWOX–p53 interaction, phosphorylation at this tyrosine residual enhances WWOX binding to p73.[44] The WWOX–p73 interaction drives the nucleus-to-cytoplasm redistribution of p73, and the cytoplasmic p73 mediates the apoptosis-inducing effect of WWOX. A recent study reveals that WWOX interacts with ITCH, an E3 ubiquitin ligase promoting polyubiquitination and degradation of p73.[7] WWOX reduces ITCH-mediated p73 polyubiquitination/degradation and enhances proapoptotic activity of p73, showing an additional effect of WWOX on p73.

The second group of WWOX-binding partners involved in tumor suppression includes oncogenic transcription factors that are functionally antagonized by WWOX. AP-2γ, c-Jun, ErbB-4, and RUNX2 belong to this category.[40,45–47] The proline-rich motif of c-Jun, as well as the PPXY motifs of other three proteins, interacts with WWOX, and the Tyr33 in the first WW domain of WWOX is required for the protein–protein interaction. Overexpressed WWOX binds to AP-2γ, c-Jun, and RUNX2 and inhibits their functions in promoter transactivation mainly through sequestering these transcription factors in the cytoplasm.[40,45,46] Overexpressed WWOX also physically associates with full-length ErbB-4 and keeps it in the cytoplasm, thus blocking nuclear translocation of the C-terminal fragment of ErbB-4 and inhibiting YAP-mediated transcriptional coactivation with this fragment.[47] On the other hand, endogenous WWOX physically interacts with CREB and co-translocates with it to the nucleus in transected sciatic nerves in rat, and this event may affect promoter activation and neuronal survival.[48]

The third group of WWOX-binding partners for tumor suppression includes various proteins regulating signal transduction. For example, WWOX binds to Dvl-2 and sequesters it in the cytoplasm, thus blocking activation of the Wnt/β-catenin signaling pathway.[49] Binding of TGF-β1 to membrane Hyal-2 facilitates interaction between the catalytic domain of Hyal-2 and the Tyr33-phosphorylated WW domain of WWOX.[33] The Hyal-2-WWOX complex is relocated into the nucleus, thereby enhancing Smad-mediated transcription activity and sensitizing TGF-β1-induced cell apoptosis.[33] In T-cell leukemia cells, WWOX physically interacts with mitogen-activated protein kinase kinase 1 (MEK1) as an inactive complex; treatment with phorbol ester releases WWOX from the complex and triggers cell apoptosis.[50]

Certain molecular mechanisms underlying WWOX-mediated inhibition of cancer metastasis have been uncovered. For the cells of breast cancers and osteosarcomas, ectopic WWOX suppresses cell invasion through functional inactivation or expressional downregulation of RUNX2. WWOX restoration reduces expression of a panel of RUNX2-regulated genes linked to metastasis.[19,40] For ovarian cancer cells, WWOX decreases membrane expression of intergrin-α3, thus reducing cell adhesion to fibronectin *in vitro* and tumorigenesis *in vivo*.[38]

How cancer cells dodge or overcome WWOX-mediated tumor suppression

As is summarized in Figure 2, WWOX can be counteracted by cancer cells in multiple ways. WWOX expression can be abrogated at genomic, transcriptional, post-transcriptional,

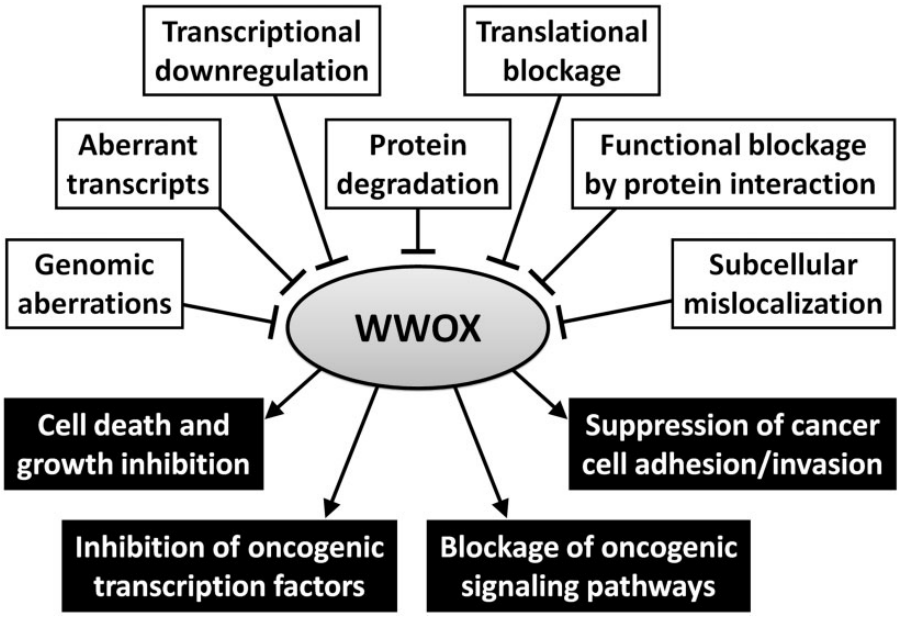

Figure 2 WWOX plays multiple roles in tumor suppression but it can be downregulated or functionally inhibited in many ways. Black boxes in the lower panel present the anticancer mechanisms of WWOX, while white boxes in the upper panel indicate the potential mechanisms of cancer cells to overcome WWOX

translational, and post-translational levels. Previous studies have also revealed some cellular proteins that physically interact with WWOX and inhibit its anticancer effects.

Although the *WWOX* gene spans the *FRA16D* common chromosomal fragile site, the cases with absence of WWOX owing truly to gene deletion or missense mutation in cancer cells are rare.[51–53] One exception is observed in primary effusion lymphoma cell lines, of which 85% (11/13) show *WWOX* gene deletion.[54] Aberrant WWOX transcripts with exon deletions have been detected in many tumor specimens and cell lines, including those of breast cancers, ovarian cancers, hepatocellular carcinomas, non-small cell lung carcinomas, and esophageal squamous cell carcinomas.[51–53,55,56] These transcripts have been associated with low expression of full-length WWOX mRNA, abnormal subcellular localization of WWOX protein, or advanced cancer stages.[30,51,56] Therefore, though it remains unclear how the aberrant transcripts are generated, they should contribute to the dysregulation of WWOX in cancer cells. Downregulation of WWOX in cancer cells can also be attributed to transcriptional silencing by epigenetic modification. For example, reduced WWOX expression has been associated with promoter hypermethylation in various cancer cells.[29,57] Treatment with a DNA demethylating reagent or a histone deacetylase inhibitor restores WWOX expression and WWOX-mediated tumor-suppressive effects on prostate cancer cells.[21] On the other hand, absence of WWOX protein in the presence of full-length WWOX mRNA has been observed in breast cancer cell lines and tumor tissues of cutaneous squamous cell carcinomas, suggesting a blockage of WWOX expression at the translational/post-transcriptional level.[20,51] In addition, loss of WWOX in cancer cells can be due to reduced protein stability. For prostate tumorigenesis, an activated tyrosine kinase Ack1 phosphorylates WWOX at tyrosine 287, resulting in WWOX polyubiquitination followed by accelerated WWOX degradation.[58]

While WWOX expression is frequently downregulated in cancer cells, there are still substantial cases showing the presence of wild-type WWOX protein in malignancies. A normal or even elevated level of WWOX expression has been reported in certain tumor tissues or cell lines of gastric carcinomas, prostate cancers, and breast cancers.[13,26,59] These observations raise a possibility that there are other strategies to antagonize WWOX's tumor suppressive functions. Some cellular proteins functionally inhibiting WWOX have been identified. Phosphorylated JNK interacts with Tyr33-phosphorylated WWOX and suppresses WWOX-mediated apoptosis.[60] Two PPXY-containing proteins, TMEM207 and COTE1, interact with WWOX and counteract WWOX-mediated inhibition of cell invasion.[36,37] Alteration of subcellular localization may be another way leading to dysfunction of WWOX. For example, overexpressed Zfra sequesters WWOX in the cytoplasm and blocks TNFα/UV light-induced nuclear translocation of WWOX, thus disturbing the proapoptotic functions of WWOX and p53.[61]

How oncogenic microbes deal with WWOX

The roles of WWOX in the cancers associated with infectious pathogens are less explored, but it is reasonably expected that the oncogenic microbes must deal with the tumor-suppressive functions of WWOX in the context of their infection-driven oncogenesis. As is illustrated in Figure 3, distinct mechanisms utilized by three cancer-associated microbes have been reported recently.

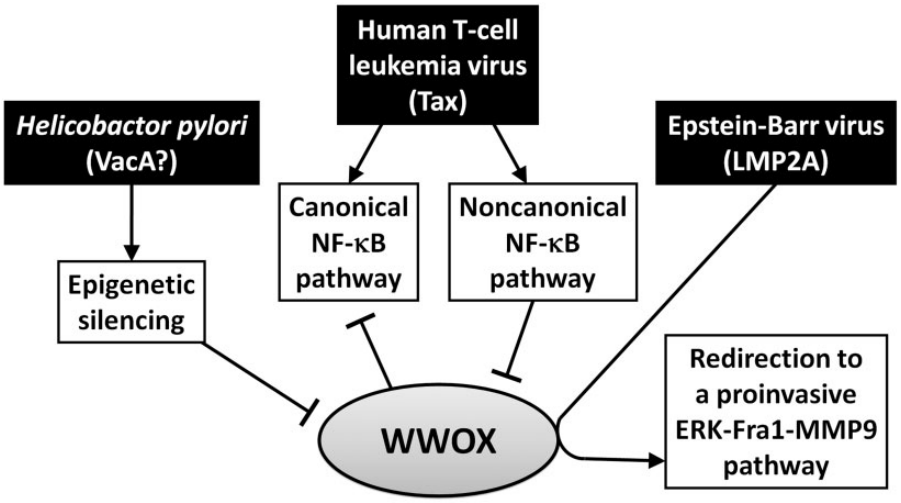

Figure 3 Oncogenic microbes use different strategies to deal with WWOX. *H. pylori* suppresses WWOX expression through epigenetic silencing. The HTLV-1 Tax-activated canonical NF-κB pathway is blocked by WWOX, while the Tax-activated non-canonical NF-κB pathway suppresses WWOX expression. EBV LMP2A interacts with WWOX and redirects WWOX's function to activation of a proinvasive ERK-Fra-1-MMP9 pathway

Epigenetic silencing of WWOX expression by Helicobacter pylori[10]

H. pylori infection is an etiologic factor for chronic gastritis, peptic ulcers, gastric adenocarcinomas, and gastric mucosa-associated lymphoid tissue lymphomas.[62] Gastric cancer cells infected with *H. pylori* or co-cultured with the bacterium-conditioned medium show alteration of cell morphology and reduced apoptosis.[63] These effects depend on VacA, a vacuolating toxin of *H. pylori*, as an isogenic strain losing the *vacA* gene exerts a mild influence on the cells. A cDNA microarray analysis reveals that *H. pylori* infection causes significant changes of expression of cellular genes involved in cytoskeleton, cell cycle, cell death, and proliferation. Among these genes, WWOX expression is dramatically decreased at 24 h postinfection.[63] Following a precedent that *H. pylori* induces promoter methylation and silencing of a tumor suppressor trefoil factor 2, the *H. pylori*-triggered downregulation of WWOX is subsequently found to be mediated by the similar epigenetic regulation.[64] *H. pylori* infection induces expression of DNA methyltransferases, DNMT1 and DNMT3A, concurrently with hypermethylation of the *WWOX* promoter and reduction of WWOX transcription.[10] In addition, *WWOX* gene hypermethylation is associated with *H. pylori* infection in primary gastric tumor tissues. Therefore, downregulation of WWOX by epigenetic silencing is a convergent anti-WWOX mechanism adapted by both *H. pylori* and microbe-free cancer cells.

Reciprocal counteraction between WWOX and Tax of human T-cell leukemia virus type 1 (HTLV-1)[11]

A relatively complex interaction is recognized between WWOX and HTLV-1, a retrovirus causing adult T-cell leukemia. HTLV-1 encodes an oncoprotein Tax, which triggers multiple signaling pathways, regulates expression of viral and cellular genes, and induces tumorigenesis in transgenic mice.[65] Tax activates both canonical and non-canonical NF-κB pathways, which are pivotal to Tax's oncogenic functions.[66–68] Notably, a recent study shows that WWOX is a negative regulator of Tax.[11] Knockdown of WWOX enhances, while overexpression of WWOX suppresses, the effects of Tax on cell transformation and tumorigenesis. It is further revealed that WWOX interacts with Tax, thus blocking the Tax-activated canonical NF-κB pathway without affecting the non-canonical NF-κB pathway. The underlying mechanism involves WWOX-mediated interference with Tax-induced recruitment of IKKα to the canonical NF-κB subunit RelA, thus inhibiting the phosphorylation of RelA at serine 536 and its activation. Interestingly, Tyr33-mutated WWOX retains the ability to bind to Tax but fails to block Tax-induced IKKα-RelA interaction, suggesting that the first WW domain may not mediate WWOX–Tax protein interaction but it contributes to the functional inhibition of Tax. Another note is that WWOX-mediated NF-κB inhibition is Tax specific, as WWOX does not affect the canonical NF-κB pathway activated by other stimuli such as TNFα. Further studies are required to identify the protein domains involved in WWOX–Tax interaction and the exact mechanisms through which WWOX interferes with the Tax-specific canonical NF-κB pathway.

To overcome WWOX's negative effect, Tax downregulates WWOX expression through activation of the non-canonical NF-κB pathway.[11] The Tax-driven, non-canonical NF-κB-mediated reduction of WWOX expression occurs at a transcriptional level, while WWOX protein is reduced by Tax more prominently. Tax fails to reduce WWOX expression in the cells with deficiency of the non-canonical NF-κB subunit p100/p52. Of note, the repression of WWOX expression by the non-canonical NF-κB pathway is not Tax specific, since the pathway activated by either p52 overexpression or CD40 signaling can also decrease WWOX expression.[11,69] The p52-mediated gene silencing is probably attributed to the fact that p52 is a DNA-binding protein lacking intrinsic transcriptional activity.[11] Considering that

hyperactivation of the non-canonical NF-κB pathway frequently occurs in various tumors, it should be a general mechanism to downregulate WWOX expression in cancer cells.[70]

Functional redirection of WWOX by latent membrane protein 2A (LMP2A) of Epstein–Barr virus (EBV)[12]

EBV is a herpesvirus associated with nasopharyngeal carcinomas and various B-cell lymphomas. LMP2A is an EBV oncoprotein contributing to growth, survival, and metastasis of tumor cells.[71,72] This membrane protein exerts oncogenic functions through triggering multiple signaling pathways.[73] The N-terminal intracellular domain of LMP2A, which is responsible for the signal-triggering events, contains two PPPPY (PY) motifs. We have found that, through the PY motifs, LMP2A activates extracelluar signal-regulated kinase (ERK) and a downstream AP-1 transcription factor Fra-1, thus upregulating matrix metalloproteinase 9 (MMP9) and MMP9-mediated cancer cell invasion.[74] As is somewhat unexpected, our recent study reveals that WWOX–LMP2A interaction is actually essential for the LMP2A-triggered ERK-Fra-1-MMP9 pathway.[12] Firstly, WWOX physically interacts with LMP2A, which requires Tyr33 in the first WW domain of WWOX and the PY motifs of LMP2A. Knockdown of endogenous WWOX by siRNA significantly inhibits the LMP2A-triggered ERK activation, Fra-1 induction, MMP9 production, and cell invasion. When endogenous WWOX is knocked down, the LMP2A-induced ERK-Fra-1-MMP9 event can be restored by exogenous wild-type WWOX but not by Tyr33-mutated WWOX. Moreover, the ERK-Fra-1-MMP9 pathway cannot be induced by WWOX overexpression in the absence of LMP2A, suggesting that WWOX positively mediates the signaling pathway only when it interacts with LMP2A.

Our data reflect that WWOX may be redirected by LMP2A to a novel role in activation of a proinvasive signaling pathway potentially linked to cancer metastasis, though the underlying mechanisms remain to be explored. In previous studies, under conditions without EBV LMP2A, WWOX exerts negative effects on the MEK1–ERK signaling pathway and cancer cell invasion.[9,19,36,50] As LMP2A serves as a signaling adaptor that recruits not only WWOX but also many other factors including protein kinases and ubiquitin ligases, it is likely that WWOX–LMP2A interaction leads WWOX to certain new partners and brings out new functions of WWOX.[75–78]

After literature reviewing, we notice more examples showing potentially opposite functions of WWOX and EBV LMP2A (Table 1). While WWOX is a proapoptotic tumor suppressor, LMP2A promotes cell survival and cell transformation.[21,27,29,35,71,79–81] Expression and activation of WWOX are positively correlated with normal keratinocyte differentiation, but LMP2A inhibits keratinocyte differentiation and contributes to an undifferentiated feature of nasopharyngeal carcinoma.[20,71,82] At a molecular level, both WWOX and LMP2A can bind to and stabilize ΔNp63α, a cellular oncoprotein driving cell proliferation and suppressing epithelial cell differentiation, but they cause opposite effects on ΔNp63α activity: inhibition by WWOX and activation by LMP2A.[34,83] As for oncogenic signaling transduction, WWOX blocks the Wnt/β-catenin pathway and the canonical NF-κB pathway, while both pathways can be activated by LMP2A.[11,49,80,81,84] Therefore, we expect that WWOX–LMP2A interaction may cause antagonism or redirection of WWOX's functions in many ways, potentially resulting in other impacts on cancer cells.

Table 1 Opposite biologic or molecular effects of WWOX and EBV LMP2A

	WWOX's effects	LMP2A's effects
Cell viability	Proapoptosis[21,27,29,35]	Prosurvival[71,79–81]
Cell invasion	Suppression[19,36]	Promotion[72,74]
Keratinocyte differentiation	Positive correlation[20]	Inhibition[71,82]
ΔNp63 activity	Inhibition[34]	Activation[83]
Wnt/β-catenin pathway	Inhibition[49]	Activation[84]
NF-κB pathway	Inhibition[11]	Activation[80,81]

Concluding remarks and perspectives

Clinical, biological, and molecular studies conclude the critical roles of WWOX in tumor suppression. Malignant cells must have strategies to withstand this cancer blocker. These strategies include downregulation of WWOX expression at multiple levels and inhibition of WWOX's functions through protein–protein interaction and/or subcellular mislocalization. An emerging study topic is how cancer-associated microbes deal with WWOX. Some oncogenic microbes may adapt the similar WWOX-targeting strategies used by microbe-free cancer cells. For example, *H. pylori* infection downregulates WWOX through promoter hypermethylation, an epigenetic mechanism known to suppress WWOX expression in many cancer cells. On the other hand, though WWOX has a potential to inhibit HTLV-1 Tax-triggered canonical NF-κB activation and tumorigenesis, Tax activates the non-canonical NF-κB pathway to successfully suppress WWOX expression. The non-canonical NF-κB-mediated WWOX downregulation is not only induced by this viral oncoprotein but may also be an anti-WWOX strategy shared with other cancers. In addition, EBV LMP2A evolves a novel strategy to redirect WWOX to a proinvasive signaling pathway. Since the N-terminal PPXY-containing domain of LMP2A mimics a cellular signaling adaptor, certain cellular oncoproteins may also twist WWOX's roles in a LMP2A-like manner.

We believe that the current reports about WWOX–microbe interaction are just "the tip of the iceberg." PPXY, LPXY, and other potential WWOX-binding motifs may exist in other microbial proteins, especially for those intracellular pathogens that closely interact with host cells. Dysregulation of WWOX at expressional or functional levels should be achieved by many cancer-associated pathogens in various ways, and further studies in this direction should expand our understanding of infection-driven oncogenesis. Meanwhile, the WWOX–microbe

interaction may not be restricted to cancer-related pathogens; the broad biologic functions of WWOX can be linked to host–microbe crosstalk in other ways. For example, PPXY-containing motifs also exist in two EBV proteins, BdRF1 (the internal scaffold protein) and BVRF2 (the maturational protease), which are essential for viral capsid assembly.[85] Whether WWOX binds to these viral proteins and is thus involved in the replicative stage of EBV is intriguing. We hope this review can trigger more study interests in the potential interactions between WWOX and microbial infection.

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